Reduced plant nutrition under elevated CO$_2$ depresses the immunocompetence of cotton bollworm against its endoparasite

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Estimating the immunocompetence of herbivore insects under elevated CO$_2$ is an important step in understanding the effects of elevated CO$_2$ on crop-herbivore-natural enemy interactions. Current study determined the effect of elevated CO$_2$ on the immune response of Helicoverpa armigera against its parasitoid Microplitis mediator. H. armigera were reared in growth chambers with ambient or elevated CO$_2$, and fed wheat grown in the concentration of CO$_2$ corresponding to their treatment levels. Our results showed that elevated CO$_2$ decreases the nutritional quality of wheat, and reduces the total hemocyte counts and impairs the capacity of hemocyte spreading of hemolymph of cotton bollworm larvae, fed wheat grown in the elevated CO$_2$, against its parasitoid; however, this effect was insufficient to change the development and parasitism traits of M. mediator. Our results suggested that lower plant nutritional quality under elevated CO$_2$ could decrease the immune response of herbivorous insects against their parasitoid natural enemies.

Global atmospheric concentration of CO$_2$ has increased from a pre-industrial value of 280 ppm to 396 ppm in 2013 (Mauna Loa Observatory; NOAA-ESRL), and are anticipated to double by the end of the 21$^{st}$ century. Elevated atmospheric CO$_2$ increases the photosynthetic rate, stimulating increases in biomass, yield, water content and carbon-to-nitrogen ratio (C: N) in most C$_3$ plants. Decreased foliar Nitrogen (N) and protein concentrations under elevated CO$_2$ reduce plant nutritional quality, diminishing the value of the foliage as a resource for insect herbivores. Most previous studies indicated that decreases in plant nutritional quality under elevated CO$_2$ result in increased development times, mortality and always associated with reduced food conversion efficiency, adult weight and population fitness of herbivore insects.

Decreased plant nutritional quality may affect the relationship between insects and their natural enemies or entomopathogens. Most previous reports have stated that plants decrease the protein concentration of their foliage in response to atmospheric CO$_2$ enrichment. Nevertheless, protein composition and content of plants tend to affect the immunocompetence of herbivorous insects in response to biotic stress. An increase in the proportion of protein in the diet of herbivorous insect larvae leads to an increase in their protein levels and improved immune defense in their hemolymph, such as melanization, phenoloxidase (PO) activity and antibacterial activity. However, the role of host plant nutrition in insect immunocompetence, which may alter the emergence of herbivorous insects, in the presence of their natural enemies under elevated CO$_2$ remains almost unexplored and requires further investigation.

Hemocytes play crucial roles in the immune response of insects against their parasites. Parasitoid eggs or larvae must avoid the immune responses of hemocyte to develop in the host larvae, and many species perform this by decreasing the total hemocyte count (THC), inhibiting hemocyte spreading and melanization of the host haemolymph. Additionally, Klemola et al. (2007) determined the strength of the immune response of autumnal moths, Epirrita autumnata (Borkhausen), by measuring their encapsulation rate to exposure to a foreign antigen and the PO activity of the pupal haemolymph. However, previous studies provided contradictory results, mainly due to the differences in methodology such as measuring a single-immune parameter rather than considering more immune parameters of the insect.
In this study, we investigated the immune response of *H. armigera* larvae to parasitization by *M. mediator* (Haliday) (Hymenoptera: Braconidae) under ambient and elevated CO2. We searched for potential variations in cellular and humoral immunocompetence in the hemolymph of cotton bollworm larvae during their development across different diets with altered nutritional quality. The solitary endoparasite, *M. mediator*, plays a key role in the natural control of cotton bollworm, which is a major agricultural pest worldwide\textsuperscript{21,22}. The bottom-up effect of plant quality on host-parasitoid immune responses was then evaluated using measures of cellular and humoral effectors. The main aims of the study were as follows: 1) to determine the immunocompetence of cotton bollworm larvae reared on wheat grown under elevated CO2 and 2) to better understand how altered plant nutritional quality and parasitization affect the immunocompetence of cotton bollworm larvae under elevated CO2.

**Results**

**Wheat ear quality.** Elevated CO2 reduces the nutritional quality of wheat grains, as a result of significant decreases in nitrogen (N) Content (F\(_{1, 8}\) = 6.283, \(P = 0.037\)), protein (F\(_{1, 8}\) = 9.207, \(P = 0.016\)) and total amino acids (F\(_{1, 8}\) = 8.368, \(P = 0.020\)) were found in wheat grains grown under elevated CO2. However, total non-structural carbohydrate (TNC) (F\(_{1, 6}\) = 13.95, \(P = 0.010\)) and TNC: N (F\(_{1, 6}\) = 20.88, \(P = 0.004\)) were increased (Fig. 1).

**Protein content of cotton bollworm larva hemolymph.** Elevated CO2 significantly increased the protein content of parasitized larvae hemolymph after 72 h (F\(_{1, 8}\) = 16.38, \(P = 0.004\)), but decreased protein content of unparasitized larvae after 96 h (F\(_{1, 8}\) = 8.251, \(P = 0.021\)). Parasitism significantly decreased the protein content of hemolymph in *H. armigera* larvae after 72 h (F\(_{1, 8}\) = 13.57, \(P = 0.006\)) and 96 h (F\(_{1, 8}\) = 12.60, \(P = 0.008\)) under ambient CO2 (Fig. 2). Sampling time significantly affected the protein content of hemolymph in *H. armigera* larvae (F\(_{3, 64}\) = 7.539, \(P < 0.001\)) (Table 1). Significant decreases were observed in the protein content of parasitized larvae hemolymph after 72 h and 96 h under ambient (F\(_{3, 16}\) = 8.742, \(P = 0.001\)) and elevated CO2 (F\(_{3, 16}\) = 3.476, \(P = 0.041\)), compared with 24 h and 48 h (Fig. 2).

![Figure 1](https://www.nature.com/scientificreports/4538) | The chemical composition of wheat grains grown under ambient CO2 (375 μL/L, open bars) and elevated CO2 (750 μL/L, closed bars). (a) Nitrogen content (mg g\(^{-1}\)), (b) Protein content (mg ml\(^{-1}\)), (c) Total non-structural carbohydrates (TNC) (mg g\(^{-1}\)), (d) The ratio of TNC: Nitrogen (%), (e) Total amino acid content (μmol ml\(^{-1}\)) and (f) The proportion of water in the wheat grain (%). Each value represents the mean (±SE). * indicates statistically significant differences (LSD test, \(P < 0.05\)), ** indicates statistically significant differences (LSD test, \(P < 0.001\)) and n.s. indicates no statistically significant difference.
parasitism significantly decreased the hemocyte spreading ratios of ambient and elevated CO2, compared with 24 h, 48 h, and 72 h (Table 1). Significant decreases were observed in the THC of parasitized insects after 72 and 96 h (F1, 22 = 5.205, P = 0.033). Sampling time significantly affected the hemocyte spreading ratios of hemoprophyl in H. armigera larvae (F1, 176 = 16.93, P < 0.001) (Table 1). Significantly higher spreading ratios were observed in the parasitized larvae hemolymph after 96 h than after 24 h and 48 h under ambient (F3, 44 = 17.07, P < 0.001) and elevated CO2 (F3, 44 = 12.96, P < 0.001) (Fig. 3B).

**Encapsulation ratio of cotton bollworm larvae hemolymph.** Elevated CO2 did not affect the encapsulation ratio of insects larvae (F1, 64 = 2.164, P = 0.146) (Table 1, Fig. 3C). Parasitism significantly decreased the encapsulation ratio after 24 and 72 h under ambient CO2 (F1, 10 = 5.295, P = 0.044 and F1, 10 = 18.22, P = 0.002, respectively) and after 72 and 96 h under elevated CO2 (F1, 10 = 5.603, P = 0.039 and F1, 10 = 7.780, P = 0.019, respectively) (Fig. 3C). Sampling time significantly affected the encapsulation ratio of hemoprophyl in H. armigera larvae (F5, 80 = 21.91, P < 0.001) (Table 1). Significantly higher encapsulation ratio were observed in the parasitized and unparasitized larvae hemolymph after 96 h than after 24 h, 48 h, and 72 h under ambient and elevated CO2 (Fig. 3C).

**Phenoloxidase activity of cotton bollworm larvae hemolymph.** Elevated CO2 did not affect the PO activity of insects larvae (F1, 64 = 2.164, P = 0.146) (Table 1, Fig. 4A). Parasitism significantly decreased PO activity during all measured time intervals under ambient CO2 (24 h: F1, 8 = 21.24, P = 0.002; 48 h: F1, 8 = 6.427, P = 0.035; 72 h: F1, 8 = 9.010, P = 0.017 and 96 h: F1, 8 = 5.910, P = 0.041, respectively) and after 48 and 72 h under elevated CO2 (F1, 8 = 12.34, P = 0.008 and F1, 8 = 5.447, P = 0.048, respectively) (Fig. 4A). Sampling time significantly affected the PO activity of hemoprophyl in H. armigera larvae (F3, 64 = 16.93, P < 0.001) (Table 1). Significantly higher PO activity were observed in the parasitized and unparasitized larvae hemolymph after 48 h, 72 h, and 96 h than after 24 h under ambient and elevated CO2 (Fig. 4A).

**Melanization ratio of cotton bollworm larvae hemolymph.** Elevated CO2 did not affect the melanization ratio of insects larvae (F1, 80 = 2.501, P = 0.118) (Table 1, Fig. 4B). Parasitism significantly decreased the melanization ratio of cotton bollworm larvae hemoprophyl during all measured time intervals under both ambient and elevated CO2 concentration (F1, 80 = 221.7, P < 0.001) (Table 1, Fig. 4B). Sampling time did not affect the melanization ratio of hemoprophyl in H. armigera larvae (F3, 80 = 1.938, P = 0.130) (Table 1).

**Development and parasitism traits of M. mediator.** 19 ± 3% and 26 ± 4% of H. armigera were parasitized by M. mediator in the ambient- and elevated-CO2 treatments, respectively. The emergency rate of parasitism, values of 72 ± 9% and 67 ± 5% were found for the

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**Table 1 | P values from ANOVA for the effect of CO2 level, time and parasitism on H. armigera**

| Measured indices | CO2 | Time | Para | CO2 × Time | CO2 × Para | Time × Para | CO2 × Time × Para |
|------------------|-----|------|------|------------|------------|-------------|------------------|
|                  | F   | P    | F    | <0.001    | 13.66      | 0.001       | 1.208           | 4.955            |
| Protein content  |     |      |      |           |            |             | 0.704            | 0.553            |
|                  | 0.028| 0.867| 7.539| <0.001    | 13.66      | 0.001       | 1.208           | 4.955            |
| Total hemocyte count | 0.762 | 0.384 | 21.17 | <0.001 | 31.13 | 0.001 | 3.781 | 6.944 | 0.009 | 0.792 | 0.500 | 2.118 | 0.100 |
| Hemocyte spreading ratio | 2.785 | 0.097 | 16.93 | <0.001 | 72.62 | <0.001 | 2.351 | 0.074 | 2.640 | 0.106 | 3.481 | 0.017 | 1.289 | 0.280 |
| Encapsulation ratio | 1.293 | 0.259 | 21.91 | <0.001 | 29.54 | <0.001 | 0.187 | 0.905 | 0.691 | 0.408 | 3.315 | 0.024 | 0.436 | 0.728 |
| Phenoloxidase activity | 2.164 | 0.146 | 14.38 | <0.001 | 40.97 | <0.001 | 0.240 | 0.868 | 0.916 | 0.342 | 0.990 | 0.403 | 1.437 | 0.240 |
| Melanization ratio | 2.501 | 0.118 | 1.938 | 0.130 | 221.7 | <0.001 | 0.614 | 0.608 | 0.027 | 0.870 | 1.700 | 0.174 | 1.534 | 0.212 |
ambient- and elevated-CO2 treatments, respectively. However, no significant differences related to the experimental conditions (CO2 levels) were found in the parasitism rate, emergence rate, cocoon weight, wasp weight, cocoon lifespan and wasp lifespan of M. mediator (P < 0.05, Fig. 5).

**Discussion**

Elevated CO2 alters the chemical composition of plant tissue. In response to atmospheric CO2 enrichment, most plants decrease the N and protein concentration of their larger foliage in order to sequester carbon, which, in turn, changes the synthesizes of nutrients and secondary metabolites in the plant. N is the most important limiting resource for herbivorous insects, and a decrease in the foliar N of host plants could limit insect growth and development, decrease the survival rates of phytophagous insects, and further depress the defense capability of herbivore insects against their natural enemies. European grape berry moths, Eupoecilia ambiguella, were
reared on five semi-artificial diets, and changes in the concentration of hemocytes and prophenoloxidase (PPO) activity were measured after a bacterial immune challenge. The result showed that the nutritional quality of diets significantly affected the immune defenses of the larvae. Dietary protein is an important determinant in the performance of herbivore insects. Ingested protein is digested into amino acids in the gut and finally absorbed into the hemolymph of herbivorous insects. Constitutive immune functions rely on the insect hemocyte and rapidly activated enzyme cascades such as PPO activity. The baseline of these immune effectors could be impaired when limited to protein-deficient food sources. Hence, herbivore insects reared on host plants differing in nutritional value are expected to differ in their baseline levels of constitutive defense. Here, in the absence of parasitism, decreased hemolymph protein concentration was detected in cotton bollworm after 96 h under elevated CO2, presumably due to the long-term feeding on a reduced protein diet. Contrarily, increased hemolymph protein concentration was detected in parasit-
tized cotton bollworms under elevated CO₂, most likely due to increased intake of protein by the caterpillars to compensate for the protein cost of resistance against their parasites\(^{12}\). Hemolymph protein concentration has been assessed as an indication of insect condition\(^{26}\). Altered hemolymph protein contents of herbivorous insects that were fed on plants grown under elevated CO₂ concentrations indicate that decreased protein concentrations in plants via "bottom-up" could have negative effects on herbivorous insects that have not been parasitized and could have positive effects at a higher level after parasitism by their natural enemies. Consistent with some previous studies, long-term feeding on plants with lower protein concentrations generates hypotrophic insect herbivores; however, the diet eaten after challenge by natural enemies can alter the likelihood of host development and the possible capacity of an insect to influence this likelihood by altering its diet to take in more protein\(^{12,26}\). All of changes mentioned above may result from intrinsic trade-offs in insects.

Reduced wheat nutritional quality due to elevated CO₂ concentration alters the cellular immune responses of cotton bollworm. Cellular responses in insects are mediated by the activity of circulating hemocytes, which participate in the encapsulation of parasite eggs and other invaders\(^{31}\). Alaix et al. (2010)\(^{14}\) indicated that hemocyte concentrations were increased in bees fed a diet without protein and further suggested that an investment in producing different types of hemocytes is costly, which would ultimately lead to an overall decrease in hemocyte numbers. In our study, we observed greater increases in THC and the spreading ability of hemocytes of cotton bollworm without parasites and fed on reduced quality wheat at the early sampled stage under elevated CO₂. Consistent with our results, autumnal moth larvae fed on poor quality food apparently suffered from moderate nutritional stress compared to larvae fed on higher quality food and then increased their immune defense to a higher level\(^{26}\). Accordingly, we suggest that unparasitized cotton bollworm larvae fed on wheat of decreased nutritional quality grown under elevated CO₂ apparently suffer from nutritional deficiency compared to larvae fed on grain grown under ambient CO₂ and may therefore exhibit a stronger immune defense. In addition, the THC and spreading ability of hemocytes were all greater at the early sampling stage under elevated CO₂, which suggests that the enhanced cellular immunocompetence is ephemeral. Longer sampling times may generate different results, and further research should be conducted on this in the future.

Most published work has shown that plant nutritional quality affects the capacity of herbivorous insect larvae to encapsulate abiotic (e.g., experimental glass needles, chromatography beads, and nylon threads) or biotic (e.g., insect eggs, larvae, and nematodes) agents\(^{10,32}\). After parasitization by \textit{M. mediator}, the encapsulation ability was decreased under elevated CO₂ during all measured time intervals, whereas the encapsulation ability was not significantly affected by decreases in the quality of wheat grown under the elevated CO₂ in this study. Different from our results, Laurent et al. (2012)\(^{26}\) showed that diet quality (increased catalpol concentrations) influences the encapsulation capability of \textit{Melittaea cynthia} to defend against parasitoids and pathogens. Clearly, plant nutritional traits including primary- and secondary-chemistry affect the immune responses of herbivore insects. Further research should be conducted on how secondary-chemistry of wheat grown under elevated CO₂ affects the immunocompetence of cotton bollworm.

Regardless of parasitization by \textit{M. mediator}, decreases in the nutritional quality of wheat grain grown under elevated CO₂ did not have a statistically significant effect on humoral immunity. We might have predicted that the greater number of hemocytes in cotton bollworm larvae that fed on higher quality food should have coincided with higher levels of PO activity because hemocytes produce some of the effector molecules used for humoral immunity including components of the PO cascade. However, we found that the baseline level of PO activity remained unchanged. Different from our results, several researches showed that individuals fed on poor-quality food exhibited higher PO activity than insects fed on higher quality food\(^{10,32}\). PO activity eventually leads to the production of melanin, a nitrogen-rich compound that may require substantial protein or nitrogen investment for its production\(^{14,34}\). Cuticular melanization are strongly dependent on the quantity of dietary protein ingested according to Lee et al. (2008)\(^{11}\). Their results implied that protein quality has a significant influence on the nitrogen pool that is potentially available for investment in melanization reaction. Based on their implications, depressed PO activity and impaired melanization reaction should be measured in our study. However, the lower N and protein contents of spring wheat grain grown under elevated CO₂ are insufficient to influence PO activity and the rate of melanization of the host hemolymph.

Altered plant nutrition under elevated CO₂ conditions affect the immune response of insect herbivores and further may influence natural enemy traits through "bottom up" effects. Several studies illustrate that plant quality can influence higher trophic levels in the same direction\(^{30-33}\), such that highly nutritional (or less defensive) plants increase the performance of both the insect herbivores and their natural enemies\(^{36}\). Other studies have shown opposite effects of plant quality on herbivorous insects and their natural enemies\(^{36,37}\); for example, nutrient deficiency and stress can reduce the general immunocompetence in insects against natural enemies\(^{36,37}\). Consistent with the above opposite effects, our results show that elevated CO₂ levels decrease the immunity of cotton bollworm larvae that have been parasitized with \textit{M. mediator} due to the reduced nutritional quality of the wheat. However, the lower immunocompetence of cotton bollworm did not change the development and parasitic traits of \textit{M. mediator}. Although decreased plant quality can in theory compromise the immune responses of herbivores and increase the fitness of parasites, poor food plant quality is a major constraint on the development of immature parasites\(^{30}\).

In summary, our study demonstrates that decreased plant quality weakens the immunocompetence of the cotton bollworm \textit{H. armiger} against its natural enemies (endoparasites) but is not sufficient to affect parasite emergence. The results of this study could have implications for the evolution of plant–herbivore–parasitoid interactions and emphasize the important role of the immune system and its variation based on host plant variation in bottom-up processes involving plants\(^{26}\). All in all, the role of elevated atmosphere CO₂ in the herbivore insect immune function is poorly known, definitely requiring further investigation.

**Methods**

\textbf{CO₂ concentration. Open-top Chamber.} This experiment was carried out using six octagonal open-top chambers (OTC), each 4.2 m in diameter, located at the Observation Station of the Global Change Biology Group, Institute of Zoology, Chinese Academy of Science (CAS) in Xiaotangshan County, Beijing, China (40°11′ N, 116°24′ E). The atmospheric CO₂ concentrations used were 1) current ambient CO₂ levels (375 ± 9 μL/L) and double the current ambient CO₂ levels (750 ± 15 μL/L) for the two elevated CO₂ treatments. During the period from seedling emergence to harvest, CO₂ concentrations were monitored continuously using an infrared CO₂ analyzer (Vontestat 8102, Telaire Company, USA) and adjusted every twenty minutes to maintain the assigned CO₂ concentrations. The automatic control system used to adjust the levels of CO₂ and the specifications for the open-top chambers are detailed in Chen et al. (2005)\(^{30}\).

\textit{Closed-dynamics CO₂ Chamber.} Insects were reared in a growth chamber (HPG280H; Orient Electronic, Harbin, China). Growth chamber conditions were maintained at 25 ± 1 °C, 60–70% relative humidity, a photoperiod of 14:10 (hours of light: hours of dark), and an active radiation of 9,000 lux (supplied by 1,260 W fluorescent lamps in each chamber). Two atmospheric CO₂ concentrations (current ambient CO₂ levels (375 ± 9 μL/L) and double the current ambient CO₂ levels (750 ± 15 μL/L)) were maintained to match the OTCs used for wheat growth. Three chambers were used for each CO₂ treatment. As previously mentioned, CO₂ concentrations were automatically monitored using an infrared CO₂ analyzer (Vontestat 8102; Telaire, Goleta, CA, USA) and adjusted. A detailed explanation of the methodology employed in the
automatic control system for maintaining and adjusting the CO₂ concentrations is described in Chen & Ge (2004)⁴⁹.

Wheat variety and growth conditions. Spring wheat (Longfu174379 variety) was sown in plastic pots (height: 35 cm, diameter: 45 cm), in the six open-top chambers previously mentioned. Thirty-five pots were placed in each OTC. Pot placement was re-randomized in each OTC weekly. Pure CO₂ was mixed with ambient air and supplied to each chamber by a CO₂ concentrator, corresponding to their treatment level. During the milky-grain stage of spring wheat, ears and grains were harvested from all six OTGs and then refrigerated at −20°C until supplied to H. armigera as food.

Insect stocks. H. armigera egg masses were obtained from a laboratory colony maintained by the Insect Physiology Laboratory, Institute of Zoology at CAS, and reared using wheat milky grains in a growth chamber. The temperature in each chamber was maintained at 25 ± 1°C, the relative humidity was 70 ± 10%, and the photoperiod/scotophase ratio was 14:10 (hours of light: hours of dark).

M. mediator specimens were obtained from the Plant Protection Institute (PPI) of Hebei Academy of Agricultural and Forestry Sciences and were reared in a growth chamber using 15% hydromel (a fermentation of honey) as a food source. Growth chamber conditions were maintained exactly as described above.

Insect feeding. Elevated CO₂ had little direct effect on cotton bollworms when fed on artificial diet. Accordingly, H. armigera larvae were reared in growth chambers with ambient or elevated CO₂ concentration, corresponding to their treatment level. Within each CO₂ concentration treatment group, larvae that reached the third larval instar were randomly divided into two lots. In the first lot, larvae were used to test chemical composition assay of wheat grains as food. The resulting drop of undiluted hemolymph was left for 20 min at ambient temperature. A change in color of the hemolymph from opaque or green to brown-black, was recorded as normal melanization, whereas the maintenance of the previous color or a change to an intermediate color was considered to reflect inhibition of melanization⁴⁹. The melanization ratio of hemocyte was calculated as follows: % melanization = (number of melanization reactions of larval hemolymph observed)/(total number of larval hemolymph sampled) × 100.

**Phenoloxidase activity.** PO activity was assayed spectrophotometrically by using L-dihydroxyphenylalanine (L-DOPA) as a substrate. 50 μl of larval hemolymph (sampled at 24, 48, 72 and 96 h post parasitization) were pre-incubated with an equal volume of the activator (1 mg/ml trypsin, 0.5 mg/ml laminarin) or for the controls, with 0.01 M sodium cacodylate buffer pH 7.7 containing 5 mM calcium chloride and 0.25 M sucrose) for 1 h at 20°C before adding 50 μl L-DOPA (3 g/L). The reaction was allowed to proceed for 10 min at 20°C. Enzyme activity was expressed as the change in absorbance at 490 nm per min and per mg protein (Huang et al. 2009)⁴⁹.

The protein concentration of H. armigera larval hemolymph was measured using the Bradford method⁴⁹, with a standard curve created from a bovine serum albumin standard.

In vitro melanization reaction. To determine the capacity of the melanization reaction in the hemolymph of the host, host larvae were selected at designated times post parasitization or control under both CO₂ levels. Hemolymph samples from larvae were collected on a glass slide by puncturing the larval proleg with a sterile insect pin. The resulting drop of undiluted hemolymph was left for 20 min at ambient temperature. A change in color of the hemolymph from opaque or green to brown-black, was recorded as normal melanization, whereas the maintenance of the previous color or a change to an intermediate color was considered to reflect inhibition of melanization⁴⁹. The melanization ratio of hemocyte was calculated as follows: % melanization = (number of melanization reactions of larval hemolymph observed)/(total number of larval hemolymph sampled) × 100.

Data analysis. One-way analysis of variance (ANOVA) tests (SPSS 17.0 for Windows; SPSS, Chicago, IL, USA) were used to analyze the effects of elevated CO₂ on the chemical composition of spring wheat grains. Differences between means were compared using the Least Significant Difference (LSD) test. The data for parasitism rate and cocoon rate of M. mediator, as well as the emergence rate, weight and adult longevity of M. mediator were also analyzed following the method described above.

The THC, hemocyte spreading ratios, encapsulation ratios, PO activity, melanization ratios and the protein concentration of H. armigera larval hemolymph were factors analyzed by ANOVA with CO₂ levels as the main factor and designated times as sub-factors deployed in a split-plot design. The differences between means were determined using LSD test.

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F.G. and J.Y. designed the experiment. J.Y. and Y.S. performed the experiment. J.Y., Y.S. and F.G. wrote the paper.

Additional information
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