Influence of elevated CO$_2$ on development and food utilization of armyworm *Mythimna separata* fed on transgenic *Bt* maize infected by nitrogen-fixing bacteria

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**ABSTRACT**

**Background:** *Bt* crops will face a new ecological risk of reduced effectiveness against target-insect pests owing to the general decrease in exogenous-toxin content in *Bt* crops grown under elevated carbon dioxide (CO$_2$). The method chosen to deal with this issue may affect the sustainability of transgenic crops as an effective pest management tool, especially under future atmospheric CO$_2$ level raising.

**Methods:** In this study, rhizobacteria, as being one potential biological regulator to enhance nitrogen utilization efficiency of crops, was selected and the effects of *Bt* maize (Line IE09S034 with *Cry1Ie* vs. its parental line of non-*Bt* maize Xianyu 335) infected by *Azospirillum brasilense* (AB) and *Azotobacter chroococcum* (AC) on the development and food utilization of the target *Mythimna separata* under ambient and double-ambient CO$_2$ in open-top chambers from 2016 to 2017.

**Results:** The results indicated that rhizobacteria infection significantly increased the larval life-span, pupal duration, relative consumption rate and approximate digestibility of *M. separata*, and significantly decreased the pupation rate, pupal weight, adult longevity, fecundity, relative growth rate, efficiency of conversion of digested food and efficiency of conversion of ingested food of *M. separata* fed on *Bt* maize, while here were opposite trends in development and food utilization of *M. separata* fed on non-*Bt* maize infected with AB and AC compared with the control buffer in 2016 and 2017 regardless of CO$_2$ level.

**Discussion:** Simultaneously, elevated CO$_2$ and *Bt* maize both had negative influence on the development and food utilization of *M. separata*. Presumably, CO$_2$ concentration arising in future significantly can increase their intake of food and harm to maize crop; however, *Bt* maize infected with rhizobacteria can reduce the field hazards from *M. separata* and the application of rhizobacteria infection can enhance the resistance of *Bt* maize against target lepidoptera pests especially under elevated CO$_2$.

**INTRODUCTION**

With increased fossil fuel combustion and drastic changes in land utilization, the concentration of atmospheric carbon dioxide (CO$_2$) has increased by more than 40%,
from 280 to 400 ppm, between the industrial revolution and now (Ciais et al., 2013). The recent forecast indicated that atmospheric CO$_2$ concentration will increase to approximately 900 ppm by 2100 (Intergovernmental Panel on Climate Change (IPCC) 2014). Increasing atmospheric CO$_2$ concentration alone can be very significant in crop production because of its direct effect on plant physiology and biochemistry (Cornelissen, 2011), and indirect effect on tri-trophic interactions involving plants, herbivores, and predators or pathogens (Robinson, Ryan & Newman, 2012; Trębicki et al., 2017). Elevated atmospheric CO$_2$ also affects the crop production via direct or indirect impact on the physiology and feeding behavior of phytophagous insects (Zvereva & Kozlov, 2006; Massad & Dyer, 2010; O’Neill et al., 2010). These changes may then lead to more severe and frequent outbreaks of pest insects in agricultural ecosystems (Percy et al., 2002).

Several studies have shown that the elevated CO$_2$ increased lepidopteran insect feeding and damage severity in agricultural crops (Ainsworth et al., 2007; Lindroth et al., 2001), because of the increased proportion of C:N in host plant tissue and lower nutritional quality caused by elevated CO$_2$ (Ainsworth et al., 2007). For example, larvae of Helicoverpa armigera fed on wheat grown in elevated CO$_2$ showed the extended larval life span and increased consumption with reduced growth rate (Chen, Wu & Ge, 2004). Transgenic maize that expresses insecticidal Cry proteins derived from the soil bacterium Bacillus thuringiensis Berliger (Bt) has been used to control target lepidopteran insects (Carrière, Crowder & Tabashnik, 2010; Huang et al., 2014; Walters et al., 2010), e.g., European corn borer Ostrinia nubilalis (Hubner), Asian corn borer O. furnacalis (Guenée) (Lepidoptera: Crambidae) and corn armyworm Mythimna separata (Lepidoptera: Noctuidae) (Guo et al., 2016; Zhang et al., 2013; Jia et al., 2016). Transgenic Bt maize has widely been adopted worldwide (Cattaneo et al., 2006; Huang et al., 2005; Hutchison et al., 2010; Lu et al., 2012). It was anticipated that the primary effect of elevated CO$_2$ on Bt toxin production would be due to differences in N concentration in plant tissues (Coviella, Stipanovic & Trumble, 2002). Biologically relevant changes in plant defensive chemistry of Bt maize are expected to have measurable effects on the target lepidopteran pests under climate change.

Additionally, many researchers found that the nitrogen metabolism of transgenic Bt crops could affect the expression of Bt toxin protein, and stimulating plant N uptake to increase in biomass N relative to C to increase the nitrate reductase activity and Bt toxin production of Bt crops. (Stitt & Krapp, 1999; Pang et al., 2005; Gao et al., 2009). Nitrogen plays the most important role for plant growth, and it is an important complement of enzymes catalyzing and controlling reactions in plants for normal physiological processes (Richardson et al., 2009). While most of nitrogen in the environment is found in a form of nitrogen gas (N$_2$) which approximately amounts to 78% in the atmosphere, plant available nitrogen found in soil is generally derived from fertilizer augmentation. As plants cannot use N$_2$ directly, soil-inhabiting microbes play a significant role in nitrogen uptake by plants as they change the N$_2$ into ammonia (Yamprai, Mala & Sinma, 2014). Azospirillum sp. and Azotobacter sp. are the two major free-living soil microbes (Biari, Gholami & Rahmani, 2008), that are economically important nitrogen-fixing bacteria in maize crop production system.
Thus, optimization of soil-nitrogen management offers significant potential in the utilization of soil rhizobacteria to increase Bt-crop nitrogen utilization to affect the expression of Bt toxin under elevated CO₂.

**MATERIALS AND METHODS**

**Setup of CO₂ levels**

A two-year study (2016–2017) was conducted in six open-top chambers (i.e., OTCs; Granted Patent: ZL201120042889.1; 2.5 m in height × 3.2 m in diameter) (Chen et al., 2011) at the Innovation Research Platforms for Climate Change, Biodiversity and Pest Management (CCBPM; http://www.ccbpm.org) field laboratory in Ningjin County, Shandong Province of China (37°38’ 30.7” N, 116°51’ 11.0” E). A total of two CO₂ levels, ambient (375 µL/L, hereafter referred to as aCO₂) and elevated (750 µL/L or double-ambient, hereafter referred to as eCO₂) were applied continuously from 10 June to 7 October in both years. A total of three OTCs were used for each CO₂ treatment, and the CO₂ concentrations in each OTC were monitored continuously and adjusted using an infrared CO₂ analyzer (Ventostat 8102; Telaire Company, Goleta, CA, USA). The OTCs of elevated CO₂ treatments were inflated with canned CO₂ gas with 95% purity and automatically controlled by the same type of infrared CO₂ analyzer (Chen, Wu & Ge, 2004). Actual mean CO₂ concentrations and temperature throughout the entire experiment for both 2016 and 2017 are provided in Table 1.

**Plant materials**

The Bt maize cultivar (Line IE09S034, hereafter referred to as Bt) and its non-Bt parental line (cv. Xianyu 335, referred to as Xy) were both obtained from the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. Both Bt and non-Bt lines used in this study had the similar maturity (approximately 102 d: from 10 June to 20 September) and were well adapted to the growing conditions of northern China (Guo et al., 2016; Zhang et al., 2013; Jia et al., 2016; Ling, 2010). Both maize accessions were planted in plastic buckets (diameter × height = 30 × 45 cm) filled with 20 kg autoclaved soil and

| Climate factors | OTC 2016 | OTC 2017 | Two-way ANOVAs (F/P values) |
|-----------------|----------|----------|----------------------------|
| CO₂ (µL/L)      | aCO₂ OTC | 744.4 ± 3.3<sup>b</sup> | 748.8 ± 4.5<sup>b</sup> | F<sub>CO₂</sub> = 399.32, P = 0.000 |
|                 | eCO₂ OTC | 372.6 ± 4.7<sup>a</sup> | 374.5 ± 3.8<sup>a</sup> | F<sub>Year</sub> = 2.85, P = 0.13 |
|                 |          | F<sub>Interaction</sub> = 0.45, P = 0.52 |
| Temperature (°C) | aCO₂ OTC | 26.01 ± 0.5<sup>a</sup> | 26.12 ± 0.3<sup>a</sup> | F<sub>CO₂</sub> = 0.006, P = 0.94 |
|                 | eCO₂ OTC | 25.99 ± 0.4<sup>a</sup> | 26.11 ± 0.4<sup>a</sup> | F<sub>Year</sub> = 3.38, P = 0.10 |
|                 |          | F<sub>Interaction</sub> = 0.057, P = 0.82 |

Notes:

OTC: ambient-CO₂ OTC (aCO₂ OTC) and elevated-CO₂ OTC (eCO₂ OTC). Different lowercase letters indicate significantly different between the eCO₂ OTC and aCO₂ OTC in same year by the Duncan test at P < 0.05, respectively. Not significantly different between 2016 and 2017 at same CO₂ level or temperature by the Duncan test at P > 0.05, respectively.
10 g compound fertilizer (N:P:K = 18:15:12), then placed them into chambers on 10 June each year.

**Soil nitrogen-fixing bacteria and infection of maize seeds**

Lyophilized *Azospirillum brasilense* (strain number ACCC 10103) and *Azotobacter chroococcum* (strain number ACCC 10006) were provided by Agriculture Culture Collection of China (ACCC) in plastic tubes (3 cm in diameter and 15 cm in height) with bacterial growth medium. Both species of rhizobacteria were grown in liquid medium at 28 °C under continuous shaking (200 rpm) until they reached an absorbance of 1.008 (*A. brasilense*) and 1.005 (*A. chroococcum*) at a wavelength of 600 nm. Before inoculation, the culture was centrifuged, and the supernatant was discarded, and the pellet of cells was re-suspended in the liquid medium to a density of $10^8$ copies per milliliter. The seeds of both Bt and non-Bt maize were infected with *A. brasilense* and *A. chroococcum* cultures each, and the inoculation doses were all adjusted to a final volume of 10 ml for each seed. After inoculation, all the treated seeds were maintained under sterile laminar air flow for 2 h at 28 °C (*Cassán et al., 2009*). Bacteria inoculation treatments consisted of three types of rhizobacteria infection, including (1) seeds infected with *A. brasilense* (referred to as AB); (2) seeds infected with *A. chroococcum* (referred to as AC); and (3) non-infected seeds (control) treated with a final volume of buffer solution (referred to as CK). The entire experiment, thus, consisted of 12 treatments, including two CO$_2$ levels (aCO$_2$ and eCO$_2$), two maize cultivars (Bt and Xy), and three rhizobacteria infections (AB, AC, and CK), replicate six time. Each pot serves as one replication. Specifically, six buckets for each maize cultivar (Bt and Xy) and three rhizobacteria inoculations (6 buckets per transgenic treatment × 2 transgenic treatments × 3 inoculation treatments = 36 buckets) were placed randomly in each CO$_2$ chamber (ambient and double-ambient CO$_2$), and three maize seeds were sown in each bucket at 2 cm soil depth. No pesticides were applied during the entire experimental period and the manual weeding keep the maize buckets weed-free during the experiment. The rhizosphere soil was sampled from each bucket one-day before planting, 14 days after planting, and at harvest and measured the relative density of *A. brasilense* and *A. chroococcum* using RT-PCR (Tables 2 and 3) (*Jiang et al., 2017*).

**Insect source and rearing**

The colony of armyworm *M. separata* was originated from a population collected in maize fields in Kangbao County, Hebei province of China (41.87°N, 114.6°E) in the
Table 3  The rhizosphere soil densities of rhizobacteria inoculated in the potted soil of transgenic Bt maize and its parental line of non-Bt maize grown under ambient and elevated CO₂ in 2016 and 2017.

| Measure matters                                       | Rhizobacteria infections | 2016 (AB; AC copies/g) | 2017 (AB; AC copies/g) |
|-------------------------------------------------------|--------------------------|-------------------------|-------------------------|
| Sampled soil before maize planting                    | AB           | aCO₂-Bt 8.46 ± 0.24 10¹¹; 4.48 ± 0.26 10⁵ | 8.40 ± 0.28 10¹¹; 4.44 ± 0.11 10⁵ |
|                                                      | AB           | aCO₂-Xy 8.25 ± 0.26 10¹¹; 4.21 ± 0.08 10⁵ | 8.69 ± 0.23 10¹¹; 4.56 ± 0.22 10⁵ |
|                                                      | AC           | aCO₂-Bt 8.36 ± 0.19 10¹¹; 4.43 ± 0.15 10⁵ | 8.59 ± 0.21 10¹¹; 4.47 ± 0.17 10⁵ |
|                                                      | AC           | aCO₂-Xy 8.70 ± 0.27 10¹¹; 4.58 ± 0.29 10⁵ | 8.24 ± 0.12 10¹¹; 4.34 ± 0.27 10⁵ |
| Sampled soil at the maize seedling after 14 days      | AB           | aCO₂-Bt 5.54 ± 0.25 10⁵; 7.37 ± 0.29 10¹¹ | 5.70 ± 0.28 10⁵; 7.40 ± 0.26 10¹¹ |
|                                                      | AB           | aCO₂-Xy 5.73 ± 0.24 10⁵; 7.29 ± 0.17 10¹¹ | 5.36 ± 0.22 10⁵; 7.66 ± 0.25 10¹¹ |
|                                                      | AC           | aCO₂-Bt 5.62 ± 0.30 10⁵; 7.71 ± 0.15 10¹¹ | 5.13 ± 0.04 10⁵; 7.32 ± 0.13 10¹¹ |
|                                                      | AC           | aCO₂-Xy 5.46 ± 0.28 10⁵; 7.59 ± 0.17 10¹¹ | 5.42 ± 0.13 10⁵; 7.57 ± 0.22 10¹¹ |
| Sampled soil at the maize harvest                     | AB           | aCO₂-Bt 5.71 ± 0.20 10⁵; 4.52 ± 0.21 10⁵ | 5.92 ± 0.08 10⁵; 4.67 ± 0.17 10⁵ |
|                                                      | AB           | aCO₂-Xy 5.50 ± 0.29 10⁵; 4.24 ± 0.15 10⁵ | 5.33 ± 0.18 10⁵; 4.31 ± 0.13 10⁵ |
|                                                      | AC           | aCO₂-Bt 5.46 ± 0.08 10⁵; 4.26 ± 0.18 10⁵ | 5.62 ± 0.31 10⁵; 4.48 ± 0.21 10⁵ |
|                                                      | AC           | aCO₂-Xy 5.46 ± 0.18 10⁵; 4.76 ± 0.23 10⁵ | 5.47 ± 0.17 10⁵; 4.21 ± 0.09 10⁵ |

Note:
Rhizobacteria infections: A. brasilense (AB) and A. chroococcum (AC) vs. the control buffer solution (CK). CO₂ levels: ambient CO₂ (aCO₂) and elevated CO₂ (eCO₂). Transgenic treatment: Bt maize (Bt) and non-Bt maize (Xy). Different lowercase letters indicate significantly different between ambient CO₂ and elevated CO₂ for same maize cultivar in same year by the Duncan test at P < 0.05, respectively.

summer of 2014, and fed on artificial diet and maintained for more than 10 generations in climate-controlled growth chambers (GDN-400D-4; Ningbo Southeast Instrument Co., Ltd., Ningbo, China) at 26 ± 1 °C, 65 ± 5% RH, and 14: 10 h L/D photoperiod. The same rearing conditions were maintained for the following experiments. Newly-hatched larvae were randomly selected from the above colony of M. separata and fed on artificial diet (Bi, 1981) until the second instar larvae, and then the third instar larvae were individually fed on excised leaves of the experimental plants growing in CO₂ chambers. Feeding trials were conducted in plastic dish (6 cm in diameter and 1.6 cm in height) and the experimental leaves were randomly selected from six buckets for each of the 12 experimental treatment combinations (2 transgenic treatments × 2 CO₂ treatments × 3 bacteria inoculations) during the tasseling stage until pupation. Sample
size for the *M. separata* larval feeding trial consisted of 20 larvae (sample unit size) with five replicates for each of the 12 treatment combinations (i.e., 1,200 larvae evaluated for the entire study). Because of the cannibalism among the late instar larvae of *M. separata* (Jiang et al., 2016; Ali et al., 2016; Liu et al., 2017), the sampled larvae were reared separately in the Petri dish until pupation.

**Development and reproduction of *M. separata***

Larval development was evaluated from third instar to pupation by way of observing each individual petri dish every 8 h and recording the timing of larval ecdysis, pupation, and emergence of *M. separata* moths. After eclosion, the newly emerged moths were paired (female: male = 1:1) for mating in a metal frame screen cage (length × width × height = 35 × 35 × 40 cm), and the paired moths were fed with a 10% honey solution provided on a large cotton wick in a single plastic cup (diameter × height = 8 × 20 cm) covered with cotton net yarn butter paper for oviposition. The cotton net yarn and butter paper were replaced every day. Moth survivorship and oviposition were recorded daily until both moths from each pair died.

**Food utilization of the larvae of *M. separata***

Each third instar test larvae of *M. separata* was weighed at the initiation of the feeding trial by using an electronic balance (AL104; METTLER-TOLEDO, Greifensee, Switzerland). Total accumulated feces from third instar until the larva entered pupal stage (sixth instar), sixth instar larval weight, and the remaining leaves were also weighed. The food utilization indices of *M. separata* included the relative growth rate (RGR), relative consumption rate (RCR), approximate digestibility (AD), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) (Chen, Ge & Parajulee, 2005a; Chen et al., 2005b). Formulas for calculation of the measured indices were adapted from Chen et al. (2005b).

**Data analysis**

All data were analyzed using the statistical software SPSS 19.0 (2015; SPSS Institute, Chicago, IL, USA). Four-way analysis of variance was used to analyze the effects of CO₂ levels (elevated vs. ambient), transgenic treatment (*Bt* maize vs. non-*Bt* maize), rhizobacteria infection (AB and AC vs. CK), sampling years (2016 vs. 2017), and the interactions on the measured indices of growth, development, and reproduction, including larval life-span, pupation rate, pupal weight, pupal duration, adult longevity and fecundity of *M. separata*. The measured food utilization indices were analyzed by using an analysis of covariance with initial weight of *M. separata* (i.e., third instar larva) as a covariate for RCR and RGR, while food consumption was a covariate for ECI and AD to correct the effect of variation in the growth and food assimilation of *M. separata* (Raubenheimer & Simpson, 1992); food assimilated was also used as a covariate to analyze the ECD parameter (Hägele & Rowell-Rahier, 1999). The assumption of a parallel slope between covariate and dependent variable was satisfied for each analysis. Treatment means were separated by using the Duncan-test to examine significant difference at *P* < 0.05.
RESULTS

Effects of CO$_2$ level, transgenic treatment, and rhizobacteria infection on the rhizosphere soil densities of *A. brasilense* and *A. chroococcum* in different sampling period

Significant effects of rhizobacteria infection ($P < 0.001$) were observed on the measured rhizosphere soil densities of both *A. brasilense* (AB) and *A. chroococcum* (AC) 14 days after maize planting. Compared with ambient CO$_2$, elevated CO$_2$ significantly increased the rhizosphere soil densities of both *A. brasilense* and *A. chroococcum*; compared with the control buffer solution (CK), rhizobacteria infection significantly increased the rhizosphere soil densities of both *A. brasilense* and *A. chroococcum* (Table 3). CO$_2$ level and rhizobacteria infection both significantly affected the densities of *A. brasilense* and *A. chroococcum* in rhizosphere soil at maize harvest (Table 4).

### Effects of CO$_2$ level, transgenic treatment, and rhizobacteria infection on the development and reproduction of *M. separata*

Carbon dioxide level and transgenic treatment both significantly affected the larval life-span, pupation rate, pupal weight and duration, adult longevity, and fecundity in *M. separata* fed on both *Bt* and non-*Bt* maize infected with *A. brasilense* and *A. chroococcum*.

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Table 4: Four-way ANOVA on the rhizosphere soil densities of rhizobacteria inoculated in the potted soil of *Bt* maize and its parental line of non-*Bt* maize grown under ambient and elevated CO$_2$ in 2016 and 2017.

| Impact factors | Sampled soil at the maize seedling after 14 days | Sampled soil at the maize harvest |
|----------------|-----------------------------------------------|----------------------------------|
|                | AB                             | AC                             | AB                             | AC                             |
| Y$^a$          | 0.00/0.99                      | 0.002/0.96                     | 0.97/0.33                      | 1.13/0.29                      |
| Cv.$^b$        | 0.32/0.574                     | 0.36/0.55                      | 0.070/0.79                     | 0.080/0.78                     |
| CO$_2$.$^c$    | 0.19/0.89                      | 0.80/0.38                      | 26.01/0.001***                 | 331.16/0.001***                |
| Rhizobacteria.$^d$ | 2555.00/0.0001***           | 1380.37/0.001***              | 1311.83/0.001***              | 2080.71/0.001***               |
| Y × Cv.        | 0.62/0.44                      | 1.96/0.17                      | 0.18/0.673                     | 0.53/0.47                      |
| Y × CO$_2$     | 1.21/0.28                      | 2.50/0.12                      | 0.74/0.40                      | 0.032/0.86                     |
| Y × Rhizobacteria | 0.00/1.00                      | 0.02/0.99                      | 0.97/0.39                      | 1.13/0.33                      |
| Cv. × CO$_2$   | 0.35/0.55                      | 0.010/0.92                     | 0.32/0.57                      | 0.36/0.55                      |
| Cv. × Rhizobacteria | 0.32/0.73                      | 0.36/0.70                      | 0.070/0.93                     | 0.80/0.93                      |
| CO$_2$ × Rhizobacteria | 0.019/0.98                     | 0.80/0.45                      | 26.01/0.001***                 | 331.16/0.001***                |
| Y × Cv. × CO$_2$ | 5.99/0.018*                    | 0.06/0.94                      | 0.82/0.37                      | 0.63/0.43                      |
| Y × Cv. × Rhizobacteria | 0.62/0.54                      | 1.96/0.15                      | 0.18/0.84                      | 0.53/0.59                      |
| Y × CO$_2$ × Rhizobacteria | 1.21/0.31                      | 2.50/0.093                     | 0.74/0.49                      | 0.032/0.97                     |
| Cv. × CO$_2$ × Rhizobacteria | 0.35/0.70                      | 0.10/0.99                      | 0.32/0.73                      | 0.36/0.70                      |
| Y × Cv. × CO$_2$ × Rhizobacteria | 5.99/0.05                      | 0.006/0.99                     | 0.82/0.45                      | 0.63/0.54                      |

Notes:

* P < 0.05;
** P < 0.01;
*** P < 0.001.

$^a$ Year (2016 vs. 2017).

$^b$ Transgenic treatment (*Bt* maize vs. non-*Bt* maize).

$^c$ CO$_2$ levels (elevated CO$_2$ vs. ambient CO$_2$).

$^d$ Rhizobacteria infection (*A. brasilense* and *A. chroococcum* vs. the control buffer), the same as in Tables 5 and 7.
A. chroococcum (P < 0.001). However, the rhizobacteria infection significantly affected the larval life-span, pupal duration (P < 0.05) and fecundity (P < 0.001) of M. separata fed on both transgenic treatments and at both CO₂ levels (Table 5).

Compared with ambient CO₂, elevated CO₂ significantly prolonged the larval life-span (+6.21%), pupal duration (+5.56%), and significantly decreased the pupation rate (Table 8).
Table 7 Four-way ANCOVA on the food utilization indices of Mythimna separata fed on Bt maize and non-Bt maize infected with A. brasilense and A. chroococcum under ambient and elevated CO2 in 2016 and 2017.

| Impact factors | The 3rd to 6th instar larvae ($n = 828$) |
|----------------|------------------------------------------|
|                | RGR (mg g$^{-1}$ day$^{-1}$) | RCR (mg g$^{-1}$ day$^{-1}$) | AD (%) | ECD (%) | ECI (%) |
| Covariate$^e$  | 1.81/0.11 | 3.83/0.067 | 0.781/0.23 | 0.580/0.41 | 0.87/0.32 |
| Y$^a$          | 1.19/0.31 | 3.96/0.12 | 4.56/0.061 | 4.82/0.059 | 5.80/0.053 |
| Cv.$^b$        | 1545.53/0.001*** | 302.67/0.001*** | 185.62/0.001*** | 716.17/0.001*** | 1038.95/0.001*** |
| CO2$^c$        | 67.09/0.001*** | 27.98/0.001*** | 9.69/0.003** | 35.90/0.001*** | 57.98/0.001*** |
| Rhizobacteria$^d$ | 12.26/0.001*** | 26.84/0.001*** | 35.28/0.001*** | 13.64/0.001*** | 7.22/0.002** |
| Y × Cv.        | 4.27/0.049* | 5.69/0.021* | 1.86/0.18 | 19.21/0.001*** | 15.64/0.001*** |
| Y × CO2        | 6.90/0.012* | 4.82/0.033* | 6.73/0.013* | 6.22/0.016* | 3.41/0.071 |
| Y × Rhizobacteria | 5.04/0.010* | 0.17/0.84 | 0.27/0.77 | 0.436/0.65 | 0.25/0.78 |
| Cv. × CO2      | 30.44/0.001*** | 7.39/0.009** | 1.40/0.043* | 1.80/0.017* | 1.61/0.011* |
| Cv. × Rhizobacteria | 213.46/0.001*** | 48.31/0.001*** | 73.42/0.001*** | 132.82/0.001*** | 144.91/0.001*** |
| CO2 × Rhizobacteria | 13.25/0.001*** | 6.70/0.003*** | 9.78/0.001*** | 13.01/0.001*** | 12.63/0.001*** |
| Y × Cv. × CO2  | 0.220/0.64 | 1.83/0.18 | 4.16/0.047* | 0.743/0.39 | 0.56/0.46 |
| Y × Cv. × Rhizobacteria | 3.27/0.047* | 0.55/0.58 | 2.23/0.12 | 4.01/0.025* | 1.41/0.25 |
| Y × CO2 × Rhizobacteria | 0.72/0.49 | 1.88/0.16 | 1.33/0.28 | 2.66/0.080 | 2.47/0.095 |
| Cv. × CO2 × Rhizobacteria | 0.62/0.043* | 13.24/0.001*** | 9.84/0.001*** | 9.59/0.001*** | 9.92/0.001*** |
| Y × Cv. × CO2 × Rhizobacteria | 0.48/0.62 | 0.087/0.92 | 0.98/0.38 | 0.59/0.56 | 0.06/0.94 |

Notes:
- $^a$ P < 0.05;
- $^b$ P < 0.01;
- $^c$ P < 0.001.
- $^d$ Year (2016 vs. 2017).
- $^e$ Transgenic treatment (Bt maize vs. non-Bt maize).
- $^f$ CO2 levels (elevated CO2 vs. ambient CO2).
- $^g$ Rhizobacteria infection (A. brasilense and A. chroococcum vs. the control buffer), the same as in Tables 4 and 5.
- $^h$ Initial weight as a covariate for RGR and RCR, and food consumption as a covariate for AD and ECI, and food assimilated as a covariate for ECD.

Impacts of CO2 level, transgenic treatment, and rhizobacteria infection on the food utilization of Mythimna separata

There were significant effects of CO2 level, transgenic treatment, and rhizobacteria infection ($P < 0.01$ or $P < 0.001$) on food utilization of M. separata fed on both Bt and non-Bt maize infected with A. brasilense and A. chroococcum at both CO2 levels in both years of the study (Table 7).
Compared with ambient CO\(_2\), elevated CO\(_2\) significantly reduced the RGR (−9.95%), ECD (−16.05%), and ECI (−17.95%) of *M. separata* (*P* < 0.05; Table 8). Compared with the CK, rhizobacteria infection with *A. brasilense* and *A. chroococcum* both significantly decreased the ECD (−9.28% and −7.48%) and ECI (−9.22% and −7.91%), and significantly increased the RGR (+4.75% and +5.56%), RCR (+6.78% and +7.53%) and AD (+5.28% and +4.93%) in *M. separata* (*P* < 0.01; Table 8). Moreover, significant decreases in RGR (−13.85%), ECD (−41.25%) and ECI (−31.97%), and significant increases in RCR (+16.60%) and AD (+7.88%) were found when *M. separata* fed on Bt maize compared to that on non-Bt maize (*P* < 0.05; Table 8).

### Interactive influence of CO\(_2\) level, transgenic treatment, and rhizobacteria infection on growth, development and reproduction of *M. separata*

In addition to the significant main effects of CO\(_2\) level, transgenic treatment, and rhizobacteria infection, there were significant two-way and three-way interaction of these three main effects on larval life-span, pupation rate, pupal weight and duration, adult longevity, and fecundity of *M. separata* fed on Bt and non-Bt maize infected with *A. brasilense* and *A. chroococcum* under both CO\(_2\) levels in both years of the study (*P* < 0.05, *P* < 0.01 or *P* < 0.001; Table 5).

### Transgenic treatment × CO\(_2\)

Similar trends were found in the measured growth, development and reproduction indexes of *M. separata* fed on both Bt and non-Bt maize cultivars grown under elevated CO\(_2\) in contrast to ambient CO\(_2\), infected with *A. brasilense* (AB) and *A. chroococcum* (AC) as well as the CK in 2016 and 2017 (Figs. 1A–1F). Compared with ambient CO\(_2\), elevated CO\(_2\) significantly prolonged the larval life-span (Bt maize: +6.44%; non-Bt maize: +8.39%) and pupal duration (non-Bt maize: +7.27%) and shortened the adult longevity (non-Bt maize: −6.19%), and significantly decreased the pupation rate (non-Bt maize: −20.81%), pupal weight (Bt maize: −7.03%; non-Bt maize: −13.73%) and fecundity (Bt maize: −29.43%; non-Bt maize: −18.85%) when *M. separata* fed on Bt maize and non-Bt maize (*P* < 0.05; Figs. 1A–1F).

| Impact factors | Factor levels | RGR (mg g\(^{-1}\) day\(^{-1}\)) | RCR (mg g\(^{-1}\) day\(^{-1}\)) | AD (%) | ECD (%) | ECI (%) |
|---------------|---------------|-------------------------------|-------------------------------|--------|--------|--------|
| Cv. Bt        | 82.79 ± 6.43\(^b\) | 1636.31 ± 13.23\(^a\) | 56.13 ± 0.72\(^a\) | 9.26 ± 0.98\(^b\) | 5.13 ± 0.57\(^b\) |
| Xy            | 94.26 ± 7.16\(^a\) | 1403.36 ± 14.80\(^b\) | 52.03 ± 0.69\(^b\) | 13.08 ± 1.23\(^a\) | 6.77 ± 0.66\(^a\) |
| CO\(_2\) Elevated | 84.33 ± 8.67\(^b\) | 1595.24 ± 16.14\(^a\) | 55.55 ± 0.87\(^a\) | 10.34 ± 1.52\(^b\) | 5.46 ± 0.76\(^b\) |
| CO\(_2\) Ambient | 92.72 ± 6.59\(^a\) | 1444.43 ± 15.01\(^b\) | 52.61 ± 0.81\(^b\) | 12.00 ± 1.21\(^a\) | 6.44 ± 0.63\(^a\) |
| Rhizobacteria infection AB | 89.64 ± 5.52\(^a\) | 1549.07 ± 9.32\(^a\) | 55.06 ± 0.85\(^a\) | 10.78 ± 1.35\(^b\) | 5.75 ± 0.67\(^b\) |
| Rhizobacteria infection AC | 90.34 ± 5.52\(^a\) | 1559.90 ± 9.32\(^a\) | 54.88 ± 0.85\(^a\) | 10.96 ± 1.35\(^b\) | 5.82 ± 0.67\(^b\) |
| Rhizobacteria infection CK | 85.58 ± 6.14\(^b\) | 1450.73 ± 9.54\(^b\) | 52.30 ± 0.62\(^b\) | 11.78 ± 1.13\(^a\) | 6.28 ± 0.66\(^a\) |

Compared with ambient CO\(_2\), elevated CO\(_2\) significantly reduced the RGR (−9.95%), ECD (−16.05%), and ECI (−17.95%), and significantly enhanced the RCR (+10.44%) and AD (+5.59%) of *M. separata* (*P* < 0.05; Table 8).
Figure 1 Effects of bi-interactions between transgenic treatment and CO$_2$, between transgenic treatment and rhizobacteria and between CO$_2$ and rhizobacteria on development and reproduction of *Mythimna separata*. Larval life-span–(A), (G), (M); Pupation rate–(B), (H), (N); Pupal weight–(C), (I), (O); Pupal duration–(D), (J), (P); Adult longevity–(E), (K), (Q); Fecundity–(F), (L), (R); Each value represents the average (±SE). Different lowercase letters indicate significant differences treatments by the Duncan test at $P < 0.05$. DOI: 10.7717/peerj.5138/fig-1
Transgenic treatment × Rhizobacteria

An inverse trend was found in the measured growth, development and reproduction indexes of *M. separata* fed on *Bt* maize and non-*Bt* maize, which were infected with *A. brasilense* (AB) and *A. chroococcum* (AC) under ambient and elevated CO$_2$ in 2016 and 2017 (Figs. 1G–1L). Compared with the CK, rhizobacteria infection significantly prolonged the larval life-span (AB: +7.63%; AC: +8.45%), pupal duration (AB: +4.53%; AC: +5.08%) and shortened the adult longevity (AB: −4.88%; AC: −6.94%), and decreased pupation rate (AB: −20.83%; AC: −30.81%), pupal weight (AB: −7.24%; AC: −10.65%) and fecundity (AB: −48.36%; AC: −64.60%) when *M. separata* larvae fed on *Bt* maize (P < 0.01; Figs. 1G–1L); and rhizobacteria infection significantly shortened the larval life-span (AB: −6.92%; AC: −7.84%) and pupal duration (AB: −5.01%; AC: −5.01%) of *M. separata* under ambient CO$_2$, and significantly shortened the larval life-span (AB: −4.98%; AC: −5.11%) and decreased the pupal weight (AC: −4.77%) under elevated CO$_2$ (P < 0.05; Figs. 1M–1P); and rhizobacteria infection significantly decreased the adult longevity (AB: −4.63%; AC: −5.09%) and fecundity (AB: −22.90%; AC: −22.58%) of *M. separata* under elevated CO$_2$ (P < 0.05; Figs. 1Q and 1R).

CO$_2$ × Rhizobacteria

Similar trends were found in the larval life-span, pupation rate, pupal weight, and pupal duration, while inverse trends were observed in adult longevity and fecundity of *M. separata* under ambient and elevated CO$_2$, which fed on *Bt* maize vs. non-*Bt* maize infected with *A. brasilense* (AB) and *A. chroococcum* (AC) as well as the CK in 2016 and 2017 (Figs. 1M–1R). Compared with the CK, rhizobacteria infection significantly shortened the larval life-span (AB: −6.92%; AC: −7.84%) and pupal duration (AB: −5.01%; AC: −5.01%) of *M. separata* under ambient CO$_2$, and significantly shortened the larval life-span (AB: −4.98%; AC: −5.11%) and decreased the pupal weight (AC: −4.77%) under elevated CO$_2$ (P < 0.05; Figs. 1M–1P); and rhizobacteria infection significantly decreased the adult longevity (AB: −4.63%; AC: −5.09%) and fecundity (AB: −22.90%; AC: −22.58%) of *M. separata* under elevated CO$_2$ (P < 0.05; Figs. 1Q and 1R).

Transgenic treatment × CO$_2$ × Rhizobacteria

There were opposite trends in the measured growth, development and reproduction indexes of *M. separata* fed on *Bt* maize and non-*Bt* maize infected with *A. brasilense* (AB) and *A. chroococcum* (AC) compared with the CK in 2016 and 2017 regardless of CO$_2$ level (Fig. 2). In comparison with the CK, rhizobacteria infection with *A. brasilense* and *A. chroococcum* both significantly prolonged the larval life-span and pupal duration of *M. separata* fed on *Bt* maize, and significantly shortened the larval life-span and pupal duration of *M. separata* fed on non-*Bt* maize under the same CO$_2$ level; and rhizobacteria infection with *A. brasilense* and *A. chroococcum* both significantly reduced the pupation rate, pupal weight, adult longevity and fecundity of *M. separata* fed on *Bt* maize, and significantly enhanced the pupation rate, pupal weight, adult longevity and fecundity of *M. separata* fed on non-*Bt* maize under the same CO$_2$ level. Moreover, compared with ambient CO$_2$, there were opposite trends in the larval life-span, pupal weight, pupal duration, adult longevity and fecundity of *M. separata* fed on *Bt* maize infected with *A. brasilense* and *A. chroococcum* compared with the CK under elevated CO$_2$ in both years;
Figure 2 Impacts of the tri-interactions among CO$_2$ level, transgenic treatment, and rhizobacteria infection on the growth, development and reproduction of $M.$ separata in 2016 (A–F) and 2017 (G–L). Each value represents the average (+SE). Different lowercase and uppercase letters, and /C3 indicated significant difference among three types of rhizobacteria infection for same type of maize under same CO$_2$ level, between Bt maize and non-Bt maize for same type of rhizobacteria infection under same CO$_2$ level, and between ambient and elevated CO$_2$ for same type of maize and rhizobacteria infection by the Duncan test at P < 0.05 respectively.

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compared with ambient CO\textsubscript{2}, elevated CO\textsubscript{2} significantly decreased the pupation rate of \textit{M. separata} fed on \textit{Bt} maize, and decreased the pupation rate, pupal weight, adult longevity and fecundity of \textit{M. separata} fed on non-\textit{Bt} maize, and prolonged the larval lifespan and pupal duration of \textit{M. separata} fed on non-\textit{Bt} maize infected with \textit{A. brasilense} and \textit{A. chroococcum} compared with the CK in both years.

**Interactive effects of CO\textsubscript{2} level, transgenic treatment, and rhizobacteria infection on food utilization of \textit{M. separata}**

In addition to significant main effects of CO\textsubscript{2} level, transgenic treatment, and rhizobacteria infection, two- and three-way interactions of these factors influenced the RGR, RCR, AD, ECD, and ECI of \textit{M. separata} larvae fed on \textit{Bt} maize and non-\textit{Bt} maize infected with \textit{A. brasilense} and \textit{A. chroococcum} under ambient and elevated CO\textsubscript{2} in both years (\(P < 0.05, P < 0.01\) or \(P < 0.001\); Table 7).

**Transgenic treatment \(\times\) CO\textsubscript{2}**

Similar trends were found in the measured food utilization indexes of \textit{M. separata} fed on \textit{Bt} maize (\textit{Bt}) and non-\textit{Bt} maize (\textit{Xy}) grown under elevated CO\textsubscript{2} in contrast to ambient CO\textsubscript{2}, infected with \textit{A. brasilense} (AB) and \textit{A. chroococcum} (AC) as well as the CK in 2016 and 2017 (Figs. 3A–3E). Compared with ambient CO\textsubscript{2}, elevated CO\textsubscript{2} significantly decreased the RGR (non-\textit{Bt} maize: \(-7.34\%\)); ECD (\textit{Bt} maize: \(-9.67\%\); non-\textit{Bt} maize: \(-10.25\%\)) and ECI (\textit{Bt} maize: \(-8.53\%\); non-\textit{Bt} maize: \(-8.89\%\)), and significantly increased the RCR (\textit{Bt} maize: +9.69\%; non-\textit{Bt} maize: +6.37\%) when \textit{M. separata} larvae fed on \textit{Bt} maize and non-\textit{Bt} maize (\(P < 0.05\); Figs. 3A–3E).

**Transgenic treatment \(\times\) Rhizobacteria**

Inverse trend was found in the measured food utilization indexes of \textit{M. separata} fed on \textit{Bt} maize and non-\textit{Bt} maize, which were infected with \textit{A. brasilense} (AB) and \textit{A. chroococcum} (AC) under ambient and elevated CO\textsubscript{2} in 2016 and 2017 (Figs. 3F–3J). Compared with the CK, rhizobacteria infection significantly enhanced the RGR (AB: +9.53\%; AC: +11.78\%), ECD (AB: +11.61\%; AC: +19.79\%) and ECI (AB: +10.08\%; AC: +15.79\%), and significantly decreased the RCR (AC: \(-6.52\%\)) and AD (AC: \(-6.19\%\)) when \textit{M. separata} larvae fed on non-\textit{Bt} maize (\(P < 0.001\); Figs. 3F–3J); and rhizobacteria infection significantly decreased the RGR (AB: \(-9.62\%\); AC: \(-10.41\%\)), ECD (AB: \(-34.32\%\); AC: \(-41.55\%\)) and ECI (AB: \(-20.16\%\); AC: \(-25.28\%\)), and significantly increased the RCR (AB: +14.99\%; AC: +19.06\%) and AD (AB: +9.60\%; AC: +10.79\%) when \textit{M. separata} larvae fed on \textit{Bt} maize (\(P < 0.001\); Figs. 3F–3J).

**CO\textsubscript{2} \(\times\) Rhizobacteria**

Similar trends were observed in RGR, RCR, and AD, while inverse trends were shown in ECD and ECI of \textit{M. separata} under ambient and elevated CO\textsubscript{2}, which fed on \textit{Bt} maize and non-\textit{Bt} maize infected with \textit{A. brasilense} (AB) and \textit{A. chroococcum} (AC) vs. CK (Figs. 3K–3O). Compared with the CK, rhizobacteria infection significantly decreased ECD (AB: \(-20.71\%\); AC: \(-22.07\%\)) and ECI (AB: \(-12.77\%\); AC: \(-12.89\%\)) of \textit{M. separata} larvae under elevated CO\textsubscript{2}, and significantly increased ECD (AB: +5.35\%; AC:
Figure 3: Effects of bi-interactions between transgenic treatment and CO$_2$, between transgenic treatment and rhizobacteria and between CO$_2$ and rhizobacteria on food utilization of *Mythimna separata* larvae. RGR–(A), (F), (K); RCR–(B), (G), (L); AD–(C), (H), (M); ECD–(D), (I), (N); ECI–(E), (J), (O); Each value represents the average (±SE). Different lowercase letters indicate significant differences treatments by the Dunnett test at $P < 0.05$. DOI: 10.7717/peerj.5138/fig-3
+8.04%) and ECI (AB: +7.43%; AC: +9.85%) of M. separata larvae under ambient CO$_2$ ($P < 0.05$; Fig. 3); and rhizobacteria infection significantly enhanced RGR (AB: +3.32% and +7.40%; AC: +5.14% and +8.67%), RCR (AB: +9.78% and +5.29%; AC: +11.32% and +5.93%) and AD (AB: +7.34% and +4.18%; AC: +7.92% and +4.66%) under elevated and ambient CO$_2$, respectively ($P < 0.01$; Figs. 3K–3O).

**Transgenic treatment × CO$_2$ × Rhizobacteria**

There were opposite trends in the measured food utilization indexes of M. separata larvae fed on Bt maize (Bt) and non-Bt maize infected with A. brasilense (AB) and A. chroococcum (AC) compared with the CK in both years regardless of CO$_2$ level (Fig. 4). In comparison with the CK, rhizobacteria infection with A. brasilense and A. chroococcum both significantly decreased RGR, ECD, and ECI of M. separata fed on Bt maize, and significantly increased RGR, ECD, and ECI of M. separata fed on non-Bt maize under the same CO$_2$ level; and rhizobacteria infection with A. brasilense and A. chroococcum both significantly enhanced RCR and AD of M. separata fed on Bt maize, and significantly reduced RCR and AD of M. separata larvae fed on non-Bt maize under the same CO$_2$ level. Moreover, compared with ambient CO$_2$, elevated CO$_2$ significantly increased RCR and AD, and significantly decreased RGR, ECD, and ECI of M. separata larvae fed on same type of maize cultivar infected with A. brasilense and A. chroococcum in both years ($P < 0.05$; Fig. 4). Furthermore, there were significant decreases in RGR, ECD, and ECI, and significant increases in RCR and AD of M. separata larvae fed on Bt maize in contrast to non-Bt maize infected with same type of rhizobacteria species within the same CO$_2$ level in both years.

**DISCUSSION**

Insects are sensitive to environmental variations, and environmental stresses can cause changes on their growth, development, fecundity, food utilization and the occurrence and distribution of populations as a result of metabolic rate fluctuation (Bloom et al., 2010). In this study, elevated CO$_2$ significantly prolonged larval and pupal duration and decreased pupation rate and pupal weight of M. separata compared to ambient CO$_2$. Elevated CO$_2$ negatively affected the larval survival, weight, duration, pupation, and adult emergence of cotton bollworm, H. armigera (Akbar et al., 2016), and reduced the egg laying by Cactus moth Cactoblastis cactorum (Stange, 1997) and Achaea Janata (Rao et al., 2013). In this study, elevated CO$_2$ significantly increased the RCR (+10.44%) and the AD (+5.59%) (i.e., AD), and significantly reduced the RGR (−9.95%), ECD (−16.05%) and ECI (−17.95%) of M. separata larvae compared with ambient CO$_2$. RGRs of Gypsy moth (Lymantria dispar) were reported to be reduced by 30% in larvae fed on Quercus petraea exposed to elevated CO$_2$ (Hattenschwiler & Schafellner, 2004). RCR was significantly higher for H. armigera larva fed maize grown at 375 and 750 ppm CO$_2$ in contrast to ambient CO$_2$ condition, and elevated CO$_2$ significantly decreased the ECI food, the ECD food, and the RGR of H. armigera larvae compared with ambient CO$_2$ (Yin et al., 2010).

According to the “Nutrition compensation hypothesis,” elevated CO$_2$ can affect the development fitness of herbivores by changing the nutritional components, above and
Figure 4  Impacts of the tri-interactions among CO$_2$, transgenic treatment, and rhizobacteria infection on the food utilization of _M. separata_ from the third to the sixth instar larvae in 2016 (A–E) and 2017 (F–J). Each value represents the average (+SE). Different lowercase and uppercase letters, and * indicated significant difference among three types of rhizobacteria infection for same type of maize under same CO$_2$ level, between _Bt_ maize and non- _Bt_ maize for same type of rhizobacteria infection under same CO$_2$ level, and between ambient and elevated CO$_2$ for same type of maize and rhizobacteria infection by the Duncan test at $P < 0.05$ respectively.
below-ground biomass, and photosynthetic rate of host plants indirectly (Ainsworth & Rogers, 2007; Jackson et al., 2009; Zavala, Nabity & Delucia, 2013), including increased C/N ratio and decreased nitrogen content etc. Declined growth rate, reproduction, and survival rate were found in the chewing mouthparts insects (e.g., H. armigera, Spodoptera exigua, M. separata), and the food consumption of which increased so that they could obtain necessary nutrition to survive (Bottomley, Rogers & Prior, 1993; Rogers et al., 2006).

Yin et al. (2010) reported that elevated CO$_2$ increased the food consumption and prolonged the development time of H. armigera, which due to the reduced nutritional quality of maize leaves, as a result of reduced nitrogen content and increased C/N ratio. Elevated CO$_2$ significantly reduced the food conversion rate and enhanced the food ingestion of H. armigera, which attribute to reduced nitrogen content of the cotton, Simian-3 (Chen, Ge & Parajulee, 2005a; Chen et al., 2005b). Thus, Chen, Ge & Parajulee (2005a) and Chen et al. (2005b) inferred that elevated CO$_2$ might be unfavorable to H. armigera. Our results in maize system appear to be similar to the study by Chen, Ge & Parajulee (2005a) and Chen et al. (2005b) in a cotton system.

Although the transgenic corn, Zea mays L., hybrids expressing the Cry insecticidal protein from Bacillus thuringiensis (Bt) were developed to control H. zeae, O. nubilalis, S. frugiperda, and M. separata (Koziel et al., 1993; Armstrong et al., 1995; Jouanin et al., 1998; Lynch, Plaisted & Warnick, 1999), few studies focused on the defense responses of transgenic cry1Ie maize to corn armyworm under elevated CO$_2$, especially on the growth, development and food utilization of the pest insects. Prutz & Dettner (2005) reported that the transgenic Bacillus thuringiensis-maize could result in decreased growth rate and increased mortality, which might attribute to the termination of larval metamorphosis. Most studies showed that adverse effects on life-table parameters of different herbivores were direct by the Cry protein (Lawo, Wacker & Romeis, 2010), which might be due to the interaction of feeding inhibitors and growth inhibitors (e.g., secondary plant substances) (Smith & Fischer, 1983). Effects of elevated CO$_2$ on the plant nutrition, metabolism and secondary defense metabolism might adverse for the growth, development and nutrition utilization of herbivores (Akbar et al., 2016). The insects possessed more nutrients to meet their growth needs and prolong the food digestion time in the midgut so that the RCR and AD increased (Reynolds, Nottingham & Stephens, 1985).

In this study, we found that some negative effects of transgenic cry1Ie maize (Bt) and Xianyu 335 (XY) grown in elevated CO$_2$ on the food utilization indices (including RGR, ECD, and ECI) of M. separata larvae and some positive effects on the RCR and AD, which indicated that the resistance responses of Bt maize might persist under elevated CO$_2$, and M. separata might ingest more food to get enough nutrition for surviving in limited developmental time under elevated CO$_2$. Meanwhile the Bt maize and its parental line (Xianyu 335) prolonged their larval life-span and pupal duration, decreased growth rate and increased mortality that might result in lowering of pests’ occurrence. According to the “carbon nutrition balance hypothesis” (Gebauer, Strain & Reynolds, 1997), elevated CO$_2$ would increase the fixed organic matter in plant while increase C-based secondary metabolites and decrease N-based secondary metabolites, thus affecting the insects resistance of plants. Robinson, Ryan & Newman (2012) indicated that
elevated CO$_2$ increased 19% phenols, 22% condensed tannins, and 27% flavonoids, while the terpenoids and NBSC decreased by 13% and 16% respectively. Coviella, Stipanovic & Trumble (2002) anticipated that the primary CO$_2$ effect on Bt toxin production would be due to differences in N concentration within the plant. In a meta-analytical review of 33 studies that simultaneously increased CO$_2$ conditions compared to ambient conditions, Zvereva & Kozlov (2006) showed that nitrogen concentration in plants was reduced under elevated CO$_2$, and this decrease was stronger for woody compared to herbaceous plants. If conditions of increased carbon (e.g., elevated CO$_2$) allow plants to allocate significantly more resources to condensed tannins and gossypol, then the enzyme composition in the insect herbivore is expected to also change. Similarly, if Bt toxin production changes due to elevated CO$_2$, then the insect herbivore’s body enzymes should also be changed in this circumstance.

Most of the nitrogen, however, is found in the form of N$_2$ which approximately amounts to 78% in the atmosphere. As plants cannot use this form of nitrogen directly, some microbes can change the N$_2$ into ammonia. Most free living microbes in soil which can fix nitrogen and whose activities in enhancing the growth of plants are bacteria namely Azotobacter sp. and Azospirillum sp. These two bacteria are particularly important in maize production system due to their greater nitrogen fixing ability. Azospirillum acquires carbohydrate directly from sieve tube as a resource of carbon which promotes its growth (Olivera et al., 2004). Azospirillum can be used to promote the growth of sprouts under normal and arid conditions (Alejandra et al., 2009). Azospirillum also provides more flexibility to cell wall which enhances the growth (Pereyra et al., 2010) and increases products of wheat in waterless plot of land (Martin, 2009). Furthermore, azospirillum had the highest efficiency in nitrogen fixation at the root of sweet corn and it would reach the highest point of nitrogen fixation in the week 4 amounting to 0.20 mgNhr$^{-1}$m$^{-2}$ (Toopakuntho, 2010). Azospirillum can also create auxin, a substance promoting growth of maize, of 53.57 mg/ml (Phookkasem, 2011). Therefore, we used techniques of rhizobacteria (A. brasilense and A. chroococcum) inoculation of maize seeds to stimulate plant N uptake to increase in biomass N relative to C under elevated CO$_2$, increase Bt toxin production for transgenic cry1Ie maize and create a substance promoting maize plant growth. In this study, we found that elevated CO$_2$ significantly enhanced the rhizosphere soil densities both A. brasilense and A. chroococcum at the maize harvest, but there was no significant difference of the rhizosphere soil densities both A. brasilense and A. chroococcum between elevated and ambient CO$_2$ at the maize seedling after 14 days. We hypothesize that the elevated CO$_2$ increased the maize root bifurcation and soil nutrition (e.g., carbohydrates, amino acids and multi-trace elements) for rhizobacteria to provide the living space and nutrition with a long-time environmental effect. Other researchers have also shown positive effects of elevated CO$_2$ on the bacterial community in the rhizosphere of maize (Chen et al., 2012). Moreover, significant adverse effects on the growth, development, reproduction, and food utilization of M. separata were observed when the host substrate maize was exposed to rhizobacteria treatments, which might be attributed to rhizobacteria stimulating plant N uptake to increase Bt toxin production.
for transgenic cry1Ie maize and promoting growth of its parental line (Xianyu 335) (Olivera et al., 2004; Stitt & Krapp, 1999).

There was no significant year-to-year variation in our field research data. Therefore, the overall results clearly indicate that increasing CO$_2$ had negative effects on *M. separata*. Resistance performance of transgenic cry1Ie maize decreased under elevated CO$_2$ as shown by decreased RGR, ECD, and ECI. The rhizobacteria treatments (*A. brasilense* and *A. chroococcum*) had positive effects on improving the effectiveness of *Bt* maize on target Lepidoptera pest management via decreased RGR, ECD, and ECI of *M. separata* that fed on transgenic cry1Ie maize and promoting growth of Xianyu 335 via increased RGR, ECD, and ECI of *M. separata*. Under future predicted climate changes (e.g., elevated CO$_2$), it is particularly important to understand the field insect resistance traits of resistant crops to target pests. In an environment of accelerated greenhouse effect, *Bt* maize may have decreased resistance performance in the field with inhibiting effect on the development and food utilization of insects. Therefore, we used techniques of rhizobacteria (*A. brasilense* & *A. chroococcum*) inoculation of maize seeds to stimulate plant N uptake to increase in biomass N relative to C under elevated CO$_2$, increase *Bt* toxin production for transgenic cry1Ie maize, and create a substance promoting maize growth.

**CONCLUSION**

Overall, our results indicated that elevated CO$_2$ and *Bt* maize were negative against development and food utilization of *M. separata*. Rhizobacteria infection significantly increased the larval life-span, pupal duration, RCR and AD of *M. separata*, and significantly decreased RGR, ECD and ECI of *M. separata* fed on *Bt* maize; there were opposite trends in development and food utilization of *M. separata* fed on non-*Bt* maize infected with rhizobacterias compared with the CK in 2016 and 2017 regardless of CO$_2$ level. This study demonstrates that the use of rhizobacteria (e.g., *A. brasilense* and *A. chroococcum*) as pest control enhancer especially under elevated CO$_2$ is significantly more beneficial in transgenic *Bt* maize system compared to that in non-transgenic system. Rhizobacteria (*A. brasilense* & *A. chroococcum*), as being one potential biological regulator to enhance nitrogen utilization efficiency of crops, could make the *Bt* maize facing lower field hazards from the target pest of *M. separate*, and finally improve the sustainability and resistance of *Bt* maize against target lepidoptera pests, especially under future CO$_2$ raising.

**ADDITIONAL INFORMATION AND DECLARATIONS**

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Competing Interests
The authors declare that they have no competing interests.

Author Contributions
- Zhuo Li performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Megha N. Parajulee conceived and designed the experiments, authored or reviewed drafts of the paper, approved the final draft.
- Fajun Chen conceived and designed the experiments, approved the final draft.

Data Availability
The following information was supplied regarding data availability:
The raw data are provided in a Supplemental File.

Supplemental Information
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REFERENCES
Ainsworth EA, Rogers A. 2007. The response of photosynthesis and stomatal conductance to rising [CO$_2$]: mechanisms and environmental interactions. *Plant, Cell & Environment* **30**(3):258–270 DOI 10.1111/j.1365-3040.2007.01641.x.

Ainsworth EA, Rogers A, Leakey ADB, Heady LE, Gibon Y, Stitt M, Schurr U. 2007. Does elevated atmospheric [CO$_2$] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves? *Journal of Experimental Botany* **58**(3):579–591 DOI 10.1093/jxb/erl233.

Akbar SM, Pavani T, Nagaraja T, Sharma HC. 2016. Influence of CO$_2$ and temperature on metabolism and development of *Helicoverpa armigera* (Noctuidae: Lepidoptera). *Environmental Entomology* **45**(1):229–236 DOI 10.1093/ee/nvv144.

Alejandra PM, Ballesteros FM, Creus CM, Sueldo RJ, Barassi CA. 2009. Seedling growth promotion by *Azospirillum brasilense* under normal and drought conditions remains unaltered in Tebuconazole-treated wheat seeds. *European Journal of Soil Biology* **45**(1):20–27 DOI 10.1016/j.ejsoilb.2008.09.015.
Ali A, Rashid MA, Huang QY, Lei CL. 2016. Effect of UV-A radiation as an environmental stress on the development, longevity, and reproduction of the oriental armyworm, *Mythimna separata* (Lepidoptera: Noctuidae). *Environmental Science & Pollution Research* 23(17):17002–17007 DOI 10.1007/s11356-016-6865-0.

Armstrong CL, Parker GB, Pershing JC, Brown SM, Sanders PR, Duncan DR, Stone T, Dean DA, DeBoer DL, Hart J, Howe AR, Morrish FM, Pajeau ME, Petersen WL, Reich BJ, Rodriguez R, Santino CG, Sato SJ, Schuler W, Sims SR, Stehling S, Tarochione IJ, Fromm ME. 1995. Field evaluation of European corn borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. *Crop Science* 35(2):550–557 DOI 10.2135/cropsci1995.0011183X003500020045x.

Biari A, Gholami A, Rahmani HA. 2008. Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. *Journal of Biological Sciences* 8(6):1015–1020 DOI 10.3923/jbs.2008.1015.1020.

Bi FC. 1981. A new artificial diet of *Mythimna separate*. *Acta Entomologica Sinica* 24(4):379–383 DOI 10.16380/j.kcxb.1981.04.006.

Bloom AJ, Burger M, Asensio JSR, Cousins AB. 2010. Carbon dioxide enrichment inhibits nitrate assimilation in wheat and Arabidopsis. *Science* 328(5980):899–903 DOI 10.1126/science.1186440.

Bottomley PA, Rogers HH, Prior SA. 1993. NMR imaging of root water distribution in intact *Vicia faba* L plants in elevated atmospheric CO$_2$. *Plant, Cell and Environment* 16(3):335–338 DOI 10.1111/j.1365-3040.1993.tb00878.x.

Carrière Y, Crowder DW, Tabashnik BE. 2010. Evolutionary ecology of insect adaptation to Bt crops. *Evolutionary Applications* 3(5–6):561–573 DOI 10.1111/j.1752-4571.2010.00129.x.

Cassán F, Perrig D, Sgroy V, Masciiarella O, Pennab C, Lunaa V. 2009. *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (Glycine max L.). *European Journal of Soil Biology* 45(1):28–35 DOI 10.1016/j.ejsobi.2008.08.005.

Cattaneo MG, Yafuso C, Schmidt C, Huang CY, Rahman M, Olson C, Ellers-Kirk C, Orr BJ, Marsh SE, Antilla L, Durtlep L, Carrière Y. 2006. Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. *Proceedings of the National Academy of Sciences of the United States of America* 103(20):7571–7576 DOI 10.1073/pnas.0508312103.

Chen FJ, Ge F, Parajulee MN. 2005a. Impact of elevated CO$_2$ on tri-trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environmental Entomology* 34(1):37–46 DOI 10.1603/0046-225X-34.1.37.

Chen SN, Gu J, Fu QX, Sun W, Qian X, Gao H, Qing QJ. 2012. Effects of inoculating azotobacter on soil enzyme activities and bacterial community functional diversity in the rhizosphere of maize (*Zea mays* L.). *Journal of Plant Nutrition and Fertilizer* 18(2):444–450.

Chen FJ, Wu G, Ge F. 2004. Growth, development and reproduction of the cotton bollworm, *Helicoverpa armigera* (Hübner) reared on milky grains of wheat grown in elevated CO$_2$ concentration. *Acta Entomologica Sinica* 47(6):774–779 DOI 10.16380/j.kcxb.2004.06.014.

Chen FJ, Wu G, Ge F, Parajulee MN. 2011. Relationships between exogenous-toxin quantity and increased biomass of transgenic Bt crops under elevated carbon dioxide. *Ecotoxicology and Environmental Safety* 74(4):1074–1080 DOI 10.1016/j.ecoenv.2011.02.001.

Chen FJ, Wu G, Ge F, Parajulee MN, Shrestha RB. 2005b. Effects of elevated CO$_2$ and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Entomologia Experimentalis et Applicata* 115(2):341–350 DOI 10.1111/j.1570-7458.2005.00258.x.
Cornelissen T. 2011. Climate change and its effects on terrestrial insects and herbivory patterns. Neotropical Entomology 40(2):155–163 DOI 10.1590/S1519-566X2011000200001.

Coviella CE, Stipanovic RD, Trumble JT. 2002. Plant allocation to defensive compounds: interactions between elevated CO$_2$ and nitrogen in transgenic cotton plants. Journal of Experimental Botany 53(367):323–331 DOI 10.1093/jexbot/53.367.323.

Ciais P, Sabine C, Bala G, Bopp L, Brovkin V, Canadell J, Chhabra A, DeFries R, Galloway J, Heimann M, Jones C, Quéré CL, Myneni RB, Piao SL, Thornton P. 2013. Carbon and other biogeochemical cycles. In: Climate change 2013. The physical science basis, working group I contribution to the fifth assessment report of the intergovernmental panel on climate change, 465–570.

Gao HJ, Xiao NW, Li JS, Chen FJ, Zhai BP. 2009. Effects of double atmospheric CO$_2$ concentration on nitrogen metabolism of transgenic Bt cotton under different nitrogen fertilization levels. Chinese Journal of Ecology 28(11):2213–2219 DOI 10.13292/j.1000-4890.2009.0378.

Gebauer RLE, Strain BR, Reynolds JF. 1997. The effect of elevated CO$_2$ and N availability on tissue concentrations and whole plant pools of carbon-based secondary compounds in loblolly pine (Pinus taeda). Oecologia 113(1):29–36 DOI 10.1007/s004420050350.

Guo JF, He KL, Hellmich RL, Bai SX, Zhang TT, Liu YJ, Ahmed T, Wang ZY. 2016. Field trials to evaluate the effects of transgenic cry1Ie maize on the community characteristics of arthropod natural enemies. Scientific Reports 6(1):22102 DOI 10.1038/srep22102.

Hägele BF, Rowell-Rahier M. 1999. Dietary mixing in three generalist herbivores: nutrient complementation or toxin dilution? Oecologia 119(4):521–533 DOI 10.1007/s004420050815.

Hattenschwiler S, Schafellner C. 2004. Gypsy moth feeding in the canopy of a CO$_2$-enriched mature forest. Global Change Climate 10(11):1899–1908 DOI 10.1111/j.1365-2486.2004.00856.x.

Huang J, Hu R, Rozelle S, Pray C. 2005. Insect-resistant GM rice in farmers’ fields: assessing productivity and health effects in China. Science 308(5722):688–690 DOI 10.1126/science.1108972.

Huang FN, Qureshi JA, Meagher JRL, Reisig DD, Head GP, Andow DA, Ni XZ, Kerns D, Buntin GD, Niu Y, Yang F, Dangal V. 2014. Cry1F resistance in fall armyworm Spodoptera frugiperda: single gene versus pyramided Bt maize. PLOS ONE 9(11):e112958 DOI 10.1371/journal.pone.0112958.

Hutchison WD, Burkness EC, Mitchell PD, Moon RD, Leslie TW, Fleischer SJ, Abrahamson M, Hamilton KL, Steffey KL, Gray ME, Hellmich RL, Kaster IV, Hunt TE, Wright RJ, Pecinovsky K, Rabaei TL, Flood BR, Raun1 ES. 2010. Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. Science 330(6001):222–225 DOI 10.1126/science.1190242.

Intergovernmental Panel on Climate Change (IPCC). 2014. Climate Change 2014: Impacts, Adaptation, and Vulnerability. Available at http://www.ipcc.ch/report/ar5/wg2/.

Jackson RB, Cook CW, Pippen JS, Palmer SM. 2009. Increased belowground biomass and soil CO$_2$ fluxes after a decade of carbon dioxide enrichment in a warm-temperate forest. Ecology 90(12):3352–3366 DOI 10.1890/08-1609.1.

Jia HR, Geng LL, Li YH, Wang Q, Diao QY, Zhou T, Dai PL. 2016. The effects of Bt Cry1Ie toxin on bacterial diversity in the midgut of Apis mellifera ligustica (Hymenoptera: Apidae). Scientific Reports 6(1):24664 DOI 10.1038/srep24664.

Jiang SL, Lu YQ, Dai Y, Qian L, Muhammad AB, Li T, Wan GJ, Parajulee MN, Chen FJ. 2017. Impacts of elevated CO$_2$ on exogenous Bacillus thuringiensis toxins and transgene expression in
transgenic rice under different levels of nitrogen. *Scientific Reports* 7(1):14716 DOI 10.1038/s41598-017-15321-9.

Jiang XF, Zhang L, Yang HX, Sappington TW, Cheng YX, Luo LZ. 2016. Biocontrol of the oriental armyworm, *Mythimna separata*, by the tachinid fly *Exorista civilis* is synergized by Cry1Ab protoxin. *Scientific Reports* 6(1):26873 DOI 10.1038/srep26873.

Jouanin L, Bonade-Bottino M, Girard C, Morrot G, Giband M. 1998. Transgenic plants for insect resistance. *Plant Science* 131(1):1–11 DOI 10.1016/S0168-9452(97)00239-2.

Koziel MG, Beland GL, Bowman C, Carozzi NB, Crenshaw R, Crossland L, Dawson J, Desai N, Hill M, Kadwell S, Launis K, Lewis K, Maddox D, McPherson K, Meghji MR, Merlin E, Rhodes R, Warren GW, Wright M, Evola SY. 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Nature Biotechnology* 11(2):194–200 DOI 10.1038/nbt0293-194.

Lawo NC, Wacker FL, Romeis J. 2010. Characterizing indirect prey-quality mediated effects of a Bt crop on predatory larvae of the green lacewing, *Chrysoperla carnea*. *Journal of Insect Physiology* 56(11):1702–1710 DOI 10.1016/j.jinsphys.2010.06.012.

Lindroth RL, Koppera BJ, Parsons WA, Bockheim JS, Karnosky DF, Hendrey GR, Pregitzer KS, Isebrandt JG, Sober J. 2001. Consequences of elevated carbon dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environmental Pollution* 115(3):395–404 DOI 10.1016/S0269-7491(01)00229-9.

Ling J. 2010. Promotion research of new maize varieties Xianyu 335 in shandong province. *Agricultural Technology Service* 27(7):943–944.

Liu Y, Fu X, Mao L, Xing Z, Wu K. 2017. Identification of host plant use of adults of a long-distance migratory insect, *Mythimna separata*. *PLOS ONE* 12(9):e0184116 DOI 10.1371/journal.pone.0184116.

Lu YH, Wu KM, Jiang YY, Guo YY, Desneux N. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* 487(7407):362–365 DOI 10.1038/nature11153.

Lynch RE, Plaisted WD, Warnick D. 1999. Evaluation of transgenic sweet corn hybrids expressing Cry1A(b) toxin for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 92(1):246–252 DOI 10.1093/jee/92.1.246.

Martin DZ. 2009. Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. *European Journal of Soil Biology* 45(1):3–11 DOI 10.1016/j.ejsobi.2008.07.001.

Massad TJ, Dyer LA. 2010. A meta-analysis of the effects of global environmental change on plant-herbivore interactions. *Arthropod-Plant Interactions* 4(3):181–188 DOI 10.1007/s11829-010-9102-7.

Olivera M, Tejera N, Iribarna C, Ocana A, Luch CL. 2004. Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorus. *Physiologia Plantarum* 121(3):498–505 DOI 10.1111/j.0031-9317.2004.00355.x.

O’Neill BF, Zangerl AR, Dermody O, Bilgin DD, Casteel CL, Zavala JA, DeLucia EH, Berenbaum MR. 2010. Impact of elevated levels of atmospheric CO₂ and herbivory on flavonoids of soybean (Glycine max Linnaeus). *Journal of Chemical Ecology* 36(1):35–45 DOI 10.1007/s10886-009-9727-0.

Pang J, Zhu JG, Xie ZB, Chen GP, Liu G, Zhang YL. 2005. Effects of elevated CO₂ on nutrient uptake by rice and nutrient contents in rice grain. *Chinese Journal of Rice* 19(4):350–354.
Percy KE, Awmack CS, Lindroth RL, Kubiske ME, Kopper BJ, Isebrands JG, Pregitzer KS, Hendrey GR, Dickson RE, Zak DR, Oksanenq E, Soberk J, Harrington R, Karnosoky DF. 2002. Altered performance of forest pests under atmospheres enriched by CO$_2$ and O$_3$. *Nature* 420(6913):403–407 DOI 10.1038/nature01028.

Pereyra CM, Ramella NA, Pereyra MA, Barassi CA, Creus CM. 2010. Changes in cucumber hypocotyl cell wall dynamics caused by *Azospirillum brasilense* inoculation. *Plant Physiology and Biochemistry* 48(1):62–69 DOI 10.1016/j.plaphy.2009.10.001.

Phookkasem C. 2011. Effects of *Azospirillum* on nitrogen fixation and growth enhancement of maize. Master’s thesis, Kasetsart University.

Prutz G, Dettner K. 2005. Effects of transgenic *Bacillus thuringiensis*-maize on larval food consumption, utilization and growth in the grass-moth species *Chilo partellus* under laboratory conditions (Lepidoptera: Crambidae). *Entomologia Generalis* 28(3):161–172 DOI 10.1127/entom.gen/28/2005/161.

Rao MS, Srinivas K, Vanaja M, Manimaniari D, Rao CAR, Venkateswarlu B. 2013. Response of multiple generations of semilooper, *Archaea janata* feeding on castor to elevated CO$_2$. *Journal of Environmental Biology* 34(5):877–883.

Raubenheimer D, Simpson SJ. 1992. Analysis of covariance: an alternative to nutritional indices. *Entomologia Experimentalis Et Applicata* 62(3):221–231 DOI 10.1111/j.1570-7458.1992.tb00662.x.

Reynolds SE, Nottingham SF, Stephens AE. 1985. Food and water economy and its relation to growth in fifth instar larvae of tobacco hornworm, *Manduca sexta*. *Journal of Insect Physiology* 31(2):119–127 DOI 10.1016/0022-1910(85)90016-2.

Richardson AE, Barea JM, Mcneill AM, Prigent-Combaret C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil* 321(1–2):305–339 DOI 10.1007/s11104-009-9895-2.

Robinson EA, Ryan GD, Newman JA. 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 Phytologist* 194(2):321–336 DOI 10.1111/j.1469-8137.2012.04074.x.

Rogers A, Gibon V, Stitt M, Morgan PB, Bernacchi CJ, Ort DR, Long SP. 2006. Increased C availability at elevated carbon dioxide concentration improves N assimilation in a legume. *Plant, Cell and Environment* 29(8):1651–1658 DOI 10.1111/j.1365-3040.2006.01549.x.

Smith CM, Fischer NH. 1983. Chemical factors of an insect resistant soybean genotype affecting growth and survival of the soybean looper. *Entomologia Experimentalis Et Applicata* 33(3):343–345 DOI 10.1111/j.1570-7458.1983.tb03278.x.

Stange G. 1997. Effects of changes in atmospheric carbon dioxide on the location of hosts by the moth, *Cactoblastis cactorum*. *Oecologia* 110(4):539–545 DOI 10.1007/s0044200500192.

Stitt M, Krapp A. 1999. The interactions between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. *Plant, Cell & Environment* 22(6):583–621 DOI 10.1046/j.1365-3040.1999.00386.x.

Toopakuntho S. 2010. Nitrogen fixation of free living microorganism in no tillage sweet corn cultivation system. M. Sci. thesis, Kasetsart University.

Trębicki P, Dáder B, Vassiliadis S, Fereres A. 2017. Insect-plant-pathogen interactions as shaped by future climate: effects on biology, distribution and implications for agriculture. *Insect Science* 24(6):975–989 DOI 10.1111/1744-7917.12531.

Walters FS, Fontes CM, Hart H, Warren GW, Chen JS. 2010. Lepidopteran-active variable-region sequence imparts Coleopteran activity in *eCry3.1Ab*, an engineered *Bacillus thuringiensis* hybrid.
insecticidal protein. *Applied and Environmental Microbiology* **76**(10):3082–3088 DOI 10.1128/AEM.00155-10.

**Yamprai A, Mala T, Sinma K. 2014.** The study on the fixed nitrogen and nitrogenase activity in the day-round of *Azotobacter* and *Azospirillum* grown with maize in *KamphaengSaen* soil series. *Modern Applied Science* **8**(6):27–36 DOI 10.5539/mas.v8n6p27.

**Yin J, Sun Y, Wu G, Ge F. 2010.** Effects of elevated CO$_2$ associated with maize on multiple generations of the cotton bollworm, *Helicoverpa armigera*. *Entomologia Experimentalis Et Applicata* **136**(1):12–20 DOI 10.1111/j.1570-7458.2010.00998.x.

**Zavala JA, Nabity PD, Delucia EH. 2013.** An emerging understanding of mechanisms governing insect herbivory under elevated CO$_2$. *Annual Review of Entomology* **58**(1):79–97 DOI 10.1146/annurev-ento-120811-153544.

**Zhang YW, Liu YJ, Ren Y, Liu Y, Liang GM, Song FP, Bai SX, Wang JH, Wang GY. 2013.** Overexpression of a novel *Cry1Ie*, gene confers resistance to *Cry1Ac*-resistant cotton bollworm in transgenic lines of maize. *Plant Cell, Tissue and Organ Culture* **115**(2):151–158 DOI 10.1007/s11240-013-0348-5.

**Zvereva EL, Kozlov MV. 2006.** Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: a meta-analysis. *Global Change Biology* **12**(1):27–41 DOI 10.1111/j.1365-2486.2005.01086.x.