Influence of CO_2 and Temperature on Metabolism and Development of *Helicoverpa armigera* (Noctuidae: Lepidoptera)

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ABSTRACT Climate change will have a major bearing on survival and development of insects as a result of increase in CO_2 and temperature. Therefore, we studied the direct effects of CO_2 and temperature on larval development and metabolism in cotton bollworm, *Helicoverpa armigera* (Hübner). The larvae were reared under a range of CO_2 (350, 550, and 750 ppm) and temperature (15, 25, 35, and 45°C) regimes on artificial diet. Elevated CO_2 negatively affected the larval survival, larval weight, larval period, pupation, and adult emergence, but showed a positive effect on pupal weight, pupal period, and fecundity. Increase in temperature exhibited a negative effect on larval survival, larval period, pupal weights, and pupal period, but a positive effect on larval growth. Pupation and adult emergence were optimum at 25°C. Elevated CO_2 and temperature increased food consumption and metabolism of larvae by enhancing the activity of midgut proteases, carbohydrases (amylase and cellulase), and mitochondrial enzymes and therefore may cause more damage to crop production. Elevated CO_2 and global warming will affect insect growth and development, which will change the interactions between the insect pests and their crop hosts. Therefore, there is need to gain an understanding of these interactions to develop strategies for mitigating the effects of climate change.

KEY WORDS *Helicoverpa armigera*, elevated CO_2, global warming, midgut protease, amylase

Global atmospheric concentration of CO_2 has increased from 280 ppm during the preindustrial era to 396 ppm in 2013 [National Oceanic & Atmospheric Administration - Earth System Research Laboratory (NOAA-ESRL)], and is anticipated to double by the end of the 21st century (Intergovernmental Panel on Climate Change [IPCC] 2007). The third IPCC report predicts that global average surface temperature will increase by 1.4 to 5.8°C by 2100, with atmospheric CO_2 concentrations expected to rise between 540 to 970 ppm (Houghton et al. 2001). Effects of elevated atmospheric CO_2 and temperature will change the nutritional quality of plants (Hunter 2001), which will change the interactions between plants, herbivores, and the natural enemies via complex mechanisms (Harrington et al. 1999, Gillespie et al. 2012).

CO_2-mediated changes in the temperature and precipitation patterns may affect the insect survival and development directly or indirectly by altering the plant chemistry. Elevated CO_2 will influence distribution, abundance, and survival and development of herbivore insects (Lincoln et al. 1984, Fajer et al. 1989, Sharma 2014). The indirect effects of CO_2 will result in reduced survival, growth, metamorphosis, and reproduction of insects (Wu et al. 2006). Buckeye butterflies (*Junonia coenia*) on *Plantago lanceolata* exhibit higher rates of mortality and increased development time when fed on plants grown under elevated CO_2 (Fajer et al. 1989). Higher rates of insect mortality have also been associated with nutritional deficiency that results from reduced foliar nitrogen concentrations under elevated CO_2 (Brooks and Whittaker 1999, Stiling et al. 1999). Climate change-mediated effects may not always be detrimental to insects, e.g., the activity of detoxification enzymes may be stimulated in insects by increased concentrations of secondary metabolites in foliage as a potential mechanism to compensate the CO_2-mediated changes in plant quality (Lindroth et al. 1993). Reduced food quality in terms of nitrogen in foliage due to elevated CO_2 increases insect performance to compensate for lower amounts of nitrogen. For example, red-headed pine sawfly, *Neodiprion lecontei* (Fitch) larvae increase nitrogen utilization efficiency in response to CO_2-mediated decline in foliar nitrogen in loblolly pine (Williams et al. 1994). Longer larval duration, increased larval survival rates, increased food consumption (from first to fourth generation), increased digestibility, and decreased efficiency of conversion of ingested food into body matter have been recorded in four successive generations of caster semi-looper *Archaea janata* (L.) under elevated CO_2 (Rao et al. 2013). The activities of total protease, trypsin-like enzymes, and weak and active alkaline trypsin-like enzymes increased in the midgut of Cotton bollworm, *Helicoverpa armigera* (Hubner), when fed on wild tomato plants grown under elevated CO_2 (Guo et al. 2012). The gut cysteine proteinase activity was greater in beetles consuming foliage of soybean grown under

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elevated CO_2 than in beetles consuming soybeans grown under ambient CO_2, which is consistent with enhanced growth and development of beetles on plants grown under elevated CO_2 (Zavala et al. 2008).

Environmental temperature has a strong bearing on ectothermic species such as insects because they have limited capabilities for thermoregulation. Global warming may lead to more frequent or more severe outbreaks of insect pests (Sharma 2014). Temperature influences herbivory and insect development directly, and also exhibits indirect effects by changing the nutritional composition of host plants (Ayres and Scriber 1994, Lombardero et al. 2000). Increased temperatures generally result in increased fitness, higher survival rates, and shorter development times (Bale et al. 2002). In contrast, survival of *Oligonychus unungus* (Jacobi) mite decreased under variable-temperature regimes (Kramer and Hain 1989). Elevated temperatures decrease larval development time during metamorphosis and have negative effects on survival of natural enemies (Ali et al. 1990, Williams et al. 2003, Zvereva and Kozlov 2006, Brose et al. 2012, de Sassi and Tylianakis 2012). Transgenic crops expressing toxin genes from *Bacillus thuringiensis* (Berliner) (Bt) have been deployed on a large scale for pest management (Sharma et al. 2005). However, their effectiveness for pest control may be influenced by climatic factors (Sharma 2014). Transgenic cotton grown under elevated CO_2 expressed reduced concentrations of Bt protein (Coviella et al. 2000). Therefore, it is important that we understand how these abiotic factors influence survival and development of individual insect species, and their interactions with the host plants.

Legume pod borer, *H. armigera*, is one of the most important constraints to crop production in Asia, Africa, Australia, and the Mediterranean Europe. It is a polyphagous pest and has been reported to attack >200 different plant species including cotton, grain legumes, cereals, vegetables, fruits, and forest trees (Sharma 2005). Therefore, it is important to understand the effects of global warming and climate change on this ubiquitous pest to develop appropriate strategies for its management. Increase in levels of CO_2 and CO_2-mediated global warming will affect the biology and population dynamics of this pest. Therefore, we studied the direct effects of varying levels of CO_2 and temperature on survival and development, and metabolism of *H. armigera*.

Materials and Methods

Insect Culture. The culture of *H. armigera* larvae was obtained from insect rearing laboratory, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India.

Insect Survival and Development Under Different Temperature and CO_2 Regimes. Neonates of *H. armigera* larvae were released into chickpea-based semisynthetic diet (Babu et al. 2014) in 25 cell-well plates under laboratory conditions at $65 \pm 5\%$ relative humidity and a photoperiod of 12:12 (L: D) h at three different concentrations of CO_2 (350, 550, and 750 ppm) at $27 \pm 1°C$ in CO_2 incubators (Percival, Bangalore, India). Another set of cell-well plates was kept at four different regimes of temperatures (15, 25, 35, and 45°C) in incubators (Percival). There were three replications for each treatment and 25 larvae in each replication in a completely randomized design. Larval weight, percentage of larvae survived, time taken to complete larval stage, pupal weight, percentage of pupae formed, time taken to complete pupal stage, percentage of adult emergence, and female fecundity were recorded for *H. armigera* kept under each treatment. Relative growth rate was determined according to Karowe (2007) as follows: Relative growth rate (RGR) = (weight gained) / (average larval weight × days).

Isolation of Midgut Extract. The third- and fourth-instar larvae collected from cell-well plates kept at different CO_2 and temperature regimes were starved for 3 h and their midguts collected by dissecting the larvae. The midguts were homogenized in 0.1 M glycine-NaOH buffer, pH 10.0. The homogenate was centrifuged at $8,000 \times g$ for 15 min, and the supernatants were collected and used for enzyme assay. Whole larval extract was used for the insects reared at 15°C; because of their prolonged larval period, they attained small size by the time larvae in other treatments reached fourth-instar stage. Amount of protein in the extracts were determined according to Lowry et al. (1951).

Assay of Midgut Proteases. Trypsin, chymotrypsin, elastase, and aminopeptidase activity was measured by incubating the gut extract with $N\alpha$-benzoyl-DL-arginine *p*-nitroanilide, *N*-glutaryl-L-phenylalanine *p*-nitroanilide, *N*-succinyl-alanine-alanine-alanine *p*-nitroanilide, and leucine *p*-nitroanilide (Sigma Aldrich, Mumbai, India), respectively (Vinod et al. 2010, Purushottam and Vandana 2013). The amount of product formed was measured at 410 nm. One unit of enzyme activity was expressed as one μmol of product formed per minute per mg of gut protein. Total protease activity was determined using azocasein (Sigma Aldrich) as substrate (Bieth et al. 1974). Total protease activity (UA) was calculated by using equation, Unit activity (UA) = ABS 450 nm/[time (min) × mg of enzyme protein].

Assay of Carbohydrases (Amylase and Cellulase). Amylase activity in midgut homogenates was assayed by the dinitrosalicylic acid (DNS) method using 1% soluble starch (Qualigens Fine Chemicals, Mumbai, India) as a substrate. The enzyme extract was incubated with soluble starch prepared in sodium phosphate buffer (pH 7.0) containing 10 mM NaCl for 20 min at 37°C. The reaction was stopped by the addition of 500 ml DNS and heating the tubes in a boiling water bath for 5 min. The absorbance was read at 540 nm after cooling on ice (Kotkar et al. 2009). A standard curve of maltose (Sigma Aldrich) was prepared to calculate amylase activity. Cellulase activity was determined by DNS method using 1% carboxymethyl cellulose sodium salt (CMC) as a substrate. Enzyme extract was mixed with CMC suspended in 50 mM sodium citrate buffer, pH 6.0, and then incubated for 0.5 h with CMC at 50°C (Oppert et al. 2010). The absorbance was read at 540 nm.

Isolation of Mitochondria. The third- and fourth-instar larvae reared at different temperature and CO_2 regimes were starved for 3 h and the midgut content removed. The cuticle was washed in cold distilled water and homogenized in Dounce homogenizer under cold conditions in the isolation media (0.25 M sucrose solution containing 0.1% defatted BSA). The homogenate was filtered through a moist muslin cloth, and the filtrate centrifuged at $800 \times g$ for 10 min at 4°C. The supernatant was centrifuged at $8,000 \times g$ for 10 min (Akbar et al. 2012). The pellet was collected and stored in isolation medium at 4°C until used.

Assay of Succinate Dehydrogenase (SDH) and Cytochrome Oxidase. SDH activity was measured using phenazinemethosulphate (PMS) as electron acceptor in the mitochondrial extract. The reaction system contained 250 mM sucrose, 50 mM potassium phosphate buffer, pH 7.2, 1 mM KCN, 5 mM $MgCl_2$, 1 mM PMS, and 200 mg mitochondrial protein in a total volume of 1 ml. The reaction was started with 70 μM 2, 6-dichlorophenol indophenol (DCPIP). The rate of reduction of DCPIP was measured at 600 nm ($€_{\mu M}$ 16.2; Akbar et al. 2012). Cytochrome oxidase was measured in a 2-ml reaction mixture containing 60 μM reduced cytochrome c in 50 mM phosphate buffer, pH 7.2. The reaction was initiated by adding mitochondrial protein, and oxidation of cytochrome c was measured at 550 nm (Akbar et al. 2012).

Statistical Analysis. The data were analysed using linear and polynomial regression. Significance of differences between the treatments was judged by F-test. The least significant difference (LSD) was used to test significance of differences between the treatment means at $P < 0.05$.

Results

Survival and Development of *H. armigera* Under Elevated CO_2. Larval survival (50%), larval period (13.67 d), pupation (38.67%), adult emergence (26%), and mean larval weight (420 mg) were optimum in insects reared at ambient CO_2 (350 ppm) than at 550 ppm (38%, 12 d, 29.33%, 19.33%, and 400 mg, respectively) and 750 ppm CO_2 (40%, 10.67 d, 32%, 20.67%, and 340 mg, respectively). Average pupal period and pupal weight were 10.67 d and 320 mg, respectively, at ambient CO_2, and the parameters significantly increased at 750 ppm CO_2 (11.33 d, 340 mg; Fig. 1). Relative growth rate was maximum at 750 ppm (0.093 g $g^{-1}d^{-1}$) compared to insects reared at 350 and 550 ppm CO_2 (0.073 and 0.083 g $g^{-1}d^{-1}$, respectively). Female fecundity was significantly higher in larvae reared at 750 ppm CO_2 (1,293 eggs female[−1]) as compared to that at 350 and 550 ppm CO_2 (831 and 824 eggs female[-1], respectively; Table 1).

Survival and Development of *H. armigera* Under Different Temperatures. Mean larval weight (370 mg) was greater at 35°C compared to the insects reared at 15 and 25°C (10 and 320 mg, respectively). Larval period (12 d), pupation (27.33%), pupal period (10.33 d), and adult emergence (20%) were optimum at 25°C as compared to the insects reared at

15°C (40.33 d, 22.67%, 61 d, 11.33%, respectively) and 35°C (10 d, 23.33%, 8.33 d, and 12.67%, respectively). Larval survival (76%) and pupal weights (370 mg) were maximum at 15°C, which decreased with an increase in temperature (62.67%, 350 mg at 25°C and 46.67%, 330 mg at 35°C, respectively). None of the larvae survived at 45°C (Fig. 2). Relative growth rate was maximum at 35°C (0.1 g $g^{-1}d^{-1}$) compared to insects reared at 15 and 25°C CO_2 (0.073 and 0.083 g $g^{-1}d^{-1}$, respectively). Female fecundity at 25°C was 864 eggs female[-1]. There was no egg laying by the insects reared at other temperature regimes (Table 2).

Activities of Midgut Proteases. Activities of trypsin, chymotrypsin, elastase, total protease, and aminopeptidase were 2.26, 0.38, 0.50, 3.43, 2.00 units, respectively, in the midgut extracts of larvae reared at ambient CO_2 (350 ppm). The activities of midgut proteases, trypsin (81.85, 80.36%), chymotrypsin (34.56, 97.59%), elastase (67.19, 82.76%), and total protease (75.36, 136.62%) increased with an increase in CO_2. Aminopeptidase activity decreased by 25.03 and 52.61% at 550 and 750 ppm, respectively, as compared to the activity in insects reared at 350 ppm (Fig. 3a).

Activities of trypsin, chymotrypsin, elastase, total protease, and aminopeptidase in insects reared at 15°C were 1.87, 0.26, 0.31, 2.55, 0.62 units/mg protein, respectively. The activities of trypsin (62.01, 86.67%), chymotrypsin (186.80, 244.57%), elastase (148.69, 194.4%), total protease (34.56, 43.53%), and aminopeptidase (208.77, 221.44%) increased significantly at 25 and 35°C, respectively (Fig. 3b).

Amylase and Cellulase Activity in the Midgut. The activities of amylase and cellulase in the midgut of *H. armigera* larvae at 350 ppm were 3.45 and 60.45 units, respectively, which increased with an increase in CO_2 (31.94, 24.81% at 550 ppm, and 68.84, 40.03% at 750 ppm). The activities of amylase and cellulase at 15°C were 1.82 and 49.76 units, respectively, which increased significantly at 25 (112.61, 23.67%) and 35°C (276.08, 78.31%; Fig. 4).

Activities of SDH and Cytochrome Oxidase. The activities of SDH and cytochrome oxidase in mitochondrial extract of *H. armigera* larvae at 350 ppm were 3.16 and 5.52 μmoles/min/mg protein, respectively, which increased at 550 (44.30, 43.20%) and 750 ppm CO_2 (85.12, 95.27%). The activities of SDH and cytochrome oxidase at 15°C were 2.55 and 3.85 μmoles/min/mg protein, respectively, which increased significantly at 25 (27.45, 51.95%) and 35°C (165.88, 124.67%; Fig. 5).

Discussion

Global rise in CO_2 levels and the CO_2-mediated changes in temperature are steadily increasing. Temperature-mediated drought will affect the plant growth and nutritional quality through changes in nitrogen content and secondary metabolites, which will indirectly influence food consumption and growth and development of insect pests. Insect survival and development is also influenced by biotic factors (natural enemies and diseases). Environmental factors affect the
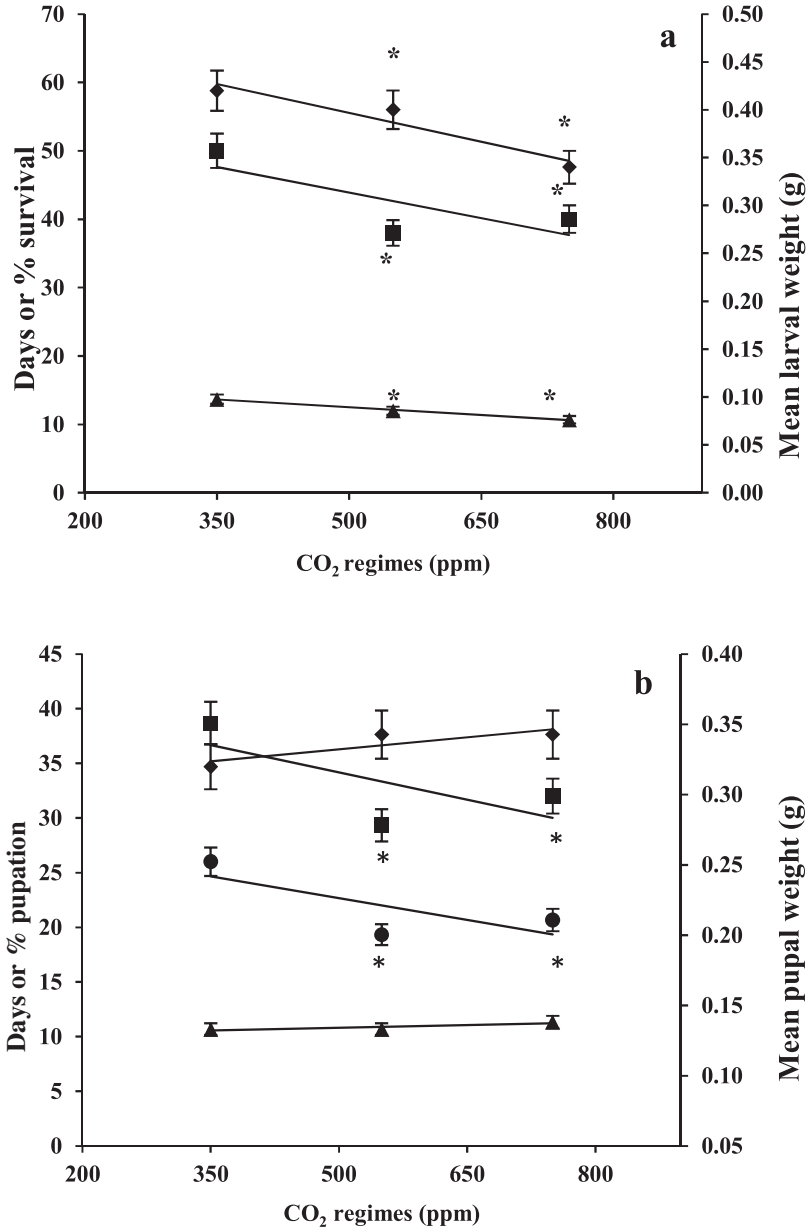
Fig. 1. Effect of different CO_2 regimes on the survival and development of *H. armigera*. (a) Weight of larvae (◆), $y = -0.0002x + 0.4967$, $R^2 = 92.3\%$; larval survival (■), $y = -0.025x + 56.417$, $R^2 = 60.5\%$; larval period (▲), $y = -0.0075x + 16.238$, $R^2 = 99.6\%$. (b) Pupal weight (◆), $y = 6E\text{-}05\,x + 0.3037$, $R^2 = 74.6\%$; pupation (■), $y = -0.0167x + 42.505$, $R^2 = 48.06\%$; pupal period (▲), $y = 0.0017x + 9.9825$, $R^2 = 75\%$; adult emergence (●), $y = -0.0133x + 29.329$, $R^2 = 57.05\%$. *Significantly different from the values at 350 ppm, at $P < 0.05$.

Table 1. Effect of CO_2 on relative growth rate and female fecundity in *H. armigera*

Treatment	Relative growth rate (g g^{-1} d^{-1})	Fecundity
350 ppm	0.073 ± 0.001a	831.3 ± 10.5b
550 ppm	0.083 ± 0.003 b	824.3 ± 15.2a
750 ppm	0.093 ± 0.002^c	1293.0 ± 20.4c

Values within the columns represent mean ± SD.
Values with same letter in a column are not significantly different at $P < 0.05$.

insect growth directly and indirectly (Berggren et al. 2009). In this study, we evaluated the direct effects of two important abiotic factors—temperature and CO_2—on survival and development of *H. armigera*. As midgut is involved in digestion of ingested food, the activity of digestive enzymes in the midgut is also influenced by changes in CO_2 and temperature which influence survival and development of the *H. armigera*. The indirect effects of abiotic factors on plant chemistry were
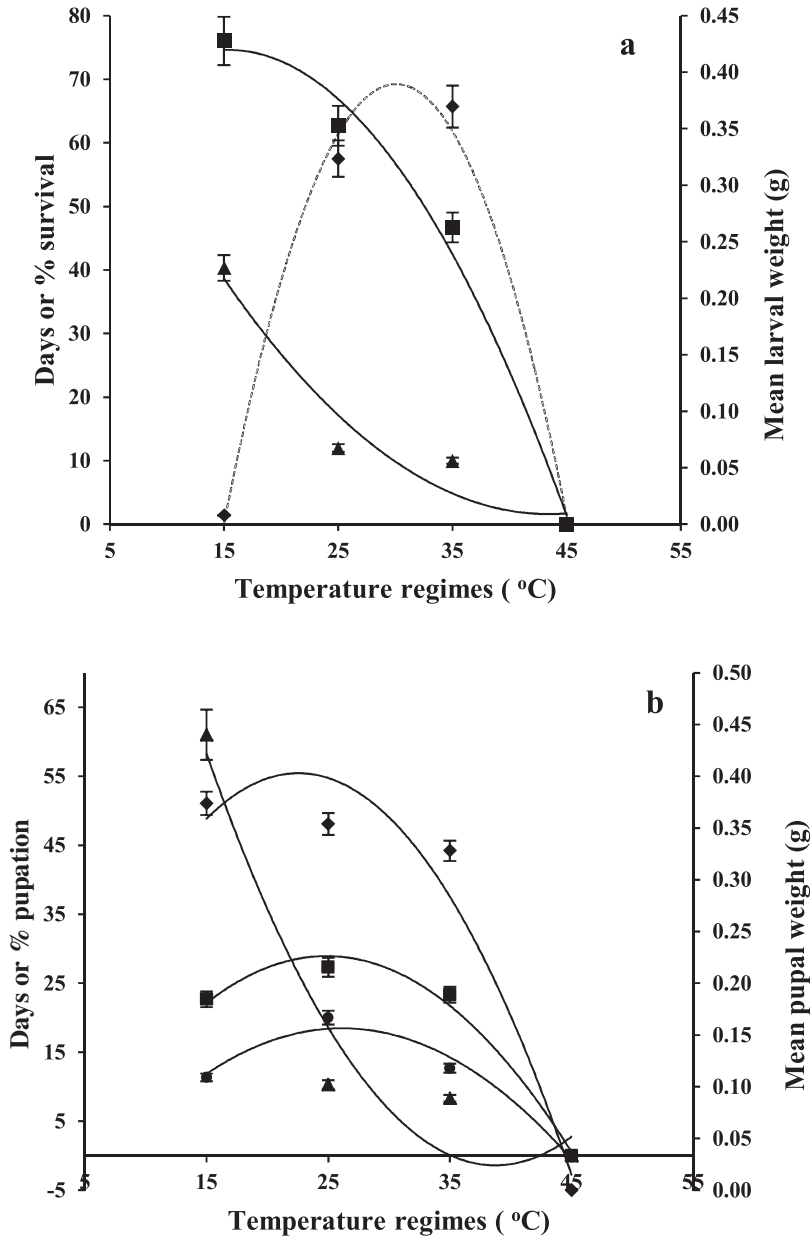
Fig. 2. Effect of different temperature regimes on survival and development of *H. armigera*. (a) Weight of larvae (◆), $y = -0.0017x^2 + 0.103x - 1.159$ ($R^2 = 99.1\%$); larval survival (■), $y = -0.0833x^2 + 2.561x + 54.939$ ($R^2 = 98.81\%$); larval period (▲), $y = 0.0458x^2 - 3.9794x + 87.994$ ($R^2 = 93.45\%$). (b) Pupal weight (◆), $y = -0.0008x^2 + 0.0347x + 0.0115$ ($R^2 = 95.33\%$); pupation (■), $y = -0.07x^2 + 3.4784x - 14.295$ ($R^2 = 98.76\%$); pupal period (▲), $y = 0.1059x^2 - 8.201x + 157.45$ ($R^2 = 93.45\%$); adult emergence (●), $y = -0.0534x^2 + 2.7878x - 17.95$ ($R^2 = 97.23\%$).

overcome by rearing the larvae on artificial diet in incubators under different CO_2 and temperature regimes.

Elevated CO_2 adversely affected the larval survival, mean larval weight, larval period, pupation, and adult emergence, but pupal weight increased under elevated CO_2. Pupation and adult emergence were in agreement with larval survival and weight. Pupal weights and female fecundity increased with an increase in CO_2 (correlation coefficient, $r = 0.85$ to 0.86). In contrast, elevated CO_2 reduced the egg laying by Cactus moth *Cactoblastis cactorum* Berg (Stange 1997) and *A. janata* (Rao et al. 2013). Elevated CO_2 did not affect the fecundity in *Manduca sexta* L. (Abrell et al. 2005). Elevated levels of CO_2 may therefore cause an outburst of *H. armigera*. Female fecundity was positively but nonsignificantly correlated with the pupal weights ($r = 0.48$). Pupal weights were strongly correlated with fecundity in *Colias philodice* Latreille in a

Table 2. Effect of temperature regimes on relative growth rate and female fecundity in *H. armigera*

Treatment	Relative growth rate (g g^{-1} d^{-1})	Fecundity
15°C	0.024 ± 0.002b	0.0 ± 0.0a
25°C	0.083 ± 0.002c	864.0 ± 12.5b
35°C	0.100 ± 0.004d	0.0 ± 0.0a
45°C	0.0 ± 0.0a	0.0 ± 0.0a

Values within the columns represent mean ± SD.
Values with same letter in a column are not significantly different at $P < 0.05$.

behavioural study predicting host plant preference (Karowe 1990). Significant increase in activities of midgut proteases (except aminopeptidase; $r = 0.86$ to 1.00), carbohydrases ($r = 0.99$ to 1.00), and mitochondrial enzymes ($r = 1.00$ to 1.00) with an increase in environmental CO_2 indicate increased digestive efficiency and metabolism of *H. armigera* larvae. But the increased activities of midgut proteases (except aminopeptidase), carbohydrases, and mitochondrial enzymes were negatively correlated with larval survival ($r = -0.66$ to -0.99) and weight gain by the larvae ($r = -0.68$ to -0.99), which indicates decreased efficiency of conversion of digested food into body matter. The reduction in larval period ($r = -0.89$ to -1.00) with increase in CO_2 levels was due to increased metabolism which in turn may lead to rapid generation turnover of *H. armigera*. CO_2-induced direct effects in *H. armigera* were similar to CO_2-mediated indirect effects observed earlier. For example, the activities of midgut proteases of *H. armigera* increased when fed on wild tomato plants grown under elevated CO_2 (Guo et al. 2012). An increase in food consumption and digestibility, and decreased efficiency of conversion of digested and ingested food into body matter, and relative growth rate in response to elevated CO_2 have earlier been observed for successive generations of *A. janata* and *H. armigera* when fed on castor and wheat, respectively (Rao et al. 2013, Wu et al. 2006).

Temperature affected the relative larval growth positively, but had a negative effect on larval survival, larval period, pupal weights, and pupal period. Although larval survival was maximum at 15°C, the larval growth was very slow. Pupation and adult emergence were maximum at 25°C; females reared at 25°C laid maximum eggs, but no egg laying was observed in insects reared at 15 and at >35°C, indicating that 25°C was optimum for the survival of *H. armigera*. The lower development threshold (LDT) for *H. armigera* was 15°C, and upper development threshold (UDT) 35°C, and the thermal optimum 25°C. The cessation of development and reproduction were observed below LDT and above UDT. At lower temperatures, larval motility decreases, but the neural and muscular activities are impaired due to loss of cell membrane fluidity and enzymatic dysfunction resulting in reduced growth due to metabolic disorders. At higher temperatures, the spiracles are wide open leading to water loss and the melting of cuticular lipids, which permits evaporation through the body surface. Exhaustion of water and

nutrients leads to rapid decrease of motility. Warming to at or above the absolute lethal temperature, which is >35°C for *H. armigera*, caused death of larvae due to irreversible tissue damage. Temperature affected the brood survival from egg to adult, reduced developmental period, maximum survival rate being only 16% at 26°C, and no eggs survived at 32°C in Argentine ant, *Linepithema humile* Mayr (Abril et al. 2010). Activities of midgut proteases ($r = 0.89$ to 0.97), carbohydrases ($r = 0.93$ to 0.99), and mitochondrial enzymes ($r = 0.97$ to 0.99) increased with an increase in temperature, enhancing rate of metabolism and digestion efficiency, which were positively correlated with the weight gain of the larvae ($r = 0.91$), but not with larval survival ($r = -0.99$). Similar to CO_2, temperature also shortened the larval period ($r = -0.99$) due to increased metabolism, which may lead to rapid generation turnover of the insect under global warming. Warming positively affects larval growth and survival due to higher metabolic rates (Bale et al. 2002, Jamieson et al. 2012). Increase in temperature accelerates the rate of development, potentially leading to more generations per year and an expansion in geographical range of the arthropods (Parmesan et al. 1999, Bale et al. 2002, Sharma 2014).

SDH and cytochrome oxidase are important components of electron transport chain in mitochondria, where SDH transfers electrons from succinate to ubiquinol, and cytochrome oxidase transfers electrons from cytochrome c to oxygen, the ultimate electron acceptor during respiration. Increased mitochondrial activity indicates increased response of mitochondria, i.e., respiration under elevated levels of CO_2 and temperature. Activity of mitochondrial enzymes increased along with that of digestive enzymes (proteases and carbohydrases, except aminopeptidase) under elevated CO_2 ($r = 0.83$ to 1.00) and temperature ($r = 0.66$ to 0.99). Increased activities of mitochondrial enzymes were not in agreement with larval weights in larvae reared under elevated CO_2 which may be due to reduced efficiency of conversion of digested food into body matter. *Colias philodice* Godart larvae fed under elevated CO_2 had increased digestion, but decreased efficiency for conversion of digested food and, consequently, had no effect on growth rate, instar duration, or pupal weight (Karowe 2007). Respiration rates were significantly higher for gypsy moth larvae reared on birch (15 to 59%) and on aspen clone 216 (36%) and lower on aspen clone 271 (26%) grown under elevated CO_2 compared with ambient conditions (Foss et al. 2013). Although it is known that elevated CO_2 caused mitochondrial dysfunction and impair cell proliferation in plant and animal cell (Gonzalez-Meler et al. 1996, Vohwinkel et al. 2011), the increase in mitochondrial activities found in insects and the ability to withstand the adverse effects of CO_2 on mitochondria could be an adaptation in them to survive at elevated levels of CO_2 which insects developed slowly during rise in the levels of environmental CO_2.

Aminopeptidase has been identified as a Bt toxin receptor in the midgut epithelium of insect pests (Gill et al. 1995, Lee et al. 1996, Luo et al. 1997, Liao et al.
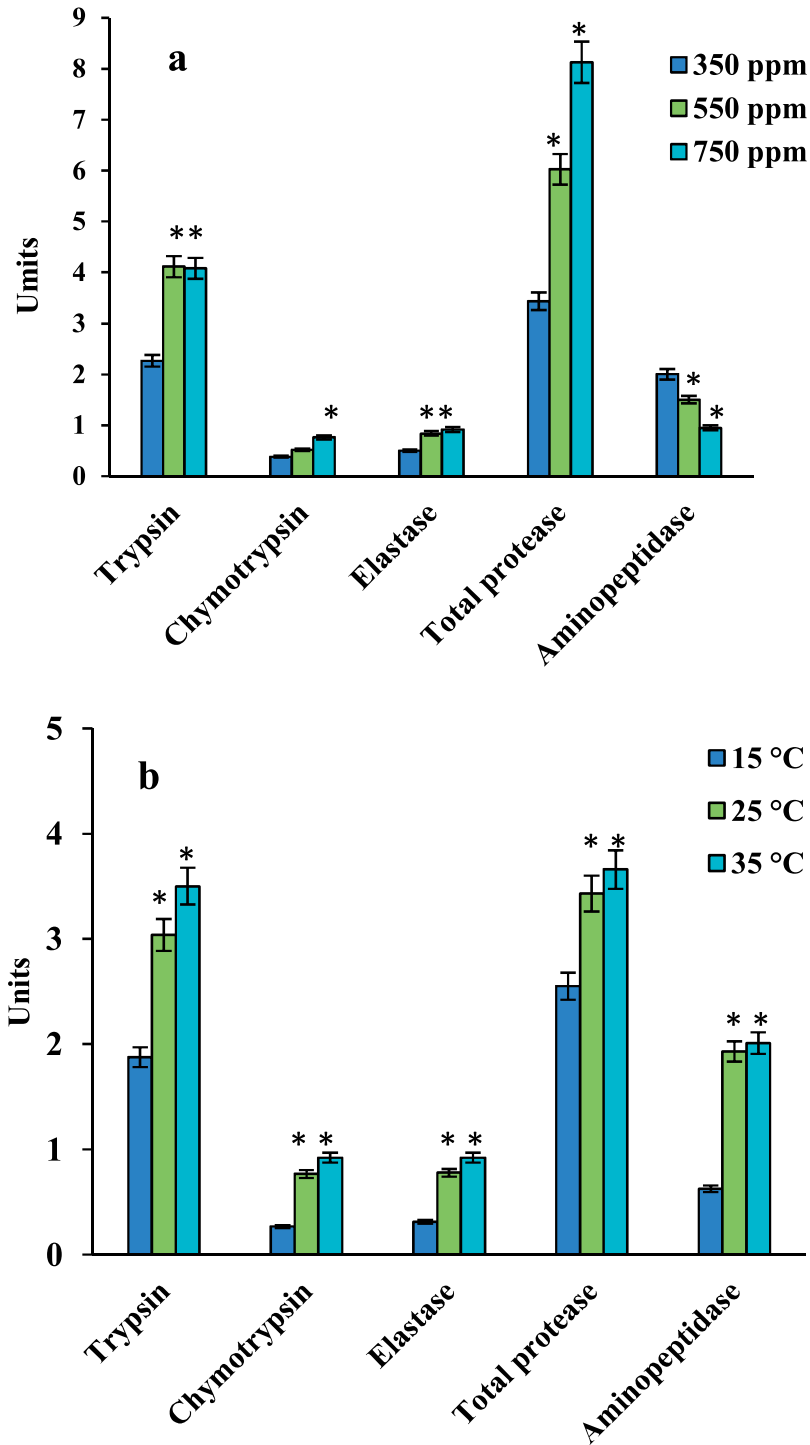
Fig. 3.   Effect of different CO_2 and temperature regimes on the midgut proteases of *H. armigera*. Activities of midgut trypsin, chymotrypsin, elastase, total protease, and aminopeptidase in *H. armigera* larvae reared at different levels of (a) CO_2 (350, 550, and 750 ppm) and (b) temperature (15, 25, and 35°C). *Values in figure a and b are significantly different from values at 350 ppm and 15°C respectively, at $P < 0.05$.
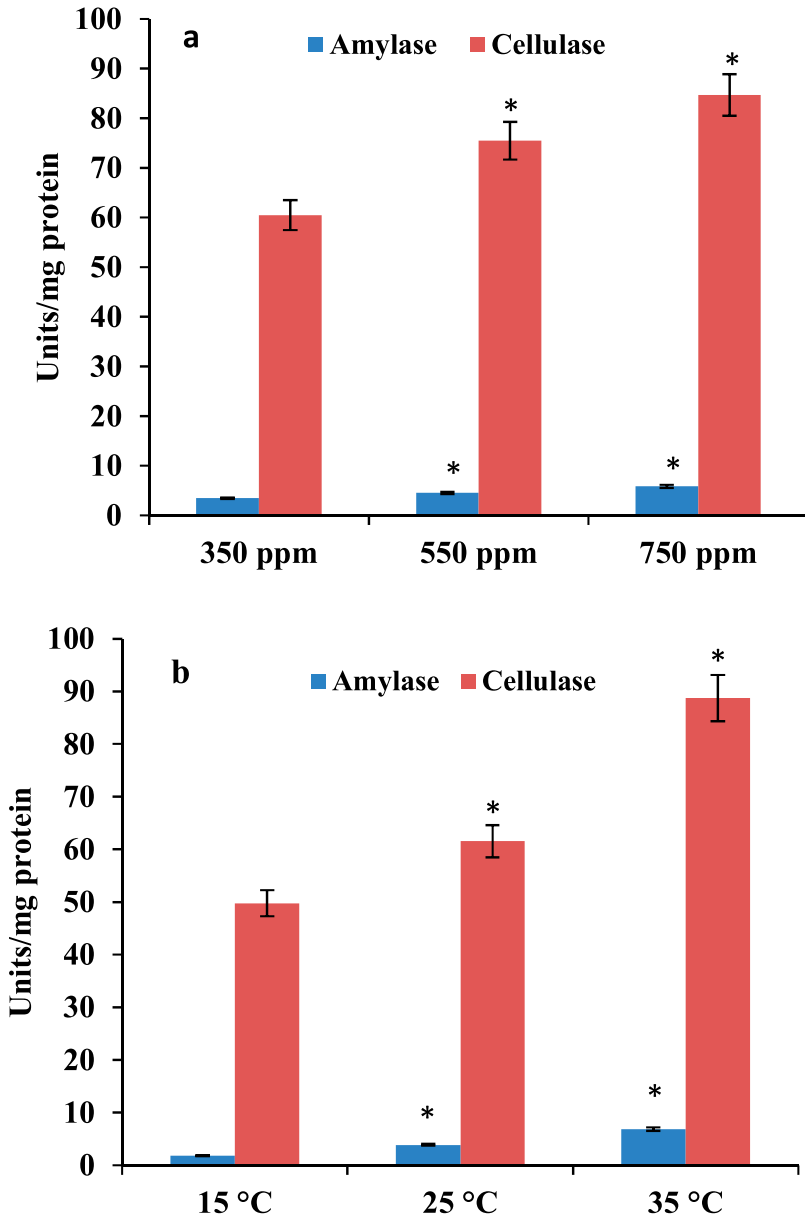
Fig. 4. Effect of CO_2 and temperature regimes on the midgut carbohydrases in *H. armigera*. Activity of amylase and cellulase in *H. armigera* larvae reared at different levels of (a) CO_2 (350, 550, and 750 ppm) and (b) temperature (15, 25, and 35°C) regimes. One unit of amylolytic activity was expressed as 1 nmol of reducing sugar produced per min per mg protein, and one unit of cellulolytic activity was expressed as 1 μmol of reducing sugar produced per min per mg protein at pH 7.0. *Values in figure a and b are significantly different from values at 350 ppm and 15°C, respectively, at $P < 0.05$.

2005). Activity of aminopeptidase decreased with an increase in CO_2 ($r = -1.00$), and this may decrease the susceptibility of *H. armigera* larvae to Bt under elevated CO_2. Crop damage by *H. armigera* is likely to increase under global warming and climate change, irrespective of the presence or absence of insecticidal genes (Sharma 2014), and this pest may become more serious under elevated CO_2 (Chen et al. 2005). However, aminopeptidase activity increased with an increase in temperature ($r = 0.89$), which may offset some of the

negative effects of elevated CO_2. Bt-transgenic cotton grown under warmer conditions caused greater mortality of *H. armigera* larvae (80%) than the plants grown under cooler conditions (35%; Mahon et al. 2002). Therefore, *H. armigera* susceptibility to Bt-transgenic crops may increase under global warming.

In conclusion, elevated CO_2 and temperature induced changes in insect metabolism by enhancing the activity of proteases and carbohydrases in the insect midgut, which influenced the survival and growth and
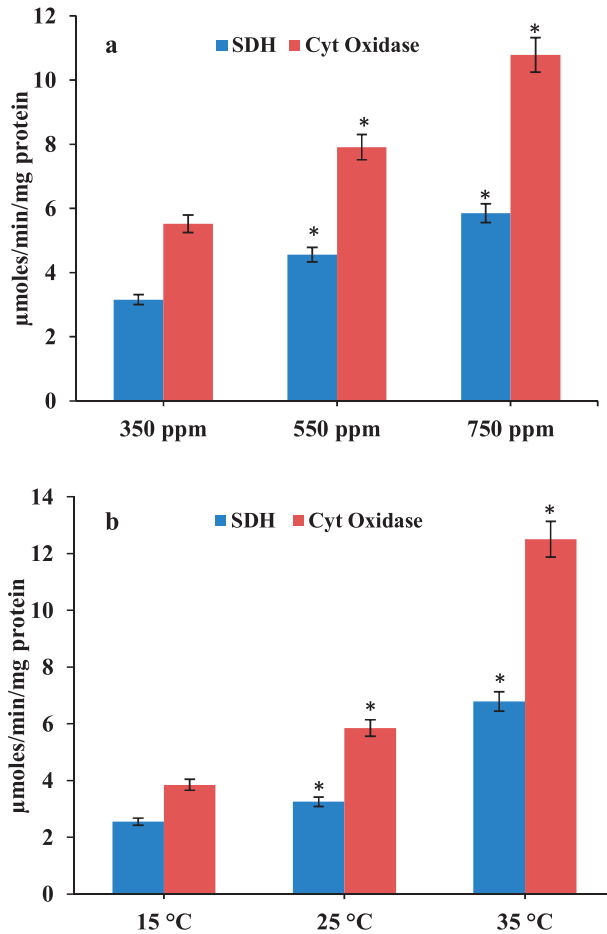
Fig. 5. Effect of CO_2 and temperature regimes on the mitochondrial enzymes in *H. armigera*. Activity of succinate dehydrogenase and cytochrome oxidase in *H. armigera* larvae reared at different levels of (a) CO_2 (350, 550, and 750 ppm) and (b) temperature (15, 25, and 35°C) regimes. *Values in figure a and b are significantly different from values at 350 ppm and 15°C, respectively, at $P < 0.05$.

development of *H. armigera*. Higher consumption rates during elevated levels of CO_2 and temperature may cause more damage to crop yields.

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