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Impact of Elevated CO_2 and Temperature on Growth and Development of *Helicoverpa armigera* Hubner

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Authors' contributions

This work was carried out in collaboration among all authors. Author DV, PV and MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PA and VS managed the analyses of the study. Author DV managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Climate change is an imminent and inevitable circumstance largely driven by increase in CO_2 and temperature. CO_2 directly affects plants through positive effects on net photosynthetic rate. Higher temperature during the crop growth phase can diminish the yield, with a longer growing season. In the present study, adverse climate conditions i.e. elevated CO_2 (550 ppm) and elevated temperatures (29, 31, 33 and 35 ± 1 °C) were simulated in carbon dioxide and temperature gradient chambers (CTG). Growth and development of *Helicoverpa armigera* on non-Bt and Bt cotton leaves from those CTG chambers were recorded and correlated with foliar carbohydrates and proteins.It

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was found that protein content decreased by almost 42 % in non-Bt cotton and by 36 % in Bt cotton, while larval weight and duration decreased significantly with elevated conditions particularly in Bt cotton. Relative Growth Rate (RGR) increased with eCO_2+ *e*Temp and is relatively less in non-Bt cotton compared to Bt cotton by 4-13 mg g^{-1} day^{-1}. Lower protein content is positively correlated significantly to larval growth rate (r = 0.9**). Effects of climate change on crops and their pests have to be further quantified precisely to develop plausible stress mitigation strategies.

Keywords: Climate change; American bollworm; Bt cotton; carbohydrates; proteins; larval duration; RGR.

1. INTRODUCTION

American bollworm, *H. armigera* is highly polyphagous with more than 100 host plants. The effect of climate change on such pests has attained a lot of significance of late. Constant emissions of greenhouse gases are supposed to increase annual average temperatures (2.5–4.3 °C) in the cultivable areas of the world by 2080 [1]. Numerous FACE (Free Air CO_2 Enrichment) studies demonstrated better cotton yield and growth with increased CO_2 concentration [2-3]. However, the concomitant increase in temperature may enhance both dark reaction and photorespiration and would certainly pose a challenge to cotton production. High CO_2 is associated with down regulation of nitrogen content of foliage [4-5] which may lead to dilution of biochemical constituents i.e. reduction in levels of proteins and inorganic ions [6]. Further, supply demand ratio for carbohydrates in cotton controls the growth rate of the various plant parts [7].

Quantity and quality of host plant foliage, has a direct bearing on the growth and development of insect herbivores. Proteins and carbohydrates are important nutrients that provide essential amino acids and energy, and influence larval development, growth, and fecundity [8]. Selection of foliage with optimum levels of protein has utmost importance as evident from the choice test, wherein *Helicoverpa zea* selected a diet with a protein-to-carbohydrate (P: C) ratio of 1.6: 1 [9]. It was estimated that the sugar content of every crop produce will rise with increase of carbon emissions [10] and as carbohydrates are phagostimulants, foliage invites more pests and result in higher crop damage. It was reported that wanting of nitrogen also stimulates insects to consume about 80 % more foliage [11]. Under nutrition of the plants grown under eCO_2 conditions, affect the development of insects indirectly often termed as ‘Host mediated effect’ [12], which can induce both increased larval duration and greater mortality [13]. However studies on the coupled influence of CO_2 + temperature on larval growth and development are meagre. Hence, studies on the interactive effect of elevated CO_2 and temperatures on cotton foliage quality and on *H.armigera*, are of immense value in planning future crop protection strategies.

2. METHODOLOGY

2.1 Experimental Study Site

The study was conducted utilizing Carbon dioxide and Temperature Gradient (CTG) Chambers and insect growth chambers at ICAR-Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad, Telangana during 2018-2020. The aforementioned facility has a provision to maintain constant levels of CO_2 and temperatures in eight chambers with 30 meters length, 6 meters width and 4 meters height at center. The experiment was planned with elevated CO_2 (eCO_2: 550 ± 25 ppm) and elevated temperatures (*e*Temp: 29, 31, 33 and 35 ± 1 °C) compared to ambient conditions (aCO_2: 380 ppm; *a*Temp: 28 °C). RCH-659 hybrids of non-Bt and Bt cotton were grown in each of the ten individual and combination treatments.

2.2 Test Insect Rearing

Larvae of the test insect, *H. armigera* were reared on standard artificial diet [14] individually in 6-cell well plates (3.5 cm diameter × 2.0 cm depth) under laboratory conditions (27 ± 1 ºC temperature and 60 % RH). Overlapping generations were maintained for continuous supply of larvae.

2.3 Leaf Biochemical Analysis

Carbohydrates and proteins were estimated in cotton foliage taken from various set conditions. The collected leaf samples were oven dried at 80

°C for 72 hours and ground into fine powder. Carbohydrates and proteins were estimated as per the standard procedures using anthrone [15] and folin-ciocalteu reagents [16], respectively.

2.4 Larval Growth and Development

Neonate larvae of *H. armigera* were allowed to feed on tender leaves of non-Bt and Bt cotton from various CO_2 and temperature conditions *viz.,* 550 and 380 ± 25 ppm CO_2 and 28, 29, 31, 33 and 35 ± 1ºC in CTGCs. Light intensity of 550 µmol/ m^2/s was maintained in the chambers during 14 hours photoperiod with 60 % relative humidity during day and 70 % at night. One larva maintained in each petri dish individually formed a replication. Twenty five such replications were maintained at each condition. Moist filter paper was kept at the bottom of the petri dishes to maintain leaf turgidity. Data regarding larval weights, larval duration and relative growth rate (RGR) were collected.

2.5 Statistical Analysis

The data collected on leaf carbohydrate and protein content and growth parameters of the test insect i.e., larval weight, duration and RGR were analyzed with two way analysis of variance (ANOVA). Correlations between various temperatures on leaf nutrition status and larval growth were performed using Pearson's correlation coefficient analysis.

3. RESULTS AND DISCUSSION

3.1 Biochemical Analysis of Cotton Foliage

Carbohydrate and protein levels were highest at 60 DAS coinciding with the rapid plant growth requirements. Irrespective of the Bt gene, carbohydrate content increased under eCO_2 and *e*Temp conditions, but the protein content decreased.

3.2 Carbohydrate Content in non-Bt Cotton

Carbohydrate content of cotton foliage under aCO_2 at 28 and 35 °C was 43.43 - 48.75 mg g^{-1} at 30 DAS; 45.03 - 51.47 mg g^{-1}at 60 DAS and 42.80 - 48.48 mg g^{-1} at 90 DAS (Table 1). Significantly higher carbohydrate content was recorded under eCO_2 with an increase of 4.83-7.01 % at 30 DAS, 7.03-5.94 % at 60 DAS and 7.59-5.65 % at 90 DAS at 28 and 35 ºC, respectively. At all the growth stages, both eCO_2 and *e*Temp significantly influenced carbohydrate content, where eCO_2 alone brought about 7.01 % rise at 30 DAS and 7.03 % increase at 60 DAS (ns) in carbohydrate content over ambient. Similarly, an increase of 8.3 % of starch in maize leaves was reported at eCO_2 [17]. The interactive effect of eCO_2 + *e*Temp has shown significant increase of 20.12 % over ambient (F = 5.28, P < .05) at 30 DAS but in later stages it was non-significant. Wheat leaf carbohydrate concentration increased 3-fold at eCO_2 [18] implying decreased carbohydrate transport from source to sink tissues due to eCO_2 [19].

3.3 Carbohydrate Content in Bt Cotton

The carbohydrate content under aCO_2 was 42.48 - 47.67 mg g^{-1}at 30 DAS followed by 45.30 - 50.52 mg g^{-1} at 60 DAS and 42.42 - 47.08 mg g^{-1} at 90 DAS. Significant increase in carbohydrate content by 3.06-4.67 % at 30 DAS, 4.34-7.64 % at 60 DAS and 2.07-2.01 % at 90 DAS was recorded in the cotton foliage at 28 and 35 ºC, respectively (Fig. 1). Interactive effect of eCO_2 + *e*Temp has shown significant increase only at 60 DAS (F = 8.515, P < .05) by 20.04 % and 90 DAS intervals (F = 3.003, P < .05) by 13.22 % (Table 2). Both non-Bt and Bt cotton had higher total carbohydrates under elevated CO_2 [20].

3.4 Protein Content in Non-Bt Cotton

The protein content of foliage under aCO_2 conditions was 25.32 - 17.37 mg g^{-1}(30 DAS), 29.71 - 20.11 mg g^{-1} (60 DAS) and 22.35 - 14.97 mg g^{-1} (90 DAS) at 28 and 35 °C. Under eCO_2, protein content decreased by 20.14-27.92 % (30 DAS), 10.23-13.97 % (60 DAS) and 1.83-10.22 % (90 DAS) at the same temperatures (Table 3). Interaction effect has significant influence on protein content of cotton foliage at all growth stages and reduced protein content by 46 % at 60 DAS. Analogously, protein content of sunflower foliage grown at 35 ºC + eCO_2 reduced by 35.90, 33.74, 28.20, 24.62 and 23.45 % at 30, 45, 60, 75 and 90 DAS, respectively compared to 28 ºC + aCO_2 [21]. The present results are also in agreement with marked decrease in protein content of sunflower at higher temperatures (33 /19 ºC) from 70 % to 40 % at 23/19 ºC [22]. Similar, reduction in protein content by 20 % under eCO_2 was reported in maize [23].

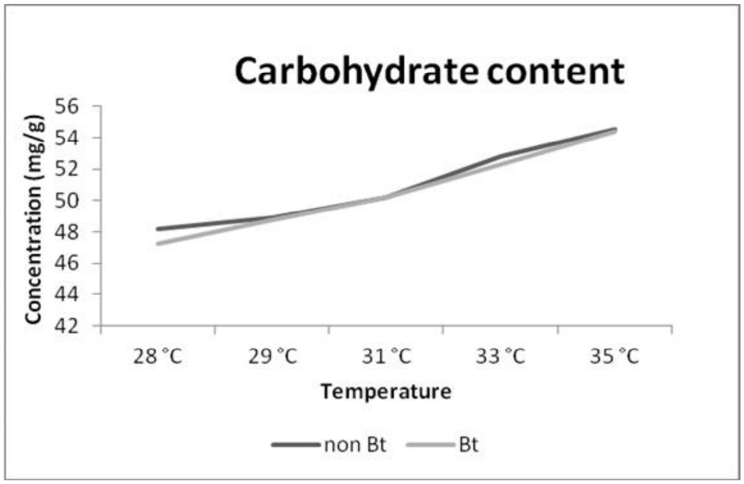

Fig. 1. Effect of *e*CO_2 and *e*Temp on carbohydrate content of non-Bt and Bt cotton

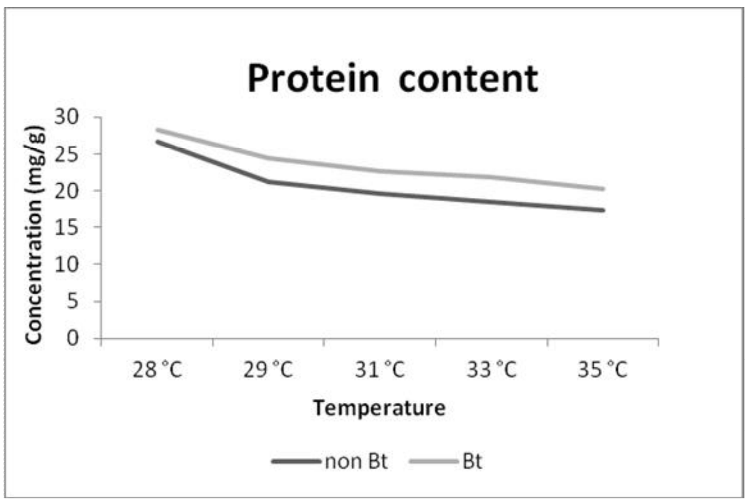

Fig. 2. Effect of *e*CO_2 and *e*Temp on protein content of non-Bt and Bt cotton

3.5 Protein Content in Bt Cotton

In Bt cotton, protein content was 22.33 to 14.09 mg g^{-1} at 30 DAS, 31.42 to 24.77 mg g^{-1}at 60 DAS and 23.26 to 16.03 mg g^{-1}at 90 DAS under *a*CO_2 conditions at 28 and 35 °C, respectively (Table 4). At the same temperatures, protein content under *e*CO_2 decreased by 8.19-0.7% (30 DAS), 10.08-18.36% (60 DAS) and 16.16 - 12.91% (90 DAS) (Fig. 2). At 60 DAS, interaction effect *e*CO_2+*e*Temp reduced the protein content by 36 % over *a*CO_2 + *a*Temp. The findings are in line with the report that high temperature reduces soluble protein content in bolls of Bt cotton [24]. Protein content was not significantly different between transgenic and non-transgenic cotton [25].

3.6 Effect on Larval Growth and Development

Larval weight and duration of *H.armigera* increased under *e*CO_2 and decreased under *e*Temp conditions in both non-Bt and Bt cotton. RGR decreased under both *e*CO_2 and *e*Temp conditions.

3.7 Larval Weight in Non-Bt Cotton

In the first generation under *a*CO_2, larval weight decreased with temperature from 28 ºC (415.08 mg) to 35 ºC (375.08 mg). Under *e*CO_2, weight increased by 19.76 % at *a*Temp and 21.90 % at *e*Temp. These present findings are in accordance with an earlier report that larval weight of *Spodoptera litura* increased significantly by 17.09 % under *e*CO_2 in castor [12]. Similar results were also reported in *H. armigera* on chickpea with increased larval weight under *e*CO_2 (384.16 mg) compared to *a*CO_2 conditions (367.91 mg) [26]. In our study, under *e*Temp, weight decreased by 9.64 and 8.01 % at *a*CO_2 and *e*CO_2 respectively. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence in the first, second and third generations. Mean larval weight increased under *e*CO_2 by 17.66 % at *a*Temp and 19.47 % at *e*Temp and decreased under *e*Temp by 9.03 % at *a*CO_2 and 7.64 % at *e*CO_2. The mean interaction effect also showed non-significant influence on larval weight. Likewise, significantly higher larval weight was recorded when *S. litura* was fed with groundnut foliage grown under *e*CO_2 + *e*Temp compared to ambient conditions [5].

3.8 Larval Weight in Bt Cotton

In the first generation, larval weight varied from 346.71 - 323.75 mg at temperatures 28 and 35 ºC, respectively. Larval weight in Bt cotton is relatively lesser than that under non-Bt cotton. Under *e*CO_2, larval weight increased by 32.06 % at *a*Temp and 31.95 % at *e*Temp. And under *e*Temp, mean weight decreased by 6.62 % at *a*CO_2 and 6.70 % at *e*CO_2. The interaction effect of *e*CO_2 and *e*Temp showed non-significant influence on larval weight in the three successive generations. Mean larval weight increased under *e*CO_2 by 32.56 % at *a*Temp and 33.49 % at *e*Temp and decreased under *e*Temp, by 6.85 % at *a*CO_2 and 6.20 % at *e*CO_2 (Fig. 3). The mean larval weight indicated non-significant influence of the interaction on larval weight (*F* = 1.05, ns). Similar results were reported in chickpea with reduced larval weight of *H.armigera* by 33.51 % at *e*CO_2+ *e*Temp [27].

3.9 Larval Duration in Non-Bt Cotton

In the first generation under *a*CO_2, larval duration ranged from 11.44-14.60 days with lowest and highest duration at 35 and 28 °C, respectively. Under *e*CO_2, duration increased by 13.15 % at *a*Temp and 17.83 % at *e*Temp. This observation is in agreement with increase in larval duration of *H. armigera* by 12.43 % under *e*CO_2 in wheat crop [28]. An increase in larval duration was reported in *Achaea janata* on castor foliage by 12.95 % at *e*CO_2 (16.13 in *a*CO_2 and 18.22 days @ 700 ppm *e*CO_2) [29]. A study on *A. janata* on castor, showed that larval duration increased by 15-20 % in four successive generations under *e*CO_2 [30]. The larval duration of *H. armigera* increased by 10.60-12.45 % in four successive generations under *e*CO_2 in chickpea compared with *a*CO_2 [26]. In the present study, larval duration decreased by 21.64 % at *a*CO_2 and 18.40 % at *e*CO_2 under *e*Temp. High temperature above 35 °C exhibited a negative effect on *H. armigera* larval period but a positive

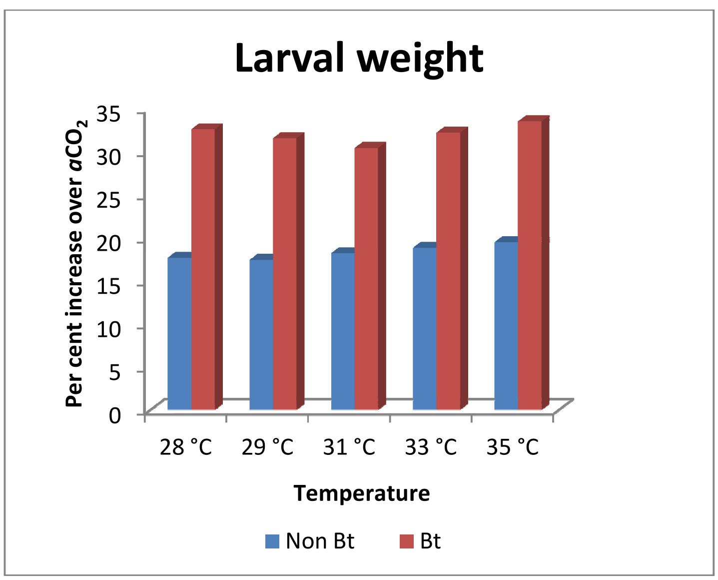

Fig. 3. Effect of *e*CO_2 and *e*Temp on larval weight of *H. armigera*

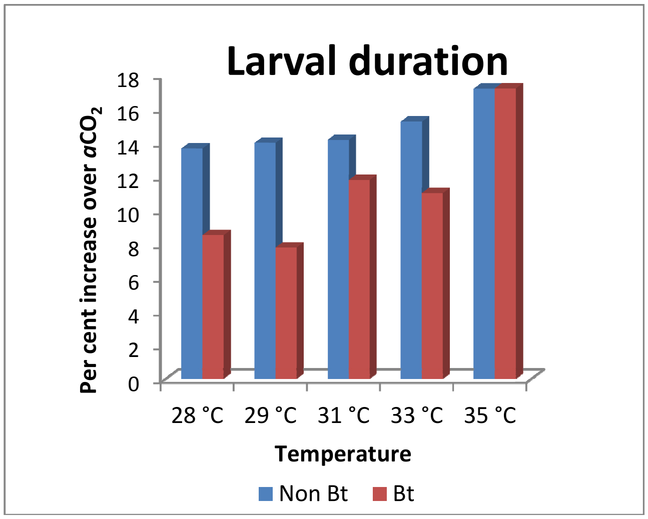

Fig. 4. Effect of eCO_2 and *e*Temp on larval duration of *H. armigera*

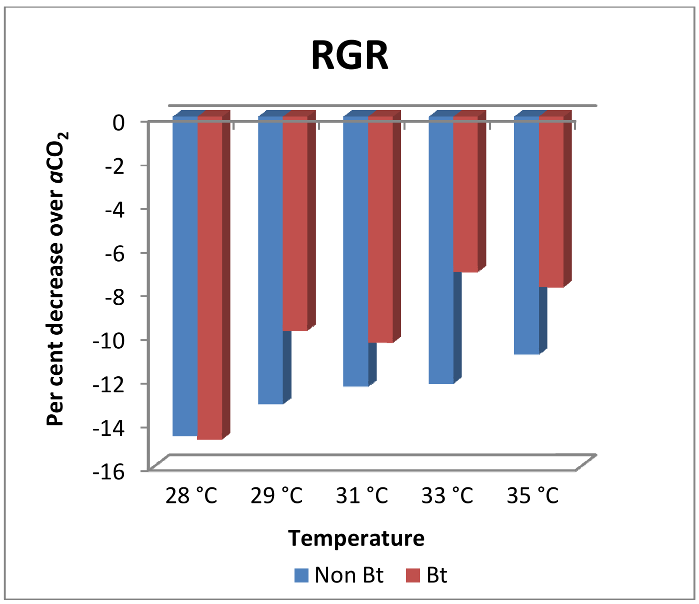

Fig. 5. Effect of eCO_2 and *e*Temp on RGR of *H. armigera*

effect on larval growth on chickpea based artificial diet [31]. Interaction effect of eCO_2 and *e*Temp showed non-significant reduction across the three generations. Mean larval duration decreased under eCO_2 and *e*Temp. Interaction effect has produced 7.02 % increase of mean larval duration, but was non-significant (F = 0.32, ns).

3.10 Larval Duration in Bt Cotton

In the first generation at aCO_2, larval duration varied from 11.15 to 13.42 days with lowest and highest duration at 35 and 28 °C, respectively. Under eCO_2, duration increased by 8.05 % at *a*Temp and 16.95 % at *e*Temp. Under *e*Temp, duration decreased by 16.92 % at aCO_2 and 10.07 % at eCO_2. The interaction effect of eCO_2 and *e*Temp caused a significant decrease in larval duration by 2.83 % in first, 3.22 % in second and 2.05 % in third generation. The interaction effect of eCO_2 and *e*Temp caused a significant decrease of 2.65 % on larval duration (F = 40.29, P < .05). *H. armigera* reared on Bt cotton grown under eCO_2 exhibited higher consumption by 46 % [20] and longer larval

Table 1. Effect of *e*CO_2 and *e*Temp on carbohydrate content of non-Bt cotton foliage

Temperature	Carbohydrate (mg g^{-1}) content in non-Bt cotton														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	43.43	45.53	**44.48**	44.55	47.15	**45.85**	45.03	48.20	**46.62**	43.38	47.75	**45.57**	42.8	46.05	**44.43**
29 ± 1°C	44.20	46.83	**45.53**	45.95	48.45	**47.20**	46.47	48.88	**47.68**	45.72	48.73	**47.23**	44.28	47.17	**45.73**
31 ± 1°C	45.70	48.40	**47.05**	47.67	49.42	**48.54**	47.97	50.20	**49.08**	47.47	50.87	**49.17**	45.2	48.67	**46.93**
33 ± 1°C	46.98	50.45	**48.72**	48.90	51.27	**50.08**	50.17	52.82	**51.49**	48.58	52.05	**50.32**	47.5	49.82	**48.66**
35 ± 1°C	48.75	52.17	**50.46**	50.17	53.20	**51.68**	51.47	54.53	**53.00**	50.20	53.65	**51.93**	48.48	51.22	**49.85**
Mean	**45.82**	**48.67**		**47.44**	**49.89**		**48.22**	**50.92**		**47.07**	**50.61**		**45.65**	**48.58**	
	F value	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**
CO_2	639.20*	0.08	0.24	395.48*	0.09	0.26	330.66*	0.11	0.31	624.49*	0.10	0.30	459.08*	0.097	0.28
Temperature (°C)	361.65*	0.13	0.37	395.48*	0.14	0.41	252.82*	0.17	0.49	250.43*	0.16	0.47	203.88*	0.153	0.45
Interaction (CO_2 + Temp)	5.28*	0.18	0.53	2.84	0.20	NS	1.47	0.24	NS	2.47	0.22	NS	2.159	0.216	NS
CV (%)	0.65			0.69			0.82			0.79			0.79		

*aCO_2 – 380 ± 25 ppm; eCO_2 – 550 ± 25 ppm; *Significant @ 5 % level of significance; NS – Non significant; DAS – Days after sowing*

Table 2. Effect of eCO_2 and eTemp on carbohydrate content of Bt cotton foliage

Temperature	Carbohydrate (mg g^{-1}) content in Bt cotton														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean
28 ± 1°C	42.48	43.78	**43.13**	43.87	46.18	**45.03**	45.30	47.27	**46.28**	43.40	44.73	**44.07**	42.42	43.3	**42.86**
29 ± 1°C	43.30	45.18	**44.24**	44.60	47.67	**46.13**	46.68	48.75	**47.72**	44.78	45.57	**45.18**	43.55	43.72	**43.63**
31 ± 1°C	44.57	47.22	**45.89**	46.62	49.23	**47.93**	48.57	50.17	**49.37**	46.23	47.20	**46.72**	44.97	44.72	**44.84**
33 ± 1°C	46.40	48.72	**47.56**	48.08	50.38	**49.23**	49.87	52.32	**51.09**	47.87	49.00	**48.43**	46.22	46.32	**46.27**
35 ± 1°C	47.67	49.90	**48.78**	49.20	51.30	**50.25**	50.52	54.38	**52.45**	49.20	50.15	**49.68**	47.08	48.03	**47.56**
Mean	**44.88**	**46.96**		**46.47**	**48.95**		**48.18**	**50.57**		**46.29**	**47.33**		**44.84**	**45.21**	
	F value	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**
CO_2	201.41*	0.10	0.31	405.22*	0.09	0.26	314.62*	0.10	0.28	51.52*	0.10	0.30	7.48*	0.09	0.28
Temperature (°C)	200.51	0.16	0.48	243.90*	0.14	0.41	272.21	0.15	0.44	203.17*	0.16	0.48	159.92*	0.15	0.44
Interaction (CO_2 + Temp)	2.46	0.23	NS	1.87	0.20	NS	8.515*	0.21	0.63	0.42	0.23	NS	3.00*	0.21	0.63
CV (%)	0.87			0.71			0.75			0.84			0.82		

*aCO_2 – 380 ± 25 ppm; eCO_2 – 550 ± 25 ppm; *Significant @ 5 % level of significance; NS – Non significant; DAS – Days after sowing*

Table 3. Effect of eCO_2 and eTemp on protein content of non-Bt cotton foliage

Temperature	Protein (mg g^{-1}) content in non-Bt cotton														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean	aCO_2	eCO_2	Mean
28 ± 1°C	25.32	20.22	**22.77**	27.50	22.23	**24.86**	29.71	26.67	**28.19**	26.58	23.15	**24.86**	22.35	21.94	**22.14**
29 ± 1°C	21.26	18.46	**19.86**	23.94	20.40	**22.17**	27.55	21.15	**24.35**	24.11	20.64	**22.37**	20.43	19.41	**19.92**
31 ± 1°C	19.92	16.95	**18.43**	21.24	19.86	**20.55**	23.92	19.57	**21.74**	22.34	17.73	**20.03**	19.51	15.48	**17.49**
33 ± 1°C	18.57	14.58	**16.57**	20.80	16.67	**18.73**	22.92	18.56	**20.74**	18.45	16.58	**17.51**	15.09	14.7	**14.89**
35 ± 1°C	17.37	12.52	**14.94**	19.21	14.35	**16.78**	20.11	17.30	**18.70**	15.28	14.14	**14.71**	14.97	13.44	**14.2**
Mean	**20.48**	**16.54**		**22.53**	**18.70**		**24.84**	**20.65**		**21.35**	**18.44**		**18.47**	**16.99**	
	F value	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**
CO_2	21120.1*	0.05	0.14	10656.7*	0.08	0.24	10408.3*	0.06	0.18	5736.0*	0.06	0.19	887.8*	0.09	0.27
Temperature (°C)	2682.5*	0.07	0.22	851.3*	0.13	0.38	1257.6*	0.10	0.28	953.4*	0.10	0.30	438.7*	0.14	0.42
Interaction (CO_2 + Temp)	522.3*	0.10	0.31	163.2*	0.18	0.54	253.7*	0.14	0.40	86.1*	0.14	0.43	20.9*	0.20	0.60
CV (%)	0.97			1.41			0.94			1.43			2.39		

*aCO_2 – 380 ± 25 ppm; eCO_2 – 550 ± 25 ppm; *Significant @ 5 % level of significance; NS – Non significant; DAS – Days after sowing*

Table 4. Effect of *e*CO_2 and *e*Temp on protein content of Bt cotton foliage

Temperature	Protein (mg g^{-1}) content in Bt cotton														
	30 DAS			45 DAS			60 DAS			75 DAS			90 DAS		
	aCO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean	*a*CO_2	*e*CO_2	Mean
28 ± 1°C	22.33	20.50	**21.41**	27.57	23.59	**25.58**	31.42	28.25	**29.83**	26.27	22.00	**24.13**	23.26	19.5	**21.38**
29 ± 1°C	19.17	17.50	**18.33**	24.71	21.24	**22.97**	29.77	24.36	**27.06**	24.72	20.05	**22.38**	22.88	17.66	**20.27**
31 ± 1°C	18.09	17.03	**17.56**	22.30	19.50	**20.90**	27.78	22.59	**25.18**	23.06	17.11	**20.08**	20.89	16.11	**18.5**
33 ± 1°C	15.43	15.57	**15.50**	19.48	17.01	**18.24**	25.16	21.87	**23.51**	20.65	16.68	**18.66**	17.55	14.49	**16.02**
35 ± 1°C	14.09	13.99	**14.04**	17.18	16.82	**17.00**	24.77	20.22	**22.49**	19.14	15.62	**17.38**	16.03	13.96	**14.99**
Mean	**17.82**	**16.91**		**22.24**	**19.63**		**27.78**	**23.45**		**22.76**	**18.29**		**20.12**	**16.34**	
	F value	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**	**F value**	**SE m**	**CD (p= .05)**
CO_2	4356.3*	0.13	0.40	250.8*	0.36	1.06	3669.8*	0.09	0.26	6460.4*	0.05	0.16	1357.7*	0.06	0.19
Temperature (°C)	240.5*	0.21	0.63	135.7*	0.57	1.67	358.8*	0.14	0.41	981.9*	0.09	0.25	816.5*	0.10	0.30
Interaction (CO_2 + Temp)	36.5*	0.30	0.88	1.0	0.80	NS	102.3*	0.20	0.58	23.5*	0.12	0.35	37.8*	0.14	0.42
CV (%)	2.13			6.29			1.25			1.05			1.42		

*aCO_2 – 380 ± 25 ppm; eCO_2 – 550 ± 25 ppm; *Significant @ 5 % level of significance; NS – Non significant; DAS – Days after sowing*

Table 5. Correlation between foliar carbohydrate and protein content with larval growth and development of *H. armigera*

Larval / Cotton leaf	Duration		Weight		RGR	
	Non-Bt	Bt	Non-Bt	Bt	Non-Bt	Bt
Carbohydrates in non-Bt	-0.99	-0.98	-0.99	-0.92	-0.93	-0.95
Carbohydrates in Bt	-0.99	-0.98	-0.99	-0.92	-0.93	-0.95
Proteins in non-Bt	0.86**	0.90**	0.88**	0.96**	0.95**	0.95**
Proteins in Bt	0.91**	0.94**	0.92**	0.98**	0.97**	0.98**

*** significant @ 1% level of significance*

duration by 1- 2 days [32]. Similarly *S. litura* larval duration was prolonged over aCO_2 + *a*Temp by two days under eCO_2 +*e*Temp on groundnut [5]. As evident from Fig. 4, at eCO_2 larval duration increased over ambient by 13.6-17.18 % in non-Bt and 8.5-17.19 % in Bt cotton. It means per cent increase in larval duration was more in non- Bt at *a*Temp. And there is no difference in per cent increase in larval duration among Bt and non-Bt at *e*Temp.

3.11 RGR in Non-Bt Cotton

In the first generation, under eCO_2, RGR decreased by 16.02 % at *a*Temp and 12.48 % at *e*Temp. Under *e*Temp, RGR decreased by 22.98 % at aCO_2 and 19.74 % at eCO_2. The interaction effect of eCO_2 and *e*Temp has significant effect of 32.59 % in the first, 27.42 % in second and 28.07 % in the third generation. Mean RGR decreased under eCO_2 by 14.63 % at *a*Temp and 10.90 % at *e*Temp and under *e*Temp decreased by 20.66 % at aCO_2 and 17.19 % at eCO_2. The mean RGR is significantly influenced by the interaction effect with 29.30 % increase at elevated over ambient conditions (F = 21.38, P < .05). RGR of *H. armigera* in tomato increased by 25.17 % with 5 °C increase in temperature [33]. RGR in *H. armigera* on chickpea, significantly reduced by 9.53, 11.03, 10.23 and 9.72 % in first, second, third and fourth generation, respectively under eCO_2 over aCO_2 [26].

3.12 RGR in Bt Cotton

In the first generation, RGR decreased under eCO_2 by 15.91 % at *a*Temp and 7.80 % at *e*Temp. Bt cotton and eCO_2 caused significant decrease in RGR of American bollworm by 5.7 % [20]. Under *e*Temp, RGR decreased by 17.91 % at aCO_2 and 9.99 % at eCO_2. The interaction effect of eCO_2 and *e*Temp showed significant influence of 24.31 % in first, 23.85 % in second and 22.08 % in the third generation. The mean RGR decreased under eCO_2 by 14.79 % at *a*Temp and by 7.81 % at *e*Temp. Mean RGR decreased under *e*Temp, by 16.89 % at aCO_2 and 10.09 % at eCO_2. Mean interaction effect showed significant decrease of 23.38 % on RGR of larvae (F = 92.01, P < .05). There is no difference in per cent decrease of RGR among Bt and non-Bt at *a*Temp (Fig. 5). Under *e*Temp, and eCO_2 RGR decreased more at non-Bt cotton than Bt cotton implicating that RGR of *H.armigera* is relatively more affected in non-Bt over Bt cotton.

3.13 Correlation Analysis of Host Plant Nutrition with Larval Growth

Correlation was worked out between leaf carbohydrates and proteins at 60 DAS under all the temperatures and eCO_2 and the mean larval growth parameters (Table 5). Mean larval duration, weight and RGR have non-significant correlation with carbohydrates (r= -0.9**). The analysis revealed a significant positive correlation with leaf protein (r= 0.9**) content at P = .01. It was reported that RGR of *H.armigera* had significant positive correlation with protein content and significant negative correlation with starch content of chickpea grown under eCO_2 condition [34].

4. CONCLUSION

In the present study *H. armigera* larval duration increased with eCO_2 and decreased under *e*Temp and also eCO_2 + *e*Temp compared to aCO_2 + *a*Temp. Further, increase of duration is relatively less in Bt cotton over non-Bt cotton by 0.4-1.5 days based on temperature, indicating that under climate change scenario, Bt cotton may get comparatively less affected by larvae. Hence non-Bt cotton may support the larvae for a longer time. Larval weight increased with eCO_2 and decreased under *e*Temp; but increased with eCO_2 + *e*Temp compared to aCO_2 + *a*Temp. Increase of weight is relatively more in Bt cotton over non-Bt cotton by almost 60-75 mg, but was not significant. Longer larval durations, higher larval weights and increased consumption of foliage were reported in *Ostrinia furnacalis* grown on maize under eCO_2 over the larvae in aCO_2 [35]. Relative growth rate (RGR) decreases with both eCO_2, *e*Temp and eCO_2 + *e*Temp. Decrease in RGR is more in Bt cotton over non-Bt cotton by 4-13 mg g^{-1} day^{-1}. It was reported that RGR decreased in Bt cotton under eCO_2 in *H. armigera* [20]. In the present investigation, carbohydrates increased under eCO_2 + *e*Temp by approximately 20 % which did not vary greatly among non-Bt and Bt cottons having a mere difference of 0.04-0.55 mg g^{-1}. It was established earlier that CO_2 increases total carbohydrates by 27.93 % and decreases N content by 2.58 % in wheat, stimulating *H. armigera* to consume more in order to obtain enough nutrients and extending their larval duration by 12.43 % [28]. Protein content decreased under eCO_2 in Bt over non-Bt cotton by 1.6-3.3 mg g^{-1}. The observation that leaf protein content has significant positive correlation with RGR (r= 0.9^{**}) explains the reason for reduced growth rate of *H. armigera* at eCO_2 + *e*Temp. Further investigations into these aspects will aid in providing comprehensive understanding of climate mediated effects on crops and their pests.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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