RESPONSE OF MULTIPLE GENERATIONS OF TOBACCO CATERPILLAR *SPODOPTERA LITURA* FAB, FEEDING ON PEANUT, TO ELEVATED CO_2

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Abstract. Changes in quality of host plants under elevated CO_2 (eCO_2) conditions likely in future can affect the survival, growth and development, and population dynamics of insect herbivores. The present study aimed to examine the effects of eCO_2 on leaf quality of peanut (*Arachis hypogaea* L.) and consequent effects on growth characteristics of leaf feeding tobacco caterpillar, *Spodoptera litura* (Fabricius) over four successive generations. The feeding trials were conducted using peanut foliage grown in open top chambers (OTCs) under two eCO_2 (550 ppm and 700 ppm) concentrations and one ambient CO_2 concentration. Significantly lower leaf nitrogen, higher carbon, higher relative proportion of carbon to nitrogen (C: N) and higher polyphenols content were observed in eCO_2 foliage. Larvae fed with eCO_2 foliage exhibited longer larval duration, higher larval weights and increased consumption in all four generations. Increased Approximate Digestibility (AD) (about 9%) and Relative Consumption Rate (RCR) (7%), decreased levels of efficiency of conversion of ingested food (ECI) (13%), efficiency of conversion of digested food (ECD) (19%), and relative growth rate (RGR) (9%) were noticed in larvae fed eCO_2 foliage. Significant and cumulative effect of CO_2 on *S.litura* was observed over four successive generations.
Keywords: *consumption, insect performance indices, successive generations, Spodoptera litura*

Introduction

Climate change, especially rise in temperature and atmospheric carbon dioxide (CO_2) concentration, is a major concern today. The concentration of CO_2 in the atmosphere may rise to 550 ppm by as early as 2035 (Stern, 2007). Changes in climate are expected to have significant impacts on crop yields through temperature and carbon dioxide (CO_2) induced changes which in turn are likely to influence insect-plant interactions in several ways. The effects of climate change on insect pests can be both direct and indirect. Temperature, which impacts the development time, longevity and fecundity of insects has a direct effect while elevated CO_2 (eCO_2) has an indirect host- mediated effect on growth and development of insect pests (Hunter, 2001 and Yadugiri, 2010). It is well known that eCO_2 conditions generally result in increased photosynthesis, increased plant growth and greater biomass. Reduced nitrogen content and increased C: N ratio were observed in the plants grown under eCO_2 conditions (Lindroth et al., 1995) indicating reduced nutritional quality of plants leading to changes in feeding pattern of insect herbivores (Hunter, 2001). Most published studies on response of insect herbivores to feeding on plants grown under eCO_2 have been short term experiments, quantifying the consumption and developmental rates of larvae of a single generation (Bezemer and Jones, 1998). Experiments conducted over multiple generations (egg to egg) can reveal the differences in responses between

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generations and also the impact of growth of the host plant on insects (Brooks and Whittaker, 1998). A few studies - *Gastrophysa viridae* on *Rumex obtusifolius* and *Neophilaenus lineatus* on *Juncus squarrosus* (Brooks and Whittaker, 1999); *Helicoverpa armigera* on wheat (Wu et al., 2006), on transgenic cotton (Chen et al., 2007) and on maize (Yin et al., 2010) - have addressed the impact of elevated CO_2 on multiple generations of herbivore insects.

Peanut (*Arachis hypogaea* L.) is one of the world's most important edible oilseed crops. Globally, peanut cultivation occupies about 23.95 million ha with an annual production of 36.46 million metric tons and an average productivity of 1522 kg/ha (http: //eands.dacnet.nc.in/). In India, the crop occupied an area of 5.48 m ha with a production of 5.43 m tons and an average productivity of 900.0 kg/ha in 2009 (GOI, 2011). The crop is attacked by many species of insects which cause damage ranging from incidental feeding to near total plant destruction and yield loss (Wightman, 1994). In India, tobacco caterpillar *Spodoptera litura* has been reported as an important pest during the rainy season causing heavy yield losses (35-55 %). Larvae feed gregariously on leaves causing severe defoliation, leaving only midrib veins. The present studies aimed to understand the effect of *e*CO_2 on leaf quality of peanut and the cumulative effect on growth characteristics of successive generations of *S. litura* fed with eCO_2 grown peanut foliage.

Materials and Methods

Open Top Chambers (OTC)

Three square type open top chambers (OTC) of 4x4x4 m dimensions, were constructed at the Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad (17.38°N; 78.47°E), for maintaining two elevated CO_2 (*e*CO_2) levels, 550 $\pm$ 25 ppm (550 CO_2 -Elevated I) and 700 $\pm$ 25 ppm (700 CO_2 - Elevated II), and an ambient CO_2 level (380 $\pm$ 25 ppm). Carbon dioxide gas was supplied to the chambers and maintained at set levels using manifold gas regulators, pressure pipelines, solenoid valves, rotameters, sampler, pump, CO_2 analyzer, PC linked Program Logic Control (PLC) and Supervisory Control and Data Acquisition (SCADA). The system continuously monitors the concentration of CO_2, temperature and relative humidity inside the OTCs. The equipment is monitored and controlling the CO_2 in OTCs is fully automatic and the desired CO_2 level can be maintained throughout the experimental period in the independent OTCs (Vanaja et al., 2006).

Peanut crop and growth conditions

Peanut was grown in the monsoon season of 2010. Seeds of variety JL 24 were sown in all three OTCs on 29th June in 6 rows at spacing of 30 cm between rows and 10 cm between plants within a row. Plants were maintained for the entire crop duration till 28th November.

Biochemical analysis of foliage

Each chamber consisted of six 4-m rows of plants. Leaf material from the same location and angle of the plant was collected simultaneously for both feeding and biochemical analysis and samples were obtained from the third or fourth leaf down from a branch apex of peanut plants in each CO_2 condition. Foliage from each plant

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thus collected in parallel was analyzed for carbon, nitrogen and polyphenols. To determine carbon and nitrogen concentrations, samples were dried at 80°C and subsequently ground to powder. Leaf carbon and nitrogen were estimated using a CHN analyzer (Jackson, 1973). Total soluble polyphenols (hydrolysable tannins, condensed tannins and non tannin polyphenols) were determined by the Folin-Denis method (Anderson and Ingram, 1993). For this, leaf samples were dried at 40°C for 48 hrs and ground to powder. Phenolics were extracted with methyl alcohol. The concentration of polyphenols in the extract was determined spectrophotometrically using tannic acid as the standard, and the results were expressed as percentage tannic acid equivalents (TAE).

Insect stocks

An insect colony was established using eggs obtained from insect culture maintained in the laboratory. Stock cultures of *S. litura were* maintained on leaves of peanut plants. The cultures were maintained in a controlled chamber maintained at 25°C with a 14-h day/10-h night cycle. Light intensity inside the chamber during the 14 h day period was maintained at 550μ mol m^{-2} s^{-1}. Relative humidity was maintained at 60% (day) and 70% (night).

Feeding trials

First generation experiments were initiated on 30th July (30 days after sowing). At 10 am on the day of initiating the feeding trial, freshly hatched neonates obtained from insect culture maintained in the lab were placed in petridishes of 110 mm diameter and 10 mm height. Ten neonates were kept in each petridish, forming one replication. Five such replications were kept for each of the three CO_2 conditions. The feeding trials were continued up to four generations and trials were conducted as per the procedure given by Srinivasa Rao et al., 2009 & 2012. Feeding trials with first to fourth generation larvae were conducted maintaining the treatment associations, i.e., all four generations received foliage from the same respective CO_2 growing condition. A weighed quantity of leaf was offered to the larvae. The petridishes were then placed in a controlled chamber maintained at 20°C. After 24 hours, at 10 am the next day, the petridishes were opened, the weight of the ten larvae together was recorded and the larvae were replaced in the petridish after preparing it in the same manner as described earlier, with a new leaf of known weight. The leaf remaining after feeding and fecal matter excreted by the ten larvae were dried to constant weight at 40°C in an oven and dry weights were recorded. Although individual larvae were weighed, the weights of the ten larvae derived from each petridish (one replication) were aggregated and the mean was calculated. Mean leaf weight consumed and fecal matter per larva were also calculated similarly. Larval life span was calculated as the period from hatching to pupation. Pupal weight was measured about 24 hrs after pupation was observed. Pupation rate and pupal duration were recorded. Emergence of adults was recorded treatment wise. Adults were sexed and the ratio of females to males was recorded. Newly emerged adults were released in a wooden cage of size (30cm X 30cm X 30cm) for two days and then paired 1:1 (Male: Female) and released in plastic jars (15x15x15 cm) closed with a muslin cloth. Adults started laying eggs within 48 hrs of release and eggs were counted daily. The muslin cloth cover was replaced daily. The hatching

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percentage of eggs per female was recorded daily and further the first instar larvae of *S. litura* obtained from each generation were reared individually and separately with six replications as per CO_2 treatment wise. The life history parameters of successive (consecutive) second, third and fourth generations of *S. litura* were measured as in the first generation described earlier.

Insect performance indices

Using the data relating to larval weight, leaf weight consumed, and fecal matter excreted, various insect performance indices (Waldbauer, 1968; Srinivasa Rao et al., 2009) viz., relative growth rate (RGR, larval weight gain per day as a fraction of body weight), relative consumption rate (RCR, weight of leaf ingested per day as a fraction of larval body weight), efficiency of conversion of ingested food (ECI, larval weight gain per unit weight of leaf ingested expressed as %), efficiency of conversion of digested food (ECD, larval weight gain per unit weight of leaf digested expressed as %) and approximate digestibility (AD, ratio of weight of leaf digested and weight of leaf ingested expressed as %) were computed. Weight of leaf digested was obtained by subtracting weight of frass from weight of leaf ingested.

Plant parameters

The data on plant parameters viz., weight shoot biomass (g/ plant) and leaf area (sq. cm/ plant) were recorded at regular intervals (10, 20, 30, 45, 60, 75, 90 & 105 DAS). Nodule numbers per plant, nodule dry weight (mg) were observed from 25 to 75 DAS at 10 days interval.

Data analysis

The effects of CO_2 conditions on larval parameters were analyzed using one-way ANOVA. Treatment means were compared and separated using least significant difference (LSD) at $p<0.05$. The data on biochemical and plant parameters were analyzed using ANOVA. The data on weight of foliage ingested, larval weight, weight of faecal matter, larval life span and pupal weight were analyzed using ANOVA with CO_2 and generations as sources of variability where CO_2 level was main factor and *S. litura* generation as sub factor deployed in a split plot design. Analysis of covariance (ANCOVA) was adopted to analyze the ratio based nutritional indices (Raubenheimer and Simpson, 1992). ANCOVA tests whether certain factors have an effect on the outcome variable after removing the variance for which quantitative predictors (covariates) account. The inclusion of covariates can increase statistical power (significance) because it accounts for some of the variability. ANCOVA adjusts scores on dependent variable for initial differences on other variables. Hence the data on insect performance indices (ratio based) were analyzed using ANCOVA with initial weight as a covariate for RCR and RGR. Food consumption was taken as a covariate for ECI to correct for the effect of variation in growth and food assimilated on intake and growth (Raubenheimer and Simpson, 1992). Food assimilated was used as a covariate to analyze ECD (Hagele and Martin, 1999). All statistical analyses were done using SPSS version 16.0.

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Results

Biochemical analysis of foliage

Significantly lower leaf nitrogen content (P =<0.01), higher carbon (P =<0.01), higher relative proportion of carbon to nitrogen (C:N) (P =<0.01) and higher polyphenols content (P =<0.01) were observed in peanut foliage grown under both the *e*CO_2 levels compared to the ambient CO_2 level (*Table 1*).

Table 1. *Effect of elevated CO_2 on bio chemical constituents of peanut foliage grown under elevated and ambient CO_2*

Biochemical constituents	CO_2 concentrations			F(P)	LSD
	Elevated I	Elevated II	Ambient		P=<0.05
Nitrogen (%)	2.95±0.05	2.95±0.09	3.20±0.04	21.19 p=<0.01	0.204
Carbon%	39.80±0.98	41.10±1.05	38.80±1.56	1.78 p=>0.01	NS
C:N ratio	13.49±0.45	13.93±0.18	12.13±0.61	10.26 p=>0.05	1.154
TAE %	1.90±0.10	1.69±0.02	1.66±0.04	13.15 p=<0.05	0.142

Plant parameters

The shoot biomass of peanut crop increased considerably ($F_{5, 10}$ = 4.48, P =<0.05) at both *e*CO_2 levels over ambient. Increased leaf area ($F_{5, 10}$ = 16.99, P =<0.01), higher nodule number ($F_{2, 4}$= 17.23, P =<0.05) and greater nodule weight ($F_{2, 4}$ = 9.60, P =<0.05) were recorded in peanut plants grown at both *e*CO_2 conditions over *a*CO_2 (*Table 2*).

Table 2. *Impact of elevated CO_2 on plant parameters of peanut*

CO_2 concentration (ppm)	Shoot biomass (g/ plant)	Leaf area (Sq cm)	Number of Nodule	Nodule dry wt. (mg/ plant)
Elevated I	2.92	402.89	115.86	99.06
Elevated II	3.42	535.65	120.26	64.81
Ambient	2.85	388.98	48.93	40.76
$F_{5,10}$	4.48	16.99	17.23	9.60
p	< 0.05	< 0.01	<0.05	< 0.05
CV (%)	11.83	10.87	17.56	24.01

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Insect primary parameters

The variation in insect primary parameters with CO_2 conditions over generations is depicted in *Figures 1-5*. Larvae consumed more of elevated CO_2 grown foliage than ambient CO_2 grown ($F_{2, 10}$ = 70.94, P =<0.01). The quantity of foliage consumed increased over generations ($F_{3, 45}$ =92.31, P =<0.01) (*Fig. 1*). The weight of larvae that consumed elevated CO2 grown foliage was higher ($F_{2, 10}$ = 26.48, P =<0.01). Larval weight varied with generations too ($F_{3, 45}$= 24.63, P =<0.01) (*Fig. 2*). Faecal matter released by *S. litura* larvae fed with elevated CO_2 grown foliage was significantly higher ($F_{2, 10}$ = 13.91, P =<0.01) and this parameter varied significantly with generations ($F_{3, 45}$ = 72.15, P =<0.01) (*Fig. 3*). Significantly longer larval life span for third and fourth generations ($F_{3, 45}$ = 18.26, P=<0.01) was observed under *e*CO_2 levels ($F_{2, 10}$= 51.90, P =<0.01) compared to ambient (*Fig. 4*). The weight of pupae did not vary among CO_2 conditions ($F_{2, 10}$= 2.17, P =>0.01) but differed with generations ($F_{3, 45}$ = 5.67, P =<0.01) (*Fig. 5*). Interactions of CO_2 levels and generations were significant for with larval consumption and pupal weight parameters and not significant for other parameters.

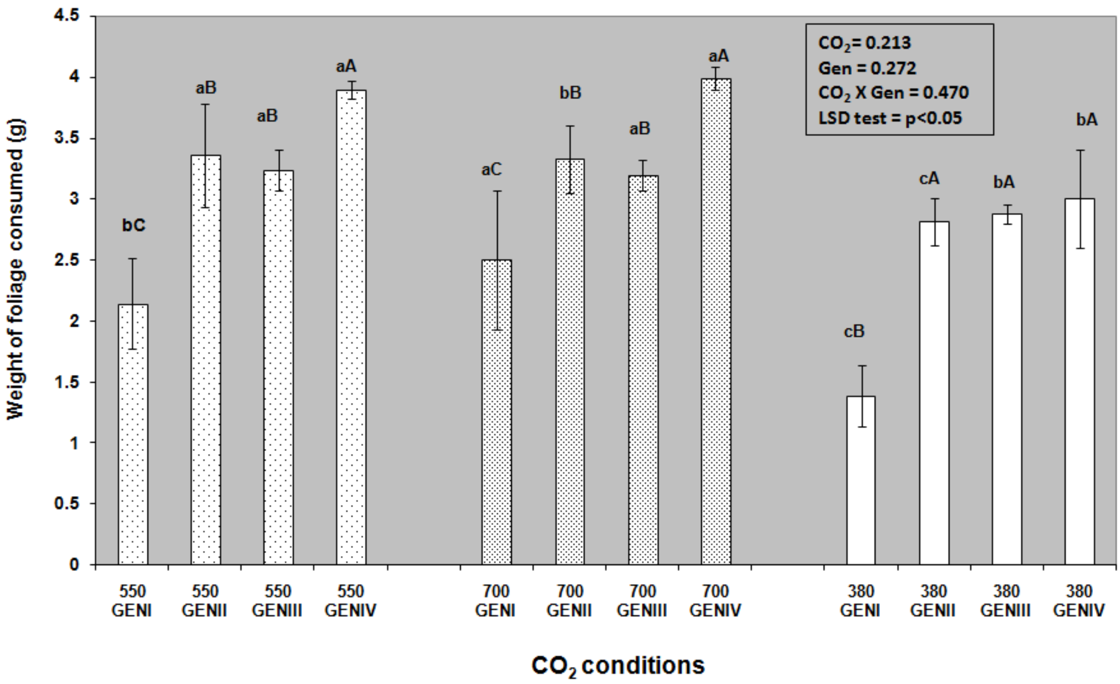

Figure 1. *Weight of foliage consumed by S. litura in four successive generations on peanut under eCO_2 conditions (lower and upper case letters indicate CO_2 levels and generations)*

Insect performance indices

The results on insect performance indices are presented in *Table 3*. Approximate digestibility (AD) of peanut foliage for *S. litura* differed with CO_2 conditions ($F_{2, 10}$ = 13.15, P = <0.01) but not with generations ($F_{3, 45}$ = 1.01, P = >0.05). The RCR of larvae was higher with both the *e*CO_2 levels ($F_{2, 10}$ = 6.947, P =<0.05) and differed with generations also ($F_{3, 45}$=11.56, P = <0.01).

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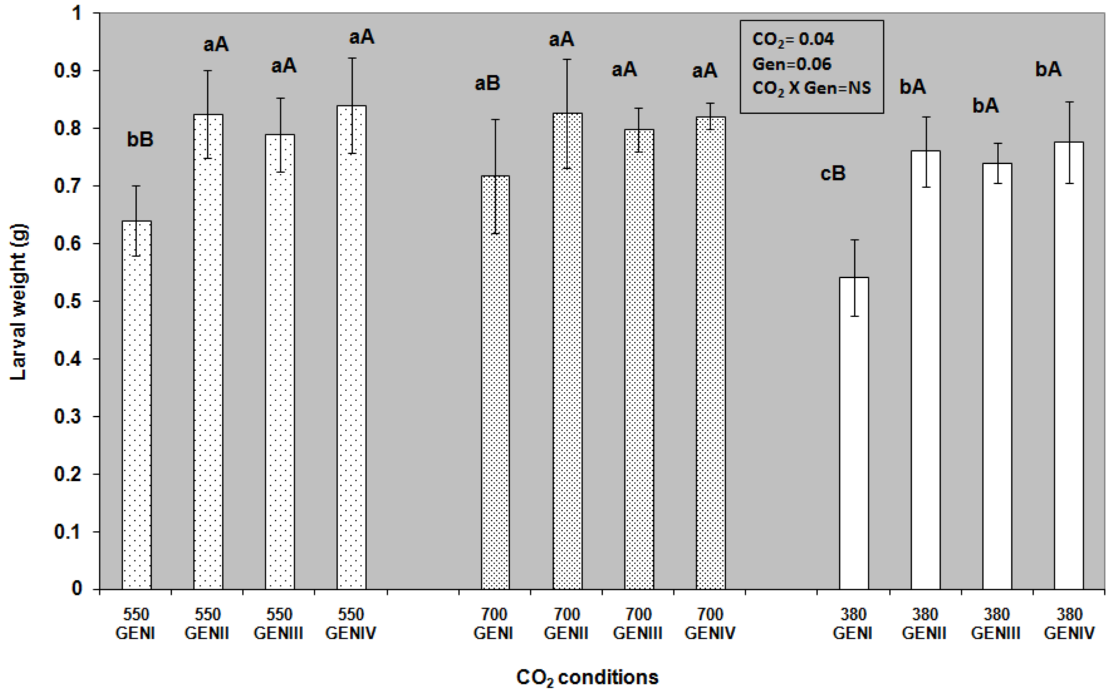

Figure 2. *Larval weight of S. litura in four successive generations on peanut under* eCO_2 *conditions*

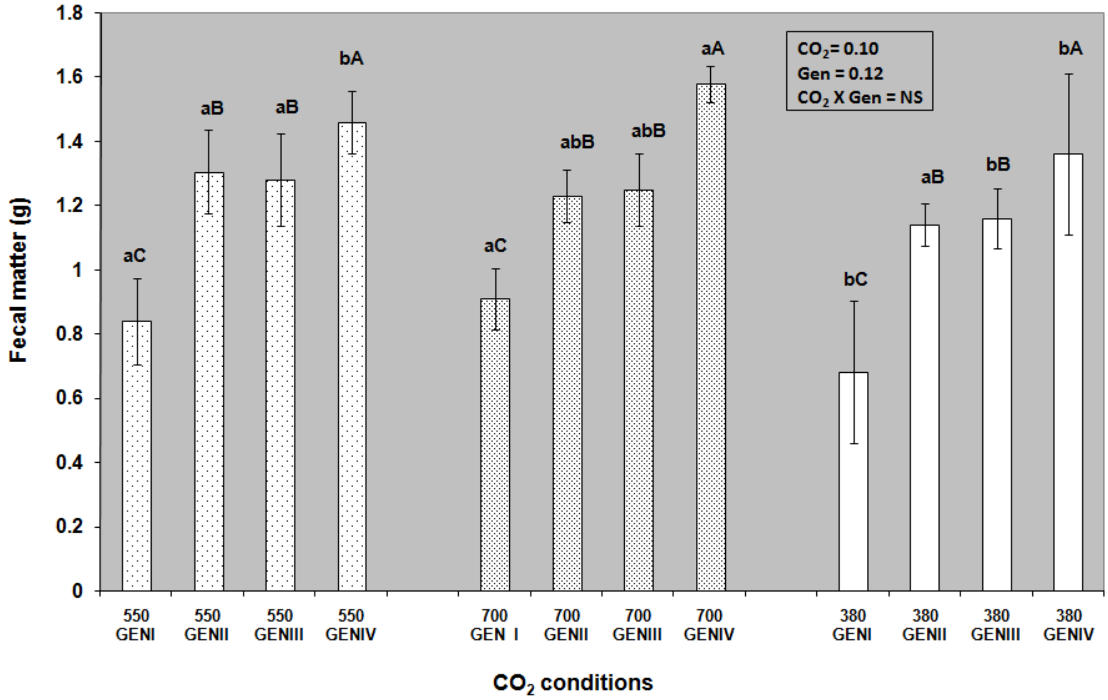

Figure 3. *Fecal matter of S. litura in four successive generations on peanut under* eCO_2 *conditions*

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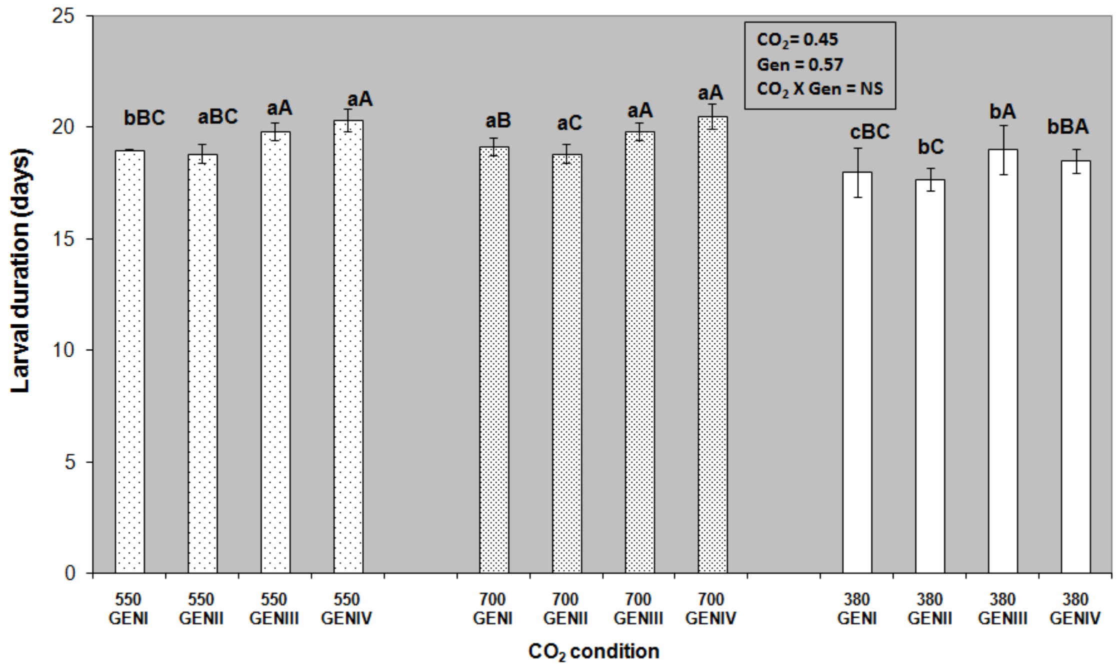

Figure 4. *Larval duration of S. litura in four successive generations on peanut under* eCO_2 *conditions*

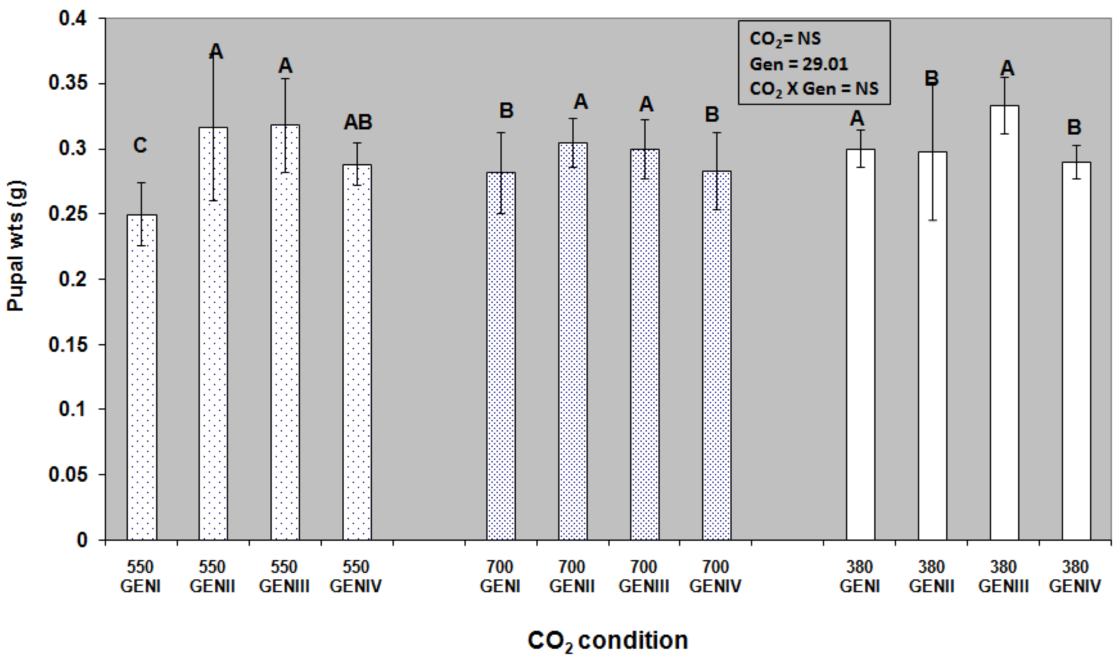

Figure 5. *Pupal weight of S. litura in four successive generations on peanut under* eCO_2 *conditions*

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Conversion of ingested food (ECI) was lower in larvae fed with *e*CO_2 foliage ($F_{2, 10}$ = 2.56, P = >0.01). ECI differed with generations ($F_{3, 45}$ =2.04, P = >0.05). The conversion of digested food (ECD) by larvae was varied with CO_2 ($F_{2, 10}$ = 3.42, P =<0.05) and generations ($F_{3, 45}$=3.33, P = <0.05). Growth rates of *S. litura* fed with *e*CO_2 foliage were lower ($F_{2, 10}$ = 13.733, P =<0.01). Growth rates did not vary with generations ($F_{3, 45}$ =9.75, P = >0.01). For most of the indices, the interactions between CO_2 and generations were not significant (*Table 3*).

Table 3. *Insect performance indices of four successive generations of S. litura fed on peanut grown under ambient and elevated CO_2 concentration*

Generation	Insect performance indices	CO_2 concentrations Elevated I	 Elevated II	 Ambient
F1	AD %	59.95 ± 6.35	61.81 ± 10.70	51.71 ±10.42
	ECI %	30.4 ± 4.56	30.28 ± 9.99	40.46 ± 10.87
	ECD%	51.43 ± 10.86	53.28 ± 32.55	78.65 ± 14.25
	RGR $mg.g^{-1}.d^{-1}$	99.54 ± 1.28	98.38 ± 2.09	109.12 ± 8.19
	RCR $mg.g^{-1}.d^{-1}$	333.55 ± 49.87	348.74 ± 91.68	281.42 ± 53.78
F2	AD %	60.65 ± 6.11	61.61 ± 4.14	59.01 ± 4.18
	ECI %	24.68 ± 1.62	25.14 ± 4.55	26.99±1.18
	ECD%	41.14 ± 5.68	40.5 ± 9.26	45.88 ± 2.85
	RGR $mg.g^{-1}.d^{-1}$	103.32 ± 2.32	101.18 ± 2.80	108.39 ± 7.82
	RCR $mg.g^{-1}.d^{-1}$	420.09 ± 28.33	415.61 ± 88.24	362.5 ± 39.29
F3	AD %	60.59 ±2.66	60.87 ± 1.98	59.42 ± 2.53
	ECI %	24.44 ± 0.96	24.96 ± 0.66	25.75± 0.86
	ECD%	40.42 ± 2.64	41.04 ± 1.82	43.42 ± 2.89
	RGR $mg.g^{-1}.d^{-1}$	97.28 ± 3.17	96.83 ± 3.41	100.76 ± 8.45
	RCR $mg.g^{-1}.d^{-1}$	398.45 ± 20.26	388.04 ± 13.60	391.27 ± 29.75
F4	AD %	62.48 ± 2.42	60.27 ± 1.46	54.67 ±5.14
	ECI %	21.57 ± 2.34	20.61 ± 0.63	26.01 ± 1.79
	ECD%	34.6 ± 4.27	34.23 ± 1.68	47.88 ± 4.97
	RGR $mg.g^{-1}.d^{-1}$	90.1 ± 11.50	87.22 ± 6.01	104.63 ± 7.19
	RCR $mg.g^{-1}.d^{-1}$	418.12 ± 42.10	423.2 ± 29.06	402.57 ± 17.68

LSD P=<0.01/0.05	CO_2	Generation	CO_2 x Gen
AD %	3.56 **	NS	NS
ECI %	3.52 **	4.51 **	NS
ECD%	7.14 **	10.84 **	14.06 *
RGR $mg.g^{-1}.d^{-1}$	6.36 **	5.57 **	NS
RCR $mg.g^{-1}.d^{-1}$	10.25**	44.81 **	NS

** and * indicate significance at 1 and 5 per cent respectively

Discussion

Elevated CO_2 levels increase photosynthesis, growth, yield and C:N ratios in most plant species, particularly C_3 plants (Pritchard et al., 1999) like peanut, *Arachis hypogaea*. Changes in plant N concentration and C: N ratio affects the quality and quantity of food available to insect herbivores (Chen et al., 2007). The impact of *e*CO_2 on phytochemistry

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of plants is well documented (Hunter, 2001; Coviella et al., 2002). In the present study, nitrogen concentration in peanut leaves decreased by about 7-8 per cent and carbon concentration increased by 2.5 - 6% in plants grown under *e*CO_2 conditions. Leaf C: N ratio increased by 11-15% of and concentration of polyphenols increased by 2-14%. These results are in agreement with the findings of Hughes and Bazzaz (1997) and Saxon et al. (2004) who reported decrease in leaf nitrogen & leaf water; and increase in C: N ratio & total phenolics in plants grown under elevated CO_2. Since nitrogen is the chief constituent of proteins, plants grown under *e*CO_2 conditions have lower protein in their tissues. Polyphenols, non-structural carbon compounds that constitute one of the defense mechanisms of plants and offer antefeedence to herbivores are also known to increase to a greater extent in leaves under *e*CO_2 conditions (Bezemer and Jones, 1998) which in turn influence the growth and development of insect herbivores.

Elevated CO_2 significantly increased the growth and biomass of the peanut plants by 11% over ambient. This trend is well documented for many species of plants (Prasad, 2005). Our results showed increased nodule number (124%) weight (101%) under *e*CO_2 conditions. Similar findings have been reported in most of the leguminous plants (Fischinger et al., 2010. Legumes capable of N fixation are less likely to suffer reduction in N under elevated CO_2 but may exhibit lower leaf N during early growth stages as observed by Rogers et al. (2006) in soybean. Cong et al. (2009) reported a 44% decrease in leaf nitrogen at mid vegetative growth stage of peanut under elevated CO_2. In our study, biochemical analysis of foliage was done on samples collected at vegetative stage. It is possible that N fixation was not fully operational by that time.

Consumption tends to increase in response to lower levels of nitrogen and higher C: N ratios, as species try to compensate the reduced nutritional quality of host tissue (Wu et al., 2006). Our results show higher consumption by *S. litura* larvae, of leaf material of peanut grown under *e*CO_2. Further, the consumption increased by 29-35% over the four generations. It was noted that leaf nitrogen content was reduced considerably (13- 21 %) under *e*CO_2 condition with an average of 13 per cent (Wang et al., 2008) Consumption and growth of larvae are influenced by nitrogen content of the foliage. As nitrogen is an important limiting factor for phytophagous insects, a reduction in nitrogen content has profound influence on insect performance. Insects increase their consumption and assimilation rates when fed with nitrogen-poor foliage. Larvae of *S. litura* were able to fully compensate for reduction in plant quality by enhanced feeding. Higher consumption of foliage of *Vigna radiata* by *S. litura* (Srivastava et al., 2002) and reduced pupal weights (Percy et al., 2002) of lepidopteran pests were reported under *e*CO_2.

Our study showed increased developmental times and reduced pupal weights of *S. litura* larvae fed with *e*CO_2 foliage. Larval duration of *S. litura* increased by 6-8% across the four generations under *e*CO_2 compared with ambient CO_2. Larval life span was up to 10% higher in the fourth generation compared to the first generation.

Insect performance indices of larvae of all four generations of *S. litura* varied with the CO_2 condition under which the plants were grown. An increase of 9% in AD and 7% in RCR was observed in all four generations under *e*CO_2 than ambient. Decrease of ECI (13%), ECD (19%) and RGR (9%) was observed in all four generations under *e*CO_2 over ambient. Larvae consumed more of peanut foliage grown under *e*CO_2 and assimilated better (higher values of RCR and AD) but grew slower (lower RGR) and took longer time (one day more than ambient) to pupation. It was reported that the efficiency of conversion of food into biomass was reduced, resulting in lower growth rate of insect larvae (Lindroth et al., 1993). A reduction in nitrogen content may be accompanied by decreased

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efficiency of conversion to body mass resulting in reduced growth rate (Masters et al., 1998). Some insects increase their consumption rate under *e*CO_2, but still exhibit significant reduction in growth rate and prolonged development time due to incomplete compensation feeding and poor food processing efficiencies, particularly efficiency conversion of digested food (ECD) (Watt et al., 1995; Agrell et al., 2000).

Reduced RGR of Gypsy moth larvae (Hattenschwiler and Schafellenr, 2004; Saxon et al., 2004), and *S. litura* on castor (Srinivasa Rao et al., 2009) under *e*CO_2 have been reported earlier. Poor food processing efficiencies are likely the consequence of decreased nitrogen and/ or increased concentrations of allelochemicals (Lindroth, 1996). Higher approximate digestibility (AD) under *e*CO_2 has been attributed to accumulation of starch content (Lindroth et al., 1993) and lower N content (Wang et al., 2008). Increased AD observed in our study might be due to increased C: N ratio. Our findings indicated that the digestive efficiency (AD) increased and conversion efficiency (ECD) decreased for larvae fed on elevated-grown foliage and similar observations were reported earlier (Lindroth, 1996; Agrell et al., 2000).

The purpose of conducting present multi-generation study was to capture the cumulative effect of *e*CO_2 on various insect parameters. The weight of the foliage consumed and weight gain by the larvae was significantly higher in fourth generation than preceding three generations under all three CO_2 conditions. Higher consumption in the fourth generation might be due to accrued effect of CO_2 over generations indicating that larvae consume more amount of foliage and gain greater weight as they feed on *e*CO_2 foliage for longer period. Fecal matter released also increasing over generations and was in tune with consumption. As mentioned earlier, most of the feeding trials with *e*CO_2 plant material are confined to a single generation, and there are no studies with *S. litura* on peanut over multiple generations. Understanding the 'adaptation' of species over generations is helpful to study the response over generations (Lindroth et al., 1995). Further multi generational studies are needed to understand the cumulative effects of *e*CO_2 levels at the individual and population level to develop realistic predictions of long-term population dynamics (Williams et al., 1997).

Conclusion

Studies on effect of peanut growing CO_2 conditions on *S. litura* over four successive generations showed a significant variation in growth and development of insect with both CO_2 conditions and generations. Nitrogen concentration in peanut leaves decreased significantly resulting in an increase of C: N ratio. In all generations, increased AD and RCR were observed under elevated CO_2 conditions over ambient. Decreased levels of efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and relative growth rate (RGR) were noticed with elevated CO_2. The data on insect development and food utilization efficiencies for multiple generations will give a better picture of effects of *e*CO_2 and can help in understanding the population dynamics of pests in the future climate change scenarios.

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