

**GROWTH AND FEEDING EFFICIENCY OF THE COTTON
LEAF WORM *SPODOPTERA LITTORALIS* (BOISD.)
(LEPIDOPTERA: NOCTUIDAE) ON COTTON PLANT
GOSSYPIUM BARBADENS (MALVACEAE) GROWN IN
ENRICHED CO₂ ATMOSPHERE**

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INTRODUCTION

Phytophagous insects are known to dominate global biodiversity (Strong *et al.*, 1984; and World Conservation Monitoring Center, 1992) and the two most considered variables in studies of the impact of global environmental change on phytophagous insects and their host plants are CO₂ and temperature (Bazzaz, 1990; Collier *et al.*, 1991; Cammel and Knight, 1992; Dewar & Watt, 1992; Lambers, 1993; Lincoln, 1993; Lincoln *et al.*, 1993 and Lindroth *et al.*, 1993). Because of carbon dioxide's critical role in photosynthesis, such increase in global atmospheric CO₂ levels will have a profound effect on the growth of crop plants (Acock, 1990). Because the composition of plant tissue often changes when plants are grown at elevated CO₂ concentrations, insect herbivore performance after feeding on such tissue may also be affected.

To investigate how the increase in atmospheric CO₂ may influence plant/insect interactions, fed on leaves from cotton plant *Gossypium barbadens* (Malvaceae) grown under ambient (350 ppm) and enriched (700 ppm) CO₂ regimes to the 3rd, 5th and 6th instar larvae of *Spodoptera littoralis* (Boisd.) and measure their food consumption and growth. The experiment also examines how the carbon supply rate of plants influences herbivore feeding and growth.

MATERIAL AND METHODS

The cotton seeds of *Gossypium barbadens* (Giza 85) were provided from the Ministry of Agriculture, Dokki, Cairo were germinated, 5 seeds / 4 liter soil, in

plastic box containing sand and clay through summer season from May till September (2000-2001) during summer season. Fifteen days after germination, plants were randomly divided into 2 groups, 20 each, one for ambient (control) CO₂ regime (350 ppm) and the other for the enriched CO₂ treatment (700 ppm).

In the first group (ambient CO₂ regime) the plastic pots were placed in the garden of the Department of Entomology, Faculty of Science, Cairo University. A certain distance was observed between successive planting pots to avoid overlapping of green shoot and to reduce mutual shading. A large muslin cloth net was set to cover most of the field area to avoid severe damage to cotton by insect pests. The mean weekly maximum and minimum temperature ranged between 40.4–45.7°C and 20-25.2°C, respectively. The mean weekly maximum and minimum relative humidity ranged between 65-76% and 20-45%, respectively. The mean monthly precipitation was zero and the photoperiod was 14D: 10L.

In the second group (enriched CO₂ regime), the pots were placed in 80 L x 80 W x 80 H m specially designed glass houses (see Carlson and Bazzaz, 1980 for details). Natural lightening which ranged from 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ to 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Fajer, 1989) was primarily used, although a white fluorescent Philips lamps, programmed to maintain a 12 D: 12 L light regime, added additional light at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ when light levels fell below 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

The temperature regime, inside the glass house, (35-38°C) was controlled automatically by a thermostat and recorded using a Traceable® Digital Thermometer (with $\pm 1^\circ\text{C}$ accuracy, 1 digit resolution). Relative humidity fluctuate between 70-85 %, CO₂ cylinders were used as a source of CO₂ to maintain the concentration inside the glass house at 700 ppm. The flow rate of inlet air stream of CO₂ was 0.1 l/min. No natural or artificial fertilizers were added to the soil. Growing cotton plants were watered with tap water every 3 days and rotated within glass house within CO₂ treatment every week in order to minimize pseudoreplication and glass houses effects. Cotton plant leaves used during feeding experiments were obtained from 45-75 day old cotton plants from both ambient (350 ppm) (control) and enriched (700 ppm) CO₂ regimes.

Leaf quality was measured to help explain the variability observed in consumption of food. The leaves used were obtained from 45-75 day-old cotton plants *G. barbadens*. For the chemical analysis, total pigments was extracted and determined according to the method described by Fadel (1962) and Sestak *et al.* (1971). Total free amino acids were determined as amino-N according to the method described by Russell (1944). Total soluble sugars was extracted from plant material

according to the method presented by Upmeyer & Koller (1973). The reducing value of soluble sugars was measured by the Nelson's test as described by Clark and Switzer (1977). The non-reducing sugar was determined according to Gaines (1973). Insoluble sugars was extracted and determined according to Streeter and Jeffers (1979). Phenolic compounds were extracted from dried tissues of shoot system according to the method of Jindal & Singh (1975).

Insect bioassays

Newly third and fifth instar larvae of *S. littoralis* were used. Each CO₂ treatment had 20 replicates of 10 larvae / box (12 W x 6 H x 18 L cm) and each replicate was treated as a single unit. Larval crowding was avoided since it may affect the rates of feeding and growth (Waldbauer, 1968). Clean and fresh cotton leaves from the two CO₂ regimes were supplied everyday to the larvae. Uneaten leaves were removed and newly fresh ones were provided. The 3rd and 5th instar larvae in each replicate were weighed daily. The average fresh weight of larvae, fresh weight of faeces, fresh weight of food ingested and the weight gained were determined. The consumption index (C.I.); the growth rate (G.R.); the relative consumption rate (R.C.R.); the efficiency of conversion of undigested food to body substance (E.C.I.); the efficiency of conversion of digested food (E.C.D.) and the approximate digestibility (A. D.) were determined according to Waldbauer (1968). Exuviae were measured with the faeces since they are not a part of the insect at the end of the experiment (Reese and Beck, 1976).

Analysis of the variance (ANOVA) and Duncan's multiple range test were used. All statistical computations were carried out using SAS program (Anonymous, 2000).

RESULTS AND DISCUSSION

Chemical composition of leaves

Phytochemical analyses revealed the significant main effects of carbon dioxide (table1). The mean total soluble sugars concentrations of the cotton leaves grown at days 45, 60 and 75 in ambient (20.612 ± 1.218 , 20.055 ± 0.415 & 15.724 ± 0.224 mg. g⁻¹ d.wt., respectively) and enriched (28.383 ± 0.636 , 25.259 ± 0.302 & 20.962 ± 1.622 mg. g⁻¹ d.wt., respectively) CO₂ were significantly ($P < 0.05$) different. The mean content of reducing sugars acquired high value at day 45 (10.253 ± 0.168 & 11.216 ± 0.303 mg. g⁻¹ d.wt.) in ambient and enriched CO₂ regimes,

respectively. Under enriched CO₂ regime a highly significant ($P < 0.01$) increase in the mean reducing sugars concentration while a significant ($P < 0.05$) increase in the non-reducing sugars was observed. Under enriched CO₂ regime, a significant ($P < 0.05$) increase was observed in the mean total carbohydrates.

The phenolic concentrations decreased as cotton plant grew older and a highly significant ($P < 0.01$) increase was detected in the mean phenolic contents (table 2). The mean nitrogen concentration significantly ($P < 0.05$) decreased as the cotton grew older under both CO₂ regimes with a significant ($P < 0.05$) lower nitrogen concentration under enriched CO₂ regimes (table 2).

Measures of plant quality during the present study are thus of importance in understanding plant-insect interactions under enriched CO₂ environment, because insect feeding and growth are a function of both the variation in nutrition between plants and the variation in the response to plant quality by insects. Results obtained in table 2, revealed that exposure of the cotton *G. barbadens* to enriched CO₂ (700 ppm) resulted in decrease in the concentration of chlorophyll a, b and a + b leading to starch accumulation as reported in several plant species by Cave *et al.* (1981); Wulff & Strain (1982); Delucia *et al.* (1985); Ehret & Jolliffe (1985); Oberbauer *et al.* (1985); Khavae-Neyad (1986) and Radoglou & Jarves (1992). Moreover, the increase availability of fixed CO₂ also increased the proportion of soluble sugars and starch as cited by Cave *et al.* (1981); Yelle *et al.* (1989); Vu *et al.* (1989); Bazzaz (1990); Kuehny *et al.* (1991) and den Hertog *et al.* (1993). The accumulation of carbohydrates is thought to dilute the concentration of foliar protein (Sionit, 1983) making them a comparatively poorer sources for phytophagous insects (Lincoln *et al.*, 1984; Kuehny *et al.*, 1991; Williams *et al.*, 1997 a & b). Also increase in phenolic concentration might affect growth and feeding responses of the cotton leaf worm *S. littoralis*.

Growth and feeding efficiency of the 3rd and 5th instar larvae

Data presented in Table (3) showed that the mean fresh weight of larvae showed no significant differences ($P > 0.05$) in the 3rd and 5th (instars. The mean fresh weight of faeces increased significantly ($P < 0.01$) in case of 5th instar larvae. Insignificant differences ($P > 0.05$) were observed between the mean fresh weights of food ingested of the 3rd and 5th larval instars when fed leaves grown under both CO₂ regimes. Although the mean weight gain increased through the larval development, a highly significant ($P < 0.01$) difference was observed in case of the 5th instar larvae when fed cotton leaves from both CO₂ regimes.

TABLE (I)

The mean carbohydrate concentrations of the cotton plant *Gossypium barbadens* grown under ambient (350 ppm) and enriched (700 ppm) CO₂ regimes at different harvest days.

Harvest days	CO ₂ regimes	Total soluble sugars Mean ± S.E. (mg. g-1 d.wt.)	Reducing sugars Mean ± S.E. (mg. g-1 d.wt.)	Non-reducing sugars Mean ± S.E. (mg. g-1 d.wt.)	Insoluble sugars Mean ± S.E. (mg. g-1 d.wt.)	Total carbohydrates Mean ± S.E. (mg. g-1 d.wt.)
45	350	20.612 ± 1.218	10.253 ± 0.168	11.646 ± 2.373	16.685 ± 0.202	37.297 ± 1.1153
	700	28.383 ± 0.636	11.216 ± 0.303	17.167 ± 0.884	17.252 ± 0.272	45.634 ± 0.4485
60	350	20.055 ± 0.415	8.966 ± 1.157	9.801 ± 0.516	15.292 ± 0.479	35.347 ± 0.1366
	700	25.259 ± 0.302	9.983 ± 0.193	15.276 ± 0.492	16.321 ± 0.223	41.58 ± 0.361
75	350	15.724 ± 0.224	6.708 ± 0.019	7.381 ± 0.372	14.15 ± 0.165	29.874 ± 0.105
	700	20.962 ± 1.622	8.343 ± 0.173	14.254 ± 1.627	15.989 ± 1.074	36.952 ± 0.75

TABLE (II)

The mean pigment fraction, nitrogen and phenolic concentrations of the cotton plant *Gossypium barbadens* grown under ambient (350 ppm) and enriched (700 ppm) CO₂ regimes at different harvest days.

Harvest days	CO ₂ regimes	Chlorophyll a Mean ± S.E. (mg. g-1 d.wt.)	Chlorophyll b Mean ± S.E. (mg. g-1 d.wt.)	Chlorophyll a+b Mean ± S.E. (mg. g-1 d.wt.)	Nitrogen Mean ± S.E. (mg. g-1 d.wt.)	Phenolics Mean ± S.E. (mg. g-1 d.wt.)
45	350	1.23 ± 0.051	0.379 ± 0.035	1.61 ± 0.86	0.689 ± 0.019	0.688 ± 0.016
	700	0.903 ± 0.005	0.301 ± 0.009	1.205 ± 0.013	0.397 ± 0.023	1.561 ± 0.016
60	350	1.275 ± 0.067	0.409 ± 0.034	1.684 ± 0.101	0.537 ± 0.035	0.649 ± 0.014
	700	0.99 ± 0.021	0.361 ± 0.009	1.351 ± 0.019	0.273 ± 0.017	1.556 0.016
75	350	1.491 ± 0.05	0.494 ± 0.02	1.986 ± 0.067	0.518 ± 0.019	0.564 ± 0.013
	700	1.281 ± 0.017	0.437 ± 0.005	1.717 ± 0.022	0.239 ± 0.017	1.351 ± 0.008

TABLE (III)

The mean values of growth and feeding responses of 3rd, 5th and 6th instar larvae of *Spodoptera littoralis* fed cotton plant leaves *Gossypium barbadens* grown under ambient (350ppm) and enriched (700 ppm) CO₂ regimes.

CO ₂ regimes (ppm)	Ambient (350 ppm) Mean ± S. E. (g.)	Enriched (700 ppm) Mean ± S. E. (g.)	(P) value
3rd instar			
Fresh weight of larvae	0.277 ± 0.009 ^a	0.251 ± 0.015 ^a	0.202
Fresh weight of faeces	0.056 ± 0.004 ^a	0.048 ± 0.009 ^a	0.443
Fresh weight of food ingested	0.176 ± 0.100 ^a	0.17 ± 0.011 ^a	0.695
Weight gain	0.116 ± 0.006 ^a	0.113 ± 0.003 ^a	0.703
5th instar			
Fresh weight of larvae	2.03 ± 0.191 ^a	1.563 ± 0.167 ^a	0.139
Fresh weight of faeces	0.476 ± 0.024 ^b	0.742 ± 0.04 ^a	0.005
Fresh weight of food ingested	1.761 ± 0.093 ^a	2.071 ± 0.117 ^a	0.106
Weight gain	1.072 ± 0.047 ^a	0.761 ± 0.046 ^b	0.009
6th instar			
Fresh weight of larvae	3.755 ± 0.069 ^a	3.134 ± 0.12 ^b	0.011
Fresh weight of faeces	1.005 ± 0.046 ^a	1.097 ± 0.025 ^a	0.154
Fresh weight of food ingested	3.923 ± 0.035 ^a	4.569 ± 0.289 ^a	0.091
Weight gain	1.613 ± 0.087 ^a	1.379 ± 0.023 ^a	0.061

-Means in rows followed by the same letters are insignificantly different (P > 0.05)

-Means in rows followed by the different letters are significantly different (P < 0.05, P < 0.01)

During this study, although the 3rd instar larvae of *S. littoralis* modified their feeding behavior, only the 5th instar larvae showed an increase in leaf consumption of significantly (P < 0.01) high leaf soluble sugars and starch, despite that during our study we did not measure the amount of leaf area consumed but leaf damage was clearly visible as compared to the control (ambient).

The significant (P < 0.01) decrease in the growth rate of the 5th instar larvae of *S. littoralis* might have probably resulted from their inability fully to compensate from the diets reduced nitrogen (Slansky & Scriber, 1985). Similar results were reported by Lincoln and Couvet (1989) for the 4th instar larvae of *S. eridiana* and by Marks and Lincoln (1996) for *S. frugiperda*. Also the mean larval faecal matter of the 5th larval instar was significantly (P < 0.07) more than in case of the 3rd instar

larvae revealing that food eaten remains undigested despite consumption were more on cotton leaves grown in enriched CO₂ regime.

TABLE (IV)

The mean values of nutritional indices of the 3rd, 5th and 6th instar larvae of *Spodoptera littoralis* fed cotton plant leaves *Gossypium barbadens* grown under ambient (350 ppm) and enriched (700 ppm) CO₂ regimes.

CO ₂ regimes (ppm) Larval stage	Ambient (350 ppm) Mean ± S.E.	Enriched (700 ppm) Mean ± S.E.	(P) value
3rd instar			
E.C.I.	65.78 ± 0.763 ^a	66.948 ± 2.413 ^a	0.669
E.C.D.	96.612 ± 0.831 ^a	93.216 ± 2.815 ^a	0.312
A.D.	62.361 ± 0.652 ^a	66.554 ± 0.638 ^a	0.338
G.R.	0.203 ± 0.003 ^a	0.201 ± 0.004 ^a	0.708
C.I.	0.305 ± 0.01 ^a	0.304 ± 0.011 ^a	0.925
R.C.R.	0.985 ± 0.032 ^a	1.022 ± 0.036 ^a	0.494
5th instar			
E.C.I.	60.941 ± 1.489 ^a	36.713 ± 0.148 ^b	0.0001
E.C.D.	83.575 ± 2.676 ^a	57.272 ± 0.8132 ^b	0.001
A.D.	72.953 ± 0.5568 ^a	66.949 ± 0.572 ^b	0.001
G.R.	0.194 ± 0.006 ^a	0.153 ± 0.006 ^b	0.008
C.I.	0.369 ± 0.035 ^a	0.421 ± 0.016 ^a	0.252
R.C.R.	1.213 ± 0.043 ^b	1.666 ± 0.048 ^a	0.002
6th instar			
E.C.I.	41.076 ± 1.843 ^a	30.351 ± 1.391 ^b	0.009
E.C.D.	55.281 ± 2.978 ^a	40.069 ± 2.343 ^b	0.016
A.D.	78.365 ± 0.538 ^a	73.317 ± 1.012 ^b	0.012
G.R.	0.132 ± 0.025 ^a	0.119 ± 0.005 ^a	0.625
C.I.	0.289 ± 0.008 ^b	0.369 ± 0.007 ^a	0.002
R.C.R.	1.354 ± 0.029 ^b	1.934 ± 0.038 ^a	0.0003

-Means in rows followed by the same letters are insignificantly different ($P > 0.05$)

-Means in rows followed by the different letters are significantly different ($P < 0.05$, $P < 0.01$)

The mean efficiency values of both conversion of ingested food (E.C.I.) and digested food (E.C.D.) showed a highly significant ($P < 0.01$) differences in case of the 5th instar larvae fed cotton leaves grown in enriched CO₂ regime. The mean values of approximate digestibility (A.D.) revealed insignificant ($P > 0.05$) difference in the case of the 3rd instar larvae and highly significant ($P < 0.01$) difference in case of the 5th instar when fed cotton leaves grown in enriched CO₂ regime.

A highly significant ($P < 0.01$) difference between the mean growth rates (G.R.) was observed only in the 5th instar larvae meanwhile insignificant ($P > 0.05$) difference was obtained in case of the 3rd instar larvae. The mean consumption index (C.I.) values did not differ significantly ($P > 0.05$) between the 3rd and 5th instar larvae. Only insignificant ($P > 0.05$) difference was observed between the mean values of the relative consumption rate (R.C.R.) for the 3rd larval instar (table 4).

From results obtained it appeared that there was a tendency for the efficiency of conversion of ingested food (E.C.I.) and the efficiency of digested food (E.C.D.) by *S. littoralis* larvae to decrease with age although insignificant ($P > 0.05$) differences in the (E.C.I.) and (E.C.D.) were observed when the 3rd instar larvae fed foliage from the two CO₂ regimes. The insignificant ($P < 0.01$) decrease observed in case of the 5th instar larvae when fed cotton leaves from the two CO₂ regimes might be attributed to the increase in leaf carbohydrate levels of *G. barbadens* which might enhance digestibility (Slansky and Rodrigue, 1987) as well as low nitrogen also account for an important proportion of the relative consumption and conversion efficiency. It appeared that the early instar (3rd instar) *S. littoralis* larvae were more selective feeders and choose more digestive foliage from the intervein regions of the leaf, also their metabolic rate was higher than older ones and hence more of the digested food is available for conversion to body substance (E.C.D.). Meanwhile, older larvae (5th instars) were more generalized in feeding and ingested different parts of foliage such as leaf vein, which contain large quantities of indigestible crude fiber. Thus, it is likely that the 5th instar larvae have lower metabolic rate than younger ones (3rd instars). The low E.C.D. in *S. littoralis* larvae suggests a less precise correspondence between its requirements and the level of nutrient balance of its diets.

Perhaps the higher approximate digestibility (A.D.) in *S. littoralis* larvae is an adaptation that might compensate for a decrease in the (E.C.D.) and (E.C.I.) resulting from nutritional imbalances (Waldbauer, 1964). Similar results were recorded by Soohoo and Fraeukel (1966) for the 5th instar *Prodenia* which compensate for its lower E.C.D. by showing higher A.D. However, Hiratsuka (1920)

reported that the E.C.I. and A.D. decline together and about the same rate in case of *Bombyx*. Srivastava *et al.* (2002) showed that A.D. of *S. littura* was almost equal in both CO₂ conditions

Thus, the present results revealed once more that the leaf nutritional quality might be reduced at enriched CO₂ ecosystems. Furthermore, herbivory was related to key measures of leaf quality, even in the most preferred plant species.

SUMMARY

Results from laboratory feeding experiments have shown that elevated atmospheric CO₂ can affect interactions between plants and insect herbivores, primarily through changes in leaf nutritional quality occurring at enriched CO₂. To understand such effect 3rd and 5th instar larvae of the cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) were fed leaves of their major host plants, cotton, *Gossypium barbadens* (Malvaceae) grown in either ambient (350 ppm) or enriched (700 ppm) CO₂ atmospheres. Despite consuming more foliage, from the two CO₂ treatments, the mean fresh weight of the 3rd and 5th instar larvae does not differ meanwhile the mean fresh weight of feces increased in case of the 5th instar larvae revealing a reduction in the efficiency of conversion of ingested food (E.C.I.) and digested food (E.C.D.) as well as in approximate digestibility (A.D.) on enriched CO₂ –grown plants. The digestive efficiency of *S. littoralis* larvae decline with growth. Moreover, the mean consumption index (C.I.) values for both 3rd and 5th instar larvae did not differ. This study suggests that feeding by herbivores on the leaves of C₃ plants may increase as the level of atmospheric carbon dioxide rises.

REFERENCES

- ACOCK, B. (1990):** Effects of carbon dioxide on photosynthesis of plant growth and other process. (*In impact of carbon dioxide. Trace gases and climate change on Global Agriculture (B. A. Kimball, N. J. Rosenberg and L. H. Allen, (eds.) American Society of Agronomy, WI).*
- ANONYMOUS (2000):** SAS program version release 6.12. (*SAS Institute Incorporation, Cary, New York).*
- BAZZAZ, F. A. (1990):** The response of natural ecosystems to the rising global CO₂ levels. (*Ann. Rev. Ecol. Syst., 21: 167-196).*

- CAMMELL, M. E. and J. D. KNIGHT (1991):** Effects of climate change on the population dynamics of crop pests. (*Adv. Ecol. Res.*, 22: 117-162).
- CARLSON, R. W. and F. A. BAZZAZ (1980):** The effects of elevated CO₂ concentration on growth, photosynthesis, transpiration and water efficiency of plants. (*In: Singh J. J., Deepark A, eds. Environmental and climatic impact of coal utilization. New York*).
- CAVE, G., L. C. TOLLEY, and B. P. STAIN (1981):** Effects of carbon dioxide on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. (*Physiol. Plant.*, 51: 171-174).
- CLARK, J. M. and R. L. SWITZER (1977):** *In: Experimental biochemistry*. 2nd ed. (*Freedenan & Company, San Francisco*).
- COLLIER, R. M., S. FINCH, K. PHELPS and A. R. THOMPSON (1991):** Possible impact of global warming on cabbage root fly (*Delia radicus*) activity in the U. K. (*Ann. Appl. Biol.* 118: 261-27).
- COVIELLA, C. E. and J.T. TRUMBLE (1999):** Effects of elevated atmospheric carbon dioxide on insect-plant interactions. (*Conservation Biology*, 18: 700 -712).
- DELUCIA, E. H., T. W. SASEK and B. R. STRAIN (1985):** Photosynthetic inhibition after long-term exposure to elevated levels of carbon dioxide. (*Photosynth. Res.*, 7: 175-184).
- DEN HERTOOG, J., I. STULEN and H. LAMBERS (1993):** Assimilation, respiration and allocation of carbon in *Plantago major* as affected by atmospheric CO₂ levels. (*Vegetatio.*, 104/105: 369-378).
- DEWAR, R. C. and A. D. WATT (1992):** Predicted changes in the synchrony of larval emergence and bud burst under climatic warming. (*Oecologia*, 89: 557-559).
- EHRET, D. H. and P. A. JOLLIFFE (1985):** Leaf injury to bean plants grown in carbon dioxide enriched atmospheres. (*Canadian Journal of Botany*. 63: 2015-2020).
- EVANS, A. C. (1939a):** The utilization of food by certain lepidopterous larvae. (*Trans. R. ent. Soc. Lond.*, 89: 13-22).
- EVANS, A. C. (1939b):** The utilization of food by the larvae of the buff-tip, *Phalera bucephala* (Linn.). (*Proc. R. ent. Soc. Lond.*, 14: 25-30).

- FADEEL, A. A. (1962):** Location and properties of chloroplast and pigment determination in roots. (*Physiol. Plant.*, 15: 130-147).
- GAINES, T. P. (1973):** Automated determination of reducing sugars, total sugars and starch in plant tissue from one weighed sample. (*J. Assoc. off. Anal. Chem.*, 56: 1419-1424).
- JINDAL, K. K. and R. N. SINGH (1975):** Phenolic content in male and female *Carica papaya*: A possible physiological marker for sex identification of vegetation seedlings. (*Physiol. Plant.*, 33: 104-107).
- KHAVARI-NEJAD, R. A. (1986):** Carbon dioxide enrichment preconditioning effects on chlorophyll contents and photosynthetic efficiency in tomato plants. (*Photosynthetica*, 20: 315-317).
- KUEHNY, J. S., M. M. PEET, P. V. NELSON and D. H. WILLITS (1991):** Nutrient dilution by starch in CO₂-enriched *Chrysanthemum*. (*J. Exp. Bot.*, 42: 711-716)
- LAMBERS, H. (1993):** Rising CO₂, secondary plant metabolism: Plant-herbivore interactions and litter composition. (*Vegetatio.*, 104/105: 263-271).
- LINCOLN, D. E. (1993):** The influence of plant carbon dioxide and nutrient supply on susceptibility to insect herbivores. (*Vegetatio.*, 104/105: 273-280).
- LINCOLN, D. E., N. SIONIT and B. R. STRAIN (1984):** Growth and feeding response of *Pseudoplusia includens* (Lepidoptera: Noctuidae) to host plants grown in controlled carbon dioxide atmospheres. (*Environm. Entomol.*, 13: 1527-1530).
- LINCOLN, D. E. and D. COUVET (1989):** The effect of carbon supply on allocation to allelochemicals and caterpillar consumption of peppermint. (*Oecologia*, 78: 112-114).
- LINCOLN, D. E., E. D. FAJER and R. H. JOHNSON (1993):** Plant-insect herbivore interactions in elevated CO₂ environments. (*Trends Ecol. Evol.*, 8: 64-68).
- LINDROTH, R. L., K. K. KINNEY and C. L. PLATZ (1993):** Responses of deciduous trees to elevated atmospheric CO₂: productivity, photosynthesis, and insect performance. (*Ecology*, 74: 763-777).
- MARKS, S. and D. E. LINCOLN (1996):** Antiherbivore defense mutualism under elevated carbon dioxide levels: a fungal endophyte and grass. (*Environ. Entomol.*, 25 (3): 618-623).

- OBERBAURE, S. F., B. R. STRAIN and N. FETCHER (1985):** Effects of CO₂ enrichment on seedling physiology and growth of two tropical trees. (*Physiologia Plantarum*. 65: 352-356).
- RADOGLOU, K. M. and P. G. JARVIS (1992):** The effects of CO₂ enrichment and nutrient supply on growth morphology and anatomy of *Phaseolus vulgaris* L. seedlings. (*Annals of Botany*. 70: 245-256).
- REESE, J. C. and S. D. BECK (1976):** Effects of allelochemicals on the black cut worm, *Agrotis ipsilon*; effects of P-benzoquinone, hydroquinone, and duroquinone on larval growth, development, and utilization of food. (*Ann. Entomol. Soc. Am.*, 69: 59-67).
- RUSSELL, J. A. (1944):** Note on the colorimetric determination of amino nitrogen. (*J. Biol. Chem.*, 156: 467-468).
- SESTAK, Z., J. CATSKY and P. G. JARVIS (1971):** Determination of chlorophylls a and b. In: plant photosynthetic production. Manual of methods. (Sestak, Z.; Catsky, J., and Jarvis, P. G. (eds.). Dr. W. Junk Publ. The Hague).
- SIONIT, N. (1983):** Response of soybean to two levels of mineral nutrition in CO₂ enriched atmosphere. (*Crop Science*. 23: 329-333).
- SLANSKY, F. and J. G. RODRIGUEZ (1987):** Nutritional Ecology of insects, Mites, Spiders and related invertebrates. (*Wiley-Inter Science, New York*).
- SLANSKY, F. and J. M. SCRIBER (1985):** Food consumption and utilization. Comprehensive insect Physiology, Biochemistry, and Pharmacology. (Kerkut, G. A., Gilbert, L. I. (eds.). Pergamon Press, Oxford).
- STREETER, J. G. and D. L. JEFFERS (1979):** Distribution of total non-structural carbohydrates in soybean plants having increased reproductive load. (*Crop Science*. 19: 729-734).
- STRONG, D. R., J. H. LAWTON and T. R. E. SOUTHWOOD (1984):** Insects on plants. Community patterns and Mechanisms. (*Blackwell Scientific, Oxford*).
- UPMEYER, D. J. and H. R. KOLLER (1973):** Diurnal trends in net photosynthetic rate and carbohydrate levels of soybean leaves. (*Plant Physiology*. 51: 871-874).
- VU, J. C. V., L. H. JR. ALLEN, and G. BOWES (1989):** Leaf ultrastructure, carbohydrates and protein of soybeans grown under CO₂ enrichment. (*Environ. Exp. Bot.*, 29: 141-147).

- WALDBAUER, G. P. (1964):** The consumption, digestion, and utilization of solanaceous and non-solanaceous plants by larvae of the tobacco horn worm, *Protoparce sexta* (Johan.) (Lepidoptera: Sphingidae). (*J. Insect Physiol.*, 7: 253-269).
- WALDBAUER, G. P. (1968):** The consumption and utilization of food by insects. (*Adv. Insect Physiol.*, 5: 229-288).
- WILLIAMS, R. S., R. B. THOMAS, B. R. STRAIN and D. E. LINCOLN (1997a):** Effects of elevated CO₂, soil nutrient levels, and foliage age on the performance of two generations of *Neodiprion lecontei* (Hymenoptera: Diprionidae) feeding on Loblolly pine. (*Environ. Entomol.*, 26 (6): 1312-1322).
- WILLIAMS, R. S., D. E. LINCOLN and R. B. THOMAS (1997b):** Effects of elevated CO₂-grown Loblolly pine needles on the growth, consumption, development, and pupal weight of red-headed pine sawfly larvae. (*Global Change Biology*. 3 (6): 501-511).
- WORLD CONSERVATION MONITORING CENTRE (1992):** Global Biodiversity: Status of the Earth's living Resources. (*Chapamn & Hall, London*).
- WULFF, R. D. and B. R. STRAIN (1982):** Effects of CO₂ enrichment on growth and photosynthesis in *Desmodium paniculatum*. (*Canadian journal of Botany*, 60: 1084-1091).
- YELLE, S., R. C. BEESON, M. J. TRUDEL and A. GOSSELIN (1989):** Acclimation of two tomato species to high atmospheric CO₂. I. Sugar and starch concentration. (*Plant Physiol.* 90: 1465-1472).