



## Tritrophic Interactions of Cowpea [*Vigna unguiculata* subsp. *unguiculata*(L.)], Aphids [*Aphis craccivora* (Koch)] and Coccinellids [*Menochilus sexmaculatus* (Fab.)] under $e\text{CO}_2$ and $e\text{Temp}$

M. Srinivasa Rao<sup>a,\*</sup>, O. Shaila<sup>a</sup>, P. Sreelakshmi<sup>a</sup>, S. Vennila<sup>b</sup>, M. Vanaja<sup>a</sup>, A.V.M. Subba Rao<sup>a</sup>, M. Maheswari<sup>a</sup>, K. Sammi Reddy<sup>a</sup>

<sup>a</sup> ICAR-Central Research Institute for Dryland Agriculture, Hyderabad 500 059, India

<sup>b</sup> ICAR-National Research Centre for Integrated Pest Management, New Delhi 110 001, India

### ARTICLE INFO

#### Keywords:

Biochemical  
Aphids  
Degree days  
Life table parameters  
Climate change  
*M. sexmaculatus*  
Predation capacity

### ABSTRACT

Experiments were conducted to understand the direct and indirect effects of temperature and elevated  $\text{CO}_2$  ( $e\text{CO}_2$ ), on tritrophic interactions of cowpea (*Vigna unguiculata* subsp. *unguiculata* L.), legume aphid *Aphis craccivora* Koch and coccinellid predator *Menochilus sexmaculatus* Fab. Reduction of the leaf nitrogen (6%), amino acid (6%) and protein (7%) of cowpea foliage with increased carbon (13%) and C:N ratio (21%) at  $e\text{CO}_2$  over  $a\text{CO}_2$  indicated the dilution of biochemical constituents at first trophic level. Shortened development time, DT and increment of reproductive rate, RR at  $e\text{CO}_2$  over ambient  $\text{CO}_2$  ( $a\text{CO}_2$ ) was significant with increase in temperature from 20 to 35 °C. Reduction of the mean degree day, DD requirement of both nymphal ( $75.79 \pm 15.163$ ) and adult stages ( $157.15 \pm 67.04$ ) at  $e\text{CO}_2$  over  $a\text{CO}_2$  and same was reflected in the summation DD for both the stages at  $e\text{CO}_2$  ( $232.96 \pm 80.32$ ) and  $a\text{CO}_2$  ( $247.07 \pm 64.77$ ) across six temperatures. The ' $r_m$ ' and ' $R_0$ ' increased gradually with increase in temperature followed the non-linear trend and reached maximum values at 27 °C with shortened ' $T$ ' across 20 to 35 °C temperatures at  $e\text{CO}_2$  indicating the significant variation of growth and development at the second trophic level. Decreased grub duration (23%) with increased predation capacity (19%) of *M. sexmaculatus* on *A. craccivora* at  $e\text{CO}_2$  over ambient was noted, indicating the incidence of *A. craccivora* is likely to be higher with increased predation in the future climate change scenario.

### Introduction

Atmospheric  $\text{CO}_2$  concentration has been steadily increasing and reached 380–400 ppm in 2013. It is anticipated to double by the end of the 21<sup>st</sup> century and an increase is predicted by 1.7–4.9 °C in mean global temperature by 2100 (IPCC, 2013). The average increase in temperature was found to be 0.78 (0.72 to 0.85) °C between the periods of 1850–1990 and 2003–2012. Ambient carbon dioxide ( $a\text{CO}_2$ ) concentration exceeded 400 ppm and future estimations predict an increase up to 550 ppm within a few decades (IPCC, 2014). The agriculture sector is more vulnerable to climate variability and climate change and the elevated  $\text{CO}_2$  and temperature would threaten the food security of the people across the globe. It is well documented that crop plants get stimulated with elevated  $\text{CO}_2$  concentrations and thus increased biomass and higher photosynthesis (Yu et al., 2012) that in turn influences the growth and development of insect herbivores (Hunter, 2001). Elevated atmospheric  $\text{CO}_2$  ( $e\text{CO}_2$ ) influences crop growth by altering C: N ratio, increasing C (Gutierrez et al., 2008) and decreasing N (Dixon

et al., 1993). The quality and quantity of the food available to the insect herbivores get changed due to  $e\text{CO}_2$  (Chen et al., 2007) and temperature; and thus the insect pests prefer to go for increased feeding rate to meet compensatory nutritional requirements (Hunter, 2001).

Cowpea (*Vigna unguiculata* subsp. *unguiculata* L.) is an important legume crop with higher protein content and is grown both in alfisols and vertisols. The crop is preferred as vegetable and even after processing (Chivenge et al., 2015). *Aphis craccivora* Koch, mainly referred as legume/cowpea aphid, is the major insect pest of legume crops including cowpea, peanut, pigeonpea etc. The incidence of aphids is common in the early vegetative stage of the crop and its infestation will continue till crop maturity. Both nymphs and adults suck the sap from plant parts (leaves, inflorescence and tender pods) and cause enormous yield losses. In the majority of the cases *A. craccivora* also acts as a vector in transmitting the important viral diseases (Wightman and Wightman, 1994).

The response of insect species to  $e\text{CO}_2$  varies individually across different species and it is understood that the multiplication of aphids is

\* Corresponding author.

E-mail address: [Ms.Rao@icar.gov.in](mailto:Ms.Rao@icar.gov.in) (M. Srinivasa Rao).

higher at  $eCO_2$  conditions (Newman, 2005) and can cause significant economic losses (Cannon, 1998). It is known (Hughes and Bazzaz, 2001) that the response of aphids to  $eCO_2$  is 'species-specific' in nature. The growth and development of aphids is influenced by the temperature and complete understanding will be of immense help to know the population dynamics. It is well known that insect performance is effected both directly and indirectly by temperature and  $eCO_2$  (Stiling and Cornelissen, 2007). Harrington et al. (2001) reported that the direct effect of temperature will be greater than rising  $CO_2$ . The interactive and concurrent effects of both parameters are more evident and complex; therefore clear understanding will be of immense help to predict insect pest dynamics in future climate change.

The occurrence of the important generalist predator, *Menochilus sexmaculatus* (Fabricius) is very common on *A. craccivora* on cowpea. Level of coccinellid predation on aphids is in the range of 110.60 to 132.53 nos. /day and is a host dependent factor (Patel, 2015). The significant effects of temperature on tritrophic interactions is well known but scarce information is available on impacts of  $eCO_2$  (Gao et al., 2010). The significant effect of  $eCO_2$  and  $eTemp$  on growth and development of *A. craccivora* and its predator (*M. sexmaculatus*) on cowpea across three trophic levels was hypothesized here by conducting an array of experiments.

## Materials and methods

Elevated atmospheric  $CO_2$  ( $eCO_2$ ) of  $550 \pm 25$  ppm and ambient  $CO_2$  ( $aCO_2$ ) concentration of  $380 \pm 25$  ppm were maintained in open top chambers (OTC) of 4X4X4 m dimensions, constructed at ICAR-Central Research Institute for Dryland Agriculture, Hyderabad (17.38°N, 78.47°E). Four OTCs were used for experimentation purpose (Srinivasa Rao et al., 2012b) with two replications of  $eCO_2$  and  $aCO_2$  in each chamber. During the month of June, 2016 cowpea (cv C 152) seeds were sown and the crop was maintained by following the recommended package of practices except plant protection. Methodology adopted for study on three trophic levels, i.e., estimation of changes in cowpea crop (trophic-I), in the growth and development of *A. craccivora* (trophic-II) and on predator (trophic-III) are given here under.

### Biochemical analyses of cowpea foliage (Trophic-I)

The carbon, N contents, C:N and polyphenols were estimated in the cowpea foliage by adopting the standard protocols. Carbon and N were measured using a CHN analyzer (Model NA 1500 N, Carlo Erba Strumentazione, Italy) after drying of leaf samples (Jackson, 1973). Condensed tannins were determined by the Folin-Denis method by estimating the tannic acid equivalents (TAE) (Anderson and Ingram, 1993). For this method, leaf samples were dried at 40 °C for 48 h. Dried leaf samples were ground to powder, and phenolics were extracted with  $CH_3OH$ . The concentration of polyphenols in the extract was determined by spectrophotometry using tannic acid as the standard, and the results were expressed as percentage tannic acid equivalents. The soluble protein was estimated by Lowry et al. (1951). Soluble proteins were extracted from 0.5 g samples (leaf and seed) using distilled water. Aliquot 0.1 and 0.2 ml were taken and made up to 1 ml with distilled water and a tube with 1 ml of water alone serving as a blank. Five ml of alkaline copper reagent was added and kept for 10 min. Then 0.5 ml of 1 N Folin Ciocalteu Reagent (FCR) was added and kept in a dark place for 30 min. Later blue color develops and read the intensity at 660 nm. Soluble proteins were calculated using Fraction V of Bovine Serum Albumin (BSA) standards (20–100 µg). Estimation of free amino acids gives an indication about physiological status and the health condition of plants. Ninhydrin method (Moore and Stein, 1949) was used for the estimation of free amino acids and is calorimetrically measured at 570 nm to explore physiological status and the health condition of plants.

### Cowpea-aphid interactions (Trophic-II)

Field collected adults of *A. craccivora* from infested plants of cowpea were maintained in insect laboratory of CRIDA at optimum conditions of temperature ( $25 \pm 2$  °C), humidity ( $75 \pm 5$  %) and 14 L/10D cycle conditions. Stock culture of *A. craccivora* was maintained continuously on cowpea plants in separate growth chambers for use in experimental studies.

### Feeding trials of *A. craccivora* on cowpea

Feeding trials were conducted in  $CO_2$  growth chambers (PERCIVAL I-36LL). Aphids were reared on leaves of potted plants of cowpea obtained from respective  $CO_2$  conditions. Aphids were reared individually in petridishes (110 mm diameter and 10 mm height) adopting the 'cut leaf' method (Srinivasa Rao et al., 2013a). A group of *A. craccivora* adults were placed in a closed petridishes and were maintained in  $CO_2$  growth chambers at six constant temperatures (20, 25, 27, 30, 33 &  $35 \pm 0.5$  °C) at  $75 \pm 5$  % relative humidity (RH) and 14L/10D hour photoperiod.

### Biology of *A. craccivora* on cowpea

Adults of *A. craccivora* used for study on biology were reared in treatment environments for two generations to rule out the maternal effects. One day old nymphs were placed singly on the leaf in single petriplate in each  $CO_2$  condition (380 & 550 ppm) and allowed to grow until the start of reproduction to record the development time (DT). Ten replicates per set and three sets were used under each treatment with each aphid serving as one replicate. It means that 30 replications were adopted. Time taken by each nymph from its birth to the onset of reproduction was accounted for development time. The freshly moulted adults of *A. craccivora* were kept individually in petridish each consisting of a cowpea leaf for observation on reproduction. Number of nymphs produced per day were also noted at 12 h interval and removed after counting to prevent overcrowding. Parameters of aphid biology viz., development time (DT), total life cycle (TLC) and fecundity were recorded as per the standard procedure (Srinivasa Rao et al., 2013a) for each of the  $eCO_2$  and  $eTemp$  conditions.

### Estimation of degree days requirement on cowpea

Data on biological parameters were analyzed to estimate thermal constant for each stage of aphid. Degree days requirement was calculated considering the average development time at six different temperatures used in the experiment. Linear regression co-efficient (b) and constant (a) were estimated after plotting the reciprocal of developmental time (developmental rate) over temperature. The standard formula of  $Y = a + bx$  was used to estimate the threshold development ( $T_0$ ) and degree day calculation was based on developmental rate (Zahiri et al., 2010) was adopted.

### Estimation of life table parameters of *A. craccivora* on cowpea

Various life table parameters were calculated using TWOSEX – MS Chart software (Chi, 2005). To facilitate raw data analysis, life table analysis, a user-friendly computer program, was used (<http://140.120197.173/Ecology/prod02.htm>). Intrinsic rate of increase ( $r_m$ ), generation time (T), net reproductive rate ( $R_0$ ) and finite rate of increase ( $\lambda$ ) were estimated using cowpea foliage from  $eCO_2$  and  $aCO_2$  separately at above mentioned six temperatures.

### Cowpea-aphid- coccinellid interactions (Trophic-III)

Culture of Predator (*M. sexmaculatus*) was maintained by following standard procedure (Solangi et al., 2007) on *A. craccivora* feeding on cowpea. Adults of *M. sexmaculatus* were collected from field and selected mating pairs were reared on *A. craccivora* nymphs in laboratory in wide mouth plastic jars. Feeding trials of *M. sexmaculatus* on cowpea reared *A. craccivora* were conducted at  $eCO_2$  and  $aCO_2$  conditions at

optimum temperature of  $27 \pm 1^\circ\text{C}$  to understand the impact of  $\text{CO}_2$  on predator. Each mating pair of coccinellids was selected from the stock culture and was kept in petridishes of 9 cm diameter per replication (10 replications/each  $\text{CO}_2$  condition).

Grubs and adults of *M. sexmaculatus* were released independently on ten *A. craccivora* adults (3 day old) in a petridish on cowpea leaves by maintaining the corresponding  $\text{CO}_2$  conditions. The predators were allowed to lay eggs. Freshly hatched *M. sexmaculatus* grubs were released on *A. craccivora* and observations viz., grub duration (days) and predation capacity (%) of *M. sexmaculatus* were recorded at 24 hour period.

**Statistical analysis**

One way ANOVA was adopted for analyzing the data on biochemical constituents. The data on DT, RT, TLC and fecundity of aphid at six constant temperatures at two  $\text{CO}_2$  conditions were analyzed by using two factorial analysis considering the  $\text{CO}_2$  and temperatures levels as main and sub-factors. Results presented are mean value of each determination (treatment)  $\pm$  standard deviation. The mean values of life table parameters of *A. craccivora* at six constant temperatures were plotted and a non linear relationship (standard quadratic form  $Y = ax^2 + bx + c$ ) was worked out taking the 'x' as temperature and  $x^2$  as square of temperature which is common with quadratic form of non linear equations. The data on grub duration and predation capacity of coccinellids were also analyzed and results were compared over  $a\text{CO}_2$ . SPSS 16.0 was used for conducting all statistical analyses.

**Results**

*Impact of  $\text{CO}_2$  on biochemical constituents of cowpea (Trophic-I)*

In this study, the leaf N concentration was lower in cowpea foliage obtained from  $e\text{CO}_2$  conditions over  $a\text{CO}_2$ . Nitrogen content was significantly ( $F_{1,4} = 26.27$ ;  $p < 0.01$ ) lower under  $e\text{CO}_2$  ( $5.08 \pm 0.08$ ) than ambient. In contrast, carbon content was significantly higher ( $38.20 \pm 1.43$ ) ( $F_{1,4} = 23.23$ ;  $p > 0.05$ ) and influenced by  $\text{CO}_2$  (Fig. 1). However, the relative C: N ratio ( $F_{1,4} = 27.78$ ;  $p < 0.05$ ) was considerably higher ( $7.52 \pm 0.256$ ) in  $e\text{CO}_2$  cowpea foliage than in ambient ( $6.23 \pm 0.70$ ). No significant difference was observed in polyphenols content measured in terms of tannic acid ( $F_{1,2} = 0.07$ ;  $p < 0.01$ ) equivalents at both  $\text{CO}_2$  conditions (Fig. 1). Amino acids and proteins ( $F_{1,2} = 5.83$ ;  $p < 0.01$ ) are lower in  $e\text{CO}_2$  (0.0016 & 27.223) when compared to ambient (0.0017 & 29.011).

**Table 1**  
Influence of  $\text{CO}_2$  and temperature on growth and development of *A. craccivora* on cowpea.

		SS	df	F	P
Model	DT	1756.77	69	179.29	< 0.001
	RT	10763.95	69	981.04	< 0.001
	TLC	18073.864	69	922.28	< 0.001
	Fecundity	204021.289	69	91.96	< 0.001
$\text{CO}_2$	DT	702.803	1	4954.26	< 0.001
	RT	220.900	1	1392.63	< 0.001
	TLC	135.669	1	477.06	< 0.001
	Fecundity	11000.278	1	342.14	< 0.001
Temp	DT	981.392	5	980.92	< 0.001
	RT	10436.589	5	8944.18	< 0.001
	TLC	17763.047	5	9106.12	< 0.001
	Fecundity	183595.156	5	1611.72	< 0.001
$\text{CO}_2 \times \text{Temp}$	DT	7.081	5	7.08	< 0.001
	RT	28.733	5	24.62	< 0.001
	TLC	46.314	5	23.74	< 0.001
	Fecundity	1284.256	5	11.27	< 0.001
Error a	DT	4.114	29	-	-
	RT	4.600	29	-	-
	TLC	8.247	29	-	-
	Fecundity	932.389	29	-	-
Error b	DT	58.028	290	-	-
	RT	67.678	290	-	-
	TLC	113.139	290	-	-
	Fecundity	6606.922	290	-	-

\*\*  $P < 0.001$ ; SS - Sum of square; df - degrees of freedom; F - F probability. Statistically significant difference between groups ( $p < 0.05$ ).

*Impact of  $e\text{CO}_2$  and temperature on *A. craccivora* (Trophic-II)*

*Variations in growth and development of *A. craccivora**

Variations in biological parameters of *A. craccivora* on cowpea at two  $\text{CO}_2$  conditions and six constant temperatures are presented in Table 1. Development time (DT) was significantly reduced by the first factor,  $\text{CO}_2$  ( $F_{1,29} = 4954.26$ ;  $P < 0.001$ ) and second factor, temperature ( $F_{5,290} = 980.92$ ;  $P < 0.001$ ) indicating the reduction of DT with elevated  $\text{CO}_2$  and an increase in temperature. Increased reproductive time (RT) ( $19.50 \pm 0.628$ ) with  $\text{CO}_2$  ( $F_{1,29} = 1392.63$ ;  $P < 0.001$ ) and a gradual reduction in temperature ( $F_{5,290} = 8944.18$ ;  $P < 0.001$ ) and reduction of total life cycle (TLC) with  $\text{CO}_2$  ( $F_{1,29} = 477.06$ ;  $P < 0.001$ ) and temperature ( $F_{5,290} = 9106.12$ ;  $P < 0.001$ ) was noticed. The interaction between both the factors was also highly significant on RT ( $F_{5,290} = 24.62$ ;  $P < 0.001$ ) and TLC ( $F_{5,290} = 23.74$ ;  $P < 0.001$ ) of *A. craccivora* on cowpea. The offspring production per

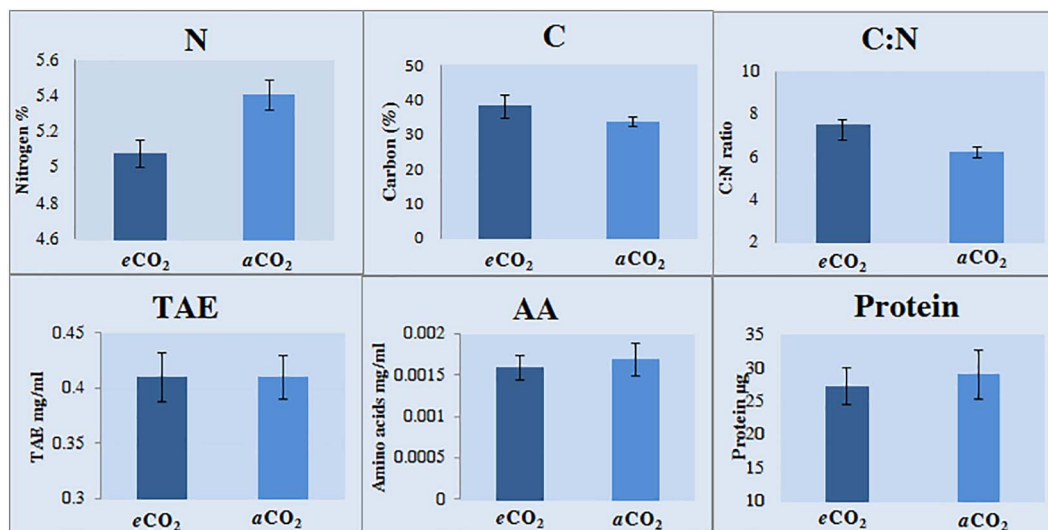


Fig. 1. Variation in biochemical constituents of cowpea foliage at  $e\text{CO}_2$  conditions.

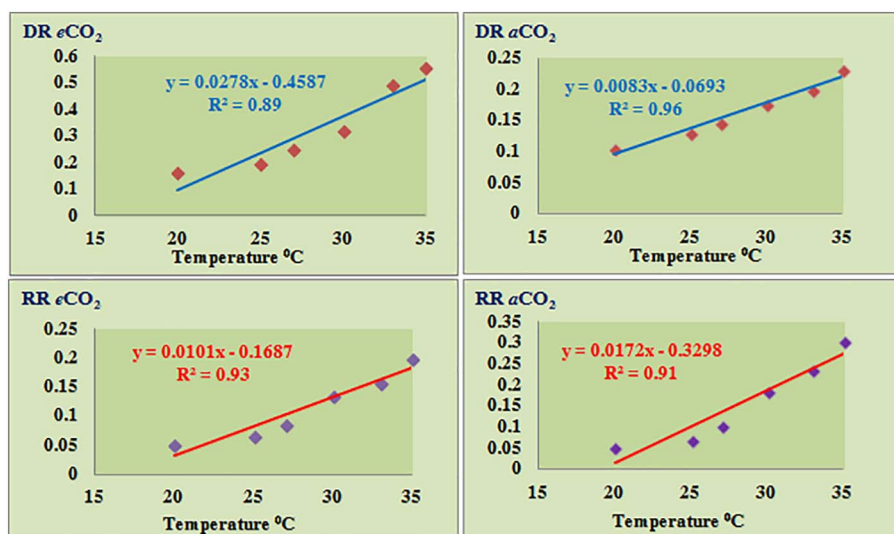


Fig. 2. Relation between Developmental Rate (DR), Reproductive rate (RR) with temperature for nymphs and adults of *A. craccivora* at  $e\text{CO}_2$  and  $a\text{CO}_2$ .

female was also impacted by  $\text{CO}_2$  ( $F_{1,29} = 342.14$ ;  $P < 0.001$ ) and temperature ( $F_{5,290} = 1611.72$ ;  $P < 0.001$ ). A substantial increase in production of offspring was noticed at  $e\text{CO}_2$  ( $74.90 \pm 6.90$ ) over  $a\text{CO}_2$  ( $60.67 \pm 8.06$ ). The highest offspring production was noticed between 20 and 27 °C and started declining with increase in temperature (30–35 °C). Significant interaction ( $F_{5,290} = 11.27$ ;  $P < 0.001$ ) of  $\text{CO}_2$  and temperature on fecundity was evident.

#### Thermal constants and degree days for *A. craccivora* stages on cowpea

Linear regression was used to estimate the temperature thresholds and thermal requirements of nymph and adult stages of *A. craccivora* independently. The best fit linear regression equation for nymph ( $Y = 0.0278x - 0.4587$ ,  $R^2 = 0.89$ ) and adult ( $Y = 0.0101x - 0.1687$ ,  $R^2 = 0.93$ ) and thus the mean lower development thresholds for both the stages were 7.7 and 20.5 °C on  $e\text{CO}_2$  foliage. In contrast, on  $a\text{CO}_2$  foliage, similar and significant trends with nymphal ( $Y = 0.0083x - 0.0693$ ,  $R^2 = 0.96$ ) and adult ( $Y = 0.0172x - 0.3298$ ,  $R^2 = 0.91$ ) stages were noted. The mean lower development thresholds for nymph were 16.50 at  $e\text{CO}_2$  than  $a\text{CO}_2$  (8.34 °C) by regressing development rate of nymphs and adults which on temperature (Fig. 2). Accumulated heat units for nymph stage varied from 58.86–95.71 DD on  $e\text{CO}_2$  as against 85.83–117.86 DD on  $a\text{CO}_2$  with temperature range of 20–35 °C. In contrast, adults required higher degree days of 88.33–264.50 at  $e\text{CO}_2$  as against 86.66–189.33 on  $a\text{CO}_2$  cowpea foliage at temperatures of 20–27 °C. The mean degree-day requirement of nymph ( $75.79 \pm 15.163$ ) and adult ( $157.15 \pm 67.04$ ) at  $e\text{CO}_2$  varied over  $a\text{CO}_2$  with similar trends reflected after summation of degree days of both nymphal and adult stages at  $e\text{CO}_2$  ( $232.96 \pm 80.32$ ) and  $a\text{CO}_2$  ( $247.07 \pm 64.77$ ).

#### Construction of life tables

Impact of  $e\text{CO}_2$  across different temperatures on life table parameters of *A. craccivora* are given in Table 2. It is evident that ' $r_m$ ' increased with increase in temperature from 20 °C showing proportional trend that started declining from 30 °C. The ' $R_o$ ' of *A. craccivora* was higher at 27 °C with 62 offspring at  $e\text{CO}_2$ . The ' $r_m$ ' and ' $R_o$ ' increased gradually with increase in temperature and reached maximum values at 27 °C. The reduction of ' $T$ ' was evident from 11 days at 20 °C with minimum of 3.29 days at 35 °C at  $e\text{CO}_2$  and followed the non-linear trend. The ' $\lambda$ ' was found to be highest 1.45 at 27 °C and followed the decreasing trend with an increase in temperature. The association between four life table parameters viz., ' $r_m$ ', ' $R_o$ ', ' $T$ ' and ' $\lambda$ ' and temperature at both  $e\text{CO}_2$  and  $a\text{CO}_2$  conditions was found to be non-linear (Fig. 3).

Quadratic form of equation with higher  $R^2$  (0.85) at  $e\text{CO}_2$  was the best noticed between ' $r_m$ ' and temperature. Other parameters viz., ' $R_o$ ', ' $T$ ' and ' $\lambda$ ' followed similar trend. When the first order derivative of equation is taken as 'zero', the temperature at which higher ' $R_o$ ' was estimated to be 24.20 °C on  $e\text{CO}_2$  foliage as against 23.29 ( $a\text{CO}_2$ ). The  $r_m$  (27.80) and  $\lambda$  (26.48) showed declining trend at 20–35 °C at  $e\text{CO}_2$  and similar non-linear trends were observed at  $a\text{CO}_2$  which are as follows ( $r_m = -0.002x^2 + 0.1138x - 1.2354$ ,  $R^2 = 0.85$ ), ( $R_o = 0.3309x^2 + 15.416x - 135.39$ ,  $R^2 = 0.83$ ), ( $T = 0.0157x^2 - 0.3799x + 12.064$ ,  $R^2 = 0.93$ ) and ( $\lambda = -0.0029x^2 + 0.1597x - 0.805$ ,  $R^2 = 0.86$ ). When the first order derivative is equated to 'zero' and to solve the 'x' (variable) the  $r_m$  and  $\lambda$  started declining at 28.45 °C and 27.22 °C on  $a\text{CO}_2$  foliage and found to be higher than  $e\text{CO}_2$  (Fig. 3).

#### Impact of $e\text{CO}_2$ on *M. sexmaculatus* (Trophic-III)

The data on impact of  $\text{CO}_2$  on two vital parameters of coccinellids which were allowed to feed on *A. craccivora* of cowpea foliage at both elevated and ambient conditions depicted in Fig. 4. Duration of grubs was found to be significantly ( $F_{1,9} = 20.43$ ;  $P < 0.001$ ) reduced at  $e\text{CO}_2$  (16) over  $a\text{CO}_2$  (18.70) and in contrast, the predation capacity of coccinellids at both nymph and adult stages increased at  $e\text{CO}_2$  (82) over  $a\text{CO}_2$  (78) condition ( $F_{1,9} = 5.88$ ;  $P < 0.001$ ).

#### Discussion

Experiments conducted to assess and quantify the impact of  $e\text{CO}_2$  and temperature across three trophic levels of cowpea, *A. craccivora*, and *M. sexmaculatus* revealed the significance of two major dimensions of climate change. Earlier studies (Cornelissen, 2011; Srinivasa Rao et al., 2014) also reported the significant effect of both  $e\text{CO}_2$  and temperature on other insect pests.

At first trophic level, reduction of leaf N content (6%) amino acid (6%) and protein (7%) in cowpea foliage with increased C (13%) and C:N ratio (21%) at  $e\text{CO}_2$  over  $a\text{CO}_2$ . A similar reduction of leaf N and protein concentrations by more than 12% (Ainsworth and Long, 2005) in  $\text{C}_3$  plants was observed. Researchers (Taub and Wang, 2008) found that N concentration within plant tissue was lower at  $e\text{CO}_2$  over  $a\text{CO}_2$ . Consumption pattern of lepidopteran and homopteran insect herbivores (Robinson et al., 2012) gets influenced due to dilution of biochemical composition of crop plants grown at  $e\text{CO}_2$  condition. The impacts of  $e\text{CO}_2$  on the host and insect herbivore interactions were well known (Hunter, 2001) indicating significant impact of  $e\text{CO}_2$  on changes in biochemical constituents of legume crop plants (Prasad et al., 2005;

**Table 2**

Mean ± standard error of life table parameters of *A. craccivora* on cowpea at six constant temperatures and two CO<sub>2</sub> conditions.

Temp ( ± °C)	<i>r<sub>m</sub></i>		<i>R<sub>0</sub></i>		<i>T</i>		$\lambda$	
	eCO <sub>2</sub>	aCO <sub>2</sub>	eCO <sub>2</sub>	aCO <sub>2</sub>	eCO <sub>2</sub>	aCO <sub>2</sub>	eCO <sub>2</sub>	aCO <sub>2</sub>
20	0.253 ± 0.006 (0.000)	0.229 ± 0.009 (0.000)	42 ± 3.27749 (10.741)	38 ± 2.391 (5.716)	11 ± 0.119 (0.014)	13 ± 0.132 (0.017)	1.3802 ± 0.009 (0.000)	1.25 ± 0.006 (0.000)
25	0.36 ± 0.006 (0.0000)	0.29 ± 0.006 (0.000)	57.6 ± 2.928 (8.578)	45.4 ± 2.981 (8.891)	10.32 ± 0.084 (0.007)	12.82 ± 0.13 (0.017)	1.4809 ± 0.009 (0.000)	1.34 ± 0.009 (0.000)
27	0.45 ± 0.003 (0.000)	0.37 ± 0.005 (0.000)	62 ± 0.803 (0.645)	50 ± 2.443 (5.971)	7.87 ± 0.053 (0.003)	10.82 ± 0.108 (0.012)	1.52 ± 0.006 (0.000)	1.45 ± 0.007 (0.000)
30	0.34 ± 0.009 (0.000)	0.42 ± 0.0146 (0.000)	22.67 ± 1.716 (2.944)	17.57 ± 1.166 (1.361)	6.02 ± 0.072 (0.005)	8.2 ± 0.112 (0.013)	1.45 ± 0.024 (0.000)	1.41 ± 0.014 (0.000)
33	0.29 ± 0.011 (0.000)	0.35 ± 0.021 (0.000)	13.9 ± 1.175 (1.381)	8.87 ± 0.698 (0.488)	4.58 ± 0.06 (0.004)	7.47 ± 0.098 (0.01)	1.35 ± 0.037 (0.001)	1.33 ± 0.014 (0.000)
35	0.23 ± 0.014 (0.000)	0.262 ± 0.025 (0.000)	6.97 ± 0.599 (0.358)	4.6 ± 0.407 (0.166)	3.29 ± 0.117 (0.014)	6.42 ± 0.12 (0.014)	1.3 ± 0.039 (0.001)	1.26 ± 0.018 (0.000)

Figures in parentheses are variance values.

(Srinivasa Rao et al., 2012a).

Impact of eCO<sub>2</sub> and temperature on second trophic level indicated that DT of *A. craccivora* decreased by 1–5 days as with increase in temperature from 20 to 35 °C (67%) and was more evident at eCO<sub>2</sub>. A similar trend was reported with other species of aphids i.e., *A. gossypii* in cotton and cucumber (Mohammad, 2010) and *Rhopalosiphum maidis* (Fitch) in barley (Xie et al., 2014) with CO<sub>2</sub> and temperature. The response of aphids to eCO<sub>2</sub> is mainly with species specificity and is able to overcome the disadvantage of eCO<sub>2</sub> by shortening DT. In addition to CO<sub>2</sub>, the temperature also influences the developmental rate of insect pests because of their physiological sensitivity (Aleosfoor and Fekrat, 2014).

Increased reproductive time of aphids and production of offsprings for longer periods was more evident in the present study with elevated CO<sub>2</sub>. Slight increment of RT in aphids with elevated CO<sub>2</sub> was observed. Insects change their physiology from time to time to get adaptation to changing climate. Earlier studies conducted by the authors (Mathukumalli et al., 2017) on *A. craccivora* in groundnut crop at both CO<sub>2</sub> levels showed the similar reduction of DT and increment of RT of aphids at eCO<sub>2</sub> over ambient across different temperatures.

It is reported that aphids proliferate in a higher number to overcome both indirect and direct effect of CO<sub>2</sub> and temperature. Increased fecundity was reported by Oehme et al. (2011) in case of *Myzus persicae*

on *Triticum aestivum*. Similar finding was reported by the authors with *A. craccivora* on cowpea (Srinivasa Rao et al., 2013a) and groundnut (Mathukumalli et al., 2017) crops. More production of offsprings with temperature in the range of 20–27 °C was noticed. Further increase in temperature caused reduction of progeny production which is in agreement with Lange et al. (2006). The present study quantifies the impact of host mediated effect of eCO<sub>2</sub> and direct effect of temperature on *A. craccivora* on cowpea and the corroborative evidences for change in growth and development of aphids were identified.

Decreased N (6%), amino acids (6%) and protein content (7%) with increased C (13%) and C:N ratio (21%) at eCO<sub>2</sub> over aCO<sub>2</sub> was noticed in cowpea foliage and these changes have significantly influenced the growth and development of *A. craccivora* on cowpea. Decreased N (3%) and increased C: N ratio (13%) and polyphenols (2%) in foliage of groundnut having significant influence on growth and development of insect herbivores have well documented (Srinivasa Rao et al., 2015) at eCO<sub>2</sub> and castor (Srinivasa Rao et al., 2013b). The variation of biological of aphids is attributed to changing nutritional quality of food due to change in biochemical composition of cowpea foliage at eCO<sub>2</sub> conditions.

Lower degree days requirement for completion of nymph and adult stages at eCO<sub>2</sub> (75.79 ± 15.16 DD) differed from those of aCO<sub>2</sub> (156.15 ± 67.04 DD) possibly due to due to shortening of DT. Similar

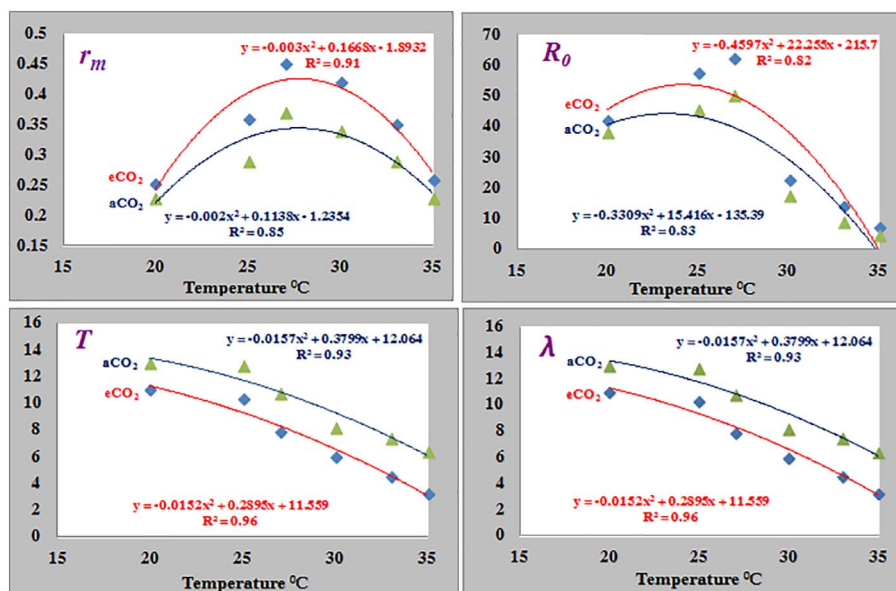


Fig. 3. Relationship between temperature and life table parameters of *A. craccivora* on cowpea at eCO<sub>2</sub> and aCO<sub>2</sub>.

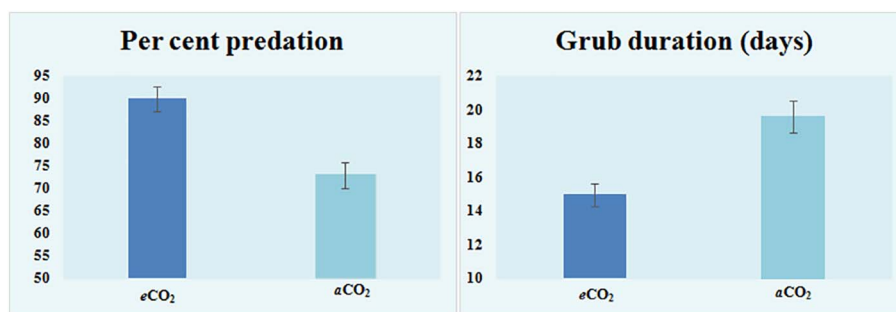


Fig. 4. Impact of eCO<sub>2</sub> on predation capacity and grub duration of *M. sexmaculatus* on *A. craccivora*.

lower thermal constants were reported for *Aphis glycines* Matsumura (McCornack et al., 2004), *R. maidis* (Kuo et al., 2006) and *A. craccivora* (Berberet et al., 2009). Summation of degree days requirement of both nymph and adult stages together also revealed a similar trend at eCO<sub>2</sub> (232.96 ± 80.32) and aCO<sub>2</sub> (247.07 ± 64.77). Findings also showed lower thermal requirement for nymphs and higher for adults feeding on foliage at eCO<sub>2</sub> over aCO<sub>2</sub> at temperatures of 20–35 °C. The overall degree day requirement of nymphs and adults of *A. craccivora* on cowpea at eCO<sub>2</sub> were similar to that of *A. craccivora* on groundnut at eCO<sub>2</sub> (Mathukumalli et al., 2017) except lower threshold levels of nymphs which were found to be higher on cowpea than groundnut. This might be due to higher C:N variation in cowpea (17%) than that of groundnut (12%) reported earlier.

Increased r<sub>m</sub> (54%) and R<sub>0</sub> (32%) with reduced T (28%) was noticed and followed an increasing trend with temperature in the range of 20 °C–27 °C and later followed descending trend and was more evident in case of eCO<sub>2</sub> than aCO<sub>2</sub>. Several workers reported the influence of CO<sub>2</sub> (Kuo et al., 2006) and temperature (Aleosfoor and Fekrat, 2014) on life table parameters of various aphids and it was found that non-linear trend was more evident. In our study eCO<sub>2</sub> influenced the 'r<sub>m</sub>' of *A. craccivora* which was contradictory to Flynn et al. (2006) who reported that eCO<sub>2</sub> did not cause the increase of 'r<sub>m</sub>' of *R. padi* and *M. persicae*.

Tritrophic interactions studied were at one temperature (27 °C) considered to be optimal. *M. sexmaculatus* were allowed to feed on *A. craccivora* reared on cowpea plants at eCO<sub>2</sub> and aCO<sub>2</sub> and the two vital parameters viz., grub duration and predation capacity calculated to vary significantly between eCO<sub>2</sub> and aCO<sub>2</sub>. The findings reflected the influence of CO<sub>2</sub> and temperature on coccinellids tentatively and further investigations are required to quantify the tritrophic interactions across six temperatures and two CO<sub>2</sub> levels.

Decreased grub duration (23%) and increased predation capacity (19%) of *M. sexmaculatus* on *A. craccivora* at elevated over aCO<sub>2</sub> in the present study was similar to increased aphid consumption rate of adult *Harmonia axyridis* (Pallas) in elevated CO<sub>2</sub> treatment reported by Chen et al. (2005). Prey preference of natural enemy varies with host quality mediated at eCO<sub>2</sub>. Chen et al. (2007) reported increased aphid abundance (*Sitobion avenae* Fab.) at elevated CO<sub>2</sub> over ambient CO<sub>2</sub> with 26–42.6% increase and with higher predation (23%). Reports of significantly increased consumption of aphids by first-instar *Chrysoperla sinica* (Tjeder) under high CO<sub>2</sub> than under low CO<sub>2</sub> levels and variation in consumption of aphids with instars of coccinellids exist (Gao et al., 2010).

## Conclusions

Direct effects of temperature at all three trophic levels and host-mediated effects of eCO<sub>2</sub> at second and third trophic levels imply an increasing incidence of aphids and its predators under anticipated changing climate with positive predator-prey density relations upholding the generalized ecological processes. However, rates of adaptation of organisms at each trophic level of crop-pest-predator and interaction thereof to changing climate accounting other abiotic variables

such as rainfall would determine the status of aphids as pests or potential of coccinellids as predator. Parallels noticed across crops relating to influence of eCO<sub>2</sub> and eTemp signify an universal trend of impact on other trophic levels and the established results of tritrophic (dicot plant-assimilate reducing insects & predator) interactions can serve as model system for similar organisms in respect of each trophic levels.

## Author's contribution

Conceived and designed the experiments-MSR, SV. Performed the experiments OS. Analyzed the data OS, PS, AVM. Contributed reagents/materials/analysis tools- MV, MM, KSR. Wrote the paper- MSR, PS, SV.

## Conflict of interest

The authors declare that they have no conflict of interest with any scientist or any firm/agency.

## Acknowledgments

This work was financially supported by grants from the Indian Council of Agricultural Research (ICAR), New Delhi in the form of National Innovations on Climate Resilient Agriculture (NICRA) Project. Authors are thankful to Prof. Dr. Hsin Chi, Laboratory of Theoretical and Applied Ecology, Department of Entomology, National Chung Hsing University, Taichung, Taiwan, Republic of China, for providing a computer programme on 'Age-Stage, Two -Sex life table analysis'.

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