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Response of three successive generations of maize aphid, *Rhopalosiphum maidis* Fitch (Aphididae: Hemiptera) reared on maize, *Zea mays* Linnaeus under elevated carbon dioxide and temperature

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Abstract
The objective of this study was to examine the response of three successive generations of maize aphid, *Rhopalosiphum maidis* Fitch (Aphididae: Hemiptera), reared on maize *Zea mays* Linnaeus under elevated Carbon dioxide (eCO_2) (550 ppm ± 25 ppm) and ambient Carbon dioxide (aCO_2) (380 ppm ± 25 ppm) concentrations at six temperatures of 20, 25, 27, 30, 33 and 35 ±1ºC in open-top chambers and CO_2 growth chambers with CO_2 and temperature regulation. Experiment was conducted by adopting agar-leaf method. Elevated CO_2 and temperature significantly extended the duration of reproductive time (19.96-23.32 days) with highest fecundity (83.20-87.68 nymphs per female) whereas the development time (6.60-7.16 days) and total life cycle (26.96-29.92 days) were declined compared to that of aCO_2 and temperatures in *R. maidis* across the three generations. The findings showed that eCO_2 and temperature significantly increased the number of generations of *R. maidis* with reduced generation time influenced the crop-insect interactions. The results indicated that the target agricultural management practices for effectively mitigating the concentrations of CO_2 and temperature.

Keywords: *Rhopalosiphum maidis*, *Zea mays*, successive generations, elevated CO_2, temperature

Introduction
Maize aphid *Rhopalosiphum maidis* Fitch (Aphididae: Hemiptera), a phloem feeding hemipteran insect and causes significant yield losses about 20.1 to 25 percent. It is a polyphagous species occurring worldwide on sorghum, barley and wheat besides maize [13]. It is now distributed worldwide in the tropics and warmer temperate regions [3]. Aphids cause mechanical harm and malnutrition to plants by the removal of phloem sap.

Agriculture is one of the most vulnerable sectors to the anticipated climate change with an adverse effect on crop yields. Temperature has an indirect influence on morphological and biochemical constituents of plants. The future estimations of ambient CO_2 concentration predict an increase up to 550 ppm within a few decades [8]. Such rise in CO_2 levels affects the biological system of living organisms, including insects [5]. Temperature has a direct influence on insect activity and their rate of development. Climate change could profoundly affect the population dynamics and the status of insect pests of crops [9]. The average increase in temperature was found to be 0.87 °C for the decade of 2006-2015 [8, 10] reported that excessively high temperatures reduce the reproductive period, fecundity, longevity and population growth. Insect generation time was strongly related to temperature [2].

Hence, in the present study the response of three successive generations of *R. maidis* were examined at two levels of CO_2 and six different temperatures to estimate the generation time and fecundity which would be useful in prediction of pest population.

Materials and Methods
Open top chambers
This experiment was conducted in open-top chambers (OTCs) of 4 X 4 X 4 m dimensions, located at ICAR-Central Research Institute for Dryland Agriculture (CRIDA), Hyderabad (17º 38' N; 78º 47' E) were utilized for raising of maize crop under eCO_2 (550 ± 25 ppm) and aCO_2 (380 ± 25 ppm) concentrations. The CO_2 concentration is maintained by pumping CO_2 diluted with air by air compressor [14]. The 100 percent CO_2 gas of commercial grade was used to elevate CO_2 levels within the chambers. CO_2 analyzer, PLC and SCADA programme with PC

were used to maintain the desired level of CO_2 within the OTCs along with temperature and relative humidity sensors.

CO_2 growth chambers with CO_2 and temperature regulation

Plants and insects were maintained in growth chambers (I 36LL; Percival Scientific, Inc. Perry, USA) under elevated and ambient concentrations of CO_2 (550 and 380 ppm ± 25 ppm, respectively) at six constant temperatures of 20, 25, 27, 30, 33 and 35 ± 1ºC and 60-70% relative humidity with a photoperiod ratio of 14L: 10D (14 hours of light: 10 hours of dark). Light illumination is provided through fluorescent lamps horizontally mounted in pairs above each shelf. Air circulation inside the chamber was maintained from a specifically designed air diffuser. The period of light, CO_2 concentrations and temperature levels were automatically monitored and controlled using Intellus Ultra Controller.

Maintenance of maize crop and growth conditions

Seeds of maize plants (DHM-117) were sown in OTCs and growth chambers and typical representative red soil type was maintained. The maize plants were raised under respective set conditions of elevated and ambient concentrations of CO_2 (550 and 380ppm ± 25 ppm, respectively) at six constant temperatures of 20, 25, 27, 30, 33 and 35 ± 1ºC. The leaves were detached from these plants and were used for the maintenance of the *R. maidis* culture for experimentation. Fully grown foliage (30 days after sowing) obtained from respective set conditions was used for feeding trials and the crop was maintained at insecticide free condition throughout the experiment to understand the impact of *e*CO_2 and temperature on insect pests.

Maintenance of *R. maidis* culture

The test insect, corn leaf aphids, *R. maidis* (family: Aphididae; Order: Hemiptera) were collected from the field and maintained in the entomology laboratory of ICAR-CRIDA. The nymphs and adults were reared individually in petridishes of 110 mm diameter of 10 mm height to obtain the mass culture for experiments. The culture was maintained for a number of generations by adopting agar-leaf method [7, 11] in growth chambers at elevated and ambient concentrations of CO_2 (550 and 380ppm ± 25 ppm, respectively) at six constant temperatures of 20, 25, 27, 30, 33 and 35 ± 1ºC and a photoperiod of 14L:10D. Agar medium was prepared with 1g of agar and 100 ml distilled water. The mixture was heated to boiling point in a microwave oven and then cooled to 45ºC by stirring. The mixture was poured in petridishes and left undisturbed until it solidified. The corn leaf from the top of corn seedlings of 1-2 months old was detached and placed in agar medium. The agar medium helpful to keep the leaf fresh and the leaves were changed on every 3 days. Light intensity of 30, 000 Lx was provided by 26 W fluorescent bulb inside the chambers during the 14 h light period with relative humidity of 60% (day) and 70% (night).

Insect feeding method

Experiments on development of *R. maidis* for three successive generations were conducted by adopting agar-leaf method at elevated and ambient concentrations of CO_2 (550 and 380ppm ± 25 ppm, respectively) at six temperatures of 20, 25, 27, 30, 33 and 35±1ºC and a photoperiod of 14L:10D. The first instar nymphs were collected carefully from the stock culture with the help of wet camel hair brush and transferred individually into each petridish containing maize leaves obtained from respective set conditions with 25 replications per each treatment. Each nymph was examined daily and the growth parameters *viz*., development time (DT), reproductive time (RT), total life cycle (TLC) and fecundity were calculated.

Statistical analysis

The data pertaining to the development of *R. maidis* (*i.e.* development time, reproductive time, total life cycle, fecundity) were analyzed using Two way ANOVA with the effect of CO_2 and temperature levels as main factor and sub factors deployed in split plot design. The growth and development of *R. maidis* with the effect of CO_2 (main factor), temperatures (sub factor) and generations as sub-sub factor deployed in split-split plot design.

Results and Discussion

Effect of *e*CO_2 and temperature on response of *R. maidis*

The results on impact of elevated and ambient concentrations of CO_2 (550 and 380ppm ± 25 ppm, respectively) at six temperatures of 20, 25, 27, 30, 33 and 35 ± 1ºC on response of *R. maidis* was found significant across its three successive generations (Table 1, 2 and 3).

Development time

The impact of *e*CO_2 at six constant temperatures on the development time of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 67.93, P = <0.01), second ($F_{11, 24}$ = 70.71, P = <0.01) and third ($F_{11, 24}$ = 30.02, P = <0.01) generation under two levels of CO_2 concentrations at six temperature conditions.

In the first generation, the development time was significantly decreased at *e*CO_2 and across the temperature conditions. Under *e*CO_2 (550 ppm ± 25 ppm) at six temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC, the developmental time decreased with temperatures and were resulted that 6.60, 5.72, 5.00, 4.20, 4.08 and 4.04 days whereas under *a*CO_2 (380 ppm ± 25 ppm) at six temperatures higher development time was recorded and followed the similar trend resulted that 10.04, 7.96, 6.84, 5.24, 4.64 and 4.24 days, respectively. A minor increase in the development time was observed at *e*CO_2 and across temperatures in second generation when compared to the first generation and values were ranging from 7.92, 6.80, 5.84, 4.52, 4.24 and 4.16 days whereas under *a*CO_2 and temperatures resulted that 11.36, 8.68, 7.16, 6.20, 4.84 and 4.44 days, respectively in the second generation. In third generation, the development time was significantly lower at *e*CO_2 and temperatures resulted that 7.16, 6.24, 5.20, 4.40, 4.16 and 4.12 days whereas under *a*CO_2 and temperatures resulted that 8.60, 7.76, 6.56, 5.24, 4.32 and 4.16 days, respectively compared to that of first and second generations.

The perusal of results of development time across three generations indicated that the reduction of development time under *e*CO_2 and increased temperatures over *a*CO_2 and increased temperature conditions. The present results indicated that the lower temperature decreased the development rate which in turn resulted in the prolonged developmental time of all stages of the aphid by decreasing the metabolic processes of aphids. The development time was significantly extended under the effect of *a*CO_2 and temperatures compared with that of *e*CO_2 and temperature conditions which might have resulted to negative effects of *a*CO_2 on metabolism rate or higher effects of decreased leaf nutritional quality at lower temperatures and also longer

developmental time may result in a reduction in the fitness of the pest. The present results are in conformity with [16], who revealed that the increase of temperature significantly decreased the duration of all developmental stages of corn leaf aphid. The findings are in agreement with the results of [15] who reported that the temperatures higher than 32 °C caused a decrease in developmental rate of spirea aphid *Aphis spiraecola* Patch (Aphididae: Hemiptera).

Reproductive time

The impact of eCO_2 at six constant temperatures on the reproductive time of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 66.39, P = <0.01), second ($F_{11, 24}$ = 37.48, P = <0.01) and third ($F_{11, 24}$ = 2.36, P = <0.01) generation under two levels of CO_2 concentrations at six temperature conditions.

In the first generation, the reproductive time was significantly increased at eCO_2 over aCO_2 across the temperature conditions. Under eCO_2 (550 ppm ± 25 ppm) at six temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC the reproductive time reduced with temperatures and were resulted that 23.32, 16.56, 14.28, 9.40, 2.52 and 1.56 days whereas under aCO_2 (380 ppm ± 25 ppm) at six temperatures shorter reproductive time and followed the similar trend resulted that 20.00, 14.72, 12.44, 8.72, 2.48 and 1.52 days, respectively. A slight decrease in the reproductive time was observed at eCO_2 and across temperatures in second generation when compared to the first generation and values were ranging from 20.16, 14.64, 12.56, 7.88, 2.36 and 1.32 days whereas under aCO_2 and temperatures reduction of reproductive time ranging from 19.80, 13.76, 12.24, 7.76, 2.16 and 1.28 days, respectively compared over eCO_2 in the second generation. A similar trend was observed in the third generation and the reproductive time was significantly lower at eCO_2 and temperatures resulted that 19.96, 14.40, 12.52, 7.84, 2.48 and 1.40 days whereas under aCO_2 and temperatures reproductive time was decreased and were resulted that 18.12, 13.72, 11.56, 7.72, 2.12 and 1.16 days, respectively compared to the second generation.

The results of reproductive time were examined across three generations indicated that the reproductive time was declined under eCO_2 and increased temperatures over aCO_2 and increased temperature conditions. An increase in the reproductive time when compared over development time under both eCO_2 and aCO_2 at six temperature conditions. Nitrogen is the single most important limiting resource for phytophagous insects. The reproductive time was significantly increased at eCO_2 and temperatures compared with that of aCO_2 and temperatures which might be attributed to the reduction in foliar N and increase in C:N ratio leading to an increase in their food consumption rates in order to compensate for the reduced nutritional quality [12].

Total life cycle

The impact of eCO_2 at six constant temperatures on the total life cycle of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 7.60, P = <0.01), second ($F_{11, 24}$ = 3.90, P = <0.01) and third ($F_{11, 24}$ = 18.53, P = <0.01) generation under two levels of CO_2 concentrations at six temperature conditions.

In the first generation, the total life cycle was significantly reduced at eCO_2 and increased temperature conditions. Under eCO_2 (550 ppm ± 25 ppm) at six constant temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC, the total life cycle decreased with temperatures and were resulted that 29.92, 22.28, 19.28, 13.60, 6.60 and 5.60 days whereas under aCO_2 at six constant temperatures total life cycle was longer and resulted that 30.04, 22.68, 19.28, 13.96, 7.12 and 5.76 days, respectively. The total life cycle was significantly decreased compared to that of first generation at eCO_2 and temperature conditions. The results were recorded that 28.08, 21.44, 18.40, 12.40, 6.60 and 5.48 days whereas under aCO_2 and temperatures an increase in the total life cycle was observed and were resulted that 29.48, 22.40, 19.08, 13.96, 7.00 and 5.68 days, respectively compared to that of second generation. A similar trend was observed in the third generation and the total life cycle was significantly gradually decreased at eCO_2 and temperature conditions and resulted that 26.96, 20.00, 17.44, 12.12, 6.28 and 5.28 days whereas under aCO_2 and temperatures increased total life cycle and were resulted that 28.56, 22.16, 19.08, 13.08, 6.80 and 5.48 days, respectively compared to that of second generation.

The results of total life cycle across three generations indicated that the total life cycle was significantly decreased under eCO_2 and increased temperatures over aCO_2 and increased temperature conditions. The present results were indicated that 27 ºC was the optimum for the biology of *R. maidis* on maize. The present results were in agreement with [4], who reported that the whole life span of the aphid was drastically reduced with the increase of temperature (15-30 ºC) due to the nymphal development time was reduced leads to shortened total life-span and natality rate per surviving female per day of parturition was increased with rather negligible deaths among the progeny.

Fecundity

The impact of eCO_2 at six constant temperatures on the fecundity of *R. maidis* was found significant in three generations. Significant differences were observed in first ($F_{11, 24}$ = 28.75, P = <0.01), second ($F_{11, 24}$ = 1014.3, P = <0.01) and third ($F_{11, 24}$ = 1769.39, P = <0.01) generation under two levels of CO_2 concentrations at six temperature conditions. In the first generation, the fecundity was significantly increased with temperature in the range of 20ºC to 27ºC but significantly declined at 30ºC to 35ºC under eCO_2 compared to that of aCO_2 conditions. Under eCO_2 (550 ppm ± 25 ppm) at six temperatures *viz*., 20, 25, 27, 30, 33 and 35 ± 1ºC highest fecundity observed and were resulted that 52.12, 72.24, 83.20, 43.04, 12.36 and 3.16 nymphs per female whereas under aCO_2 (380 ppm ± 25 ppm) and six temperatures lowest fecundity was recorded and were resulted that 43.08, 60.16, 65.32, 39.68, 8.68 and 2.60 nymphs per female, respectively. The fecundity was significantly more with temperature in the range of 20ºC to 27ºC but significantly declined at 30ºC to 35ºC under eCO_2 and aCO_2 conditions compared to that of first generation. Under eCO_2 and temperatures resulted that 57.44, 75.12, 87.04, 41.44, 9.88 and 1.64 nymphs per female whereas under aCO_2 and temperatures decreased fecundity was recorded and were resulted that 47.24, 63.24, 70.48, 41.08, 8.92 and 1.40 nymphs per female, respectively. The gradual increase in the fecundity was observed under eCO_2 and aCO_2 conditions in the third generation compared to that of second generation except at the temperature of 35ºC due to the highest temperature might have resulted that the reduced fecundity in *R. maidis*. Under eCO_2 and temperatures increased fecundity was noticed and were resulted that 58.80, 75.96, 87.68, 35.32, 6.64 and 1.28 nymphs per female whereas under aCO_2 and

temperatures resulted that 48.48, 64.68, 71.96, 31.08, 5.52 and 1.12 nymphs per female, respectively.

The results indicated that significantly increase in the fecundity across the three generations under both eCO_2 and aCO_2 at six temperature conditions. The results are in agreement with [1] found that eCO_2 increased the fecundity of cereal aphid, *Sitobion avenae* when reared on winter wheat. According to [6] reported that increased fecundity or production of offspring was more under eCO_2 and temperature conditions meaning that proliferation or multiplication will be higher during future climate change scenarios. This might be due to the variations of biochemical constituents of the crop plants under eCO_2 and *e*temperature conditions implying that crop plants may experience severe infestation by aphid population.

Effect of elevated CO_2, temperature and generations on response of *R. maidis* on maize

The effect of eCO_2, temperature and three generations on response of *R. maidis* on maize was furnished (Table 4). The reproductive time of *R. maidis* was significantly varied by CO_2 conditions ($F_{1, 24}$ = 4.67, P = <0.01). The reproductive time of *R. maidis* was significantly varied by temperature conditions ($F_{11, 24}$ = 6.75, P = <0.01). The reproductive time of *R. maidis* was significantly varied by three generations ($F_{3, 24}$ = 205.65, P = <0.01). The interaction between CO_2, temperature and three generations was found significant ($F_{14, 24}$ = 11.49, P = <0.01). The fecundity of *R. maidis* showed non-significant difference by CO_2 conditions ($F_{1, 24}$ = 1.24NS, P = <0.01). The fecundity of *R. maidis* was significantly varied by temperature conditions ($F_{11, 24}$ = 37.39, P = <0.01). The fecundity of *R. maidis* was significantly varied by three generations ($F_{3, 24}$ = 185.69, P = <0.01). The interaction between CO_2, temperature and three generations was found significant ($F_{14, 24}$ = 14.60, P =<0.01).

Table 1: Effect of elevated CO_2 and temperature on development time, reproductive time, total life cycle and fecundity of *R. maidis* on maize in first generation

Temperatures (ºC)	Development time (days)		Reproductive time (days)		Total life cycle (days)		Fecundity (nymphs/female)	
	***a*CO₂ (380 ppm)**	***e*CO₂ (550 ppm)**	***a*CO₂ (380 ppm)**	***e*CO₂ (550 ppm)**	***a*CO₂ (380 ppm)**	***e*CO₂ (550 ppm)**	***a*CO₂ (380 ppm)**	***e*CO₂ (550 ppm)**
20	10.04±0.54	6.60±0.5	20.00±0.41	23.32±0.80	30.04±0.61	29.92±0.81	43.08±3.94	52.12±3.76
25	7.96±0.73	5.72±0.48	14.72±0.74	16.56±0.58	22.68±1.11	22.28±0.74	60.16±5.10	72.24±5.48
27	6.84±0.62	5.00±0.71	12.44±0.71	14.28±0.46	19.28±0.84	19.28±0.79	65.32±4.99	83.20±5.56
30	5.24±0.52	4.20±0.41	8.72±0.46	9.40±1.12	13.96±1.22	13.60±0.64	39.68±5.20	43.04±5.11
33	4.64±0.49	4.08±0.33	2.48±0.71	2.52±0.51	7.12±0.75	6.60±0.76	8.68±2.67	12.36±3.13
35	4.24±0.44	4.04±0.20	1.52±0.59	1.56±0.58	5.76±0.74	5.60±0.7	2.60±0.96	3.16±1.31
F test	67.93**		66.39**		7.60**		28.75**	
S.Em ±	0.144		0.184		0.227		1.179	
LSD(p = 0.05)	0.286		0.365		0.450		2.339	
LSD (p = 0.01)	0.378		0.484		0.596		3.094	
CV (%)	9.00		6.26		4.99		10.57	
Factor 1 (CO_2)								
aCO_2 (380 ppm)	6.493		10.10		16.58		36.58	
eCO_2 (550 ppm)	4.940		11.14		16.08		44.53	
F test	796.42**		247.07**		34.62**		356.19**	
S.Em ±	0.055		0.066		0.085		0.412	
LSD(p = 0.05)	0.114		0.137		0.175		0.849	
LSD (p = 0.01)	0.154		0.185		0.238		1.151	
CV (%)	8.34		5.39		4.51		8.81	
Factor 2 (Temperature (ºC))								
20	8.320		21.66		29.98		47.60	
25	6.840		15.64		22.48		66.20	
27	5.920		13.36		19.28		74.26	
30	4.720		9.060		13.78		41.36	
33	4.360		2.500		6.860		10.52	
35	4.140		1.540		5.640		2.88	
F. test	506.10**		6891.40**		6680.22**		2274.65**	
S.Em±	0.103		0.133		0.163		0.856	
LSD(p = 0.05)	0.203		0.263		0.321		1.688	
LSD (p = 0.01)	0.268		0.346		0.424		2.225	

All values are mean ± standard deviation, ** Significant @ 1% level of significance; NS = Not-significant

Table 2: Effect of elevated CO_2 and temperature on development time, reproductive time, total life cycle and fecundity of *R. maidis* on maize in second generation

Temperatures (ºC)	Development time (days)		Reproductive time (days)		Total life cycle (days)		Fecundity (nymphs/female)	
	aCO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)
20	11.36±0.49	7.92±0.57	19.80±0.33	20.16±0.37	29.48±0.50	28.08±0.45	47.24±0.52	57.44±0.77
25	8.68±0.48	6.80±0.41	13.76±0.46	14.64±0.49	22.40±0.71	21.44±0.71	63.24±1.67	75.12±0.78
27	7.16±0.47	5.84±0.55	12.24±0.51	12.56±0.50	19.08±0.57	18.40±0.58	70.48±0.51	87.04±0.82
30	6.20±0.41	4.52±0.51	7.76±0.44	7.88±0.33	13.96±0.70	12.40±0.73	41.08±0.64	41.44±0.76
33	4.84±0.37	4.24±0.44	2.16±0.37	2.36±0.49	7.00±0.71	6.60±0.49	8.92±0.49	9.88±0.88
35	4.44±0.51	4.16±0.37	1.28±0.46	1.32±0.48	5.68±0.59	5.48±0.56	1.40±0.50	1.64±0.49
F test	70.71**		37.48**		3.90**		1014.3**	
S.Em ±	0.133		0.126		0.257		0.229	
LSD (p = 0.05)	0.265		0.251		0.509		0.457	
LSD (p = 0.01)	0.351		0.332		0.673		0.606	
CV (%)	7.42		4.69		5.91		1.87	
Factor 1 (CO_2)								
aCO_2 (380 ppm)	7.113		9.100		16.13		38.72	
eCO_2 (550 ppm)	5.580		9.820		15.40		45.42	
F test	793.50		188.38**		67.53**		4005.42**	
S.Em ±	0.054		0.089		0.089		0.106	
LSD(p = 0.05)	0.112		0.108		0.184		0.219	
LSD (p = 0.01)	0.152		0.147		0.250		0.296	
CV (%)	7.43		4.80		4.90		2.18	
Factor 2 (Temperature (ºC))								
20	9.640		19.14		28.56		52.34	
25	7.740		14.18		21.92		69.16	
27	6.500		12.06		18.56		78.76	
30	5.360		7.820		13.18		41.26	
33	4.540		2.260		6.800		9.400	
35	4.300		1.300		5.580		1.520	
F. test	958.80**		12378.48**		4610.85**		78961.15**	
S.Em±	0.094		0.089		0.186		0.158	
LSD(p = 0.05)	0.186		0.175		0.367		0.311	
LSD (p = 0.01)	0.245		0.231		0.484		0.410	

All values are mean ± standard deviation; ** Significant @ 1% level of significance; NS = Not-significant

Table 3: Effect of elevated CO_2 and temperature on development time, reproductive time, total life cycle and fecundity of *R. maidis* on maize in third generation

Temperatures (ºC)	Development time (days)		Reproductive time (days)		Total life cycle (days)		Fecundity (nymphs/female)	
	aCO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380 ppm)	*e*CO_2 (550 ppm)
20	8.60±0.49	7.16±0.37	18.12±0.41	19.96±0.35	28.56±0.50	26.96±0.45	48.48±0.51	58.80±0.41
25	7.76±0.52	6.24±0.44	13.72±0.44	14.40±0.50	22.16±0.71	20.00±0.71	64.68±0.48	75.96±0.35
27	6.56±0.51	5.20±0.41	11.56±0.44	12.52±0.51	19.08±0.57	17.44±0.58	71.96±0.93	87.68±0.48
30	5.24±0.52	4.40±0.50	7.72±0.46	7.84±0.37	13.08±0.70	12.12±0.73	31.08±0.64	35.32±0.48
33	4.32±0.48	4.16±0.37	2.12±0.332	2.48±0.51	6.80±0.71	6.28±0.54	5.52±0.50	6.64±0.49
35	4.16±0.37	4.12±0.33	1.16±0.48	1.40±0.50	5.48±0.59	5.28±0.56	1.12±0.33	1.28±0.46
F test	30.02**		2.36**		18.53**		1769.39**	
S.Em ±	0.129		0.122		0.176		0.152	
LSD (p = 0.05)	0.257		0.243		0.351		0.302	
LSD (p = 0.01)	0.342		0.322		0.465		0.400	
CV (%)	7.52		4.48		4.02		1.29	
Factor 1 (CO_2)								
aCO_2 (380 ppm)	6.107		9.767		15.86		37.14	
eCO_2 (550 ppm)	5.213		9.467		14.68		44.28	
F test	176.33**		34.71**		235.26**		10863.85**	
S.Em ±	0.067		0.051		0.077		0.069	
LSD(p = 0.05)	0.139		0.105		0.159		0.141	
LSD (p = 0.01)	0.188		0.142		0.215		0.192	
CV (%)	10.29		4.59		4.36		1.46	
Factor 2 (Temperature (ºC))								
20	7.880		19.88		27.76		53.64	
25	7.000		14.08		21.08		70.32	
27	5.880		12.38		18.26		79.82	
30	4.820		7.780		12.60		33.20	

33	4.240	2.300	6.540	6.080
35	4.140	1.280	5.380	1.200
F. test	650.80**	13495.04**	10083.03**	196404.27**
S.Em±	0.085	0.086	0.123	0.105
LSD(p = 0.05)	0.168	0.170	0.242	0.207
LSD (p = 0.01)	0.221	0.224	0.319	0.272
All values are mean ± standard deviation; ** Significant @ 1% level of significance; NS = Not-significant				

Table 4: Effect of elevated CO_2, temperature and generations on development time, reproductive time, total life cycle and fecundity of *R. maidis* on maize

Temp (ºC) X Generations		Development time (days)		Reproductive time (days)		Total life cycle (days)		Fecundity	
		aCO_2 (380ppm)	*e*CO_2 (550 ppm)	*a*CO_2 (380ppm)	*e*CO_2 (550ppm)	*a*CO_2 (380ppm)	*e*CO_2 (550ppm)	*a*CO_2 (380ppm)	*e*CO_2 (550ppm)
F1	20	10.04±0.54	6.60±0.5	20.00±0.41	23.32±0.80	30.04±0.61	29.92±0.81	43.08±3.94	52.12±3.76
	25	7.96±0.73	5.72±0.48	14.72±0.74	16.56±0.58	22.68±1.11	22.28±0.74	60.16±5.10	72.24±5.48
	27	6.84±0.62	5.00±0.71	12.44±0.71	14.28±0.46	19.28±0.84	19.28±0.79	65.32±4.99	83.20±5.56
	30	5.24±0.52	4.20±0.41	8.72±0.46	9.40±1.12	13.96±1.22	13.60±0.64	39.68±5.20	43.04±5.11
	33	4.64±0.49	4.08±0.33	2.48±0.71	2.52±0.51	7.12±0.75	6.60±0.76	8.68±2.67	12.36±3.13
	35	4.24±0.44	4.04±0.20	1.52±0.59	1.56±0.58	5.76±0.74	5.60±0.7	2.60±0.96	3.16±1.31
F2	20	11.36±0.49	7.92±0.57	19.80±0.33	20.16±0.37	29.48±2.37	28.08±0.70	47.24±0.52	57.44±0.77
	25	8.68±0.48	6.80±0.41	13.76±0.46	14.64±0.49	22.40±0.65	21.44±0.65	63.24±1.67	75.12±0.78
	27	7.16±0.47	5.84±0.55	12.24±0.51	12.56±0.50	19.08±0.61	18.40±0.69	70.48±0.51	87.04±0.82
	30	6.20±0.41	4.52±0.51	7.76±0.44	7.88±0.33	13.96±0.61	12.40±0.57	41.08±0.64	41.44±0.76
	33	4.84±0.37	4.24±0.44	2.16±0.37	2.36±0.49	7.00±0.58	6.6±0.65	8.92±0.49	9.88±0.88
	35	4.44±0.51	4.16±0.37	1.28±0.46	1.32±0.48	5.68±0.69	5.48±0.59	1.40±0.50	1.64±0.49
F3	20	8.60±0.49	7.16±0.37	18.12±0.35	19.80±0.41	28.56±0.50	26.96±0.45	48.48±0.51	58.80±0.41
	25	7.76±0.52	6.24±0.44	13.72±0.50	14.40±0.44	22.16±0.75	20.00±0.71	64.68±0.48	75.96±0.35
	27	6.56±0.51	5.20±0.41	11.56±0.51	12.52±0.44	19.08±0.57	17.44±0.58	71.96±0.93	87.68±0.48
	30	5.24±0.52	4.40±0.50	7.72±0.46	7.84±0.37	13.08±0.70	12.12±0.73	31.08±0.64	35.32±0.48
	33	4.32±0.48	4.16±0.37	2.12±0.332	2.48±0.51	6.80±0.71	6.28±0.542	5.52±0.50	6.64±0.49
	35	4.16±0.37	4.12±0.33	1.16±0.48	1.40±0.50	5.48±0.59	5.28±0.46	1.12±0.33	1.28±0.46
F test		0.73^{NS}		11.49**		0.84^{NS}		14.60**	
S.Em ±		0.181		0.292		0.332		1.762	
LSD (p = 0.05)		0.373		0.603		0.686		3.636	
LSD (p = 0.01)		0.506		0.816		0.929		4.926	
CV (%)		10.16		7.68		5.38		9.48	
Factor 1 (CO_2)									
aCO_2 (380 ppm)		5.967		9.593		16.13		42.09	
eCO_2 (550 ppm)		5.849		10.16		15.44		40.08	
F test		0.15^{NS}		4.67*		0.15 ^{NS}		1.24^{NS}	
S.Em ±		0.088		0.246		0.310		1.840	
LSD (p = 0.05)		0.182		0.507		0.640		3.797	
LSD (p = 0.01)		0.247		NS		0.867		5.145	
CV (%)		7.02		13.91		9.90		8.89	
Factor 2 (Temperature (ºC))									
20		6.640		9.893		16.46		37.62	
25		5.287		10.30		15.58		44.90	
27		6.500		9.60		16.10		37.51	
30		5.273		10.04		15.31		44.36	
33		6.513		9.527		16.00		37.52	
35		5.233		10.03		15.26		44.60	
F test		5.91*		6.75**		3.22^{NS}		37.39**	
S.Em ±		0.120		0.152		0.170		1.099	
LSD (p = 0.05)		0.236		0.299		NS		2.268	
LSD (p = 0.01)		NS		0.394		NS		3.073	
CV (%)		7.22		13.22		8.45		5.81	
Factor 3 (Generations)									
F_1		5.717		10.62		16.33		40.48	
F_2		6.347		9.46		15.76		40.71	
F_3		5.660		9.62		15.27		42.07	
F test		121.00**		205.65**		117.08**		185.69**	
S.Em ±		0.049		0.062		0.069		1.099	
LSD (p = 0.05)		NS		NS		NS		2.268	
LSD (p = 0.01)		NS		NS		NS		3.073	

All values are mean ± standard deviation; ** Significant @ 1% level of significance; NS = Not-significant

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