Indian Journal of Agronomy **60** (4): 594–600 (December 2015)

Research Paper

Influence of seasonal variability and conservation tillage on flower production and boll retention of Bt cotton (*Gossypium hirsutum*)

D. BLAISE[1]

ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra 440 010

Received : July 2015; Revised accepted : December 2015

ABSTRACT

An experiment was conducted at Central Institute for Cotton Research, Nagpur, Maharashtra, to monitor flower and boll production in Bt transgenic cotton (*Gossypium hirsutum* L.) hybrid ('RCH 2 Bt. hybrid') as influenced by climatic factors and conservation tillage practices in selected treatments. Two reduced tillage (RT) systems, with limited soil disturbance in the form of inter-culture operations (RT1) and no soil disturbance (RT2), were compared with the conventional tillage treatment (CT). The tillage treatments comprised subplots with and without green manure. Conservation-tillage treatments had 12–15% more bolls retained on a plant than the conventional tillage. Irrespective of the tillage systems, flower and boll production varied among years. Flower production correlated negatively with the maximum and minimum temperature ($P<0.0001$) and maximum and minimum relative humidity ($P<0.001$) during both the seasons. In general, sunshine hours and evaporation rate showed a non-significant positive correlation with flower production. In both the seasons, negative correlation was observed for boll production with maximum and minimum temperature ($P<0.0001$) and minimum humidity ($P<0.40$). Boll production correlated positively with evaporation rate, but the relationship was significant in the second season ($P<0.01$). Boll retention was 54.3% higher in the first season compared to 43.6% in the second season due to lower mean maximum and minimum temperature. In general, temperature, relative humidity and evaporation rate were positively correlated with the number of bolls shed. These results indicate that seasonal variability has a greater impact on flower and boll production, irrespective of tillage treatments. Conservation-tillage systems may modulate and modify the adverse climatic effects such as high temperature and increasing moisture stress.

Key words : Conventional tillage, Cotton, *Gossypium hirsutum,* Green manure, Reduced tillage, Temperature, Vertisols

Transgenic Bt (*Bacillus thuringiensis*) cotton is grown on nearly 12 million ha in India and is the only country where Bt transgenic hybrids are cultivated (Blaise *et al*., 2014). Conventional tillage is a widespread practice in the sub-continent wherein frequent tillage operations are done to create a suitable seedbed and facilitate effective weed control. Furthermore, recycling of crop residues is negligible. As a result of this old tradition, a decline in soil organic carbon has occurred because the soil is mixed and aerated and decomposition of organic matter is enhanced (Prasad and Power, 1991). Therefore, to improve the soil health and productivity, conservation-tillage systems evolved and were considered as an acceptable technology by the cotton-growers (Blaise *et al*., 2005). These systems were found to improve resource-use efficiency as well as productivity (Sharma *et al*., 2012). Conservation-tillage systems are now considered as an integral part of sustainable agricultural practice since soils not only sequesters more carbon compared to the conventional tillage system, but also releases less of CO_2 to the atmosphere. To offset the problem of limited supplies of manure and competing uses of crop residue (Prasad and Power, 1991), an application of green manure to soil is a good option in any agricultural production system.

Atmospheric factors also play an important role in cotton productivity. Extreme temperatures are associated with low and variable cotton yields (Oosterhuis, 2002). High night temperatures reduce pollen viability and increase the boll shedding late in the fruiting season (Gipson and Joham, 1968). Although cotton is easily adapted to tropical climates; an ideal temperature range for its growth and biomass accumulation is 20–30°C (Reddy *et al*., 1992). Since cotton is an indeterminate plant, a balance between

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[1]**Corresponding author Email:** blaise_123@rediffmail.com; blaise_agron@yahoo.co.in

[1]Head, Division of Crop Production, ICAR-Central Institute for Cotton Research, Nagpur, Maharashtra 440 010

vegetative and reproductive development is strongly influenced by soil fertility, soil moisture, and atmospheric factors.

Conservation-tillage systems can mitigate adverse environmental effects by modulating temperature (Sharma *et al*., 2012). Further, such systems possibly reduce soil-moisture stress as a result of improved weed control (Chopra and Chopra, 2010; Blaise, 2011). At present, no information is available on the flower and boll production of Bt transgenic cotton hybrids in response to tillage systems and green manure. We hypothesize that tillage and green manure modulates the flower and boll retention of cotton. The objectives of the study were to elucidate the role of tillage systems with a green manure in association with the climatic factors on flower and boll production in transgenic Bt cotton hybrids. Information from this study could serve as a reference point or a baseline for future studies.

MATERIALS AND METHODS

The field experiment was conducted at the Central Institute for Cotton Research, Nagpur, Maharashtra in a split-plot design with 3 tillage treatments as main plots with a fixed layout since the start of the experiment in 1996. Four subplots comprised 100% recommended dose of fertilizer-N (RDN) and green manure with 3 levels of fertilizer N (60, 80 and 100% of RDN). Briefly, the tillage treatments were conventional tillage (CT), reduced tillage-1 (RT1) and reduced tillage-2 (RT2). In the CT treatment, mouldboard ploughing (0.15–0.25 m) was done in alternate years, after the harvesting of the previous cotton crop. Three to four inter-row cultivations were done followed by manual hand-weeding during the cropping season. The RT1 treatment involved 2 shallow inter-row cultivations (0.05 m) with a bullock-drawn blade hoe. Two rows of sunnhemp (*Crotalaria juncea* L.) as a green manure (GM) cover crop was sown between the cotton rows after the first inter-row cultivation [20–30 days after planting (DAP) of cotton] and the second inter-row culture (80–90 DAP of cotton) was done to incorporate the green-manure crop. Nitrogen (100 kg/ha) was applied in the GM plots. Two manual hand-weeding operation was performed to control the late-emerging weeds. No inter-row cultivation was done in the treatment RT2. In this treatment, GM was harvested using a sickle and left on the soil surface as a mulch (average dry weight of 990 kg/ha). Three to four manual hand-weedings were done to control the weeds. The soil type is classified as a fine, montmorillonitic, hyperthermic, Typic Haplustert. The soil was slightly alkaline (*p*H 8.1), low in organic C (4.1 g/kg), available N (115 mg/kg) and extractable P (6.1 mg/kg).

Rainfed cotton (cv. 'RCH 2' Bt hybrid) was sown following onset of rain (24 June 2005 and 23 June 2006) in all the treatments. The field individual plot size of 36 m^2; 6 m long and 6 m wide, each consisting of 10 cotton rows spaced at 0.60 m was first leveled to obtain a gentle slope and avoid run-off with a wooden planker followed by running a bullock-drawn line marker having wooden pegs spaced 0.60 m apart. Cotton hybrid ('RCH 2 Bt') was manually dibbled with 2 seeds/hill at the intersections made by the line marker. Thus, cotton was spaced 0.6 m between rows and 0.6 m between plants within a row which is a common practice of square planting of cotton hybrids in central India (Bhalerao and Gaikwad, 2010; Blaise *et al*., 2014). At 10–15 days after sowing (DAS), plants were thinned to a single seedling/hill. Subsequently, the first dose of fertilizer was applied; half of N as urea along with entire P as single superphosphate (19 kg/ha) and K through muriate of potash (37 kg/ha) were spot placed about 0.02 to 0.03 m away from the plant at a depth of approximately 0.025 m. The remaining N was applied equally in 2 split doses– at 45 and 75 DAS at square-formation and boll-formation stages.

Six selected treatment combinations of tillage with and without GM were used for monitoring the fruiting pattern. These included CT with GM (CT+GM) and CT without GM (CT-GM); RT1 with GM (RT1+GM) and RT1 without GM (RT1-GM); RT2 with GM (RT2+GM) and RT2 without GM (RT2-GM). Five plants were tagged at random in each plot of the selected treatments. The tagged plants were monitored daily for flowers appearing on a tagged plant. Each individual open flower was tagged and monitored for boll formation. The abscised bolls were counted as bolls shed. When the boll was fully opened, each individual boll was picked, boll weight recorded and single plant yield were also recorded.

The atmospheric factors (independent variables) considered for this study were maximum air temperature (^{o}C), minimum air temperature (^{o}C), evaporation (mm), sunshine duration (h/d), maximum humidity (%) and minimum humidity (%). These data were collected from the Meteorological Observatory located within the Institute.

Rainfall received during the cropping season was also collected. In the first year, 1,042 mm rainfall was received with 52 rainy days and well distributed till mid- October. In the second season, total rainfall received was 901 mm with 45 rainy days until September. Long-term average rainfall for Nagpur during June to December is 1,002 mm with 49 rainy days.

Heat unit is a simple index for relating plant growth, development and maturity with air temperature. Heat units were calculated by the following formula given by Ritchie *et al.* (2004). The threshold temperature considered here is 12.8^{o}C.

Heat Units = (Max. Temperature + Min. temperature)/2)– Threshold temperature

Statistical analysis was conducted according to Draper and Smith (1966) following the procedures outlined in the general linear model (GLM, SAS Institute, Inc. 1985). Simple correlation coefficients were also computed between the dependent variables (flower and boll production) and the independent variables (atmospheric factors). The significance was tested at a probability level of 0.05 to determine the factors affecting the dependent variable. Multiple-linear regressions were also computed for peak flower and boll production with the independent variables.

RESULTS AND DISCUSSION

Flower and boll production

Effect of tillage and green manure: Though flower production was significantly higher in the GM plots, tillage effects were not statistically significant (Fig. 1a,b). Similarly, the GM plots also had higher number of bolls than those without GM, irrespective of the tillage systems. In the first season, flower production peaked during mid-September (Fig. 2a), whereas in the second season, peak flower production was observed at the end of September (Fig. 2c). Greater numbers of flowers produced in the first season might be attributed to a favourable effect of maximum temperature coupled with sufficient rainfall during September (253 mm during 2005 compared to 118 mm in 2006) that facilitated decomposition of GM. An increase in activity of soil microorganisms with a rise in temperature was reported to hasten decomposition of crop residues (Liu *et al*., 2008). Consequently, nutrients released would become available to the plants. This explains the greater flower and boll production, ultimately leading to higher seed-cotton yield in the GM plots than those without (Blaise, 2011). In addition, reduced weed density in the GM plots resulted in water being conserved (Blaise, 2011). This may have contributed to improving boll set and size. Furthermore, the water-conserving ability of GM might have also helped the plants further in reducing the severity of water stress that occurred as a result of less rainfall that was received and high evaporation rate during the second season.

Among the tillage systems, the RT treatments had higher number of bolls than the CT (Fig. 1). Averaged over seasons, the RT treatments had 12–15% more bolls retained on a plant than the CT treatment. Peak boll production during the first season was observed during mid November irrespective of the treatments (Fig. 2b). In the second season, boll production peaked during the end of November (Fig. 2d) which coincided with a greater moisture stress due to the receding rains (80 mm received in October of 2005 compared to only 9 mm in 2006). As a result, boll retention was higher (54.3%) during the first season, irrespective of the tillage treatments, than the second season (43.4%).

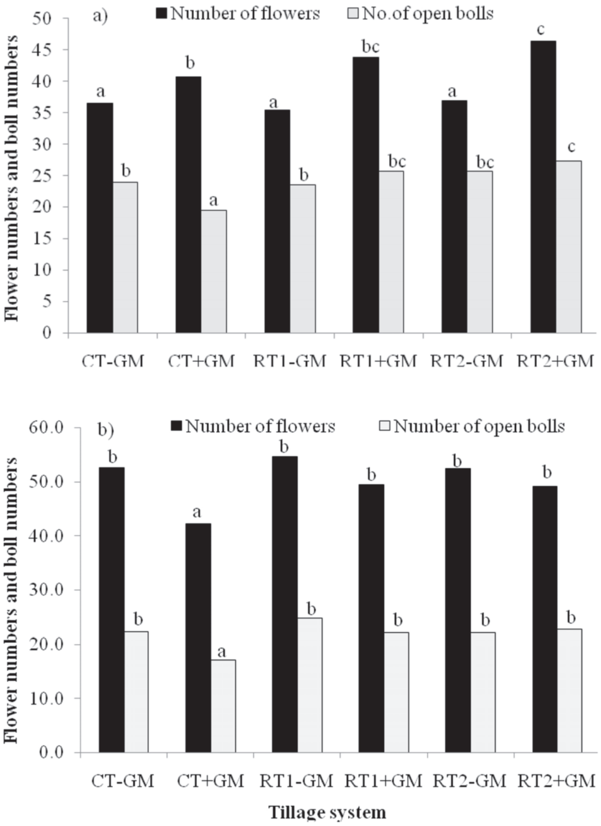

Fig. 1. Effect of tillage on flower and boll production per plant in 'RCH 2 Bt' cotton during 2005–06 (1a) and 2006–07 (1b) (for the flowers and bolls, treatment differences are not significant if values are followed by the same letter)

Effect of climatic factors: Mean maximum temperature and minimum temperatures during flowering and boll formation stages (60 to 120 DAP of cotton) of first season were lower by 2.3 and 2.4°C respectively, than the corresponding temperatures during the second season (Table 1). Mean maximum humidity was 1.1% higher during the first season and mean minimum humidity was lower by 1.7% when compared to the second season.

Regression models were computed to elucidate the influence of atmospheric factors on flower and boll production in Bt cotton hybrids, irrespective of the tillage and green-manure treatments (Table 2). In the first season, atmospheric factors accounted for 46% of the variation in flower production, 62% for boll production and 68% for boll shedding. In the second season, the corresponding values were 82, 51 and 82% respectively. Cumulative heat units showed highly significant positive correlation with flower production in 2005 ($R^2 = 0.96$, $P<0.0001$) and 2006 ($R^2 = 0.97$, $P<0.0001$) (Fig. 3a, 4a).. Similarly, boll

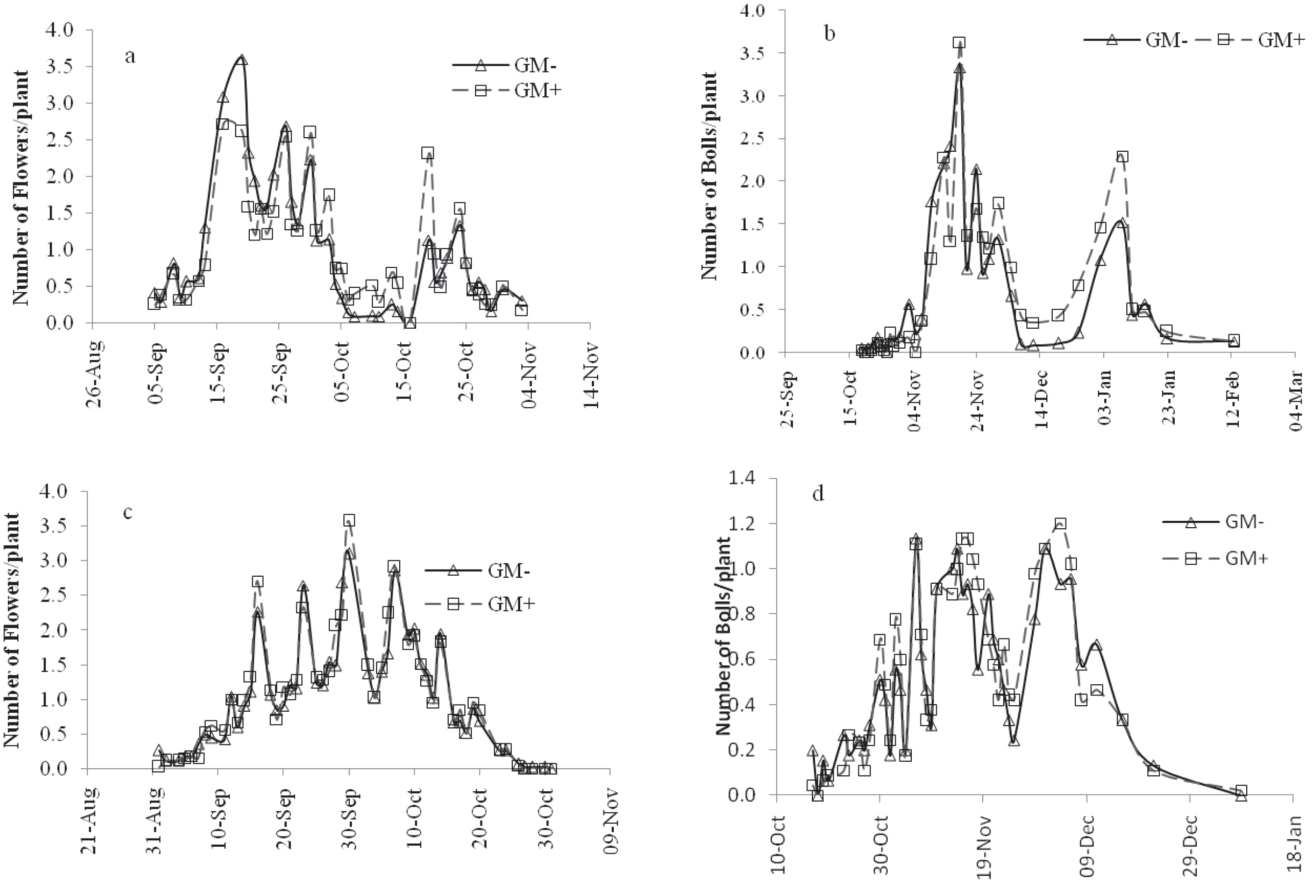

Fig. 2. Effect of green manure on flower and boll production per plant during (a, b) 2005 and (c, d) 2006

production had significant positive correlation with cumulative heat units in 2005 ($R^2 = 0.84$, $P<0.001$) and 2006 ($R^2 = 0.95$, $P<0.0001$) (Fig. 3b, 4b).

Among the different atmospheric factors, negative correlation was observed for flower production during both the seasons with maximum and minimum temperature ($P<0.0001$) and maximum and minimum humidity ($P<0.001$) (Table 2). Sunshine hours and evaporation rate showed a positive correlation with flower production in the first season, but was not significant ($P<0.9$). However, in the second season, evaporation rate was negatively correlated, however, it was not significant ($P<0.71$). A negative correlation of flower production with temperature has been reported in the upland cotton variety 'DPL 51' (Reddy *et al*., 1998) and Egyptian cotton variety 'Giza 75' (Sawan, 2013).

Boll production, during both the seasons, showed a significant negative correlation ($P<0.0001$) with maximum and minimum temperature. Positive correlation was observed with evaporation rate in both the seasons, but was significant in 2006 ($P<0.01$). Minimum humidity correlated negatively with boll production in 2005 ($P<0.92$) and 2006 ($P<0.40$). However, maximum humidity showed a negative correlation during 2005 ($P<0.91$) and positively during 2006 ($P<0.44$). Reproductive growth is particularly sensitive to high temperature before and after anthesis (Oosterhuis, 2002). The optimal temperature range that permits normal enzyme function for upland cotton growth is 23.5 to 32^{o}C with an optimum temperature of 28^{o}C (Reddy *et al*., 1992). Any temperature above or below the optimal values would impose stress on cotton plants and limit plant growth and fruiting (Hodges *et al*., 1993).

Higher temperatures in the second season favoured flowering and boll formation resulting on an average, 50.6 fruiting structures compared to only 40 in the first season. Reddy *et al*. (1992), too, reported an increase in number of fruiting sites but the number of squares and bolls decreased greatly as temperatures increased above 35^{o}C. With regard to bolls shed, in general, it was positively correlated with temperature, humidity and evaporation rate. Average temperatures more than 36^{o}C were observed for 15 days in the second season compared to only 4 days in the first season. Thus, boll shedding was greater in season 2 than in season 1. A negative correlation between

Table 1. Climatic factors during flowering and boll-formation stages of the cropping seasons

Climatic factors	Flowering stage 9 Sep. to 3 Oct. 2005				Boll-formation 7 Nov. to 8 Dec. 2005				Flowering stage 26 Sep. to 19 Oct. 2006				Boll-formation 8 Nov. to 22 Dec. 2006			
	Max.	Min.	Mean	S.D.	Max.	Min.	Mean	S.D.	Max.	Min.	Mean	S.D.	Max.	Min.	Mean	S.D.
Maximum temperature (°C)	36.0	24.0	31.6	2.5	36.0	14.4	28.7	3.0	36.0	27.6	33.3	2.0	36.0	26.6	31.6	1.9
Minimum temperature (°C)	30.8	13.8	22.1	3.1	23.8	8.0	12.9	3.4	25.0	18.6	22.8	1.7	22.6	11.5	17.01	3.3
Evaporation (mm/day)	4.4	2.2	3.1	0.6	3.7	2.7	3.3	0.3	4.7	2.8	3.5	0.41	4.1	2.5	3.18	0.3
Sunshine (h/day)	9.8	1.0	7.4	2.4	10.0	4.6	9.3	1.4	ND	ND	ND^2	ND	ND	ND	ND	ND
Maximum humidity (%)	96.1	66.2	84.7	17.6	90.0	57.0	74.3	7.8	95.0	65.0	83.6	7.9	93.0	45.0	76.0	9.4
Minimum humidity (%)	92.0	28.0	59.1	15.5	70.0	20.1	36.1	12.4	85.0	22.0	55.3	16.2	84.0	26.0	84.0	13.1

S.D., Standard deviation; ND, not determined; Max., maximum; Min., minimum

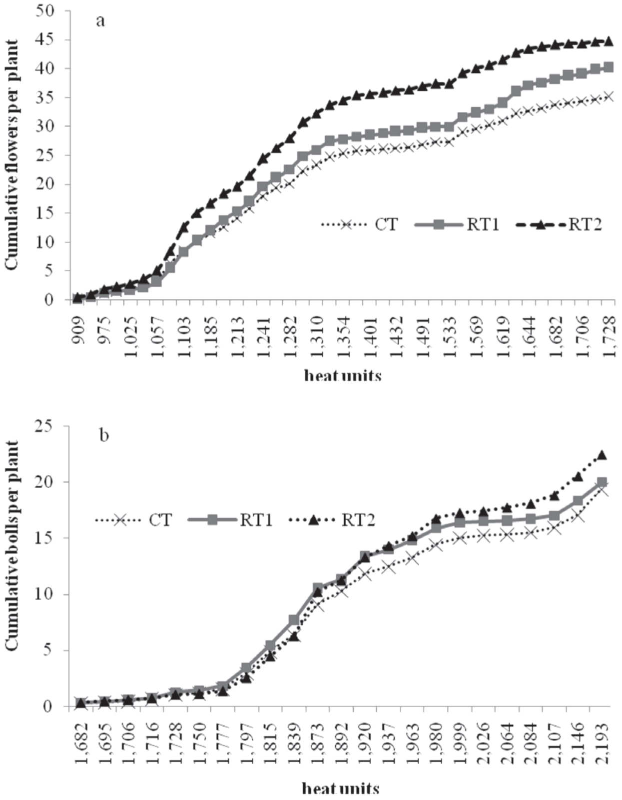

Fig. 3. Relationship of tillage system and cumulative heat units on (3a) cumulative flowers and (3b) cumulative bolls produced/plant during 2005

minimum humidity and boll retention during the second season could be due to a reduction in pollen-tube growth under less humid conditions. Relative humidity below 50% was found to significantly reduce pollen-tube elongation in cotton (Burke *et al*., 2004). Furthermore, high night temperature is responsible for increased fruit shedding (Reddy *et al*., 1992). Similarly, Zhao *et al*. (2005) observed a 70% reduction in boll retention when plants were exposed to 36/28°C day/night temperatures than those experiencing 30/22°C temperature regimes. These results support our findings. An increased boll shedding contributed to low boll retention in the second season because of high temperature-induced boll abscission. Temperatures higher than the optimal values are normally experienced in Central India, during flowering and boll development. Such high temperatures have been found to compromise the reproductive efficiency of the crop (Reddy *et al*., 2004). In the present study, Bt hybrid belonged to the Upland cotton types. These cottons have been found to be more sensitive to temperature than Pima cotton (Hodges *et al*., 1993). Researchers have also documented genotypic thermo-tolerance (Snider *et al*., 2010). At present, nearly 95% of the cotton hybrids cultivated in India belong to the

Upland cotton and there are very little genotypic differences among them. Brown and Oosterhuis (2010) also observed that the obsolete varieties were less sensitive to temperature than the modern day cultivars that were bred to perform well under ideal temperature environments. Furthermore, a high evaporation rate during the second season coupled with low and poorly distributed rainfall might have imposed water stress in cotton. High evaporation rate was found to impose water stress on plants (Sawan, 2013) and a resultant feedback inhibition of photosynthesis in cotton (Reddy *et al*., 1997). Consequently, the flow of assimilates to the developing bolls is limited causing the bolls to abscise.

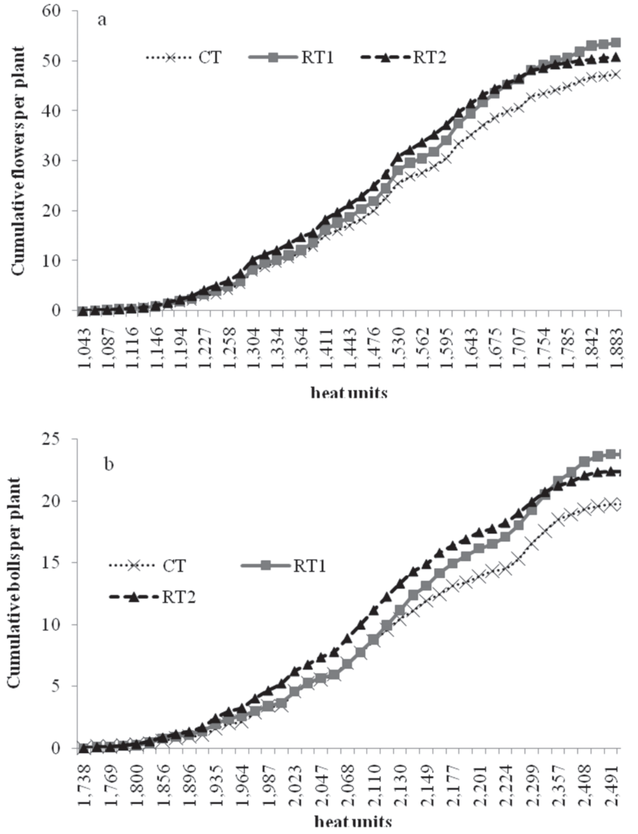

Fig. 4. Relationship of tillage system and cumulative heat units on (4a) cumulative flowers and (4b) cumulative bolls retained/plant in cotton during 2006

Sunshine duration was positively correlated with flower and boll production in cotton during the present study. Generally, reduced light reduces photosynthesis and increases boll abscission (Zhao and Oosterhuis, 1998). Among the different climatic factors, temperature, humidity and evaporation rate were major determinants that influenced flower and boll production and boll retention.

Fruiting behavior was monitored for the first time in Bt transgenic hybrids as affected by conservation tillage treatments and a green manure. Fruiting was also correlated with the weather parameters. Results of the present study indicate that green-manure application along with reduced tillage improved boll retention by 12–15% of the Bt cotton hybrids compared to the conventional-tillage treatment. Among the atmospheric factors, flower and boll production were negatively correlated with maximum and minimum temperature, whereas the numbers of bolls shed were positively correlated. Thus, boll retention declined with an increase in temperature coupled with low humidity and high light intensity. Conservation-tillage systems and use of green manure may modify and modulate the effects due to a change in climate. In the future, the widely adapted Asiatic cotton cultivars might be needed to fit for

Table 2. Multiple-regression models for peak flower, peak boll production, and boll shed with atmospheric factors and cumulative flowers and bolls with heat units

Parameter	Model	R^2
2005		
Flower production	$Y = 20.3 - 0.06X_1 - 0.37 X_2 - 0.03X_3 - 0.03X_4 + 0.004X_5 + 0.17X_6 + e$*	0.46
Boll production	$Y = 3.38 - 0.07X_1 - 0.13X_2 - 0.007X_3 + 0.03X_4 - 0.07X_5 + 0.44X_6 + e$	0.62
Bolls shed	$Y = 0.11 + 0.007X_1 + 0.039X_2 - 0.007X_3 + 0.0004X_4 + 0.023X_5 - 0.031X_6 + e$	0.68
Cumulative flowers produced vs. cumulative heat units	$Y = -53.2 + 0.056X_1 + e$	0.96
Cumulative bolls vs. cumulative heat units	$Y = -48.3 + 0.029X_1 + e$	0.84
2006		
Flower production	$Y = 17.3 - 0.06 X_1 - 0.33 X_2 - 0.06X_3 - 0.07 X_4 - 0.281X_5 + e$	0.82
Boll production	$Y = 3.03 - 0.04X_1 - 0.01X_2 + 0.001X_3 - 0.006X_4 + 0.4X_5 + e$	0.51
Bolls shed	$Y = 2.178 - 0.168X_1 + 0.252X_2 + 0.008X_3 - 0.004X_4 + 0.434X_5 + e$	0.82
Cumulative flowers produced vs cumulative heat units	$Y = -95.2 + 0.085X_1 + e$	0.97
Cumulative bolls vs. cumulative heat units	$Y = -69.0 + 0.037X_1 + e$	0.95

*where X_1, Max. temperature (°C); X_2, min. temperature (°C); X_3, max. relative humidity (%); X_4, min. relative humidity (%); X_5, sunshine hour; X_6, evaporation rate (mm/day) in 2005; evaporation (mm/day) in 2006 the changed climate scenario. Studies on these aspects will be essential to understand how these perform vis-à-vis the modern cultivars.

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