



## Article

# Extent of Bollworm and Sucking Pest Damage on Modern and Traditional Cotton Species and Potential for Breeding in Organic Cotton

Seraina Vonzun <sup>1,2</sup>, Monika M. Messmer <sup>1</sup> , Thomas Boller <sup>2</sup> , Yogendra Shrivastava <sup>3</sup>,  
Shreekant S. Patil <sup>4</sup> and Amritbir Riar <sup>5,\*</sup>

<sup>1</sup> Department of Crop Sciences, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, CH-5070 Frick, Switzerland; Seraina.Vonzun@fibl.org (S.V.); monika.messmer@fibl.org (M.M.M.)

<sup>2</sup> Plant Physiology, University of Basel, 4056 Basel, Switzerland; thomas.boller@unibas.ch

<sup>3</sup> bioRe Research, bioRe Association India, Kasrawad, 451228 Madhya Pradesh, India; biore.yogendra@gmail.com

<sup>4</sup> Department of Genetics and Plant Breeding, University of Agricultural Sciences (UAS) Dharwad, 580005 Karnataka, India; sspadvance@gmail.com

<sup>5</sup> Department of International Cooperation, Research Institute of Organic Agriculture (FiBL), Ackerstrasse 113, CH-5070 Frick, Switzerland

\* Correspondence: amritbir.riar@fibl.org

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**Abstract:** Resistance against cotton bollworm is one of the main arguments for the use of genetically modified (GM) Bt cotton around the globe. The use of GM is prohibited in organic systems and thus the remunerative value of organic cotton cultivation depends on effective bollworm control. In this study, we investigated the extent of bollworm and sucking pest damage in 68 different hybrid and varietal lines of *Gossypium hirsutum* and varietal lines of *G. arboreum* at two different locations with contrasting soil fertility and water dynamics. The damage potential of bollworms was assessed from open capsules at two time points. Sucking pests were assessed at three time points using a scoring method. *G. arboreum* varietal lines and *G. hirsutum* hybrids were on average significantly more tolerant than *G. hirsutum* varietal lines to bollworm under fertile and irrigated situations. For sucking pests, the *G. arboreum* varietal lines were clearly more tolerant than *G. hirsutum* hybrids and varietal lines. Since, recently, pink bollworm (*Pectinophora gossypiella*) became resistant against Bt cotton and pressure of sucking pests severely increased, screening of genetic resources and systems-based cotton breeding for bollworm and sucking pest tolerance will improve sustainability of organic and conventional cotton production.

**Keywords:** Bollworm; organic farming; *Gossypium* spp.; Bt toxin; smallholder farmer; breeding

## 1. Introduction

The growing awareness of the environmental impacts within the last decade and increased demand for non-genetically modified (non-GM) cotton fiber in Europe (labelled as clean, green, or organic cotton) created a niche for an organic cotton production [1]. Organic agriculture offers a low financial risk, low-input alternatives, and allows sustainable management of natural resources [2]. Presently, India is the major organic cotton supplier producing about 51% of world's organic cotton [3]. However, organic cotton accounts for less than 1% of the total cotton production worldwide [3].

Cotton is a significant cash crop accounting for 30% of India's agricultural gross domestic product [4]. The majority of Indian farmers are smallholders with less than two hectares of land to cultivate [5,6]. There are several factors that influence the low production levels and increase poverty

of Indian smallholder farmers. The most important ones include the constantly rising production costs (seeds and pesticides), the pressure of cosmopolitan pests [7], the growing risks for the environment and worker's safety as well as the intensive cultivation leading to decreasing soil fertility. Moreover, the considerably low cotton prices, which are bound to high price fluctuation on the world market put the farmer at risk.

The cotton bollworm is a major pest limiting cotton productivity worldwide. Three bollworm species cause main damage on cotton in India: the pink bollworm (*Pectinophora gossypiella*), the spotted bollworm (*Earias vitella*), and the American bollworm (*Helicoverpa armigera*). The term bollworm usually represents all different species.

The Bt cotton hybrid (GM cotton) was introduced to India in 2002 followed by a radical transformation of cotton cultivation [8]. Initially with the introduction of GM cotton with Bollgard I, bollworm became a minor problem in GM cotton fields. A decrease of bollworm population since the introduction of Bt cotton has previously been reported by Kranthi [9]. However, in the present scenario farmers experienced severe bollworm attacks due to irregular monsoon rains and unusually warm growing conditions. In addition, bollworm is still present in other alternative host crops and infests organic cotton fields. On top of that, some species have developed resistances against GM cotton [10,11]. In a recent study widespread infestation of 40 to 95% of pink bollworm on Bt cotton was reported for Maharashtra State [8]. However, information on the extent of damage caused by bollworm in non-GM and GM cotton fields in India is lacking or inconsistent. Furthermore, the introduction of Bt hybrids came along with a progressive population size of sucking pests such as cotton aphids (*Aphis gossypii*), cotton jassids (*Amrasca biguttula biguttula*), thrips (*Thrips spp.*), and whiteflies (*Bemisia tabaci*) [12]. These pests were controlled by pesticides and are not sensitive to the Bt toxins [12]. Organic farming cultivation abandons the use of GM cotton and pesticides. Cultivation of organic cotton is challenging as it relies on effective pest control based on inherent tolerance, treatments with botanical pesticides, and other system-based approaches. Today, many GM *G. hirsutum* Bt hybrids dominate the cotton seed market in India and have displaced other traditional cotton species such as *G. arboreum* and non-GM *G. hirsutum* cotton cultivars. Consequently, breeding non-GM cultivars with high bollworm resistance declined significantly. This puts organic cotton farmers under severe threat, as they can no longer obtain non-GM cotton seeds on the local markets and are disconnected from breeding progress. We hypothesize that *G. arboreum* can be a better choice for organic farming as it is known for higher genetic diversity than *G. hirsutum* [13] better adaptation to low fertile and rain-fed conditions (abiotic stresses) [14], drought and disease resistance [13], sucking pest resistance [9,15–17], salt tolerance [18] and nematode resistance [19].

To re-establish a GM-free seed chain and to develop high performing cultivars participatory breeding programs under organic conditions on non-GM cotton cultivars were initiated in India. The objectives of this study were to (i) investigate yield potential, fiber quality and resistance level against bollworms and sucking pests of different cotton species under contrasting organic on-farm growing conditions, (ii) explore correlation of morphological traits as indirect selection parameters for resistance and (iii) identify non-GM cotton cultivars with high level of resistance against bollworm and sucking pest as parental lines for participatory breeding. Breeding and selection of cultivars including *G. arboreum* species with high resistance levels to bollworms and sucking will not only increase yield and yield stability of organic cotton, but it can also reduce the use of pesticides in conventional cotton production.

## 2. Materials and Methods

### 2.1. Location and Climatic Conditions

The study was conducted in the plains of the Nimar Valley, Khargone district, Madhya Pradesh (MP) in Central India (22°8'30.3"N, 75°4'49.0"E). The altitude of the area is 250 m above sea level. The climate is subtropical, semi-arid with an average annual precipitation of 800 mm and an average

temperature of 25 °C. The year consists of three seasons: The monsoon (Kharif), which accounts for almost all the rain and usually lasts from June to September, the winter season (Rabi), characterized by its lowest temperatures in December/January and the hot and dry summer season (Zaid), which lasts from March to June.

Two sites typical for irrigated and rain-fed cotton growing conditions in Central India were chosen. The heavy soil site (HS) was a representative of clay rich and highly fertile soils categorized as Vertisol and often have irrigation facilities from either ground water or Narmada river basin. The light soil site (LS), was chosen on Inceptisol without any irrigation facilities. The LS was situated on a characteristic rain-fed area since many farmers in the area have limited to no access to water. The soil was sandy and shallow with low fertility. Both sites were under organic management at the time of study. The cotton seeds were sown manually at HS between the 6/6/2013 and 9/6/2013 (summer sowing) and watered by a drip irrigation system. The sowing at LS, also performed manually, started later at the beginning of the Monsoon from the 26/6/2013–29/6/2013 (monsoon sowing). Three seeds were sown per planting hole and after 2.5 weeks of germination, thinning was done to one plant. The organic farming system on both sites is based on the local farmers' practice [2]. The fields were fertilized with farmyard manure (1236 kg ha<sup>-1</sup>) before one week of planting and the crops were only treated with homemade organic products at early stages. These include Jeevamrat to promote healthy and vigorous plant growth and Neem oil to control sucking, and biting pests at vegetative stage.

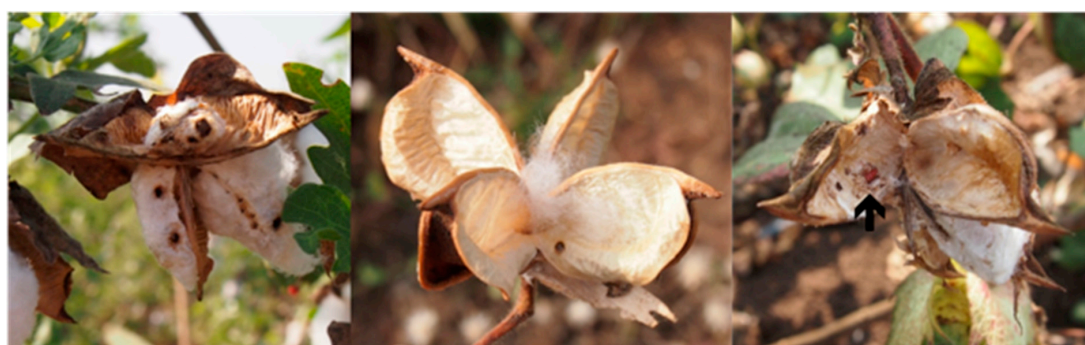
## 2.2. Experimental Design

Non-GM cotton cultivars including breeding lines and commercial hybrids were used for this study. In total 68 different cultivars with 7 diploid endemic *G. arboreum* varietal lines (AV), 25 tetraploid *G. hirsutum* hybrids (HH) and 36 tetraploid *G. hirsutum* varietal lines (HV) were used at two contrasting sites. The experiment was set up in a randomized complete block design with two replicates per site. The replicated plots with different cotton cultivars were surrounded by okra (*Abelmoschus esculentus*). Each plot had four rows of cotton and one row of okra as separator row. Each row comprised 9 and 17 cotton plants with 0.61 m (2 feet) and 0.30 m (1 feet) plant to plant distance within row for hybrids (HH) and varietal lines (HV, AV), respectively. Row to row distance was 0.92 (3 feet) and 0.61 m (2 feet) for HS and LS, respectively.

## 2.3. Assessment of Damage by Bollworm and Sucking Pest Incidence

The ratings of bollworm damage were conducted at both sites two times during the harvest season on five systematically selected plants per plot. To represent the average health condition and plant development of the plot, very sick, superiorly tall, or noticeable little plants were excluded to avoid any bias. Only middle rows of the plots and no plants close to the border were used to avoid border effects. These five plants were marked following a zigzag approach and used for all observations. The first rating took place during the time period between the 19/11/2013–27/11/2013 (after the first picking) and the second rating during the 29/12/2013–31/12/2013 (after the second picking). The damage of an entire replication was assessed, within the same day. Assessed capsules were cut to avoid repetitive counting of a capsule.

To calculate the combined average damage caused by pink bollworm *Pectinophora gossypiella* (PBW), spotted bollworm *Earias vittella* (SBW) and American bollworm *Helicoverpa armigera* (ABW) on the different cultivars, the number of open and healthy capsules, the number of open and damaged capsules and the number of open capsules damaged by bollworms were counted during the first and second assessment. The following symptoms on cotton capsules were defined as damage caused by bollworms: Eaten away or damaged seeds, bore marks and presence of bollworm larvae (Figure 1).



**Figure 1.** Symptoms defined as bollworm damage on capsules. Left: Capsule with eaten and damaged seeds. Middle: Capsule with bore mark. Right: Presence of bollworm, indicated by the black arrow.

In addition, a deeper evaluation on the susceptibility of the cotton cultivars was carried out by considering the cotton yield parameters, sucking pests' incidence and morphological traits (leaf and stem hairiness) of the plants (Table 1). Correlations between bollworm damage, cotton yield, earliness, sucking pests, and hairiness were assessed to identify indirect selection parameters. To avoid the observation variation with time, all bollworm damage assessments for an entire replication were taken within the same day.

Seed cotton yield containing seed plus fiber (yield) was assessed three times on a plot basis by measuring the total weight of picked cotton, the number of harvested bolls and the number of plants per plot. The first picking took place during the period of 22/10/2013–17/11/2013, the second picking during 16/11/2013–18/12/2013 and the third picking during 23/1/2013–19/2/2014.

**Table 1.** Assessed parameters of the different cotton cultivars with the basis, unit, and number of the ratings. Scores were 1 = healthy, 2 = little attack, 3 = medium attack, 4 = high attack and 5 = very strong attack/almost killed. For the hairiness, the ratings were 1 = very hairy, 3 = medium, 5 = not hairy.

Parameter		Observation Basis	Unit	Number of Observation Timing
Bollworm damage		five marked plants	number	3
Yield data	Yield per plot	Plot	g	3
	No. of harvested bolls	Plot	number	3
	No. of plants per plot	Plot	number	3
Earliness	First picking (%)	Plot	percentage	1
Sucking pest	Aphid	Plot	Score 1-5 <sup>1</sup>	3
	Jassid	Plot	Score 1-5 <sup>1</sup>	3
	Thrips	Plot	Score 1-5 <sup>1</sup>	3
	Whitefly	Plot	Score 1-5 <sup>1</sup>	3
Morphological traits	Leaf hairiness	five single plants per plot	Score 1-5 <sup>2</sup>	1
	Stem hairiness	five single plants per plot	Score 1-5 <sup>2</sup>	1

The earliness refers to the time of the boll maturation of the different cultivars. Early cultivars have a high percentage of the yield of the first picking in relation to the total yield throughout the complete cotton season ((yield of the first picking / yield of all three pickings) × 100). Sucking pest pressure was assessed on plot-level by a visual scoring from 1 to 5 (1 = healthy, 2 = little attack, 3 = medium attack, 4 = high attack and 5 = very strong attack/almost killed). The assessments for cotton jassids (*Amrasca biguttula biguttula*), cotton aphids (*Aphis gossypii*), whiteflies (*Bemisia tabaci*), and thrips (*Thrips spp.*) were performed separately three times at the HS (12/08/13, 19/09/2013, 09/12/2013) and the LS (25/09/2013, 26/11/2013, 12/12/2013). For the assessments of morphological traits, leaf and stem

hairiness by a 1–5 scale (1 = very hairy, 3 = medium, 5 = not hairy) on the five marked plants of HS only. All morphological traits were assessed between the beginning and middle of December 2013.

#### 2.4. Statistical Analysis

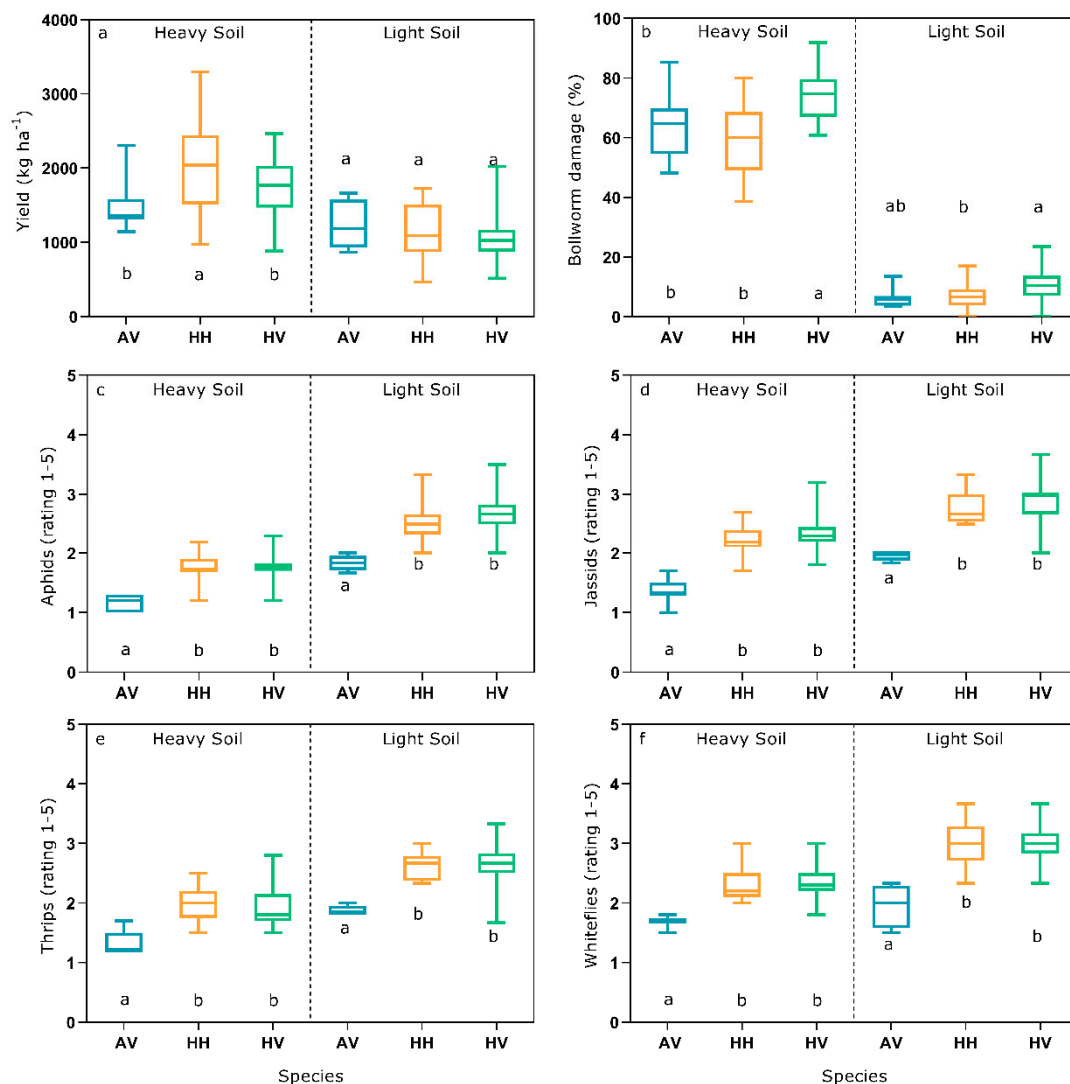
The assessed data were statistically analyzed by ANOVA (analysis of variance) using the software JMP®Pro 14.1.0 (SAS Institute, Cary, USA). Two standard least square models including different block effects as random factors were used. Model A was performed across sites and involved the factors site  $\times$  species/cultivar types (AV, HH, HV)  $\times$  cultivar. In case of site  $\times$  species/cultivar types  $\times$  cultivar interactions, Model B was applied for each site separately and involved species/cultivar types and cultivar within species/cultivar types as effect. The bollworm damage values were calculated in percentage of damage per plant as separate ratings, as the sum of the first and second ratings. Statistical analyses were done with the sum of the first and second ratings. Residuals were tested for normality by Shapiro-Wilk test. Furthermore, the Arcsine transformations were used for the bollworm damage rating (%) to reach normal distribution at  $W > 0.05$ . The transformation Arrhenius was used for the 1 picking (%) and for jassids. For aphids, reciprocal square root transformation was used. With normally distributed variables and their interactions, an ANOVA with adjacent Tukey's HSD (Honestly Significant Difference) test was conducted. Significant interactions were determined if  $p < 0.05$ . The relationship of bollworm damage and the additional parameters was tested by the non-parametric Spearman's rank correlation and reported as extremely significant when  $p < 0.001$ , highly significant when  $p < 0.01$ , significant when  $p < 0.05$ , and not significant when  $p > 0.1$ .

### 3. Results

Site by species/cultivar type interactions were found for yield and bollworm damage (Figure 2a,b). There was no site by species/cultivar type interactions found for sucking pests (Figure 2c–f). A significant difference between yield of the two sites was observed ( $F = 81.33$ ,  $p < 0.001$ ). The yield of HH was significantly higher ( $F = 20.36$ ,  $p < 0.0001$ ) compared to varietal lines AV and HV at HS (Figure 2a).

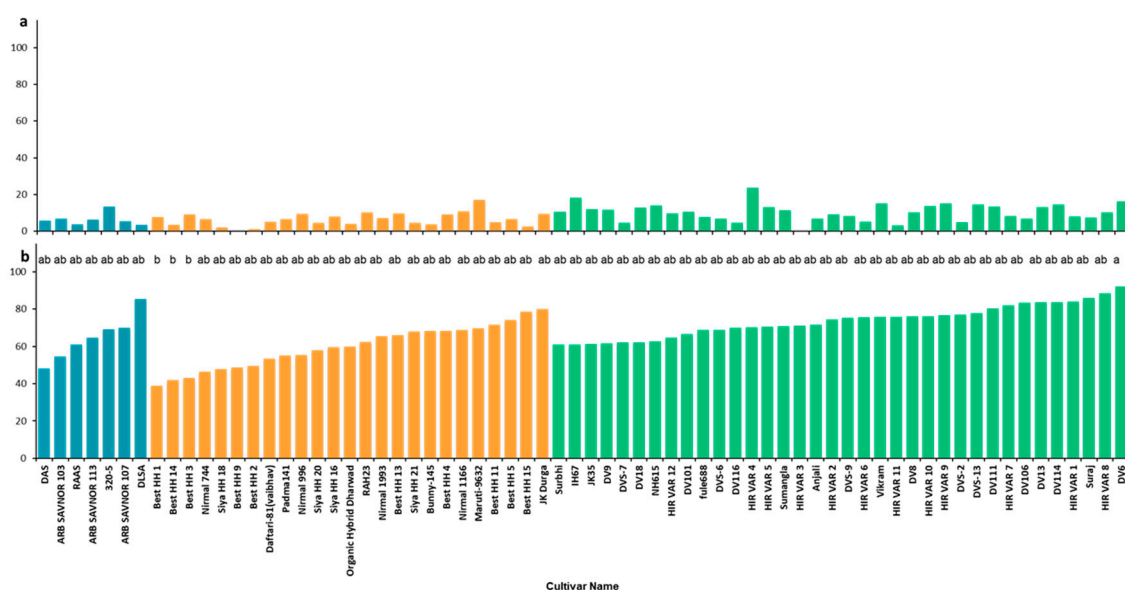
A significant difference between the bollworm incidences of the two sites was observed ( $F = 94.64$ ,  $p < 0.01$ ). The average rate of damage caused by bollworm was 67.66% ( $N = 68$ ) at HS compared to 8.62% at LS ( $N = 68$ ). The species/cultivar types were significantly different to bollworm damage at both sites ( $F = 19.94$ ,  $p < 0.0001$  for HS and  $F = 6.27$ ,  $p < 0.0001$ ). HV were significantly more susceptible to bollworms than HH and AV on HS and significantly than HH in LS (Figure 2b). The cultivars were significantly different for bollworm damage at the HS ( $F = 1.52$ ,  $p < 0.05$ ) but not significant at the LS ( $F = 1.16$ ,  $p > 0.1$ ). In summary, only a few cultivars with very low or high bollworm damage differed significantly at HS (Figure 3). The sucking pest incidence was significantly lower on AV (aphid;  $p < 0.0001$ , jassid;  $p < 0.0001$ , thrips;  $p < 0.0001$ , and whiteflies;  $p < 0.0001$ ) than on HH and HV at both sites (Figure 2c–f).





**Figure 2.** Boxplots on (a) total yield; (b) percentage of bollworm damage; (c) aphids; (d) jassids; (e) thrips and (f) whiteflies from different species/cultivar types at heavy soil site (HS) and light soil site (LS). Tukey's HSD were done for both sites separately on 7 *G. arboreum* varietal lines (AV), 25 tetraploid *G. hirsutum* hybrids (HH) and 36 tetraploid *G. hirsutum* varietal lines (HV). The same small letters indicate no statistically relevant differences at  $p > 0.05$ .

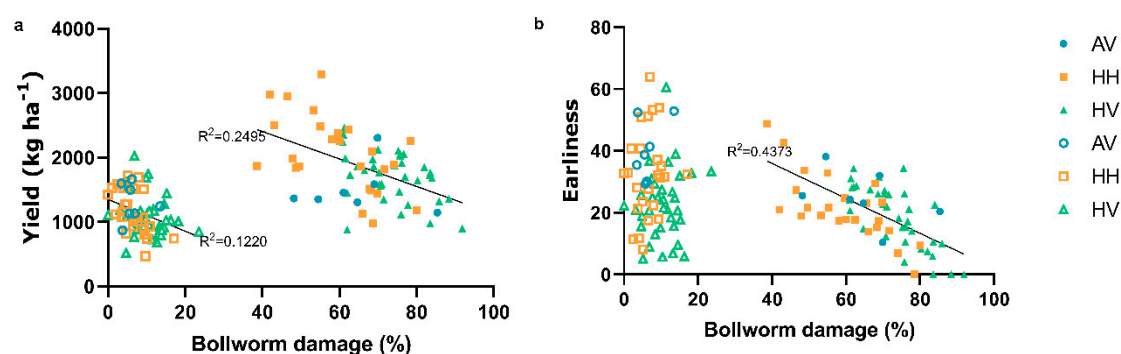
With the non-parametric Spearman's rank correlation, the association of different parameters was assessed (Table 2 and Figure 4). Bollworm damage was highly negatively correlated with the yield and earliness at the HS ( $p < 0.001$ ). Thus, high infestation rate of bollworms reduced seed cotton yield and early maturing cultivars were much less affected by bollworms than late cultivars (Figure 4). A significant positive correlation ( $p < 0.05$ ) was observed between bollworm damage and leaf, and stem hairiness at the HS. For instance, genotypes with very hairy leaves and stems were less affected by bollworms. There was no correlation between bollworm damage and any of the sucking pests at both site. However, a significant negative correlation ( $p < 0.05$ ) was observed between the earliness and jassids and thrips at LS. This indicates that early cultivars are less affected by jassids and thrips than late maturing cultivars under low fertile conditions. Leaf hairiness positively correlated with aphids ( $p < 0.05$ ) and whiteflies ( $p < 0.05$ ) at HS and LS as well as with jassids ( $p < 0.05$ ) at LS (Table 2).



**Figure 3.** Average bollworm damage of the first and second ratings (untransformed data) of cultivars at (a) the light soil site (LS) and (b) the heavy soil site (HS) both on organic managed on-farm trials. *G. arboreum* varietal lines are represented with blue bars, *G. hirsutum* hybrids with yellow bars, and *G. hirsutum* varietal lines with green bars. The ANOVA and Tukey's HSD tests performed with Model A. At both sites N = 68.

**Table 2.** Non-parametric Spearman's rank correlations (Spearman  $\rho$ ) of cotton parameters within site. Values below than the grey sections are derived from heavy soil trial (HS) the ones above are derived from light soil trial (LS) (the green section). Significant levels were: \* for  $p < 0.05$ , \*\* for  $p < 0.01$  and \*\*\* for  $p < 0.001$ .

Parameter	Bollworm Damage	Yield	Earliness	Jassids	Aphids	Whiteflies	Thrips	Leaf Hairiness	Stem Hairiness
Bollworm damage		−0.15	0.02	−0.06	0.05	0.13	0.11	0.11	0.15
Yield	−0.37***		−0.38***	−0.08	−0.02	0.07	−0.08	−0.06	−0.14
First picking	−0.62***	0.13		−0.23*	−0.15	−0.13	−0.19*	0.01	0.08
Jassids	−0.01	−0.03	0.03		0.36***	0.41***	0.45***	0.21*	0.09
Aphids	−0.09	0.11	0.12	0.66***		0.28**	0.28**	0.23*	−0.00
Whiteflies	−0.13	−0.07	0.13	0.61***	0.35***		0.30**	0.21*	0.03
Thrips	−0.01	0.20*	−0.04	0.65***	0.60***	0.37***		0.07	0.04
Leaf hairiness	0.20*	−0.13	0.02	0.12	0.19*	0.18*	0.14		0.62***
Stem hairiness	0.19*	−0.30	−0.07	0.07	0.10	0.12	0.06	0.62***	



**Figure 4.** Correlation of bollworm damage (%) with (a) yield and (b) earliness at the heavy soil site (solid symbols) and light soil site (open symbols). Each data point represents the mean of two replications (N = 68). Spearman's rank correlation for yield:  $\rho = -0.37$ ,  $p < 0.001$  and earliness  $\rho = -0.62$ ,  $p < 0.001$ .

## 4. Discussion

### 4.1. Importance of Bollworm Attack and Damage at Contrasting Sites

While severity of sucking pests was equal or slightly higher at light soil, the cotton bollworm incidence was much higher at the irrigated highly fertile site (HS) with 67% damaged bolls across all genotypes compared to 9% at the rain-fed low fertile site (LS). One reason for the differences in bollworm damage at the HS and the LS might be the availability of water and nutrients at the HS, which induced vigorous plant growth throughout the entire harvest season (up to March) and offered an ideal reproductive environment for the different bollworm populations. Furthermore, irrigated sites allow cultivation of various crops throughout the year. Bollworms are not only attacking cotton but also other crops such as okra, pigeon pea, and chickpea which can serve as alternative host crops for bollworms. This results in multiple generations of bollworms per year [7], whereas under rain-fed conditions there is bare soil for five to six months before the onset of the next monsoon interrupting the bollworm multiplication cycle [7]. A previous study on coevolving pests of Indian cotton confirmed that the pink bollworm (*Pectinophora gossypiella*) causes high damage in irrigated cotton and no significant damage in rain-fed cotton unless populations infest from irrigated fields [6]. Moreover, during peak square formation it happens that not only local but also immigrating populations (from other crops) infest cotton fields [20]. Consequently, there might have been a much higher population density of bollworms at the HS, which has also been observed in following years.

It was striking that the potential for damage of the bollworm species at the fertile site was clearly underestimated by the farmers during an evaluation workshop. Bollworm damages, unlike to the clearly visible symptoms of sucking insects are difficult to see before the opening of the capsules. It is also important to consider that this study only assessed bollworm damage at capsules, thus, the extent of damage caused by bollworms throughout the season can be expected to be even higher. Especially the early attack of bollworms on young plants (June–September) leads to boll dropping and reduced harvest in the first picking period [21]. Under heavy attack, the seed cotton yield was considerably affected by bollworms at HS, and damage was most detrimental for late maturing genotypes. Bollworm damage could cause yield losses of up to 85% [18], while sucking pest can account for 35% [22]. Thus, breeding for resistance is key for improved yield under irrigated and rain-fed organic conditions.

### 4.2. Potential of Different Cotton Species and Cultivar Types for Organic Production

Through comparisons of the different cotton species and cultivar types (AV, HH, HV) it has been shown that F<sub>1</sub> hybrids of *G. hirsutum* were on average higher yielding, earlier, and more tolerant to bollworm than the varietal lines of both *G. hirsutum* and *G. arboreum* species under irrigated organic farming at HS. The better performance of the hybrids compared to the varietal lines within *G. hirsutum* species was expected since hybrids often result in earlier and more robust plants. However, there was no yield advantage of HH versus HV and AV under marginal rain-fed conditions at LS. It is interesting to note that AV lines were equally tolerant to bollworms as HH and significantly more tolerant than HV under high bollworm incidence at HS. *G. arboreum* lines were significantly more tolerant to four main sucking pests (aphids, jassids, thrips and whiteflies) than *G. hirsutum* varietal lines or hybrids. While a strong ability of *G. arboreum* species to resist biotic stresses has been documented in previous studies [9,15,16,23], to our knowledge no studies so far proved higher resistance to bollworm of *G. arboreum* compared to *G. hirsutum* lines. The long coevolution of cotton pests and the native *G. arboreum* species might have resulted in the inherent ability to resist bollworms. Most hybrids are selected under high input (fertilizer, herbicide, pesticide and irrigation) conditions to obtain maximum yield. This could be a reason for their inability to realize their full yield potential under rain-fed and low-input conditions at the LS. AV lines, on the other hand, are more adapted to the harsh growing conditions in Central India, particularly against sucking pests. However, the biggest disadvantages of *G. arboreum* are (i) short fiber lengths (16–27mm) [24] and (ii) more time-consuming picking compared to *G. hirsutum*. In this study, we included only such AV breeding lines that reached a fiber length of



28 mm as requested by the textile industry. This has been achieved through introgression breeding with *G. hirsutum* [25], and opens a large potential for the reintroduction and upscaling of the traditional cotton species in India.

Considering the higher yield, earliness of *G. hirsutum* hybrids and the higher tolerance of *G. arboreum* varietal lines against sucking pests, these cultivar types seem to be very promising for cultivation in fertile irrigated areas. At rain-fed sites with low fertility, the use of *G. arboreum* species might be advantageous because they performed well concerning bollworm and sucking pest resistance and yields. Furthermore, they have been reported to have high drought tolerance [26,27]. In comparison to hybrids, the use of *G. arboreum* varietal lines might constitute a cheaper solution and contributes to the sovereignty of the smallholder farmers allowing the saving of seeds from the own harvest instead of buying F<sub>1</sub> hybrid seeds every year. Comparing *G. arboreum* with Bt hybrid cotton of *G. hirsutum* in Maharashtra, Romeu-Dalmau et. al. concluded that *G. arboreum* has same economic benefit as Bt cotton under rain-fed conditions and might also be a valuable option under irrigated conditions [17]. Therefore, a special interest of organic and low-input production lies in the breeding and selection of *G. arboreum* species with their high adaptive capacity.

#### 4.3. Indirect Parameters for Selection of Resistance to Bollworm and Sucking Pests

As the repeated assessment of bollworm damage of each boll of five predefined plants is very time-consuming we explored linkage to other traits that can be easier assessed. A strong correlation ( $r = -0.62, p < 0.001$ ) was found between the average bollworm damage and earliness (percentage of the first picking). Maturation of early cultivars takes place after the last monsoon rains in September. Especially at rain-fed conditions where terminal water stress frequently occurs, this is an important trait. Selection for early cultivars as well as early sowing might reduce the bollworm damage because plants with early fiber ripening escape the later bollworm attack (escape mechanism). The later attack often occurs in big populations with bolls and seeds as the main target. This might also be the reason for the higher tolerance of the early maturing HH than the later ripening HV. In addition, Esquivel and Lori found boll wall thickness of *G. arboreum* and *G. hirsutum* was thicker at 14 and 21 days after flowering compare to 3, 7 and 28 days at flowering [28]. Moreover, farmers pest management starts often at the beginning of the season and late season crop protection is weak [29]. Earliness was to a smaller extent also linked with increased tolerance to jassids and thrips, but also to reduced yield in LS. Tolerance to bollworm was not correlated with sucking pests, whereas tolerance to aphids, jassids, thrips, and whitefly was significantly correlated with each other.

Three different types of host plant resistance mechanisms related to leaf hairiness, glabrous leaf traits, and possible biochemical traits in *G. arboreum* has been identified [17]. Leaf and stem hairiness were positively correlated with the bollworm damage; i.e., hairy plants were less affected by bollworms. A previous study reported that *G. arboreum* with high hair density is favored by spiny bollworm (*Earias vittella*) because they provide a suitable environment for oviposition [30]. Similarly, leaf hairiness was linked with increased tolerance to jassids, aphids, and whiteflies, but not to thrips. The benefit of hairy leaves has been described in several studies on sucking pests of cotton [31–33]. However, these rank correlations are quite low and, thus, leaf hairiness as indirect parameter for selection towards increased pest resistance will result in low selection efficiency. For such time-consuming but important traits marker assisted selection (MAS) might be a tool to integrate into participatory breeding approaches. First quantitative loci (QTL) for tolerance to spiny bollworm [34] and to jassids [35] were recently detected and pave the way for MAS.

#### 4.4. Selection of Cultivars and Breeding Lines for Cultivation and Participatory Breeding

One objective of the study was to identify superior genotypes that are suited for organic cultivation and/or can be used as crossing parent for the breeding project. Although *G. arboreum* is on average superior to *G. hirsutum* with respect to tolerance to sucking pests, and *vice versa* for fiber length, we found a big variation for all traits allowing targeted selection within each cultivar types. Due to

the significant genotype  $\times$  environment interaction for yield it became obvious that selection need to be conducted separately for the contrasting sites to identify those cultivars that work best under irrigated, fertile soil, and those more suited for rain-fed marginal conditions. Selection for bollworm tolerance should be carried out under irrigation to expect high incidence of bollworms and better differentiation among genotypes. This should be done in replicated field trials in different years; however, this is hampered by the time-consuming assessment. The selection of early and hairy cultivars might reduce bollworm damage and sucking pest to a certain extent. Furthermore, the fiber length must be considered and selection should be adapted to the industry requirements. Three to five genotypes of each cultivar type with high resistance were selected for further multi-location field trials and for intra specific crosses of both species *G. hirsutum* and *G. arboreum* to combine best traits. This is part of a participatory breeding project on non-GM cotton in collaboration with organic farmers, universities, researchers, and the textile industry to promote organic cotton and safeguard the availability of non-GM cotton seed. Most promising commercial hybrids and varietal lines were further tested in larger on-farm pilot trails. The need for pest-resistant non-GM cultivars is very urgent for organic cotton farmers, and great financial profits can be achieved by carefully choosing suitable high-quality cultivars.

## 5. Conclusions and Outlook

Organic agriculture depends on an effective bollworm and sucking pest control in which breeding for more tolerant cultivars plays an important role. This study proved that *G. arboreum* has similar yield potential and bollworm resistance but clearly higher tolerance level to most devastating sucking pests compare to *G. hirsutum* under irrigated and rain-fed conditions. Even though yield of *G. arboreum* was on average lower than for F<sub>1</sub> hybrids of *G. hirsutum* under irrigated fertile soil, there was no yield difference between species and cultivar types under rain-fed conditions. The high variation within cultivar type is encouraging to start breeding for a broad portfolio of different cultivar types for contrasting growing conditions. As most organic cotton cultivation in India is under rain-fed conditions special emphasis should be put on the breeding of the neglected cotton species *G. arboreum* as an interesting source for natural pest and drought resistance considering the required fiber length of the textile industry. However, as the field selection is very time and resource consuming it would also be interesting to perform genome wide association studies for the different pests to pave the way for MAS, and to check for cytoplasm effects on bollworm tolerance [36]. Breeding for pest resistance gains importance for GM cotton as well. The increasing sucking pest populations, which are not controlled by the Bt toxin, still must be encountered by pesticides in conventional farming. Furthermore, the first cases of Bt-resistant bollworm occurred [10,11]. Thus, screening of cotton genetic resources for tolerance to various biotic and abiotic stress will improve sustainability of organic and conventional cotton production.

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