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News

Game On! ICAC World Cotton Day Contests with in Prizes Now Open \$9,000



**INTERNATIONAL
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ADVISORY
COMMITTEE**

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**COTTON
FOR GOOD™**

Dear Friends of Cotton,

This message was sent out in August, but now the contests are open and with one month to go before the winners are announced, there's plenty of time to get your share of the \$6,000 that will be awarded in the four ICAC World Cotton Day Twitter contests! See below for the details.

With barely one month to go until 7 October, the International Cotton Advisory Committee (ICAC) has announced that it will hold a number of contests to celebrate World Cotton Day, totalling \$9,000 in prize money. There will be six contests, with each awarding the winner \$1,000 and the runner-up \$500.

Four of them will be held on Twitter; winners will be those whose tweets get the most points (1 point for each like and 3 points for each retweet) for tweets sent between 1 September and 6 October, with the winners announced on World Cotton Day. Each tweet must include three hashtags — #worldcottonday; #ICAC_Cotton; #cottonforgood (the official theme of World Cotton Day) — as well as a fourth that indicates which contest the tweet is for:

- 1. #cotton: Tweets about the cotton plant, including the raw fibre.**
- 2. #madewithcotton: Tweets about products made from cotton.**
- 3. #sustainablecotton: Tweets about some aspect of cotton's sustainability.**
- 4. #cottonresearch: Tweets about the science of cotton.**

In addition, two contests will be held at the [World Cotton Research Conference](#), which begins on 4 October in Cairo and concludes on World Cotton Day:

- 1. Best live presentation, and**
- 2. Best poster.**

'Because cotton alleviates poverty in many of the world's least-developed countries and helps fight climate change globally, the more visibility cotton gets on 7 October, the better', said Kai Hughes, ICAC Executive Director. 'We want to motivate people to spread the good word about cotton with the Twitter contests, and want to reward the scientists whose research is the lifeblood of the cotton industry's development at the World Cotton Research Conference'.

For more information about the ICAC's World Cotton Day contests, and see an example of a tweet for the #madewithcotton contest, please [click here](#).

ABOUT WORLD COTTON DAY

World Cotton Day (7 October each year) is a concept that was born at the ICAC in 2018 and the first-ever World Cotton Day celebration was held at World Trade Organisation headquarters the following year. More than 800 people from around the world travelled to Geneva for the event; the WTO, FAO, ITC and UNCTAD are the other founding organisations of World Cotton Day.

World Cotton Day continued in 2020 and 2021, with cotton and textile professionals around the world organising their own online and in-person events, and last year the United Nations — in recognition of the critical role played by the world's most important natural fibre — announced that it would reserve 7 October as World Cotton Day on its permanent calendar.

To see what some companies have done to celebrate, and to get ideas for arranging your own World Cotton Day event, please visit the official World Cotton Day website, www.worldcottonday.com. Other resources, including educational materials, event ideas and an overview of World Cotton Day, are available here.

The official World Cotton Day logos are available in any language for people who would like to use them. In addition, ICAC Communications Director Mike McCue (mike@icac.org) is standing by to help anyone who needs assistance with planning their World Cotton Day activities.





Recent Advances in Integrated Pest Management in Cotton Crops in Brazil

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Cotton (*Gossypium hirsutum* L.) is an important crop for Brazil, the fourth-largest world producer of this fiber, with a production of 2,024 thousand tons of lint obtained in an area of 1,414 thousand hectares in the 2018/2019 season. Brazil has the highest productivity of cotton in the rainfed system in the world. In addition, the quality of Brazilian fiber is internationally recognized (Abrapa, 2018). However, extensive areas, crop succession, and tropical climate favor pest populations (Belot et al., 2016).

In Brazilian cotton crops, several species of insects and mites are responsible for many plant injuries and loss of productivity. The most damaging pests include aphid (*Aphis gossypii*) and whitefly (*Bemisia tabaci* biotype b) early in the season; mites (*Tetranychus urticae* and *Polyphagotarsonemus latus*) and lepidopteran larvae (*Spodoptera* spp., *Helicoverpa* spp., and *Chrysodeixis includens*) at mid- to late-season; boll weevil (*Anthonomus grandis*) from flowering until open bolls; and stink bugs (mainly *Euschistus heros*) from boll filling until harvesting (Torres & Bueno, 2018).

The effective adoption of integrated pest management (IPM) in Brazil depends on overcoming some bottlenecks. The same monitoring tools by pheromone attraction devices can be used in behavioral control; however, their use is still restricted to control remaining populations. In chemical control, the inefficiency of insecticides, escape from reaching and the evolution of insect resistance are factors that need to be solved. Biological control is an unattractive alternative, given the difficulty of implementation in such high-input agriculture. Cultural control is not fully explored, there are interesting techniques that need to be adopted. For the improvement of IPM techniques, several studies have been carried out, which will be discussed here.

Cultural control

The cotton stalk destruction aims to eliminate shelter and reproduction sites for various pests and diseases, mainly boll weevil. At the end of cotton season, the remaining adults of boll weevils leave the field and migrate to natural vegetation to spend the overwintering period. In the next season, when the reproductive period of the

cotton plants begins, the remaining adults of the boll weevil return to the cotton field. The displacement of the boll weevil population forward to out of the field and the attract-and-kill effect of migrant populations on the crop border was investigated (Miranda et al., 2016). The closer to the edge, the greater the number of infested reproductive structures by boll weevils. In the inside field, the population was smaller and homogeneously distributed. In the border field, the authors found live and dead boll weevils inside the reproductive structures, proving to be an important feeding and oviposition site.

Trap cropping installed after the cotton season exerted a strong influence of the attraction of the remaining individuals of the boll weevil, greater than in the area where stalk destruction was done shortly after the harvest (Miranda et al., 2017). In the trap crop, those insects were eliminated through spraying, with a consequent population reduction up to eight weeks after harvest. Based on this finding, trap cropping is an interesting alternative to

reduce the boll weevil population at the end season.

Cultural control is also an alternative for controlling other cotton pests. The main tactic employed for the control of burrower bugs (*Scaptocoris castanea*) in cotton has been the use of synthetic insecticides applied to the seeds. However, several unsuccessful cases have already been detected in Brazil, probably due to the high selection pressure that has been imposed and possibly triggering cases of resistance. Alternative management was suggested by Nascimento et al. (2014), by using sulfur sources to reduce the burrower bugs population. The study concluded that the use of sulfur sources (calcium sulfate and ammonium sulfate) promotes the tolerance of cotton plants to insect attacks. Some cover crops are suppressive to populations of burrower bugs, highlighting *Crotalaria* sp., pigeon pea, sesame, and sunflower. On the other hand, the authors strongly recommend avoiding the use of *Urochloa ruziziensis* as a cover crop in infested areas (Figure 1).

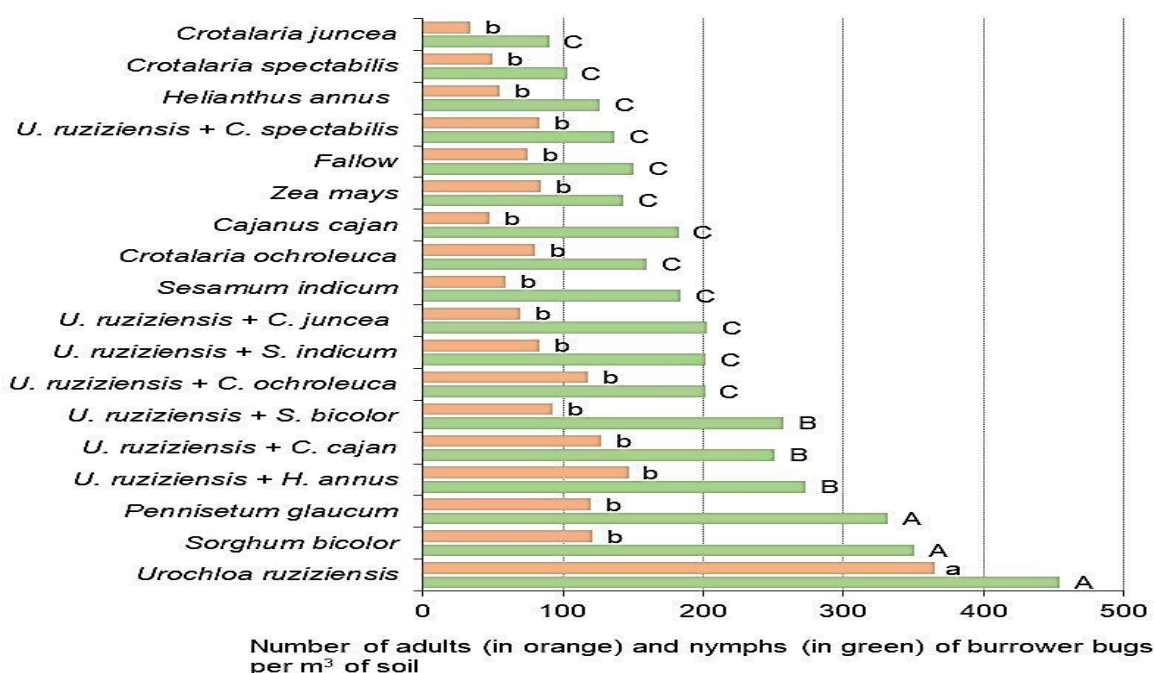


Figure 1. Effect of the presence of cover plants-cotton succession on the infestation of burrower bugs. Equal lower case letters do not differ for the number of adults and equal upper case letters do not differ for the number of adults and equal upper case letters do not differ for the number of nymphs by the Scott Knott test ($P < 0.05$).

Biological control

Recently, several major agricultural companies are investing in fungus-based products to achieve more sustainable IPM programs for major pests in both organic and conventional crops. Due to the large underexplored Brazilian microorganisms diversity, there is potential for new entomopathogens to be developed for new targets.

Government and private research groups are pursuing innovative technologies for mass production, formulation, product stability, and quality control, which will support cost-effective commercial mycoinsecticides. Mass production is replacing production by solid substrate fermentation, a time and labor-consuming process, with biphasic fermentation, a faster and low-contamination model. In addition, some industries are starting to utilize liquid culture fermentation, which permits low

labor inputs, precise control over growth conditions, and improved fitness. Improved quality control of mycoinsecticides is being achieved by increasing the proportions of fast-germinating conidia, which are responsible for most mortality of treated insects (Faria et al., 2015; Mascarin et al., 2019).

Populations of whiteflies and stink bugs have been favored by the Brazilian agricultural system, where it is cultivated for up to three growing seasons per year, added to the presence of host plants and the occurrence of tropical climate (Quintela et al 2016). The potential of biological control by fungus is an important pest control component in cotton crops. The susceptibility of whiteflies to isolates *Cordyceps* sp. was reported by Boaventura et al. (2021). The authors verified mortalities ranged from 63.7 to 87.8%. Furthermore, 60.0 to 99.5% of adults that emerged from fourth instar nymphs

previously treated with the fungus succumbed to the infection.

Metarhizium anisopliae and *Beauveria bassiana* are other fungi with a high potential to control cotton pests. Two strains (ESALQ PL63 and IBCB 66) are used in 13 commercial products targeting *B. tabaci* (Mascarin et al., 2019). One isolate

M. anisopliae and two isolates *B. bassiana* caused mortality of 82.5%, 73.2%, and 65.1% in adults of the boll weevil, respectively (Figure 2). The same isolate *M. anisopliae* killed 75% of the nymphs of stink bugs (Sousa et al., 2020).

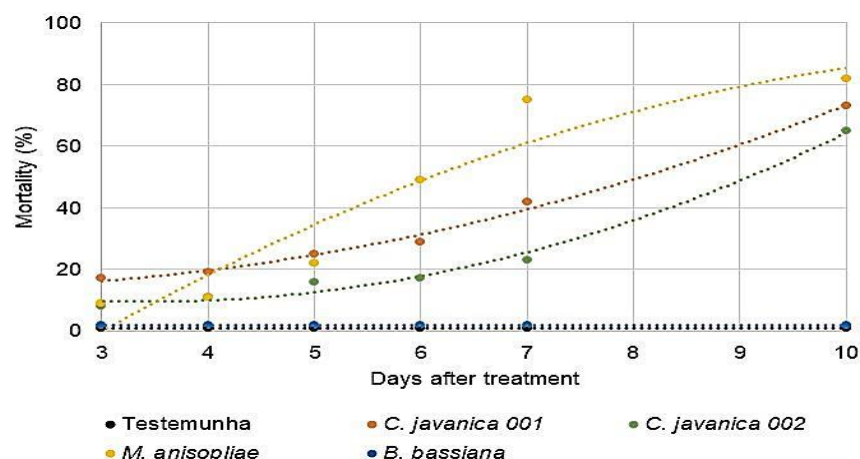


Figure 2. Mortality of boll weevil adults by entomopathogenic fungi. Adapted from Sousa et al., 2020.

The epicuticle layer of some pest species is a physiological barrier to insecticides. One strategy to overcome the barrier includes sublethal doses of chemical insecticides applied in combination with fungal entomopathogens. Quintela et al. (2013) reported a mixture of *M. anisopliae* conidial with a sublethal dose of thiamethoxam that significantly increased the mortality of stink bugs. This strategy to improve the activity of mycoinsecticides could also be applied to other stinkbugs to delay or prevent insecticide resistance.

Others bioinsecticides based on nucleopolyhedrovirus and *Bacillus thuringiensis* formulations are available against lepidopterans. A study conducted by Vivan et al., (2017) assessed the control potential of *Helicoverpa armigera* by baculovirus (NPV) and Bt insecticides,

especially causing high mortality in first and second instar young larvae.

Transgenic events

The large-scale introduction of transgenic events has recently modified the cotton crop in Brazil, mainly because of adjustments in field operational procedures. Field assessments showed that stacked GM cultivars resulted in a cost reduction of 10%, with expressive savings in insecticide (44%) and herbicide (57%) applications (Figueiredo Filho, 2015).

The most important commercial traits for resistance to insects are carriers of Cry proteins, obtained from the bacterium *Bacillus thuringiensis* (Bt toxins). In Brazil, The first generation of crops carrying this transgenic trait had only one toxin in each plant, Cry1Ac. A second generation extended resistance to a greater number of

species, combining two or more toxins (Cry1Ab, Cry1Ac, Cry1F, Cry2Ab2, Cry2Ae) in a single plant with different modes of action (Tokel et al., 2021). A third-generation is recently being used, adding the vip3A genes. These biotechnologies allow the control of the diverse species of lepidopterans that occur in the cotton crop. Studies report up to 10% cost reduction through the use of Bt cultivars, and around 40% insecticide savings in insecticide applications (Raphael, 2019), although some complementary applications of insecticides are made in greater or lesser numbers, depending on the event (Figure 3).

While transgenic varieties require up to 15 years to be developed, some pest species, e.g. fall armyworm, have quickly evolved resistance to most commercially released Bt crops. Strategies for Bt trait durability are paramount. Most of the Bt

corn hybrids cultivated in Brazil lost their ability to control fall armyworm in just three years after release (Favoretto et al., 2017), and the same is happening in cotton crops. To manage resistance and ensure trait durability, insect resistance management (IRM) practices for Bt crops must be a priority. IRM strategies include refuge (non-Bt crops), high-dose, and pyramid products.

In the last years, biotechnologists have been working on the development of boll weevil-resistant cotton plants, using two approaches: Cry-proteins and RNA interference. The first events were built with Cry proteins from *B. thuringiensis* and promising results were obtained (Oliveira et al., 2016; Silva et al., 2016; Ribeiro et al., 2017). Recent studies conducted in Brazil aim to develop a transgenic cotton genotype resistant to boll weevil (Monnerat et al., 2015).

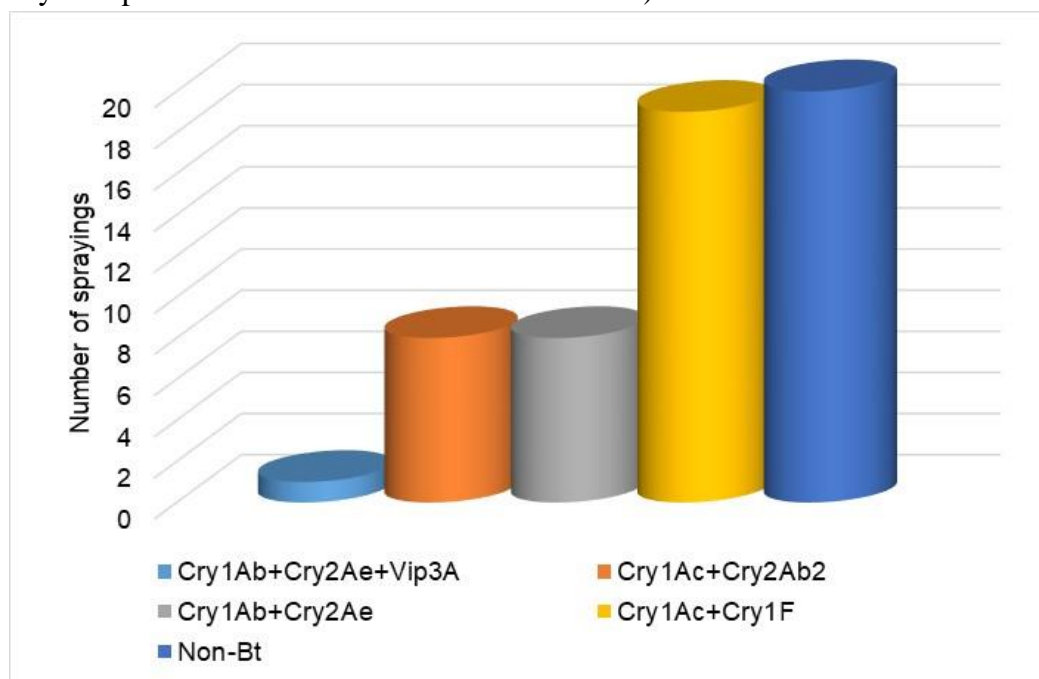


Figure 3. Biotechnological events used in Brazilian cotton crops and number of complementary sprays to control lepidopteran pests during 2017/2018 season in Brazil.

The introgression of the genes of interest and the phenotyping of the transformed plants to assess the biological effect on the target insects are ongoing, with some preliminary results obtained (Table 1).

Table 1. Mortality of boll weevils on transgenic cotton plants containing cry-proteins and/or dsRNA.

Construction	Introduced gene	Event	Selected plant	Generation	Mortality (%)
pCottonBt2	Cry10mod	A31-15	A31-15.9.1	T2	54.0
pCottonBt2	Cry10mod	A12-2	A12-2.16.6.13	T3	52.0
pCottonBt2	Cry10mod	A28-3	A28-3.6.38	T2	44.0
pCottonBt2	Cry10mod	A28-63	A28-63.25	T1	42.9
pCottonBt2	Cry10mod	A28-73	A28-73.22	T1	39.1
pCottonBt2	Cry10mod	A28-3	A28-3.6.23	T2	37.5
pCottonBt2	Cry10mod	A28-3	A28-3.6.1	T2	37.5
pCottonAgra3	dsRNA	A70-7	A70-7.14	T1	35.3
pCottonAgra3	dsRNA	A70-7	A70-7.14.4	T2	33.3
pCottonBt2	Cry10mod	A28-63	A28-63.33	T1	32.0
pCottonAgra2	Cry10mod+ dsRNA	A60-5	A60-5.4	T1	31.3
pCottonBt2	Cry10mod	A31-15	A31-15.9.3	T3	31.0
pCottonAgra3	dsRNA	A70-46	A70-46.4	T1	30.8
pCottonBt2	Cry10mod	A28-73	A28-73.14	T1	30.0

Stable inheritance and expression of transgenes in transgenic plants are of paramount importance in the successful application of genetic engineering in crop improvement (Miryeganeh and Saze, 2019). Genome stability, the capability to preserve and transmit the genetic material from generation to generation, is an frequent obstacle. In transgenic cotton plants, chimeric variegation, where there is a mixture of two or more genetically different types of cells, may occur.

An alternative approach for making GM cotton resistant to insect pests is RNA interference (RNAi) technology. RNAi is a promising alternative strategy for

controlling insects that shows the advantage of using the insect's systemic gene-silencing machinery to suppress essential gene expression (Younis et al., 2014). Double-stranded RNA (dsRNA) is the RNAi trigger molecule that primes the post-transcriptional downregulation of a target gene. The main advantage of RNA interference technology in controlling crop insect pests is the high specificity for the target gene. Studies using this technology showed efficient gene knockdown of several target genes into larvae and adult boll weevil insects (Firmino et al., 2013; Coelho et al., 2016; Macedo et al., 2017).

Chemical control

More than 300 insecticide formulations belonging to 72 active ingredients are registered to spray cotton fields in Brazil. According to Machado et al. (2019), only a part of this list of insecticides is recognized to provide efficient control. These authors recommend the use of non-selective insecticides, like insect growth regulators, spinosyns, and diamides, to minimize the risk to natural enemies and the environment. Insecticide products with low impact on natural enemies are also reported by Barros et al. (2018), including chlorantraniliprole and pymetrozine.

More selective products have been tested against the pest, and spinosyns, commonly used against lepidopteran pest species in cotton, are low risk to nontargets insects (Barros et al., 2018). The study recorded >80% mortality of boll weevil promoted by spinosad and spinetoram, in

addition to residual toxicity of up to eight days (Rolim et al., 2019).

The constant use of pyrethroids in cotton crops to control other pests may be contributing to the selection of boll weevil populations capable of resisting the recommended doses of this group of insecticides. Quintão et al. (2020) noticed that several pyrethroids, such as etofenprox and alpha-cypermethrin, did not achieve 50% control. Similar results were obtained by Rolim & Netto (2020). Rolim et al. (2021) reported field-evolved resistance of boll weevils to beta-cyfluthrin.

Insecticide spray run-off promoted by rainfall may reduce their efficiency against pests. Quintão et al. (2020) reported that products that promoted high boll weevil mortality, such as malathion and fipronil (92- and 70% mortality, respectively) had their efficiency reduced to 54- and 49% due to run-off (Figure 4).

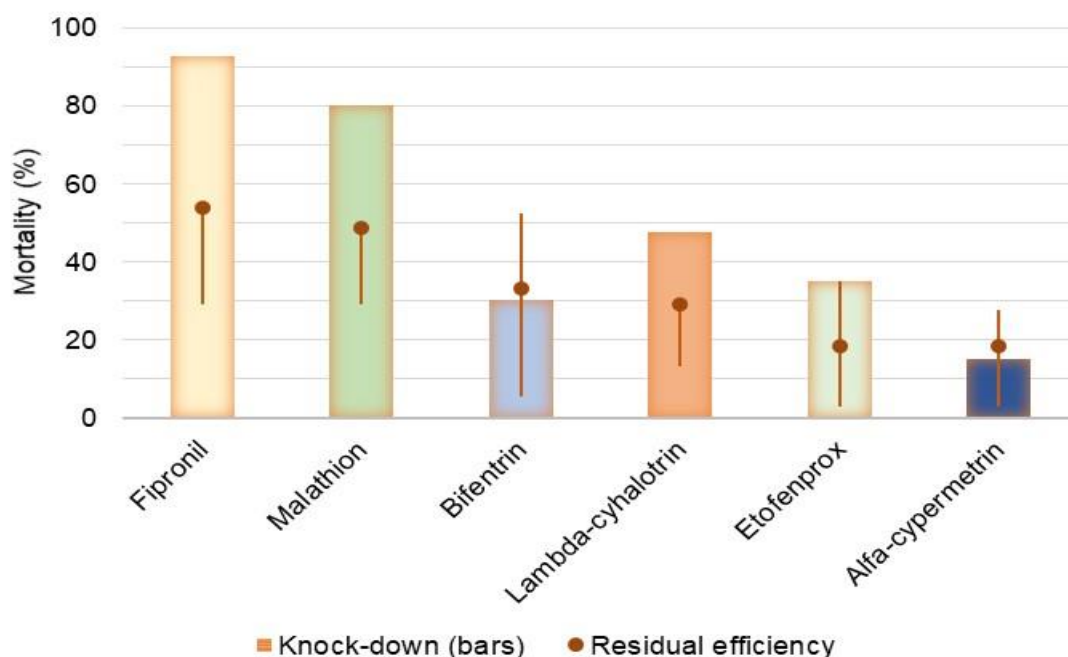


Figure 4. Knock-down effect and efficiency of control of boll weevil by insecticides in a period of 10 days after treatment, 7 days after run-off. Adapted from Quintão et al., 2020.

Semiochemicals as tools for monitoring pests

The pest monitoring in the cotton crop can be done through a visual sampling of the presence of the insects or their damage caused in the cotton plant, or even with the use of pheromone traps. On-farm practice, false-negative sampling errors are obstacles to efficient monitoring. Semiochemicals such as pheromones and kairomones are being increasingly used and contribute to reducing visual sampling errors.

Pheromone traps have been widely used in Brazil to monitor lepidopterans and weevils populations (Azambuja & Degrande, 2014). For boll weevils, pheromone traps are installed in all cotton production areas, during the off-season. Pheromones secreted in the feces by male boll weevils have the function of aggregating individuals during the pest migration period (off-season). During the

fruiting period of cotton plants, grandlure functions as a short-acting sex pheromone. (Grigolli et al., 2015). At this time, pheromone trap tools have their efficiency compromised by the volatile organic compounds released by the plants (Magalhães et al. al., 2018). When the cotton flowering period begins, the profile of volatile substances emitted by the plants, changes. The boll weevils notice this and respond by returning from the refuge areas to the cotton field and the number of insects captured in the pheromone traps is drastically reduced (Magalhães et al., 2012). Magalhães et al. (2016) showed that the reduction in the production and release of acyclic homoterpenes stimulates the attraction of boll weevils by plants (Figure 5). From these findings, a synthetic blend comprising six semiochemicals was developed (Magalhães et al., 2018). Chemical industries have shown interest in the large-scale production of this synthetic

blend, which will improve boll weevil monitoring in cotton fields.

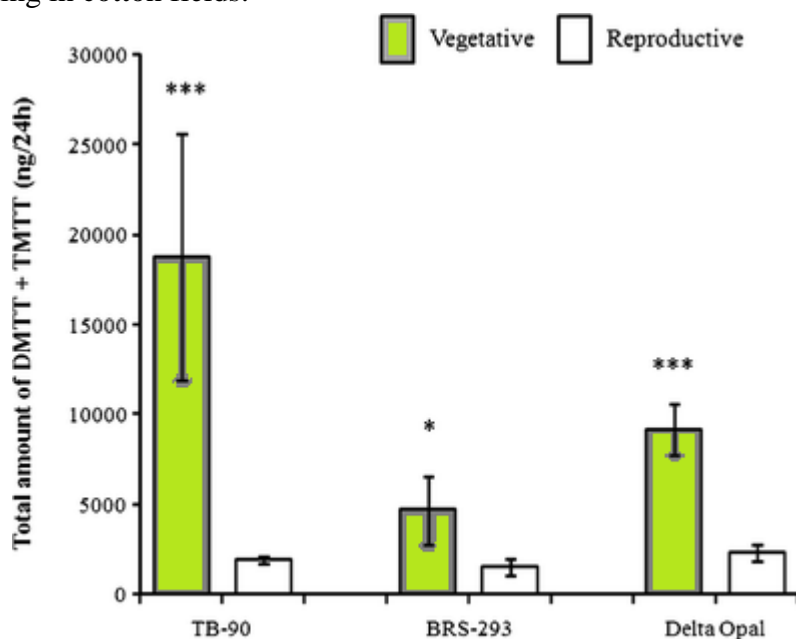


Figure 5. Total amount (ng/hr) of the acyclic homoterpenes, (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E,E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT), emitted by CNPA TB-90, BRS-293 and Delta Opal cotton at vegetative and reproductive stages. In each genotype, asterisks represent differences between phenological stages ($0.05 > P > 0.01$, $0.01 > P > 0.001$, $P < 0.001$). Adapted from Magalhães et al., 2016.

Digital Farming

Although unpredictable, before causing economic losses in crops, arthropod pest outbreaks need to be early detected in the fields. The recent evolution of digital farming technologies is helping producers to assure high productivity in the field (Iost Filho et al., 2020). In Brazil, cotton cultivation has been at the forefront in the use of information technologies aimed at increasing productivity. Digital technologies already available contribute to a reduction of up to 35% in the cost of pest control in cotton. The integration of sophisticated data acquisition systems, including sensors, drones, georeferencing systems, and automated mobile devices allowed increased control of different pests,

which has resulted in cost savings, increased profitability, and reduced environmental impact (Oliveira et al., 2020).

There are already devices with specific cameras that emit infrared rays to analyze the health of the plant and obtain information about pest incidence (Liu et al., 2021). Promising image-based systems aim to send the insect image to a specialist and then the insects could be identified and counted remotely in real-time (Lima et al., 2020).

The infrared sensor traps were shown to be useful for counting insects, but are limited because they cannot identify the species, which can result in misleading data in the survey. To solve this restraint, the use

of stationary traps baited with pheromones combined with infrared sensor technology seems to be ideal. When the automatic stationary traps, strategically installed in georeferenced sampling points, attract the target insects, an electronic circuit activates the counting system and sends the data for processing in a central computer, generating a report to the user about the presence of the insect, the location and time of report (Bieganowski et al., 2020).

Automated stationary traps can cover extensive areas, generating heat maps, determining critical infestation periods, indicating potential refuge areas and alternative surviving sites. The automation of data collection on the main pests of cotton crops, such as the boll weevil and lepidopterans like *Spodoptera frugiperda* and *Helicoverpa armigera* will promptly optimize control decision-making. Reduction percentages are not yet available, as such technologies are still under development. However, considering only these three pests mentioned, which are responsible for about 30% of the cotton production cost, the efficiency in monitoring and population control of these pests can save from US\$300 to US\$400 per hectare in the cotton cost production (Belot et al., 2016). Increasing efficiency can save 8 to 15 insecticide sprays per season, which reduces environmental impact.

Early localized spraying of chemical or biological inputs can dispense full-area pest control, saving 10- to 12 sprayings per cotton season. A study affirmed that localized management using spatial distribution maps and spraying of parasitoids *Telenomus podisi* to control stink bugs *Euschistus heros* reduced by 50% the demand for spraying to control this

pest (Rogia et al., 2021). A major challenge for the use of drones in precision pest management is the initial steep costs of the material: the drone itself, the various sensors or application technologies, mounting equipment, and analysis software. Although costs are decreasing with improving technology, sums are still relatively high. In 2017, the costs of a fixed-wing drone with a hyperspectral sensor were estimated at US\$144,000, while costs of a multi-rotor drone with a multispectral sensor were estimated at US\$12,000 (Pádua et al. 2017). Therefore, various companies are offering drone-related services, such as renting out drones with remote sensing equipment or offering parasitoids dispersal services. Furthermore, consulting companies offer remote sensing and data analysis services for a reasonable fee, even combined with other agriculture-related services, to provide one platform for efficient record-keeping and planning.

Final considerations

The recent discoveries and advances in knowledge listed here are contributing to improving the IPM of cotton crops in Brazil. The availability of bio-inputs to replace synthetic insecticides is an irreversible trend. Allied to the development of transgenic cultivars, resistance monitoring is necessary to ensure the durability of transgenic cultivars, and insect resistance management practices must be prioritized. Advances in semiochemical research, regarding pheromones or allelochemicals (plant volatiles) for control of key pest species, will help reduce insecticide applications or enhance the efficacy of selective delivery

methods, such as trap cropping and attract-and-kill control. The use of digital technology is increasingly helping the monitoring and localized control of pests in cotton. Improving timeliness and accuracy in pest management will lead to a reduction in the use of insecticides and the production cost. The sustainability of cotton cultivation involves this modernization.

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Marker-Assisted Selection for Disease Resistance in Brazilian Cotton Germplasm

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Abstract

Originated from Mexico semi-arid highlands of, upland cotton (*Gossypium hirsutum* L.) accounts for more than 90% of world cotton fiber production. Intensively cultivated in Brazilian Cerrado since the early 1980s, the upland cotton faces a wet and warm environment that favors the development of several pathogens (fungal, bacteria, viruses and nematodes). Disease resistance is an important trait in public and commercial plant breeding. Embrapa's cotton breeding program focus on developing high-yielding cotton cultivars with superior fiber quality with resistance to the main diseases and adapted to the Brazilian cerrado. Applying marker-assisted selection (MAS), in the last 10 years, several cotton cultivars have been developed and released with resistance to bacterial blight (BB), cotton blue disease (CBD) and ramularia leaf spot (RLS). In upcoming years, cultivars with simultaneous resistance to BB, CBD, RLS, and Root-knot nematode will be released.

Keywords: *Gossypium hirsutum*, Genetic resistance, Molecular marker

The genus *Gossypium* L. (collectively termed cotton) comprises more than 50 species dispersed globally. Independently, four species were domesticated - two allopolyploids from the Americas, *G. hirsutum* L. and *G. barbadense* L., and two diploids from Africa-Asia, *G. arboreum* L. and *G. herbaceum* L. (Wendel and Grover 2015). *Gossypium hirsutum* L. *latifolium* Hutch, commonly known as upland cotton, is an important commodity in the world's agricultural economy and accounts for over 90% of cotton fiber production. The extra-long staple, *Gossypium barbadense*, represents approximately 5% of world fiber production (Adams 2015).

Since the 1990s, upland cotton growing area has increased substantially within the Cerrado, a savanna biome that covers the central Brazilian plateau. Currently, the cotton cultivated area in this biome is approximately 1,600,000 hectares and yields are the highest on the planet for rainfed conditions, 4224.45 kg.ha⁻¹ of seed cotton or 1760.77 kg.ha⁻¹ of lint (CONAB, 2022). These higher numbers are achieved mainly due to the very predictable rain period in the Cerrado, the breeding efforts for developing regionally adapted high-

yielding cultivars, as well the improvements in agronomic practices (Morello et al. 2020; Silva Neto et al. 2016; Suassuna et al., 2021).

However, the warm and humid weather during cotton growing is also a perfect environment to the development of several cotton diseases, leading to emerging diseases that reaches epidemic levels quite often. Nowadays, in terms of economic impact, the main cotton diseases in Brazil are: *Ramularia* leaf spot, Target spot, Root-knot nematode, Cotton blue disease, Reniform nematode, Damping off, *Fusarium* wilt and Bacterial blight. Minor diseases can also occur occasionally, but with no economic importance.

Embrapa's cotton breeding program has developed or adapted pre-existing molecular marker linked to genes conferring disease resistance in cotton to *Ramularia* leaf spot (RLS), Root-knot nematode (RKN), Cotton blue disease (CBD) and Bacterial blight (BB). Sources of resistance to all the above-mentioned diseases are governed by one or few major genes, making possible the use of marker-assisted selection (MAS), enhancing the response from selection with high precision and reductions in cost. Additionally, MAS is suitable to avoid error associated in selecting traits phenotypically difficult to measure, like nematodes responses.

***Ramularia* leaf spot**

Ramularia leaf spot (RLS) is a disease of major importance on cotton fields in Brazil due to its effects on yield and cotton fiber quality. RLS can be caused by either *Ramulariopsis pseudoglycines* or *R. gossypii* (Videira et al., 2016). In Central Brazil *R. pseudoglycines* is prevalent

(Mathioni et al., 2021). This main fungal disease causes early defoliation and photosynthetic area reduction, reducing lint yield and negatively affecting fiber quality (Silva et al., 2019).

A pre-breeding effort along the years 2003 to 2011 allowed to characterize several cotton accessions from the Embrapa's cotton germoplasm collection and identify resistance sources. Recombining partial resistant cotton genotypes and subsequent selection in segregating populations allow us to develop the cotton cultivars BRS 372 and BRS 416 with high resistance level to RLS (Suassuna et al., 2020).

Using a segregant population derived from BRS 372 and the susceptible cultivar CD 408 and a cotton 63K single nucleotide polymorphism (SNP) array developed by Hulse-Kemp et al. (2015), the resistance was mapped, and SNPs markers were identified linked to two QTLs, one in chromosome A01 and other in chromosome A04 (Boldt & Suassuna, unpublished). It was developed a TaqMan real-time PCR assay for RLS marker-assisted selection. When both QTLs are in homozygous condition, the same RLS resistance level of BRS 372 is achieved.

Root-knot nematode

The Root-knot nematode (RKN), *Meloidogyne incognita*, is one of the most important economic pests of cotton worldwide. Host plant resistance is the most economical, practical, and environmentally sound method to provide protection against this pathogen. The resistant line Auburn 623RNR and a number of elite breeding lines derived from it remain the most important source of root-knot nematode

(RKN) resistance. Genetic analysis has identified two epistatically interacting RKN resistance QTLs, *qMi-C11* and *qMi-C14*. The *qMi-C11* locus predominantly affects root gall suppression whereas the *qMi-C14* locus largely reduces egg production but has little effect on galling (He et al., 2014). Further, while the main effects of each QTL appeared to serve as the major genetic basis in conferring resistance for both galling and egg production phenotypes, resulting in a near-immunity to infection when both QTLs are present (Lopes et al., 2020). The origin of the resistance loci was traced to the two moderately resistant parents of Auburn 623 RNR, with the *qMi-C11* locus on Chromosome 11 inherited from Clewilt 6 (Gutierrez et al., 2010) and the *qMi-C14* locus on Chromosome 14 inherited from Wild Mexican Jack Jones (Shen et al., 2006).

Two simple sequence repeat (SSR) markers are closely linked with both QTLs: CIR316 associated with resistance on chromosome 11 (Shen et al., 2006) and marker BNL3661 on chromosome 14 (Ynturi et al., 2006). The SSR markers CIR316 and BNL3661 were converted to SNPs markers and have been routinely used in Embrapa's cotton breeding program.

Cotton blue disease

Cotton leafroll dwarf virus (CLRDV) (Genus: *Polyomavirus*; Family: *Luteoviridae*) causes the cotton blue disease (CBD), and occurs in Africa, Asia, South America and North America (Tabassum et al., 2021). CBD was named for the dark green to bluish color, inward rolling, and leathery texture of leaves on the infected plants. In early-season infections, epinasty can be severe, with reddened petioles and veins,

and pronounced stunting of plants (Cauquil, 1977). In Brazil and other South American countries, CBD is a limiting problem for cotton production when susceptible cultivars are used. This disease can reduce cotton yield of susceptible varieties by up to 80% if cotton aphids are not properly controlled during the early growing season (Silva et al. 2008). The development and use of resistant variety offers the best management tool for CBD control. Commercial production in Brazil depends heavily on having highly resistant varieties available.

The resistance to CBD controlled by one single dominant gene, designated *Cbd*, in the resistant cultivar 'Delta Opal' and two simple sequence repeat (SSR) markers were identified as linked to *Cbd* at the telomere region of chromosome 10 (Fang et al., 2010). SSR marker DC20027 was mapped very close to *Cbd*. Additionally, four SNPs were identified and mapped on chromosome 10 associated with *Cbd*. Along with SSR marker DC20027, Embrapa's cotton breeding program uses the SSRs NG0204310 and NG0203481 markers, flanking *Cbd*, to select resistant genotypes. Since 2010, all cotton cultivars developed by Embrapa is resistant to CBD (Barroso et al., 2017; Morello et al., 2010; Morello et al., 2012; Morello et al., 2015; Morello et al., 2020; Suassuna et al., 2018; Suassuna et al., 2020; Suassuna et al., 2021).

A less aggressive resistant-breaking genotype of CLRDV was observed in Brazil on cotton varieties known to be resistant against CBD (Silva et al., 2015). This new disease was referred to as 'atypical' cotton blue disease (ACBD) to differentiate from the 'typical' CBD (Silva

et al., 2015). So far, no complete resistant cotton genotype were identified to ACBD.

Bacterial blight

Bacterial blight, caused by *Xanthomonas citri* subsp. *malvacearum* (*Xcm*), is a major disease of cotton occurring in most cotton producing countries of the world, causing significant yield losses. The bacteria penetrates the host plants through open stomata or wounds, and creates water-soaked lesions on cotton leaves, stems, and bolls, followed by premature leaf senescence and reduced lint yield. The disease management includes sanitary practices during ginning and seed processing, planting of acid-delinted and fungicide-treated seeds, destruction of residues from the previous crop, crop rotation, and use of resistant varieties. The deployment of resistant varieties is the most effective and economical means to control the disease and minimize yield loss.

At least 20 *Xcm* races occurs worldwide and race 18 is the most virulent and is prevalent in Brazil (Braga et al., 2016). Resistance to bacterial blight has been studied extensively and there are at least 22 reported resistance genes or gene complexes in cotton that confer differing degrees of resistance to various *Xcm* races carrying different avirulence genes in a typical gene-for-gene manner (Delannoy et al. 2005). Of these 22 genes, *B₁₂* confers a high level of resistance to all *Xcm* races presently found in Brazil (Silva et al., 2013).

SSR markers were identified as closely linked to the resistance gene *B₁₂*: CIR246, BNL3545 and BNL3644 on chromosome 14 [D02] (Xiao et al., 2009).

Additionally, SNPs markers NG0207069 and NG0210142, flanking *B₁₂*, can be used to select genotypes harboring the entire region on chromosome 14.

Conclusion

Applying marker-assisted selection (MAS), in the last 10 years, the Embrapa's cotton breeding program developed and released several cotton cultivars owning resistance to bacterial blight (BB), cotton blue disease (CBD) and ramularia leaf spot (RLS). In the coming years, cultivars with simultaneous resistance to BB, CBD, RLS, and Root-knot nematode will be released.

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Revival of Cotton Crop in Pakistan by Using Four Brothers (Fb) Next Generation (Ng) Cotton Technologies

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Abstract:

Agriculture sector plays an important role in the economic development of the country. The private sector of Pakistan is playing pivotal role in developing innovative technologies to increase productivity of the field crops. For the silver lining nothing would have been more suitable than white gold, the cotton. Cotton is crucial for economy and effects more than 70 million farmers directly. The FB Genetics is the first Agri biotech Private Sector Plant Biotechnology lab which is ISO 17025-2017 accredited by Pakistan National Accreditation Council (PNAC), continuously thriving to provide excellent quality solutions to combat biotic and abiotic stresses to the field crops. Genetic engineering enables molecular biologists to reshuffle genes in combinations otherwise not possible conventionally, opening a vast new source of genetic diversity for crop improvement. FB Genetics research focuses on the crop improvement through advanced molecular biology tools especially cotton. FB Genetics developed

plant transformation systems for gene transfer in cotton and other important field crops using a current state of the art and unified methodologies. FB Genetics research work centres around the positive improvement of crop plants that can resist to both biotic and abiotic stresses. In doing that FB Genetics pursued several lines of work that span multiple aspects including yield improvement, tolerance against chewing and sucking insect/pests, heat stress and improving plant immune system.

An outstanding achievement is the development of Next Generation (NG) Cotton Technology which is Sucking insect resistant especially whitefly, Pink Bollworm, Glyphosate and heat resistant multiple genes cotton technology. A tetra, triple and separate two double gene constructs were designed and developed to combat devastating effects of whitefly, Pink bollworm, heat and drought on cotton crop. It is the modern, cost-effective, prosperous and advanced herbicide tolerant cotton field crop solution for millions of farmers worldwide including Pakistan. The

best performing events of NG technology are being evaluated at lab and contained field trials. This has been approved by the National Biosafety Committee (NBC) and patented by Intellectual Property Organisation (IPO) of Pakistan. Patenting of the NG Technologies of the FB group is also in process in the USA, India, China, East African.

FB Genetics has established genome-wide SNP markers of local cotton varieties and also developed DNA barcodes for variety identification. The CRISPR-Cas9 system open new avenues to exploit the disease susceptible genes and negative

regulators of immune system for crop protection. FB Genetics has engineered cotton plant with enhanced immune system by knocking out negative regulators with cutting edge CRISPR-Cas9 system. The CRISPR -Cas9 edited cotton plants with strong immune system are being tested under contained field trials. Four Brothers Group Pakistan is the only private sector company in Pakistan which have its own nine commercialized GM cotton varieties including first ever triple gene cotton variety i.e., Hataf-3 along with six more in the approval process.



Fig:1: Whitefly resistant plant. After transformation sucking insect resistant genes, *Agrobacterium* mediated method of transformation was used and plant were generated through tissue culture.



Fig:2: Pink resistant plant. After transformation of advance pink insect resistant genes, *Agrobacterium* mediated method of transformation was used and plant were generated through tissue culture.



Fig:3: Climate resilient plant. After transformation of advance Heat resistant genes, Agrobacterium mediated method of transformation was used and plant were generated through tissue culture.



Fig:4: Drought Tolerant Cotton sown at Chakwal-Pakistan which is upper Punjab rainfed area. Excellent tolerance again drought was observed as there was rain after 42 days from the first one. In figure “B” excellent fruiting was observed almost 50 bolls per plant with excellent boll weight.



Fig:5: Hataf-3 approved commercialized triple gene high yielding cotton variety. Left Ghauri -3 Advance Triple gene herbicide and insect resistant cotton varieties. has completed all mandatory approval trials and soon will be with Farmers