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Source: Journal of Economic Entomology, 114(4) : 1817-1822

Published By: Entomological Society of America

URL: https://doi.org/10.1093/jee/toab109

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Journal of Economic Entomology, 114(4), 2021, 1817–1822
doi: 10.1093/jee/toab109
Advance Access Publication Date 9 June 2021
Research

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Plant Resistance

Water Deprivation Induces Biochemical Changes Without Reduction in the Insecticidal Activity of Maize and Soybean Transgenic Plants

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Subject Editor: Louis Hesler

Received 18 October 2020; Editorial decision 9 May 2021

Abstract

Like conventional crops, transgenic plants expressing insecticidal toxins from *Bacillus thuringiensis* (Bt) are subjected to water deprivation. However, the effects of water deprivation over the insecticidal activity of Bt plants are not well understood. We submitted Bt maize and Bt soybean to water deprivation and evaluated biochemical stress markers and the insecticidal activity of plants against target insects. Bt maize (DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-ØØ6Ø3–6 × SYN-IR162-4) containing the PowerCore Ultra traits, Bt soybean (DAS-444Ø6-6 × DAS-81419-2) with the Conkesta E3 traits, and commercial non-Bt cultivars were cultivated and exposed to water deprivation in the greenhouse. Leaves were harvested for quantification of hydrogen peroxide, malondialdeyde (MDA), and total phenolics and insecticidal activity. Maize or soybean leaf disks were used to evaluate the insecticidal activity against, respectively, *Spodoptera frugiperda* (J.E Smith) and *Chrysodeixis includens* (Walker) neonates. Except for Bt soybean, water deprivation increased hydrogen peroxide and MDA contents in Bt and non-Bt plants. Both biochemical markers of water deficit were observed in lower concentrations in Bt plants than in non-Bt commercial cultivars. Water deprivation did not result in changes of phenolic contents in Bt and non-Bt maize. For Bt or non-Bt soybean, phenolic contents were similar despite plants being exposed or not to water deprivation. Water deprivation did not alter substantially insect survival in non-Bt maize or non-Bt soybean. Despite water deprivation-induced biochemical changes in plants, both Bt plants maintained their insecticidal activity (100% mortality) against the target species.

Key words: Bt crops, insect management, abiotic stress, hydrogen peroxide, plant stress

Maize and soybean transgenic plants with resistance to insect pests were first approved for commercialization in 2007 and 2010, respectively, in Brazil (CTNBio 2020). Since then, farmer adoption of maize and soybean transgenic plants expressing insecticidal proteins from *Bacillus thuringiensis* Berliner (Bt plants) for pest control has been increasing in Brazil and follows trends observed worldwide (ISAAA 2018). Today, maize and soybean transgenic technologies are cultivated on 15.38 and 34.86 million hectares, respectively, in Brazil (ISAAA 2018).

Among the maize transgenic technologies, hybrids containing PowerCore Ultra (events DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-ØØ6Ø3–6 × SYN-IR162-4) express 2 proteins for herbicide tolerance (PAT and CP4 EPSPS) and 4 insecticidal Bt proteins (Cry1F, Cry1A.105, Cry2Ab2, and Vip3Aa20) targeting the insect pests *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae), *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) and the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Marques et al. 2019, Moscardini et al. 2020). The fall armyworm is considered one of the most important pests in South America due to its polyphagous habit, high reproductive rate, migratory behavior and high phenotypic plasticity which allows

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this species to feed on different hosts as well as develop resistance to multiple control tactics (Nagoshi et al. 2015, Blanco et al. 2016, Silva-Brandão et al. 2017, Okuma et al. 2018, Arias et al. 2019).

Conkesta E3 (events DAS-444Ø6-6 × DAS-81419-2) is a transgenic technology of soybean which express three proteins for herbicide tolerance (2mEPSPS, AAD-12, PAT) and two insecticidal Bt proteins (Cry1Ac and Cry1F) for the control of the soybean lepidopteran (Lepidoptera: Noctuidae) pests *Anticarsia gemmatalis* (Hübner), *Chloridea virescens* (F.), *Helicoverpa armigera* (Hübner), *Elasmopalpus lignosellus* (Zeller) and the soybean looper, *Chrysodeixis includens* (Walker) (Marques et al. 2016; 2017). The soybean looper is a polyphagous pest widely distributed across the Americas, and is considered one of the main insect pests of soybean (Specht et al. 2015, Santos et al. 2017).

Maize and soybean plants cultivated in non-irrigated production fields are frequently exposed to conditions of water deprivation (Bergamaschi et al. 2004, Nóia Júnior and Sentelhas 2019). Water deprivation often results in biochemical changes that reduce photosynthetic efficiency and increase the production of reactive oxygen species (ROS) with consequent cell membrane lipid peroxidation and cell damage (Ramachandra Reddy et al. 2004). For example, maize and soybean plants under drought are known to have increased hydrogen peroxide and malondialdehyde (MDA), a lipid peroxidation marker, contents, two indicators commonly used as stress markers (Anjum et al. 2017, Rao and Chaitanya 2020).

Part of the harmful effects to plants caused by the increase of ROS can be attenuated by enzymatic and nonenzymatic antioxidant systems as well with adjustments in the concentration of soluble sugars and free amino acids (Rejeb et al. 2014). Biochemical changes induced by water deprivation in plants could also alter plant protein expression (Mohammadi et al. 2012, Blein-Nicolas et al. 2019) and increase proteolysis in plants (Simova-Stoilova et al. 2010). These two types of responses could reduce the expression and stability of transgenic insecticidal proteins with a consequent reduction in target pest control (Parimala and Muthuchelian 2010).

Questions remain regarding how water deprivation may affect the insecticidal efficacy of Bt plants (Traore et al. 2000, Jiang et al. 2006, Luo et al. 2008, Martins et al. 2008, Girón-Calva et al. 2020). We evaluated in this study if biochemical modulations of plants under water deprivation affect the efficacy of Bt plants against target pests.

Materials and Methods

Plants

Seeds of PowerCore Ultra Bt maize (events DAS-Ø15Ø7-1 × MON-89Ø34-3 × MON-ØØ6Ø3–6 × SYN-IR162-4; commercial hybrid B2401PWU with the maturity of 125 d) and a non-Bt glyphosate-tolerant commercial hybrid (30A37RR), as well as seeds of Conkesta E3 Bt soybean (events DAS-444Ø6-6 × DAS-81419-2) and a non-Bt commercial cultivar (Maverick crossed with experimental variety—maturity group 5.0) were sourced from Corteva Agriscience (Wilmington, DE). Seeds (at least 3 per pot) were sown in 5-liter pots filled with a mixture of sand, soil, and manure (3:1:1) and grown in the greenhouse. After germination, seedlings were thinned to 1 per pot. Greenhouse experiments were conducted at São Paulo State University (UNESP), Jaboticabal, Brazil from May until June 2018 with mean monthly solar irradiation of 203.6 ± 34.5 h and compensated temperature average of 20.2 ± 2.38°C (mean±SD). Plants were maintained inside a greenhouse and irrigated daily with 500 ml water until the beginning of water deprivation.

Water Deprivation

Water deprivation started for maize plants at vegetative stage V6 (Ritchie et al. 1982a) by witholding water for 9 d. For soybean plants, water deprivation started at reproductive stage R1 (Ritchie et al. 1982b) for 6 d. From the beginning of water deprivation until leaf collection for assays, control maize and soybean plants (not water-deprived) were irrigated daily with 300 ml of water (Meyer and Gee 1999). Ten plants of maize (Bt and non-Bt) or five plants of soybean (Bt and non-Bt) were water-deprived and ten plants of maize (Bt and non-Bt) or five of soybean (Bt and non-Bt) were kept well-watered. Treatments were randomized in the greenhouse and each plant was considered one replicate for each treatment.

Leaf Collection for Plant Physiological Assays

The second fully expanded leaf immediately below the whorl of maize plants and the second fully expanded trifoliate leaf from the apex of soybean plants were collected for biochemical analysis. Leaves for biochemical assays were collected, inserted into perforated aluminum foil envelopes (16 cm height; 8 cm width), and immediately dipped in liquid nitrogen at the greenhouse. Samples were kept in the freezer (–20°C) for less than one month until analysis.

Hydrogen Peroxide

For hydrogen peroxide quantification, leaf samples of maize (400 mg) or soybean (100 mg), were ground in liquid nitrogen using mortar and pestles. A fine powder was obtained and transferred to 2 ml Eppendorf tubes. Next, solid polyvinylpolypyrrolidone (PVPP) was added to a final concentration of 20% (w/v) and 2 ml of 0.1% (w/v) trichloroacetic acid (TCA) were added. The solutions were briefly homogenized, centrifuged (7,155×*g*; 15 min; 4°C) and the supernatants (plant extracts) were collected. Plant extracts (200 µl) were transferred to Eppendorf tubes (2 ml) containing 200 µl of 100 mM pH 7.5 potassium phosphate buffer and 800 µl of 1 M potassium iodate. Samples were incubated on ice and in total darkness. After 1-h, tubes were maintained in dark conditions at room temperature and absorbance measurements of samples were conducted at 390 nm in a spectrophotometer using quartz cuvettes. A blank was prepared with 200 µl 0.1% TCA instead of plant extract. Ten replicates were analyzed for each maize treatment and five replicates were analyzed for each soybean treatment, with tissue from one plant considered as a replicate. Hydrogen peroxide content was expressed in µmol g^{-1} of fresh leaves using a standard curve of hydrogen peroxide as reference (Alexieva et al. 2001).

MDA Quantification

MDA content was determined using the same plant extract previously obtained for hydrogen peroxide quantification. For this, an aliquot of 250 µl of plant extract was transferred to new Eppendorf tubes (2 ml) containing 1 ml TCA 20% (w/v) plus 0.5% (w/v) 2-thiobarbituric (TBA). Samples were incubated in a thermal block (30 min; 95°C) and then kept in ice for 10 min before another centrifugation (7,155×*g*; 5 min; 4°C). The supernatants were collected and read at 535nm in a spectrophotometer. A blank sample containing 250 µl 0.1% TCA instead of plant extract was also prepared. Absorbance reading of samples was also conducted at 600 nm to account for nonspecific values of absorbance. Ten replicates were analyzed for each maize treatment and five replicates were analyzed for each soybean treatment, with tissue from one plant considered as a replicate. MDA content in plant extracts was expressed in nmol MDA.g^{-1} of fresh leaves using the molar extinction coefficient 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

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Total Phenolics

To evaluate total phenolics, leaf tissue (20 mg) from maize and soybean plants were ground to a fine powder in liquid nitrogen using mortar and pestle. The ground tissue was transferred to Eppendorf tubes (2 ml) and 1.7 ml of 95% methanol was added. Tubes were vortexed for 5 min, covered with aluminum foil for 48-h and centrifuged (13,000×*g*; 5 min; 25°C). The supernatant was collected and 100 µl of the supernatant was transferred to a new Eppendorf tube (2 ml) containing 200 µl of 10% Folin–Ciocalteu (F-C) reagent and 800 µl of 700 mM sodium carbonate. Samples were homogenized and analyzed after 2-h in a spectrophotometer at 765 nm. A blank was prepared with 100 µl 95% methanol instead of plant extract. Ten replicates were conducted for each maize treatment and five replicates were conducted for each soybean treatment, with the tissues from one plant considered a replicate. A standard curve using gallic acid was prepared as a reference for the determination of phenolic content (µg mg^{-1} of fresh leaves) (Ainsworth and Gillespie 2007).

Insects and Bioassays for Insecticidal Activity

Neonates of *S. frugiperda* and *C. includens* from Bt-susceptible populations maintained in the laboratory and not exposed to transgenic events or Bt toxins were obtained at Applied Ecology Lab (APECOLAB, UNESP, Jaboticabal) and Laboratório de Biologia de Insetos (ESALQ/USP, Piracicaba), respectively. Leaves immediately above those used for biochemical analysis were collected, inserted in paper bags (32.5 cm height; 25 cm width), and taken to the laboratory at environmental temperature until processing leaves for insect bioassays. Before bioassays, leaves were washed by bathing in 0.01% sodium hypochlorite for 30 s followed by another bath in tap water for 1 min. Leaves were superficially dried with paper towels and leaf disks of 2 cm diameter were cut with a cork borer.

One leaf disk per treatment of maize or soybean was inserted in each well of cell culture plates (12 wells each 2 cm in diameter, SPL Life Sciences Co. Ltd., Pocheon-si, Korea) over a paper disk deposited over 1 ml of 1% agar/water solidified in the bottom of the wells. One *S. frugiperda* neonate was inserted into each well containing the leaf disk of treatments of maize plants and one *C. includens* neonate was inserted into each well containing the leaf disk of the treatments of soybean plants. Plates were then covered with a polypropylene sheet and the plate's original cover. Plates were randomly arranged in an incubator at controlled conditions (25 ± 1°C; 12h photophase) and larval mortality was evaluated after 4 d. Each plate containing 12 insects feeding on leaf disks was considered a replicate, and 10 replicates using maize leaves for feeding larval *S. frugiperda* or five replicates with soybean leaves for feeding *C. includens* were prepared for each treatment.

Statistics

Means obtained from treatments (Bt vs non-Bt; water-deprived vs well-watered) of maize or soybean plants were presented as means ± standard error of means (SEM). Homoscedasticity and normality of data (MDA, hydrogen peroxide, and phenolics) were checked using, respectively, Bartlett and Cramer-von Mises tests. When data did not fit these assumptions, data were transformed using the coefficient indicated by Box-Cox test. Data were analyzed by two-way ANOVA using water condition (water-deprived or well-watered) as one factor and plant genotype (Bt or non-Bt) as a second factor. Means of MDA and hydrogen peroxide obtained for all treatments (Bt water deprived, Bt well-watered, non-Bt water deprived, and non-Bt well-watered) in maize or soybean plants were compared by Tukey test ($\alpha = 0.05$). For mortality results, data were analyzed using the non-parametric Kruskal–Wallis Rank Sum Test followed by comparisons of means obtained among all treatments (Bt water deprived, Bt well-watered, non-Bt water deprived, and non-Bt well-watered) in maize or soybean plants using Dunn's Multiple Comparison Test ($\alpha = 0.05$). All statistical analyses were made using the R software (R Core Team 2020). For maize data, the total degree of freedom was 39 [(10 replications × 2 water conditions × 2 genotypes) −1] and the residual degree of freedom was 36 [(total degree of freedom: 39)-(degree of freedom of factor A: 2-1 = 1)-(degree of freedom of factor B: 2-1 = 1)-degree of freedom of the interaction between factor A (2-1) × factor B (2-1) = 1]. For soybean data total degree of freedom was 19 [(5 replications × 2 water conditions × 2 genotypes)-1], and residual degree of freedom was 16 [(total degree of freedom: 19)-(degree of freedom of factor A: 2-1 = 1)-(degree of freedom of factor B: 2-1 = 1)]-degree of freedom of the interaction between factor A (2-1) × factor B (2-1) = 1].

Results

Bt and Non-Bt Maize Assays

After water deprivation, hydrogen peroxide content significantly increased in Bt and non-Bt maize ($F = 469.81$; df = 1, 36; $P < 0.001$). However, the hydrogen peroxide content in Bt maize was significantly lower than that observed in non-Bt maize after water deprivation ($F = 31.41$; df = 1, 36; $P < 0.001$) An interaction between the factors (maize genotype and water condition) was observed for the hydrogen peroxide content in maize leaves ($F = 4.98$; df = 1, 36; $P = 0.03$) (Fig. 1A). Changes in MDA contents in maize plants exposed or not to water deprivation followed the same pattern observed for hydrogen peroxide: water-deprived maize plants had an increased content of MDA ($F = 194.38$; df = 1, 36; $P < 0.001$) but water-deprived Bt maize presented a lower content of MDA than water deprived non-Bt maize ($F = 31.41$; df = 1, 36; $P < 0.001$). An interaction among the factors genotype and water condition was also detected acting on MDA content of maize leaves ($F = 5.01$; df = 1, 36; $P = 0.03$) (Fig. 1B).

Water deprivation did not influence total phenolics in Bt or non-Bt maize ($F = 4.12$; df = 1, 36; $P = 0.05$). No interaction between maize genotype and water condition was observed influencing total phenolics concentration ($F = 0.3125$; df = 1, 36; $P = 0.58$). An increased concentration of total phenolics was observed in non-Bt maize than in Bt maize in both water treatments ($F = 64.89$; df = 1, 36; $P < 0.001$). A post hoc analysis (Tukey test) indicated that phenolic content (mg g^{-1} of fresh leaves) in well-watered Bt maize (0.30 ± 0.07) and water-deprived Bt maize (0.21 ± 0.05) did not differ among each other but were both inferior to the phenolic content in well-watered non-Bt maize (1.12 ± 0.13) and water-deprived Bt maize (0.82 ± 0.12), which mean values did not differ among each other.

Independent of whether plants were well-watered or water-deprived, feeding *S. frugiperda* larvae with Bt maize resulted in superior larval mortality (100%) than that observed for *S. frugiperda* larvae fed with well-watered or water-deprived non-Bt maize (mortality below 3.5%) (Kruskal–Wallis $\chi^2 = 36.29$, df = 3, $P < 0.001$). After Dunn's test, we observed no differences for the mortality (%) of well-watered Bt maize (100 ± 0.0) and water-deprived Bt maize (100 ± 0.0), and significantly inferior values were observed for larval mortality of well-watered non-Bt maize (3.3 ± 1.8) that did not differ from mortality values observed for larvae fed with water-deprived non-Bt maize (0.8 ± 0.8).

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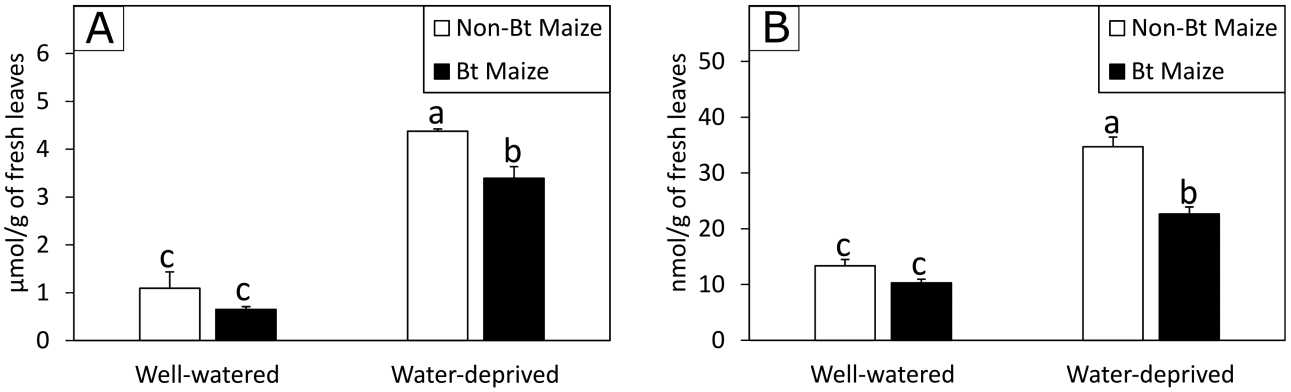

Fig. 1. Effects of water deprivation in the contents of (A) hydrogen peroxide, (B) malondialdehyde (MDA) of a Bt maize hybrid, and in a non-Bt commercial maize hybrid. Bars with different letters present significant differences among all treatments (Bt water deprived, Bt well-watered, non-Bt water deprived, non-Bt wellwatered) for hydrogen peroxide (A) or MDA (B) according to the Tukey test ($P < 0.05$).

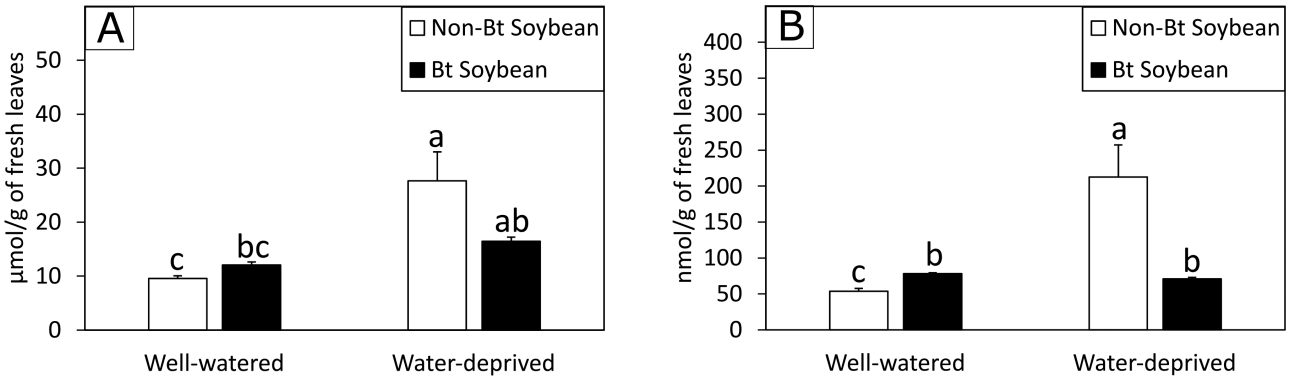

Fig. 2. Effects of water deprivation in the contents of (A) hydrogen peroxide, (B) malondialdehyde (MDA) in a Bt soybean cultivar and a non-Bt soybean commercial cultivar. Bars with different letters present significant difference among all treatments (Bt water deprived, Bt well-watered, non-Bt water deprived, non-Bt well-watered) for hydrogen peroxide (A) or MDA (B) according to the Tukey test ($P < 0.05$).

Bt and Non-Bt Soybean Assays

Under the same water treatments, soybean genotypes presented the same content of hydrogen peroxide ($F = 0.96$; df = 1, 16; $P = 0.34$). Water deprivation induced a significant increase (~three-fold) of hydrogen peroxide content in non-Bt plants, but not in Bt plants ($F = 27.76$; df = 1, 16; $P < 0.001$). An interaction between the factors (soybean genotype and water condition) was observed for the hydrogen peroxide content in soybean leaves highlighting the response of non-Bt soybean plants to water deprivation ($F = 27.76$; df = 1, 16; $P < 0.001$) (Fig. 2A). Different from hydrogen peroxide content, MDA content in soybean leaves was influenced by the soybean genotype ($F = 2.78$; df = 1, 16; $P < 0.001$), but not only by the factor water deprivation ($F = 40.14$; df = 1, 16; $P = 0.12$). An interaction among water treatment and soybean genotype was detected highlighting the increase of MDA content in non-Bt soybean leaves after water deprivation ($F = 59.08$; df = 1, 16; $P < 0.001$) (Fig. 2B).

The phenolic content in Bt or non-Bt soybean was not influenced by the soybean cultivar ($F = 0.38$; df = 1, 16; $P = 0.55$), water condition ($F = 0.40$; df = 1, 16; $P = 0.54$), and no interaction among the factors was observed ($F = 1.13$; df = 1, 16; $P = 0.30$). Phenolic content in well-watered Bt soybean was 0.33 ± 0.11 mg g^{-1}, in water-deprived Bt soybean was 0.30 ± 0.10 mg g^{-1}, in well-watered non-Bt soybean was 0.21 ± 0.06 mg g^{-1}, and in water-deprived non-Bt soybean was 0.37 ± 0.04 mg g^{-1}.

Larvae of *C. includens* fed with Bt soybean exposed or not to water deprivation presented a mortality rate of 100%. Mortality rates of *C. includens* larvae fed with non-Bt soybean plants exposed or not to water deprivation did not differ among water treatments (<15%), values much lower than that observed for Bt plants (Kruskal–Wallis $\chi^2 = 17.31$; df = 3; $P < 0.001$). After Dunn's test, we observed no differences in larval mortality (%) caused by consumption of well-watered Bt soybean (100 ± 0.0) and water-deprived Bt soybean (100 ± 0.0), and significantly inferior values were observed for larval mortality caused by consumption of well-watered non-Bt soybean (1.7 ± 1.7) that did not differ from mortality values observed for larvae fed with water-deprived non-Bt soybean (13.3 ± 6.8).

Discussion

Water deprivation commonly induces an increase in hydrogen peroxide content, a reactive oxygen species (ROS), in plants. Consequently, the increase in hydrogen peroxide increases MDA contents in plant tissues, which indicates cell membrane damage mediated by ROS under stressful conditions (Apel and Hirt 2004, Rao and Chaitanya 2020). In our experiments, we observed this pattern most clearly for non-Bt plants.

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Interestingly, our results showed that, along with a less pronounced increase in hydrogen peroxide content after water deprivation, maize and soybean Bt plants also produced lower amounts of MDA than water-deprived non-Bt commercial cultivars. Although the difference in the genetic background of non-Bt cultivars could explain indirect effects associated with antioxidant systems not related to Bt proteins, antioxidant activity associated with Cry and Vip proteins expressed in transgenic plants should also be considered (Kavitha et al. 2018).

The most proximal results indicating that Bt proteins may be related with the reduction of oxidative stress in plants come from studies that evaluated the role of *B. thuringiensis* as a growth promoter microorganism in plants (Armada et al. 2016). However, no studies investigating Cry or Vip proteins from *B. thuringiensis* acting directly as antioxidants or activators of ROS scavenging in plants were found and require complementary studies to further explore this question.

Despite the damage caused by hydrogen peroxide in cell membranes, this ROS is also recognized as an important component of plant cell signaling in response to abiotic stresses such as water deprivation (Neill et al. 2002, Sewelam et al. 2016). For example, hydrogen peroxide can trigger stomatal closure, a common response of plants exposed to water deprivation to reduce water loss (Pei et al. 2000) as well as acting in cell signaling to increase the production of secondary metabolites in plants after abiotic stresses (Nakabayashi and Saito 2015).

Secondary metabolism triggered by hydrogen peroxide, or other signaling molecules, may produce phenolic compounds such as chlorogenic acid and rutin (Niggeweg et al. 2004, Agati et al. 2013). Both molecules are also known for their antagonistic effects on the development of Lepidopteran larvae (Stamp and Osier 1998). In our results, we did not observe an increase in total phenolics after water deprivation in Bt or non-Bt maize or soybean plants, suggesting that phenolics with antioxidant and insecticidal activity were not being accumulated after water deprivation. However, deeper investigations to detect the concentration of specific phenolics compounds after water deprivation may aid the comprehension of the responses of plants in terms of secondary metabolism.

During water-deprived periods, plants change their gene expression and some proteins are up-regulated, e.g., enzymes associated with antioxidant responses to stresses, while other proteins may be down-regulated (Benešová et al. 2012, Blein-Nicolas et al. 2019). These changes in gene expression can influence, for instance, the expression of transgenic proteins, affecting the insecticidal efficacy of the transgenic plants (Traore et al. 2000, Parimala and Muthuchelian 2010).

In our experiments, both target species, *S. frugiperda* larvae for Bt maize plants or *C. includens* larvae for Bt soybean plants, showed high mortality rates (~100%) after consuming Bt plants exposed or not to water deprivation. High larval mortality indicates that even if water deprivation modulates gene expression and influences the production of transgenic proteins and/or produces molecules that could negatively impact the insecticidal activity of transgenic proteins, the insecticidal efficacy of transgenic plants exposed to water deprivation in this study was maintained against the pest species tested. Similar results indicating the maintenance of insecticidal activity of stressed transgenic plants were observed in salt-stressed Bt cotton (Jiang et al. 2006).

Finally, we argue the importance of understanding how transgenic plants with insecticidal activity interact with target pests in the scenario of transgenic cropping and insect resistance management (IRM). Despite the high control rate (100%) observed in this work, the use of a high-dose Bt event, i.e., a transgenic Bt plant that produces the insecticidal protein in a concentration sufficient to kill at least 95% of the heterozygous insects with the resistance genes, is a key feature in IRM (Bates et al. 2005, Huang et al. 2011).

Attention must be given if transgenic insecticidal proteins may be reduced below high-dose levels in water deprived plants. If this reduction happens, heterozygous pests may be maintained in the production fields, fostering the evolution of resistance in the pest population under selection pressure. Future work for the evaluation of larval mortality in bioassays using different concentrations of lyophilized leaf tissue of Bt plants exposed or not to water deprivation incorporated into artificial diet may be an informative way to measure if Bt plants under water deprivation keep their high-dose status against insect pests such as the fall armyworm or the soybean looper. These studies will give a better understanding of changes in Bt protein expression in the field during water deprivation and will increase the knowledge of pest population dynamics and, consequently, increase the durability of Bt crops in commercial fields.

Author Contributions

G.D.R., R.M. and L.H.M. designed the idea. J.B.S. and R.M. performed previous and published experiments and prepared the manuscript drafts. P.L.G. supervised plant biochemical assays. All authors contributed in writing and approved the final manuscript.

Conflict of interests

L.H.M., A.C.S., T.N., M.L.D. and J.B. are employees of Corteva Agriscience and declare no competing financial or non-financial interests. The remaining authors have no conflict of interests to declare.

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