INHIBITION OF ELECTRON TRANSPORT RATE IN COTTON AND WEEDS BY AMMONIUM GLUFOSINATE

I. P. Freitas e Silva Mato Grosso do Sul State University Cassilandia, Mato Grosso do Sul J. F. Silva Junior E. H. C. B. Van Cleef Triangulo Mineiro Federal University Iturama, Minas Gerais C. A. Carbonari E. D. Velini N. Corniani I. P. F. S. Brito R. Araldi G. L. G. C. Gomes Sao Paulo State University Botucatu, Sao Paulo L. Tropaldi Sao Paulo State University Dracena, Sao Paulo

Abstract

The glufosinate herbicide promotes plant death by indirectly inhibiting the action of the enzyme glutamine synthetase, causing inhibition of photorespiration and photosynthesis. The present study aimed to evaluate the effect of glufosinate on the electron transport rate of photosystem II in cotton and weeds. Cotton plants, *Urochloa decumbens* and *Ipomoea grandifolia* were cultivated in five-liter pots filled with substrate. The trial was conducted in a completely randomized design with four replicates. The treatments consisted of the application of ammonium glufosinate (2.0 L p.c. ha⁻¹) and five periods without rainfall: 1, 3, 6, 24 and 48 h after application and a control treatment (without application). The evaluations of photosystem II electron transport rate (ETR) were performed at 0, 1, 2, 3, 4, 5, 6, and 7 days after application (DAA). For cotton, there was a greater inhibition of ETR in the treatment 48 h without rain at 1 DAA. At 8 DAA, there was an inhibition of ETR greater than 95% for all the treatments. For *U. decumbens*, an inhibition of ETR greater than 50% was observed for all treatments at 2 DAA, reaching 98% inhibition at 8 DAA. In *I. grandifolia*, at 2 DAA, all treatments promoted inhibition of ETR greater than 70% and, at 8 DAA, the inhibition was greater than 80% for all treatments. It was concluded that the amount of herbicide absorbed 1 hour after application is enough to promote inhibition of ETR in cotton plants, as well as in *U. decumbens* and in *I. grandifolia*.

Introduction

Brazil is among the five largest cotton producing countries in the world, which are: India, China, the United States, Brazil and Pakistan (ABRAPA, 2019). As a result of the strong worldwide campaign to reduce the consumption of non-biodegradable plastic materials, there is an opportunity for natural fiber from cotton to regain a market that was being lost to synthetic fibers, with Brazil having land and technology to supply the world demand of cotton (SEVERINO et al., 2019). Cotton is the raw material of various products used daily, such as clothing, vegetable oils, animal feed, and crop residues used as fertilizers (JABRAN, 2016).

Cotton is sensitive to biotic and abiotic stresses, with the first being more harmful (JABRAN, 2016). Abiotic stress is related to water deficiency, heat stress and salinity (MASSACCI et al., 2008) and biotic stress is related to damages caused by pests, diseases and weeds, which can cause losses greater than 80% in yield (OERKE, 2006) and reduce the quality of cotton fiber (JABRAN, 2016). Regarding weeds, there are many species that interfere with cotton production fields (DOGAN et al., 2014), which can also cause indirect damage to the crop, which are alternative hosts of pests and diseases (JABRAN, 2016).

Chemical weed control is of great importance for cotton cultivation, since pre-emergent herbicides, post-emergent herbicides and non-selective herbicides facilitate management, are efficient (OWEN et al., 2015). The weed control revolution was driven by the introduction of dicamba, glyphosate and glufosinate-resistant cotton (JABRAN, 2016).

Ammonium glufosinate is derived from phosphinothricin and it is a degradation product of bialaphos, produced by the bacterial species *Streptomyces viridochromogene* and *S. hygroscopius* (DAYAN; DUKE, 2014). It is a non-selective broad-spectrum herbicide, used as an alternative to glyphosate, especially in fields with resistant weeds (BRUNHARO; CHRISTOFFOLETI; NICOLAI, 2014). This herbicide inhibits the enzyme glutamine synthetase (GS) by interfering with ammonium assimilation and nitrogen regulation in plants (SUNDAR; SAKTHIVEL, 2008). Ammonium accumulation is not the only factor that causes plant death, but the depletion of carbon skeletons such as glutamine, which inhibits photorespiration and photosynthesis (SEELYE et al., 1995).

The GS inhibition caused by glufosinate blocks glutamine synthesis through the glutamate route (CARBONARI et al., 2016). This herbicide can also interfere with the synthesis of some amino acids, with increases in free radical production and blocking photosynthesis (CARVALHO 2013). The symptoms occur mainly in leaves because the herbicide is not readily translocated into the plant (DAVIS et al., 2013), which becomes chlorotic and dries out within 2 to 5 days after treatment (SHIN et al., 2011). For *Lolium rigidum* and *Avena sterilis* species, glufosinate absorption occurs within the first 24 hours after treatment (KUMARATILAKE et al., 2002). According to Rodrigues and Almeida (2011), glufosinate needs 6 hours without rain after application to have an efficient action.

Under stress conditions, the reduction in photosynthetic rate may be caused by lower energy dissipation through electron transport, which results in lower quantum efficiency of photosystem II (PSII) and electron transport rate (ETR) and may be associated with increased non-photochemical fluorescence extent and zeaxanthin pool (HAVAUX; NIYOGI, 1999).

The evaluation of chlorophyll fluorescence alterations is useful for determining the site of inhibition of electron transport by herbicides and for relating herbicide injuries to the absorption rate or pulverized concentration, having a particularity with post-emergent herbicides, since their performance may be interfered by the presence of adjuvants, environmental conditions, and leaf age (RICHARD Jr et al., 1983).

The intense use of the ammonium glufosinate for weed control in cotton crop and the need for information on the rate of absorption of this herbicide and its effects on weed and cotton metabolism, the current study aimed to evaluate the inhibition caused by ammonium glufosinate in the electron transport rate of photosystem II in cotton and weeds.

Materials and Methods

The trial was conducted in a greenhouse with a temperature ranging from 15 to 28°C and relative humidity ranging from 70 to 90% at the Center for Advanced Research in Matology - NUPAM, from the Department of Plant Production, School of Agronomic Sciences of São Paulo State University, Botucatu, Brazil.

Five-liter pots (n = 72) were filled with Tropstrato HT – Vegetables, composed of simple superphosphate, potassium nitrate, peat, expanded vermiculite and pine bark, enriched with macro and micronutrients, with pH 5.8 (\pm 0.5). Eight cotton plants (cv. FiberMax 910), twenty *Urochloa decumens* and twenty *Ipomoea grandifolia* plants were used per pot. Thinning was performed 21 days after planting, leaving four cotton plants and ten of each weed per pot.

The trial was set up in a completely randomized design with four replications. The treatments consisted of the use of the herbicide ammonium glufosinate (2.0 L p.c. ha⁻¹) at five periods without rainfall: 1, 3, 6, 24 and 48 h after application and a control treatment without herbicide. The electron transport rate (ETR) was evaluated.

At the time of application, the cotton plants had the second true leaf fully expanded and the third expanding at approximately 30 cm high, the *U. decumbens* was approximately 36 cm high and the *I. grandifolia* was approximately 18 cm high and presented tendrils.

The application of the herbicide ammonium glufosinate was performed using a stationary sprayer (Figure 1) equipped with a spray bar consisting of four tips (XR 110.02) spaced by 0.5 m and positioned 0.5 m above the plants. The system was operated with a travel speed of 1 m s⁻¹, which corresponds to 45 Hertz in the frequency modulator, with herbicide solution consumption corresponding to 200 L ha-1. The equipment was operated at a constant pressure of 1.5 bar using compressed air. The same equipment was used to simulate 40 mm of rain in each treatment.



Figure 1. Stationary sprayer used for application of herbicide solution and rain simulation.

The ETR evaluations were performed at 0, 1, 2, 3, 6 and 8 days after herbicide application (DAA). The device used was the portable fluorometer (Multi-Mode Chlorophyll Fluorometer OS 5p - Opti Sciences) (Figure 2), and the readings occurred in the completely open and photosynthetically active leaves. The protocol used for the measurements was the Yield.



Figure 2. Portable fluorometer Multi-Mode Chlorophyll Fluorometer OS5p.

Data were subjected to analysis of variance and means were compared by Tukey test at 5% probability.

Results and Discussion

There was a great reduction of ETR in the first two days of evaluation, with inhibition of approximately 80%, in the treatment "48 hours rain" in cotton plants, differing significantly from the other treatments. At 8 DAA, an inhibition of ETR greater than 95% was observed, with no significant differences among treatments (Figure 3). Thus, the amount of herbicide absorbed within 1 hour after application is enough to cause phytotoxicity in cotton plants.

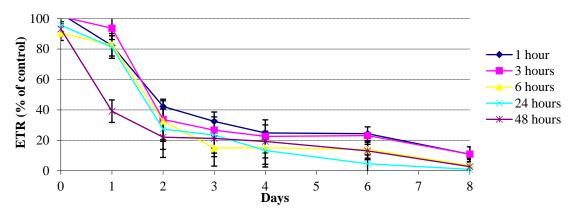


Figure 3. Electron Transport Rate (ETR) over time for cotton plants. The bars indicate the DMS of the evaluated periods.

There was an inhibition greater than 50% of ETR for *U. decumbens* at 2 DAA (Figure 4). During the evaluation time, ETR was similarly inhibited for all treatments, reaching, at 8 DAA, an inhibition of approximately 98%.

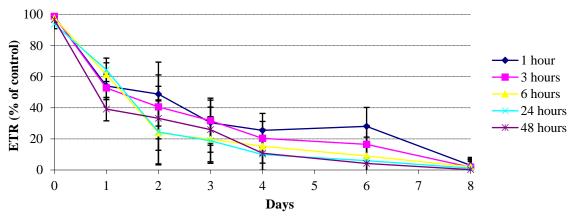


Figure 4. Electron Transport Rate (ETR) over time for *U. decumbens* plants. The bars indicate the DMS of the evaluated periods.

All treatments evaluated promoted an inhibition of ETR greater than 70%, at 2 DAA for *I. grandifolia* (Figure 5). At 8 DAA an inhibition greater than 80% was observed for all treatments.

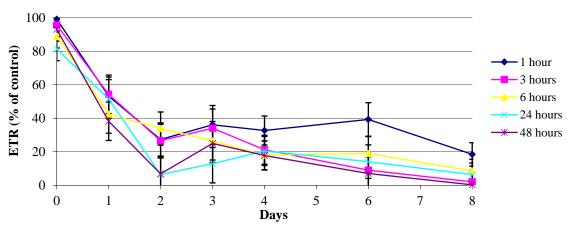


Figure 5. Electron Transport Rate (ETR) over time for *I. grandifolia* plants. The bars indicate the DMS of the evaluated periods.

Summary

Doses greater than 400g i.a. ha⁻¹ of glufosinate in *Sisymbrium loeselii* L. caused an inhibition in the quantum yield of photosystem II, and at 900g i.a. ha⁻¹ a reduction of approximately 100% of this quantum yield, with the same effect found in *Symphytum officinale* L., *Sisymbrium altissimum* L. and *Descurainia sophia* L. (MERKEL et al., 2004). Coetzer and Al-Khatib (2001) reported a rapid inhibition of photosynthetic rate and an increase in stomatal conductance 2 hours after glufosinate application and, after 6 hours of application, there was a 63% reduction in photosynthetic rate.

Chlorophyll fluorescence monitoring is widely used to measure phytotoxicity caused by herbicides (KLEM et al. 2002). Lacuesta et al. (1992) found reductions in chlorophyll fluorescence and photochemical fluorescence inhibition in glufosinate-treated plants. Inhibition of the enzyme glutathione synthetase present in the nitrogen uptake pathway in plants causes side effects on the electron transport rate in Photosystem II and irreversible plant damage (SAUER et al. 1987).

Freitas e Silva et al. (2016), researching the absorption rate in weeds and cotton, observed that the internal increase of ammonium glufosinate occurred in the first 24 h without rain after application in cotton plants, and it was found that the absorption after 2 to 5 hours without rain promoted great accumulation of ammonium in plants. According to those authors, the amount of ammonium glufosinate absorbed by *I. grandifolia* increased up to 48 hours without rain and by *U. decumbens*, the absorption was faster and more intense in the first 6 hours without rain and there were increments of this herbicide in the leaves up to 48 hours without rain after application.

In the current study, there were reductions greater than 50% of ETR for all treatments for cotton and *I. grandifolia* plants 2 days after application. The same inhibition was observed 3 days after application for *U. decumbens*. Eight days after the application, cotton, *U. decumbens* and *I. grandifolia* presented ETR inhibitions greater than 80%. The amount of herbicide absorbed 1 hour after application is enough to promote ETR inhibition in cotton plants, as well as in *U. decumbens* and in *I. grandifolia*.

Acknowledgements

Authors thank the Agronomy (Agriculture) Graduate Program from Sao Paulo State University – Unesp/FCA, for the infrastructure provided for the conduction of this trial; the Coordination for the Improvement of Higher Education Personnel (Capes), for the Ph.D. scholarship; the supervisors Caio Antonio Carbonari and Edivaldo Domingues Velini, for the technical-scientific support; the Brazilian Association of Cotton Producers – ABRAPA for sponsoring for the participation of the first author in the Cotton Beltwide Conference, 2020; and the Mato Grosso do Sul State University - UEMS for the partnership in the development of scientific research.

References

ABRAPA. Cotton in Brazil. 2019. Available in: https://www.abrapa.com.br/Paginas/dados/algodao-no-brasil.aspx. Access in: 26 nov. 2019.

Brunharo, C.A.C.G., P.J. Christoffoleti, and M. Nicolai. 2014. Aspects of the mechanism of action of ammonium glufosinate: resistant crops and weed resistance. Rev. Brasileira de Herbic. 13:163-177.

Carbonari, C.A., D.O. Latorre, G.L. Gomes, E.D. Velini, D.K. Owens, Z. Pan, and F.E. Dayan. Resistance to glufosinate is proportional to phosphinothricin acetyltransferase expression and activity in LibertyLink® and WideStrike® cotton. Planta, Berlin, v. 243, n. 4, p. 1-9, 2016.

Carvalho, L.B. 2013. Herbicides. p. 21-52. In L.B. Carvalho (eds.) Physiological dynamics. Lages, SC.

Coetzer, E., and K. Al-Khatib. 2001. Photosynthetic inhibition and ammonium accumulation in *Palmer amaranth* after glufosinate application. Weed Sci. 49:454-459.

Dayan, F.E., and S.O. Duke. 2014. Natural compounds as next-generation herbicides. Plant Physiology. 166:1090-

1105.

Davis, B.R.C.C., and J.K. Norworthy. 2013. Response of wheat (*Tritium aestivum*) to low rates of glyphosate and glufosinate. Crop Prot. 54:181-184.

Dogan, M.N., K. JABRAN and A. UNA., 2014. Integrated weed management in cotton. In: Recent Advances in Weed Management, CHAUHAN, B.S. and G. MAHAJAN, The Netherlands, Springer, 197-222.

Freitas e Silva, I.P., C.A. Carbonari, E.D. Velini, J.F. Silva Junior, L. Tropaldi, G.L.G.C. Gomes. 2016. Absorption velocity of glufosinate and its effects on weeds and cotton. Agrociencia. 50:239-249.

Havaux, M., and K.K. Niyogi. 1999. The violaxanthin cycle protects plants from photooxidative damage by more than one mechanism. Proceedings of the National Academy of Sciences of the United States of America. 96:8762-8767.

Jabran, K. 2016. Weed flora, yield losses and weed control in cotton cropWeed flora, yield losses and weed control in cotton crop. Julius. Kühn. Archive. 452:177-182.

Klem, K., M. Pundova, H. Hrabaloba, L. Nau, M. Vaova, J. Masojidek, and P. Tomek. 2002. Comparison of chlorophyll fluorescence and whole-plant bioassays of isoproturon. Weed Res. 42:335–341.

Lacuesta, M., A. Munoz-Rueda, C. Gonzalez-Murua, and M.N. Sivak. 1992. Effect of phosphinothricin (glufosinate) on photosynthesis and chlorophyll fluorescence by barley leaves illuminated under photorespiratory and non-photorespiratory conditions. J. Exp. Bot. 43:159–165.

Massacci, A., S.M. Nabiev, L. Pietrosanti, S.K. Nematov, T.N. Chernikova, K. Thor, and J, Leipner. 2008. Response of the photosynthetic apparatus of cotton (*Gossypium hirsutum*) to the onset of drought stress under field conditions studied by gas-exchange analysis and chlorophyll fluorescence imaging. Plant Physiol. Biochem. 46:189-195.

Merkel, U., G.W. Rathike, C. Schuster, K. Warnstorff, and W. Diepenbrock. 2004. Use of glufosinate-ammonium to control cruciferous weed species in glufosinate-resistent winter oilseed rape. Field Crop Reseacher. 85:237-249.

Oerke, E.C., 2006. Crop losses to pests. J. Agric. Sci. 144:31–43.

Owen, M.D., H.J Beckie, J.Y. Leeson, J.K. Norsworthy, and L.E. Steckel. 2015. Integrated pest management and weed management in the United States and Canada. Pest Manage. Sci. 71:357-376.

Richard Jr, E.P., J.R. Goss, C.J, Arntzen, F.W. Slife. 1983. Determination of herbicide inhibition of photosynthetic electron transport by fluorescence. Weed Science. 31:361-367.

Rodrigues, B.N., and F.S. Almeida. 2011. Herbicide Guide. p. 697. *In* B.N. Rodrigues and F.S. Almeida (eds Herbicide Guide, Londrina, PR.

Sauer, H., A. Wild, and W. Ruhle. 1987. The effect of phosphinothricin (glufosinate) on photosynthesis. II. The causes of inhibition of photosynthesis. Zeitschrift fuer Naturforschung. 42:270-278.

Seelye, J.F., W.M Brost, G.A. King, P.J. Hannan, and D. Maddocks. 1995. Glutamine synthetase activity ammonium accumulation and growth of callus cultures of *Asparagus officinalis* L. exposed to high ammonium or phosphinothricin, J. Plant Physiol. 146:686-692.

Severino, L.S., S.M.M. Rodrigues, L.G. Chitarra, J. Lima Filho, E.C.M Mota, R. Marra, and A. Araujo. 2019. Product: Cotton: Part 01: Characterization and technological challenges. 2019. Available in: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/198192/1/SerieDesafiosAgronegocioBrasileiroNT3Algodao.pdf. Access in: 26 nov. 2019.

Shin, J.S. K.M. Kim, D.J. Lee, S.B Lee, N.R. Burgose, and Y.I. Kuka. 2011. Resistance levels and fitness of glufosinate resistant transgenic sweet potato in field experiments. Field Crops Res. 121:324–332.

Sundar, I. K. and Sakthivel, N. 2008. Advances in selectable marker genes for plant transformation. Journal of Plant Physiology. 165:1698-1716.