Communications in PLANT SCIENCES

http://cpsjournal.org
e-ISSN 2237-4027
Volume 10 (2020)

RESEARCH ARTICLE

Influence of K salts in enhanced herbicide activity

Deivid Araújo Magano[1], **Jerson V. Carús Guedes**[2], **Shyam Pariyar**[3], **Juergen Burkhardt**[3], **Ivan Ricardo Carvalho**[1]*, **José Antonio Gonzalez da Silva**[1] and **Mauricio Hunsche**[3]

[1] Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Ijuí, RS, Brasil.
[2] Universidade Federal de Santa Maria, Santa Maria, RS, Brasil.
[3] University of Bonn, Bonn, Alemanha.
*Author for correspondence: carvalho.irc@gmail.com

Submitted on February 03, 2020
Accepted on August 18, 2020
Early View on August 21, 2020
Final Publication on August 21, 2020

Authors declare no conflict of interest

KEYWORDS:
Paraquat
Bromoxynil
Damage
PAM-fluorescence
Multidroplets

ABSTRACT

Maximize process of pest control reducing loss in the agriculture process is fundamental. The aim this research was to achieve a different way to increase the herbicide activity, using potassium salts. Some experiments were conduct to analyze the efficiency of salts in enhanced the activity of two selected herbicides (paraquat and bromoxynil). Firstly, we checked the effects of chaotropic and kosmotropic potassium salts and their capability to cause damage in the foliar tissue, and made a dosage adequate to avoid these undesirable effects. Later on, we analyze the physiological impairments due salts using PAM-Fluorecence techniques, employing different kind of arrays to verify, the response and ability to increase the real damage in foliar tissues. The best performance was obtained with multiple droplets applied 48 hours earlier than herbicides, promoting an increase of 2 times more efficacy, for paraquat and bromoxynil.

Highlighted Conclusions

1. There is interaction between K salt and herbicides.
2. Microencapsulated herbicides consist in a strategy to combine with K salts.

INTRODUCTION

The Brazilian agriculture has shown great highlight in the production of food, fibers and industrial products, with interesting results for economy, being the country a higher export of grains and vegetal resources. In this scenario, the area of crop protection works hardly, improving the efficacy of pesticides, due to susceptible agriculture crops are to damage caused by plagues (weeds, insects and pathogens), that if not treated could be economic loss, reducing the profit of producers (Cruz et al. 2020). Historically, pest management is carried out with a limited number of mechanisms of action (MoA). Problems with resistance increases due to the repetitive application of pesticide with the same (MoA) making the control of pests more complex, requiring new strategies to maximize the efficiency of existing compounds enhanced chemical pest management (Hawkins et al. 2018).

An alternative to improve the efficacy of contact or systemic foliar-applied agrochemicals is improving the ability of these compounds to penetrate in the leaf cuticle (Basi et al. 2013). Usually, helped by tank-mix adjuvants increasing the amount of pesticides deposited on hydrophobic leaf surfaces (Stevens 1993, Kirkwood 1999, Baur 1999, Forster et al. 2015). Nevertheless, foliar uptake may principally happen via the cuticular or stomatal pathway. A new approach, proposed by Burkhardt (2013), called hydraulic activation of stomata (HAS) broken with a paradigm that was present by nearly 40 years. According HAS, it is possible that hygroscopic salts will enable the stomatal uptake of compounds, even if the surface tension is well above 30 mN m^{-1} (Burkhardt et al. 2012).

In this sense, make it necessary begin to exploit the use of salt solutions, like a way of maximizing the efficacy of compounds that already exist in the market, promoting an increase in the control of these different biological targets (Burkhardt et al. 2012, Basi et al. 2013). Other way is act under mechanisms that can change the ''*status quo*'' of foliar tissues, modifying characteristics such as roughness, adhesion and other parameters (Burkhardt et al. 2013, Kautz et al. 2015). Hence, we conducted a series of experiments to analyze the efficiency of salts in different combinations. Our guiding hypothesis was that chaotropic and kosmotropic salts classified in the Hofmeister series, and we assumed that salts with higher potential for cuticular permeation would support the co-penetration of other ions contained in the treatment solution. This research aims to achieve a different way to enhance the herbicide activity, using potassium salts.

MATERIAL AND METHODS

Trials. In the first trial, we analyzed the possible role of kosmotropic and chaotropic salts applied in solution, directly on the plant tissues and check possible effects under plant models (beans and apple seedlings). We did a screening with six concentrations (25, 20, 15, 10, 5, 1 mM and water) of five different salts (KSCN, KI, KIO_3, KH_2PO_4, K_2SO_4), and we applied these solutions in beans and apple seedlings and checked visual effects of this salt promote damage in these leaves. At the same time as, we analyzed the physiological response with PAM Fluorescence, detecting changes in the tissues treated. In the second trial, we verify the pattern of deposition of these salts. In the third trial, we verify the effects of KSCN in solution mixing with two different herbicides (*Bromoxynil* and *Paraquat*), using different arrays with simple droplets (SD) and multiple droplets (MD), changing the time of application and analyzing the different visual effects on damage area (Table 1).

Table 1. Surface tension and contact angle of sessile droplets by different potassium salts (25 mM).

Solutions	Surface Tension (mN m^{-1})	Contact Angle (°)
H_2O	72.28 b	99.66 a
KSCN	72.35 a	98.60 a
KIO_3	71.89 bc	95.83 b
KH_2PO_4	71.57 bc	96.73 b
K_2SO_4	71.83 bc	95.81 b
C.V. (%)	0.68	1.12

Values: mean, n = 10 for surface tension; n= 20 for contact angle). Statistical significance ($p \leq 0.05$, Tukey test, and denoted by different letters.

Experiments were done under semi-controlled environmental conditions in a glasshouse (50°43'46.9"N 7°04'21.6"E, Bonn, Germany), with an average temperature of 25 °C and a relative humidity between 60 – 80 %. Experiments were conducted with apple (*Malus domestica* Borkh.) seedlings (seeds originated from the cultivar Golden Delicious) and common bean (*Phaseolus vulgaris* L.) seedlings (cultivar Negra, Kiepernkerl, Volmary GmbH, Muenster, Germany). Plants were cultivated in pots filled with substrate (soil:peat:perlite), and fertilized and irrigated according to their needs.

Experiment 1: Screening trial. In this approach, treatment solutions were applied with a Hamilton microsyringe as single micro-droplets (1 µL) to the leaf surfaces. Occurrence of phytotoxic symptoms was assessed visually on ten individual plants (apples and common bean) per treatment at 5, 24, 48, 72 and 96 hours after application (HAT).

Experiment 1: Contact angle and surface tension. Contact angle (CA) and surface tension (ST) measurement were performed using a goniometer DSA30E (Krüss GmbH, Hamburg, Germany), employing the pedant – drop method (Table 1).

Experiment 1: Characterization of salts in contact with plant tissues. The pattern of deposition for each droplet of salts were analyzed using an environmental scanning electron microscopy (XL 30 ESEM, FEI-Phillips Co., Kassel, Germany).

Experiment 2: Physiological impairments due Salts. PAM chlorophyll fluorescence was recorded with an imaging system (Imaging PAM, Heinz-Walz GmbH, Effeltrich, Germany) enabling the precise identification of the affected tissues. Recordings were performed in the laboratory on the adaxial leaf side of dark-adapted leaves (30 min). First, ground fluorescence (*Fo*) was recorded after leaf illumination with blue light-emitting diodes (0.5 µmol m^{-2} s^{-1}). Maximum chlorophyll fluorescence (*Fm*) was measured after a saturation pulse of 1000 µmol m^{-2} s^{-1}. The variable chlorophyll fluorescence (*Fv*) was calculated as *Fm* – *Fo* and the maximum photochemical efficiency as *Fv/Fm*. Saturation pulses were applied at 20 s interval within a time frame of 320 s to determine the kinetic curves. The evaluation of data was performed with the software ImagingWin v2.40b (Heinz-Walz GmbH, Effeltrich, Germany).

Experiment 2: Pictures of damage. Records were realize using a digital camera Nikon, with a basis to apply the scale in the background. The results were evaluated with software Gimp 2.8, and analyzed pixel by pixel. Processing digital of image was effectuated with software IMAGEJ, editing the background with a natural scale and proceeding the damage area analysis, using excel sheets.

Data evaluation and statistics. Quantitative data were analysed with the software SPSS (IBM SPSS, V22, Ehningen, Germany). Graphs were drawn with the software SigmaPlot (Systat, Erkrath, Germany).

RESULTS AND DISCUSSION

Screening solutions were accessed based on damage observed in foliar tissues. For KIO_3 and KI, all concentrations applied were aggressive for beans seedlings, causing strong havoc. In the weakest concentration (1mM), the effects of these salts were measured using PAM technique, and revealed with 5 HAT, changes in the natural behaviour on the parameter chlorophyll fluorescence (data not showed). KSCN with 25mM and 20mM showed low effects in the foliar tissues, but when analysed by PAM, during 48 HAT appear hard signals in the bean seedlings, being necessary verify 72 hours to consider visual effects. With 15 mM we did not checked foliar phytotoxic effects for beans. However, these effects in apple seedlings appear clearly in 15 days after application, but was detected by PAM technic with 48 hours (data not showed). The KSCN with concentration of 1, 5 and 10 mM were elected to continuous in this research, because no offer damage to foliar tissue (Figures 1-9).

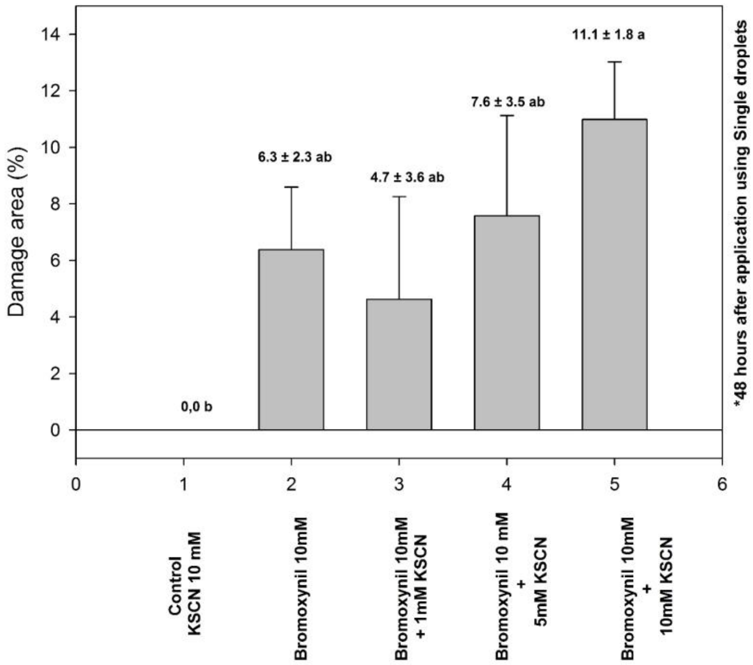

Figure 1. Damage (%) caused by different treatments, using potassium salts in array of Single droplets.

The KH_2S0_4 and K_2S0_4 did not showed any effects or influence for co-penetration. Analysing ESEM microphotography (Electronic Microscope), left clear that characteristics of these compounds were inert, and we cannot find any kind of difference with your addition in the leaves. Based on the anions present in the salts, they were classified as chaotropic (structure breakers) or kosmotropic (structure makers) following the Hofmeister classification system. Accordingly, the salts KSCN, KI and KIO_3 had a rather chaotropic, and the salts KH_2PO_4 and K_2SO4 a rather kosmotropic nature.

Surface tension (ST) of the liquids and the contact angle (CA) of the sessile droplets were only slightly affected by the salts (25 mM), as demonstrated in Table 1. ST of treatment solutions was lower than water (ST = 72.28 mN m^{-1}), except KSCN (ST = 72.28 mN m^{-1}) was slightly lowered particularly with addition of KH_2PO4 (71.57 mN m^{-1}). The contact angle of sessile droplets was significantly ($P \leq 0.05$) lower (CA = 95-96°), except for KSCN (CA = 98°), as compared to pure water (CA = 99°).

The residues of single droplets on beans seedlings were characterized with scanning electron microscopy (ESEM), and representative images are shown in Figure 10. Droplets containing KI showed a huge crystalized cube structure, describing the higher values of deliquescence/efflorescence (Figures 10A, 10D and 10L). KIO_3 in the solution, deposits formed similar to single droplets areas. However, at higher amplification, small crystal needles made by the salts could be clearly identified cover totally the vegetal tissue (Figures 10B, 10F, 10G and 10H). KSCN spread well over the cuticular membranes, some residues of the salt remaining as isolated small islands, mainly close to the boarder of the residue area (Figures 10C, 10E and 10K). K_2SO_4 showed a massive structure, condensing all particles of salt, not interacting with foliar tissue (Figure 10H). Similar behavior was founded for KH_2SO_4, crystalizing a big structure without any interaction with tissue surface, however without any spreading (Figure 10I). Factors as spreading, roughness and ability of expand through plant tissue are very important, because permit increase the specific contact area warranting a better wetting, including different cycles of deliquescence/efflorescence, facilitating the uptake of crystals or solutions in contact with stomata, infer in regulatory processes. PAM technique was efficient to quantify the damage, without visual symptoms, and low values for all treatments containing K salts, reducing the values of chlorophyll fluorescence. Both herbicides needed of energy for feed their PSI or PSII and with the tissues damaged not is possible access normal indices.

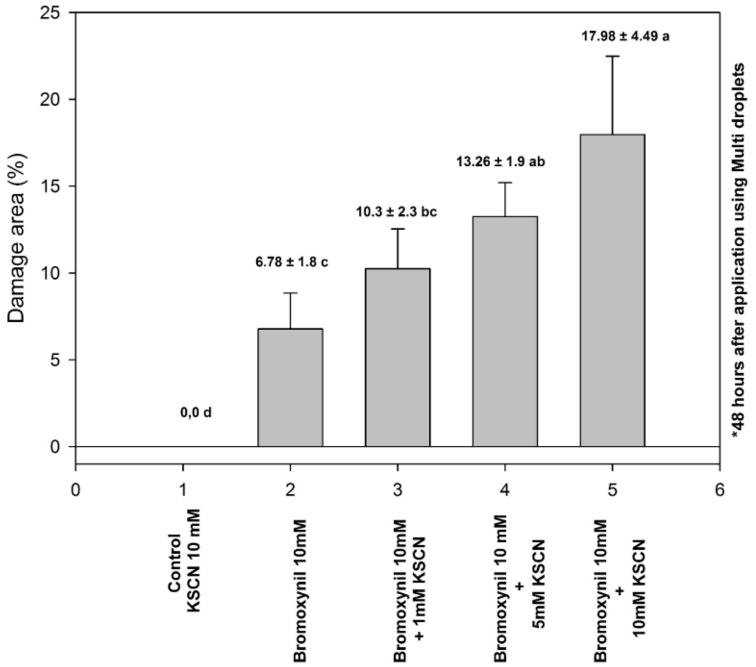

Figure 2. Damage (%) caused by different treatments, using potassium salts in array of Multi droplets.

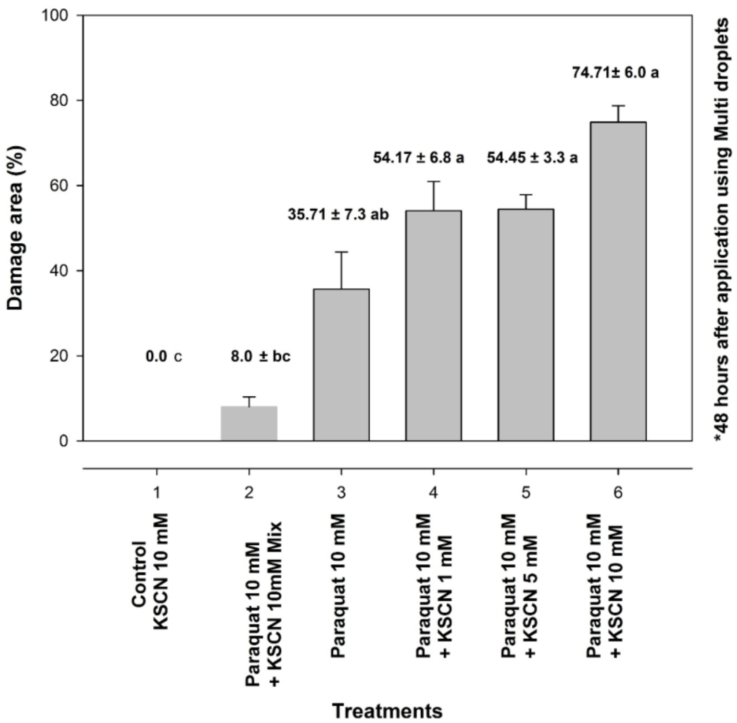

Figure 3. Damage (%) caused by different treatments, using potassium salts in array of Multi droplets.

Changing the array, using multi-droplets (MD, 1 mM, nearly 25 droplets in the whole AOI), these parameters takes on other configuration, bromoxynil alone promote a damage area close to 7%. Surprisingly, when applied other concentrations, the damage caused increase quickly according biggest concentration of the salt reaching in the maximum (10 mM) that was possible reach around 18% in the area affected. How the difference was not meaningful, we solve to apply for the next array, only MD by the similarity with the real situations of application to the field. The paraquat alone promote 35% of damage, being very aggressive, being possible to verify visual symptoms 3 hours after application in beans. We do not find differences between 1 and 5 mM in the percentage of damage, both around 55%, that can be correspondent to almost 1.6 times more than paraquat alone. When we checked paraquat applied over 10 mM KSCN, the values increase for around 75%, a real increase of 2.1 times, enhanced the efficacy of this product.

Mix solutions were used but both herbicides paraquat and bromoxynil mixed directly in the KSCN solution, but were presented incompatibility, which can be better understood when performed microphotography, which reveal the modification in the pattern of crystals. Paraquat and KSCN together in the same solution reduced the damage to 8%, a huge interference. With bromoxynil, the effects were strongly, promoting total reduction in the potential to cause damage, becoming totally without effect. We can observe the antagonist behavior of bromoxynil in the Figure 4. Regardless of dosage or commercial product or active ingredient pure, the results were the same for both experiments.

In previous studies, Burkhardt (2012) puts an end to a paradigm that lasted for 40 years, through the discovery of Hydraulic Activation of Stomata (HAS), a process that enables the bi-directional movement of liquids along the internal wall of the guard cells, significantly supports the uptake of agrochemicals (Basi et al. 2013, Burkhardt et al. 2013). In addition, we demonstrate that ions, depending on their chaotropic or kosmotropic behavior as classified according to the Hofmeister series, might support the formation paths from the outer to the inner side of the stomata (Burkhardt et al. 2012).

HAS describes the establishment of a hydraulic connection between the exterior and the interior of the stomatal cavity along the stomatal walls. Typically, salts might creep over the hydrophobic leaf surface towards those regions with higher relative humidity, the stomata (Burkhardt et al. 2012). When the path is formed, it allows the bidirectional movement of dissolved compounds, e.g. microscopic particles and molecules from the surface to the interior of the stomata. The formation of the paths against the gravity might also be favored by the comparatively low surface tension of supersaturate salt solutions, as it is the case when solution droplets of 'normal' concentration loss water to the surrounding environment.

In our results, it is possible verify the creeping of KSCN and your ability to spread easily, across the vegetal tissue. This is an important characteristic, because factors as spreading, roughness and ability of expand through plant tissue are very important, in order to permit increase the specific contact area warranting a better wetting. Puri et al. (2008) explains that the effectiveness of herbicides is enhanced when the spray solution is uniformly spread over the leaves. However, only the uniform coverage does not guarantee enhanced phytotoxicity (Gaskin 1993). When we applied KSCN in array of MD, we changed this potential including different cycles of deliquescence/efflorescence, an important propriety of this K salt, that which may facilitate the uptake of crystals or solutions in contact with stomata, inferring in regulatory processes. Burkhardt et al. (2012) explore the question of different cycles of deliquescence/efflorescence and their importance to enable stomatal entry.

We would stress that the point of surface tension in this discussion. The measured surface tension does not show differences, but this was measured at low concentration. When droplets dry, they will get to high concentration, and only at ionic strength higher than 0.1 M there will be significant Hofmeister effects. In a plant surface, there is cuticular transpiration, the humidity right at the surface may be higher than the deliquescence point and might prevent complete drying of the droplets for a while, and by this point of view, surface tension and Hofmeister effects may play a role for penetration.

Our attempts were focused in to access the stomata efficiently. For this reason, we tried to work with two different plants models, apples and common bean seedlings due to the presence/absence of stomata in the abaxial region of the leaves. However, the constitution with an excessive layer of wax on the apples, produced in the common beans results much more expressive, due to the presence of these structures on the vegetal surface. A characterization of these different surfaces, on the adaxial leaf surface of apple seedlings containing 412 ng.cm^{-2} waxes distributed in acids (8.4%), primary alcohols (41.5%), alkanes (14.1%), triterpenes (25.0%) and esters (10.9%). For bean seedling leaves 926 ng.cm^{-2} waxes on the adaxial surface and consisted of primary alcohols (85.4%), alkanes (12.7%), triterpenes (0.004%) and esters (1.4%). This different composition of waxes may be responsible to produce these results.

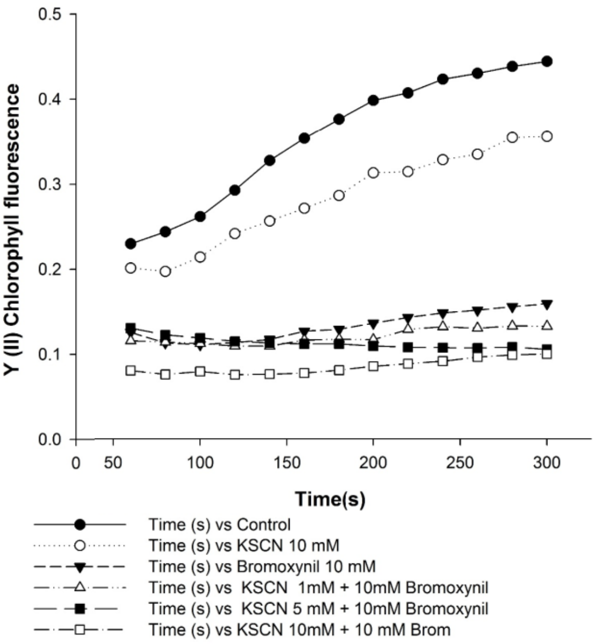

Figure 4. Changes in the behavior of Parameter YII (Chlorophyll Fluorescence) in selected time for different treatments for bromoxynil.

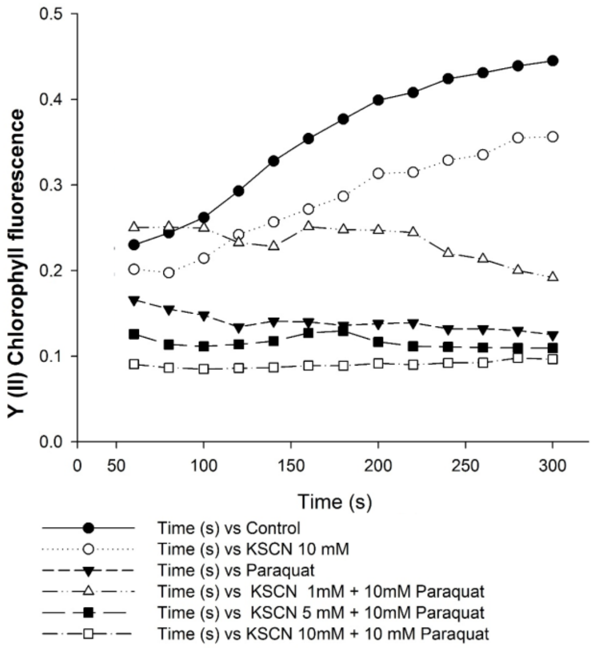

Figure 5. Changes in the behavior of Parameter YII (Chlorophyll Fluoresce) in selected time for different treatments for paraquat.

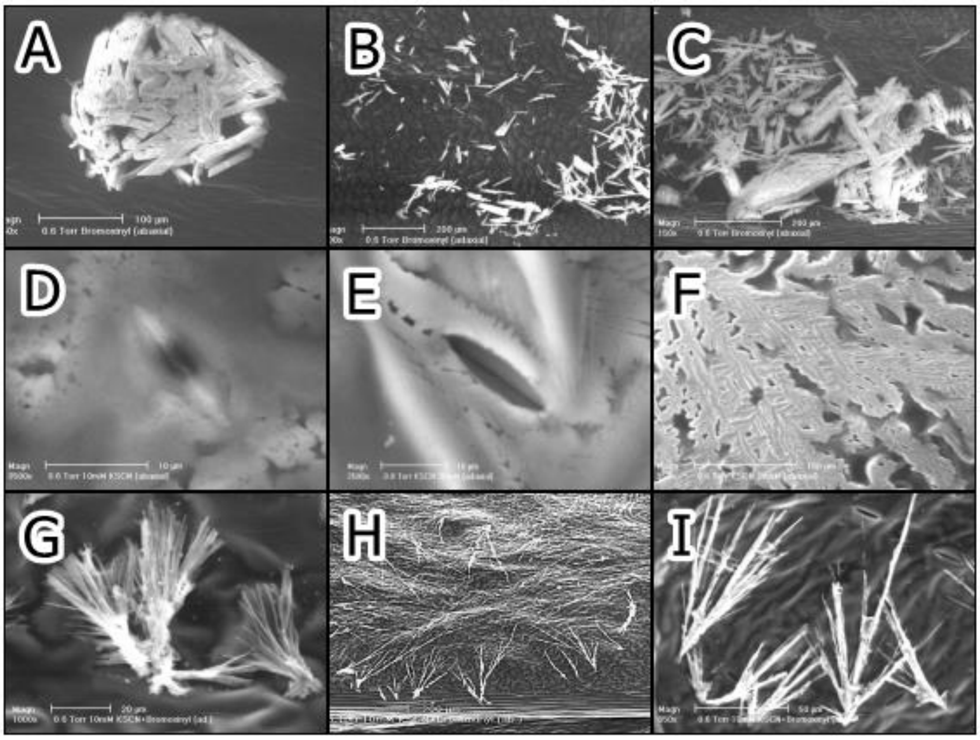

Figure 6. Characterization of changes in the deposition pattern with different magnification for selected k salts and mixture of Bromoxynil and KSCN. (A) Bromoxynil alone, (B) KSCN alone, (C) Mix between KSCN+Bromoxynil.

The analysis and behavior of different kind of solutions were fundamental importance to explain many responses in our work. Chlorophyll fluorescence is a technique universally accepted to evaluate the physiological status of the plants, promoting a rapid and non-destructive technique to evaluate the activity of photosynthetically active herbicides on the plant tissue. We accessed this parameter using the pulse amplitude modulated (PAM). The use of paraquat and bromoxynil can be explain because both herbicides acting showed mechanisms photosensitive, using the energy obtained by light to increase their mode of action (MOA) (Beyer et al. 1988), belongs possible detect the transference by chlorophyll fluorescence using PAM technique. The effects of paraquat

MOA involves shifting the energy flux from sunlight into photosynthesis to produce highly reactive free radicals that destroy cell membranes and dehydrate leaves rapidly. This happens in a few hours in bright sunlight, due to the high levels of energy that escapes control. Almost all green plants are affected by paraquat, which makes it a non-selective broad-spectrum herbicide. Similar behavior can be assessed by bromoxynil, that works with other MOA, but too make use energy that comes to the sun, respectively photosystem-I-electron diversion (PSI) and Inhibition of photosynthesis at photosystem II (PSII) (Bartels 1985a, 1985b).

Chlorophyll fluorescence happens in a defined spectral band (usually 680–690 nm). Based on the ground and the maximum fluorescence of dark-adapted leaves, as well as the fluorescence intensity during kinetic measurements, specific parameters allow conclusions about the current photosynthetic activity and the physiological state of the tissues. The results using this tool were very efficient, because e.g. apple seedlings showed a delay nearly 15 days to demonstrate visual effects, and using this resource was possible to detect changes in the behavior of parameter YII, a couple of hours after application. It was possible to access the influence of K salts interacting with both pesticides applied, allowed the quantification and qualification of damages containing.

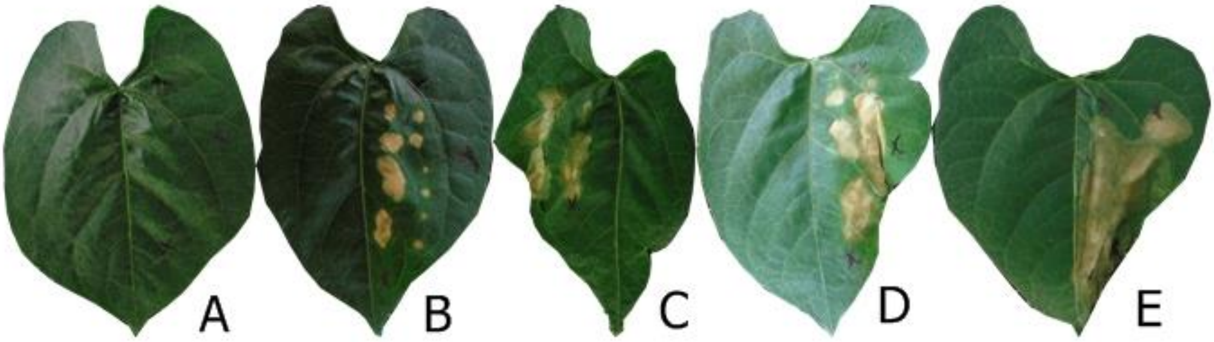

Figure 7. Visual effects of damage. (A) KSCN 10 mM; (B) bromoxynil 10 mM; (C) 1mM KSCN+ 10 mM bromoxynil; (D) 5mM KSCN + 10 mM bromoxynil; (E) 10 mM KSCN + 10 mM bromoxynil.

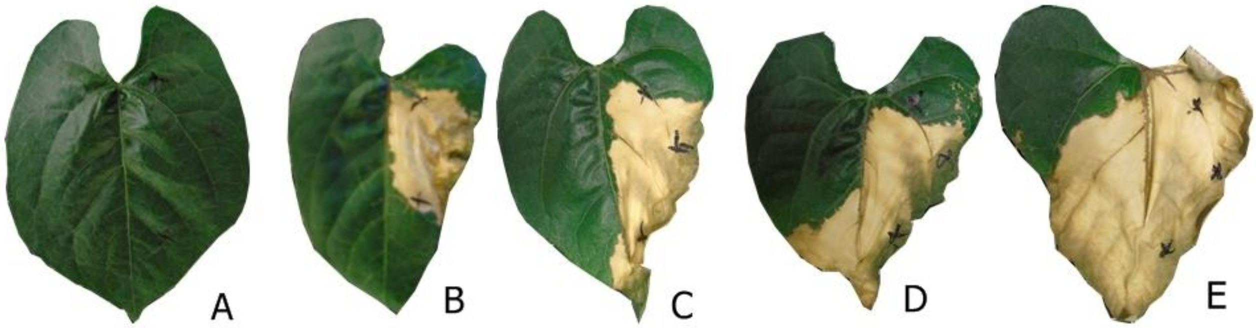

Figure 8. Visual effects of damage. (A) KSCN 10 mM; (B) paraquat 10 mM; (C) 1mM KSCN+ 10 mM paraquat; (D) 5mM KSCN + 10 mM paraquat; (E) 10 mM KSCN + 10 mM paraquat.

Figure 9. Visual effects of reduction on damage. (A) KSCN 10 mM (no damage), 10 mM KSCN + 10 mM paraquat, 10 mM KSCN + 10mM bromoxynil.

DOI: 10.26814/cps2020010 | 9 pages

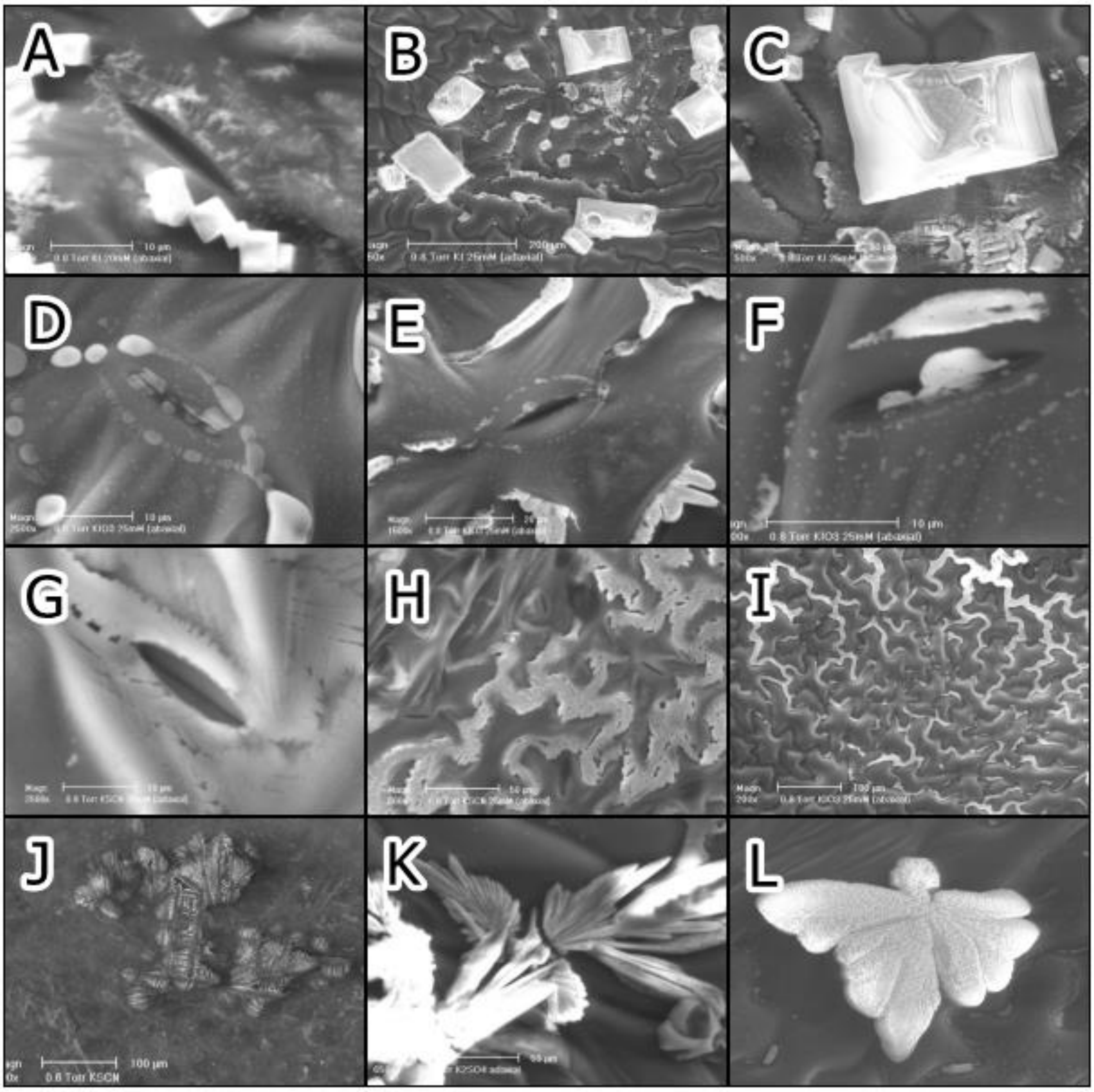

Figure 10. Characterization of deposition pattern with different magnification for selected k salts. (A), (B) and (C) KI pattern. (D), (E) and (F) pattern of KIO_3. (G) and (H) pattern of KSCN and their differences between KIO_3 (H x I). (J) Pattern of KSCN using 10 mM applied in tomato cuticle. (K) pattern of K_2so_4 and (L) pattern of KH_2SO_4.

In this research, an unexpected result was the interaction between salt and both herbicides, reaction that works with any kind of energy or catalyzer, freely. We just check this result using ESEM microphotography that showed clearly the changes in the deposition pattern. However, we can prove that is possible improve the efficacy of herbicides using potassium salts, through of benefits of HAS. Maybe our work brings a new contribution for development of formulations and using this background, problems with antagonist effects as showed in our experiments, could be surpassed. It is a challenge for companies that are working with crop protection. Microencapsulated herbicides like clomazone 360, maybe could be a strategy to make the difference using k salts.

Acknowledgements

The authors acknowledge Libeth Schwager and Britta Albinus for her valuable support in the lab activities, Knut Wichterich for preparing the ESEM micrographs and Antje Fiebig for support. The authors acknowledge also the Brazilian CAPES foundation for providing the first author with a scholarship for his stay at Bonn University (Process PDSE 99999.010628/2014-02), and Professor Dr. Georg Noga for his support and partnership.

References

Basi S et al. 2013. Relevance of the deposit structure for the uptake and bio-efficacy of diquat, as monitored by the spatially resolved chlorophyll fluorescence. Pesticide Biochemistry and Physiology 107: 218-225.

Baur P. 1999. Surfactant Effects on Cuticular Penetration of Neutral Polar Compounds: Dependence on Humidity and Temperature. Journal of Agricultural and Food Chemistry 47: 753-761.

Bartels PG. 1985a. Effects of herbicides on chloroplast and cellular development. In: Duke SO. (Ed.). Weed Physiology. Boca Raton, EUA: CRC Press, II. pp.64-91.
Bartels PG. 1985b. Effects of herbicides on photosynthesis. In: Duke, S.O. (Ed.). Weed Physiology. Boca Raton, EUA: CRC Press, II. pp.2-36.
Beyer Jr E et al. 1988. In: Kearney PC & Kaufman DD. (Eds.), Herbicides chemistry, degradation and mode of action. New York, EUA: Marcel Dekker, 117-189.
Burkhardt J et al. 2012. Stomatal penetration by aqueous solutions - an update involving leaf surface particles. New Phytologist 196: 774-787.
Burkhardt J et al. 2013. "Breath figures" on leaf surfaces-formation and effects of microscopic leaf wetness. Frontiers in Plant Science 4:422.
Cruz AA et al. 2020. Herbicide Resistance in Brazil: Status, Impacts, and Future Challenges. In: Kassio Ferreira Mendes. (Org.). Herbicides - Current Research and Case Studies in Use. IntechOpen, 2020. pp.1-25.
Forster WA et al. 2015. The contribution of spray formulation component variables to foliar uptake of agrichemicals. Pest Management Science 71: 1324-1334.
Gaskin RE et al. 1993. Antagonism of the foliar uptake of glyphosate into grasses by organosilicone surfactants. Part 1: Effects of plant species, formulation, concentrations and timing of application. Pesticide Science 38:185-192.
Hawkins NJ et al. 2018. The evolutionary origins of pesticide resistance. Biological Reviews of the Cambridge Philosophical Society 94:135-155.
Kautz B et al. 2015. PEG and drought cause distinct changes in biochemical, physiological and morphological parameters of apple seedlings. Acta Physiologiae Plantarum 37:162.
Kirkwood RC. 1999. Recent developments in our understanding of the plant cuticle as a barrier to the foliar uptake of pesticides. Pesticide Science 55:69-77.
Puri A. et al. 2008. Effect of the foliar application of diquat herbicide on selected natural area and field crop species. Weed Biology and Management 8:133-138.
Stevens PJG. 1993. Organosilicone Surfactants as Adjuvants for Agrochemicals. Pesticide Science 38: 103-122.