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S-metolachlor e atrazine afetam a cinética de absorção de água e a emissão radicular de sementes de milho: análise de imagem

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Orientador: Prof. Dr. Anderson Rodrigo da Silva

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SUMÁRIO

RESUMO

Os herbicidas pré-emergentes podem ter efeitos negativos nas sementes de milho. O objetivo deste estudo foi modelar curvas de embebição de sementes de milho por meio do processamento de imagens RGB, sob o efeito das concentrações de s-metolachlor + atrazine na cinética de embebição e na emissão da raiz primária. As sementes foram acondicionadas em placas de Petri contendo soluções aquosas do herbicida S-metolachlor (290 g L-1) + atrazine (370 g L-1) por 114 horas nas concentrações: 0% (somente água), 2%, 5%, 10%, 20% e 50 %, com base na dose de recomendação (4,0 litros do produto comercial por hectare). As imagens foram obtidas sistematicamente de um scanner de mesa com controle de luz artificial. O índice de excesso de vermelho foi adaptado para melhorar a segmentação da imagem. A partir de máscaras binárias, as curvas de embebição para cada concentração de herbicida foram obtidas usando estimativas de intumescência das sementes por tempo. As curvas de embebição foram descritas ajustando o modelo de Peleg. A concentração do herbicida tem efeitos significativos tanto na taxa de absorção quanto na emissão da raiz primária, reduzindo-os. Vinte por cento, ou mais, de concentração de s-metolachlor (290 g L^{-1}) + atrazine (370 g L^{-1}) na solução aquosa pode inibir totalmente a germinação das sementes.

Palavras-chave: *Zea mays* L., embebição de sementes, herbicida pré-emergente.

ABSTRACT

Preemergent herbicides can have negative effects on maize seeds. The objective of this study was to model seed soaking curves through the processing of RGB imagery of maize seeds under the effect of concentrations of s-metolachlor + atrazine on both the soaking kinetics and primary root emission. Seeds were placed to soak in Petri dishes containing aqueous solutions of the herbicide for 114 hours containing *s*-metolachlor (290 g L^{-1}) + atrazine (370 g L^{-1}) with the concentrations: 0% (water only), 2%, 5%, 10%, 20% and 50%, based on the recommended dose (4.0 L of the commercial product per hectare). Images were systematically taken from a flatbed scanner with artificial light control. We adapted the red excess index to improve image segmentation. From the binary masks, the soaking curves for each herbicide concentration were obtained using estimates of seed intumescence in time. The soaking curves were described by fitting Peleg's model. The herbicide concentration has significative effects on both the absorption rate and primary root emission, reducing them. Twenty percent, or more, concentration of s-metolachlor (290 g L^{-1}) + atrazine (370 g L^{-1}) in the aqueous solution can fully inhibit seed germination.

Key words: *Zea mays* L., seed imbibition, preemergence herbicide.

S-metolachlor plus atrazine affects the soaking kinetics and primary root emission of maize seeds: an imagery analysis
(Image analysis of maize seed soaking kinetics)

Abstract

Preemergent herbicides can have negative effects on maize seeds. The objective of this study was to model seed soaking curves through the processing of RGB imagery of maize seeds under the effect of concentrations of *s*-metolachlor + atrazine on both the soaking kinetics and primary root emission. Seeds were placed to soak in Petri dishes containing aqueous solutions of the herbicide for 114 hours containing *s*-metolachlor (290 g L^{-1}) + atrazine (370 g L^{-1}) with the concentrations: 0% (water only), 2%, 5%, 10%, 20% and 50%, based on the recommended dose (4.0 L of the commercial product per hectare). Images were systematically taken from a flatbed scanner with artificial light control. We adapted the red excess index to improve image segmentation. From the binary masks, the soaking curves for each herbicide concentration were obtained using estimates of seed intumescence in time. The soaking curves were described by fitting Peleg's model. The herbicide concentration has significative effects on both the absorption rate and primary root emission, reducing them. Twenty percent, or more, concentration of *s*-metolachlor (290 g L^{-1}) + atrazine (370 g L^{-1}) in the aqueous solution can fully inhibit seed germination.

Key words: *Zea mays* L., seed imbibition, preemergence herbicide.

Introduction

Maize (*Zea mays* L.) is an important world crop, as it can be used by humans and animals (cattle, poultry and pigs) as an important food source (FAOSTAT, 2017). Approximately 65% of the world's annual maize consumption is supplied by these main maize-producing countries: United States (320 million tons of grain), China (241 million tons of grain), European Union (76 million tons grain) and Brazil (62.5 million tons of grain) (de Oliveira et al., 2019). Agricultural practices, mainly aimed at maize, have evolved considerably in the last decades, with the adoption of integrated pest management. This trend triggered the use of herbicides, often in mixtures, in order to improve their efficiency and the range of treated weeds (Carles et al., 2018). However, this can cause negative effects on the seeds, altering the kinetics of absorption of water and/or solutes by the seeds, a process commonly studied through the imbibition curve.

The imbibition or rehydration process of viable maize seeds takes place, under the right condition of temperature and humidity, according to a three-phases pattern. The first phase consists of water absorption for the production of germination-inducing metabolites during the second phase, when the water absorption is drastically reduced or practically null. The third phase consists of the growth of the embryonic axis, again needing water absorption for cell division (Noblet et al., 2017).

The standard method for determining the imbibition curve consists of systematically weighing seed samples in imbibition (ISTA, 1985). Nevertheless, systems consisting of software and high-resolution digital imaging sensors have been developed for seed analysis (Halcro et al., 2020; Tanabata et al., 2012). Boelt et al. (2018) reviewed how physiological characteristics of seeds can be assessed using RGB (red, green, blue) and multispectral image data. Thus, it is possible to automate the analysis of the seed imbibition process through computational image processing. In this sense, Miller et al. (2018) developed a system based on RGB scanner imaging for automating measurements related to imbibition of corn seeds.

Silva and Lima (2017) present models for the seed imbibition curve of different cultivated species, including maize, showing how to calculate important parameters such as absorption rates, considering the presence or absence of the third phase. Modelling the imbibition curve allows the comparison of seed lots with differences in physiological potential or subjected to different imbibition conditions.

Environmental factors such as temperature and humidity affect the duration of the imbibition phases and germination itself, thus allowing to define, for example, ideal planting conditions (Meyers et al., 1984). It is also known that phytosanitary products such as herbicides can affect the final stage of imbibition – primary root emission (Subedi et al., 2017). But the explanation for this lies in physiological changes in the first and second phases of the imbibition process.

Gomes et al. (2017) observed that doses of glyphosate caused disturbances in the electron transport chain, promoting H_2O_2 accumulation in seeds of non-genetically modified soybean and, consequently, reduced germination. Moore and Locke (2012) found significant reductions in root growth of *Typha latifolia* (L.) subjected to *s*-metolachlor + atrazine. In sorghum hybrids, reductions of up to 69% in plant stand were caused by preemergence application of *s*-metolachlor + atrazine (Pimentel et al., 2019).

Atrazine is an herbicide of the triazine group (C1) used extensively throughout the world for the control of broadleaf plants and some narrow-leaved species, being used predominantly in the cultivation of maize, given its selectivity for this crop and its broad spectrum of control of different weed plants (Lerro et al., 2017). This herbicide has been usually applied in a mixture with acetochlor and s-metolachlor (Bedmar et al., 2011). Atrazine is known for its mobility in the soil, which makes it one of the most detected herbicides in surface waters (de Oliveira et al., 2019). Its mechanism of action involves the photosystem II, whose site of action is the thylakoid membrane, resulting in electron transport block and interruption of the fixation of CO_2 and, consequently, the reduction of ATP and $NADH_2$ production; in addition, this herbicide also induces oxidative stress and causes lipid peroxidation (Lerro et al., 2017; Heck et al., 2020).

The herbicide *s*-metolachlor ([2-chloro-N- (2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide]) has also been widely used in the maize crops, applied in pre-mergence or in pre-planting incorporated for weed control, being one of the most sold herbicides for maize and soybean crop due to its selective performance (Carles et al., 2018; Song et al., 2019). *s*-Metolachlor belongs to the acetamide group (k3) and is formed by two R isomers and two S isomers, which are present in equal proportions in the herbicide, with the S isomer with higher herbicidal activity than the R isomer (Lowry et al., 2013). The use of *s*-metolachlor stands out, mainly for its effectiveness in controlling *Commelia benghalensis* L. (Pimentel et al., 2019). This herbicide is able to inhibit the synthesis of proteins, chlorophyll and very long chain fatty acids, in addition to acts on cell division, inhibiting the growth of target plants (Lowry et al., 2013). Moreover, the phytotoxic effect of *s*-metolachlor is observed after the germination of the seedlings, culminating in the non-opening of the coleoptile and wrinkling of the definitive leaves, caused by the lower growth of the central vein in relation to the growth of the leaf blade (Karam et al., 2006).

According to Barnes et al. (2019), *s*-metolachlor + atrazine can provide broad-spectrum weed control compared with either herbicide applied alone. With this perspective and considering the current need for preemergent herbicides to control weeds in maize crops, studying the seed soaking in herbicide solutions is important to reveal potentially harmful effects of a given dose to the crop considered effective to control weeds (Mueller et al. 2014). In addition, understanding the effect of preemergent herbicides on both absorption of soil solution and germination is essential to carry out chemical management of weeds according to edaphic conditions, considering in particular the clay and organic matter content, given the sorption of preemergent herbicides in these particles of soil. Thus, it is possible to consider the feasibility of changing the timing of application or sowing to guarantee the plant stand.

We aimed to model the maize seed soaking curves by processing RGB images, as well as to quantify the effect of concentrations of the preemergent herbicide *s*-metolachlor + atrazine on the imbibition kinetics (initial imbibition phase) and on the primary root emission (final phase).

Materials and methods

Vegetal material and germination analysis

Commercial maize seeds, hybrid Syngenta Status Vip3, were obtained during the 2018/2019 harvest in an experimental area located at the geographic coordinates 18º 55' 32.9" S and 48º 09' 50.8" W, Mid-Western Brazil. An industrial phytosanitary treatment of the seeds was carried out with fungicide (100 mL/100 kg Metalaxyl-M 20 g/L + Thiabendazole 150 g/L + Fludioxonil 25 g/L) and insecticide (120 mL/60,000 seeds of 350 g/L Thiametoxam).

Assessments of the seed lot physiological quality were done in terms of:

Germination: four replications of 50 seeds were taken randomly. A sheet of germination paper was moistened with distilled water, the equivalent to 2.5 times the weight of the dry paper. The seeds were arranged on a paper roll and kept in a germination chamber, Mangelsdorf type, at a constant temperature of 25 ºC. The count of normal seedlings was done after 8 days and the result was expressed as a percentage.

Water content: two replications of 10 seeds each were placed to dry in an oven at 105 ± 1 ºC for 24 hours. The initial and final weight of the samples was computed on a precision electronic scale (0.01 g precision) and the difference was expressed as a percentage.

Weight of a thousand seeds: 8 replications of 100 seeds each were weighed on a precision electronic scale (0.01 g precision) to estimate the weight of a thousand seeds.

Soaking curves: image processing

The seeds were soaked in aqueous solutions containing *s*-metolachlor (290 g L^{-1}) + atrazine (370 g L^{-1}) with the concentrations: 0% (water only), 2%, 5%, 10%, 20% and 50%, based on the recommended dose (4.0 L of the commercial product per hectare) and volume of 100 L of water (syrup) per hectare, aiming to simulate a wide gradient of herbicide concentration reaching the seeds through the soil solution. The doses were chosen according to the pesticide description leaflet from the registration system of the Ministry of Agriculture of Brazil, which presents products together with their recommended doses, toxicological classification and environmental risk (AGROFIT, 2020). In addition, the concentrations were based on studies by de Oliveira et al. (2019), Joly et al. (2013) and Moore and Locke (2012), with adaptations.

Three replications of ten seeds each were placed to soak in Petri dishes of 90 mm diameter containing 20 mL of the solution and kept in a germination chamber, Mangelsdorf type, at 25 ºC during the entire imbibition process. RGB images of the dishes were captured using a flatbed scanner model Epson Perfection V19, with resolution of 600 pixels per inch and dimensions 100 × 100 mm, every hour during the first 6 hours, and then in two-hour intervals until 12 hours of imbibition; afterwards, the images were acquired at intervals of four hours up to 24 hours of imbibition and, finally, at intervals of 6 hours until 50% of the seeds of each sample presented emission of primary root. To control light variation, a box coated with opaque black paper was placed over the scanner platform, covering it completely.

The images were processed as follows:

i) normalization of the intensity values of pixels of the spectral bands to the interval [0, 1] (Eq. 1);

$$r = \frac{R}{R+G+B}, \quad g = \frac{G}{R+G+B}, \quad b = \frac{B}{R+G+B} \tag{1}$$

ii) seed segmentation through an adaptation (Eq. 2) of the red excess index (Meyer et al., 1999) and application of the Otsu (1979) method for thresholding;

$$ExR^{*} = 3r - 1.2g - 1.2b \tag{2}$$

iii) application of the median filter with a radius of 12 pixels (0.5%) to remove noise.

iv) calculation of individual and total (10 seeds) area by computing the number of binary pixels in the segmented image.

v) calculation of the intumescence (Eq. 3) of the soaking seeds at every time *t*:

$$IN(t) = \frac{100(A_t - A_0)}{A_0} \tag{3}$$

where A_t is the sum of the individual areas at time *t*, A_0 is the sum of the areas at time $t = 0$ of imbibition. *IN*(*t*) was used as an indirect measure of water absorption, or relative increase in area, so that differences in shape and size of individual seeds could not affect water absorption estimates (Figure 1). The curves of each Petri dish were obtained, that is, for the each one of the three replications of each treatment.

The codes for obtaining the image-based curves were implemented in R language (www.R-project.org), with the use of the *EBImage* package (Pau et al., 2010).

Statistical analysis and modelling

The modelling of imbibition curves was performed according to Silva *et al*. (2018), using the *seedwater*® package version 2.0 (Silva, 2020) of software R. Peleg's (1988) model (Eq. 4) was fitted to describe the phase I.

$$IN(t) = \frac{t}{k_1 + k_2 t} + \varepsilon \tag{4}$$

Where k_1 and k_2 are the model parameters, representing the kinetic rate of hydration in phase I and the capacity constant, respectively. The inverse of k_1 was used to analyse the absorption rate of the treatments. The absorption rate was subjected to linear regression analysis to model the effect of herbicide concentrations by applying the Student's t-test and by evaluating the model goodness-of-fit of the through the coefficient of determination. 95% confidence bands were built as suggested by Silva et al. (2017) to compare the curves obtained with each herbicide concentration.

A generalized linear model with a binomial-type response with logit link was fitted for the analysis of primary root count. The goodness-of-fit of this model was assessed using the mean absolute error. An analysis of deviance was performed considering the nominal level of 5% of significance. The statistical analyses were performed using software R version 3.4.3.

Results and discussion

The seed lot presented water content of 8.5%, germination of 94% and an average weight of a thousand seeds of 355 g. These values are similar to those found by Silva et al. (2017), studying commercial seed lots in Brazil.

With the adaptation of the red excess index, giving a greater weight to the "Red" channel, effective segmentations were obtained, with little noise to be removed by the median filter. Herbicide solutions in higher concentrations showed a milky appearance. Nevertheless, we did not see any loss in segmentation quality (Figure 1). Some published works endorse our findings: Miller et al. (2018) achieved excellent results with the segmentation of maize seeds based on thresholding hue the histogram with the Otsu method (Otsu, 1979). Yan et al. (2011) performed the segmentation of maize seeds with the "Red" channel and fixed threshold (55/255).

With a "Blue" channel and fixed threshold (140/255), Lev and Blahovec (2017) obtained effective segmentation of wheat seeds in imbibition keeping light conditions controlled throughout the experiment. In the present study, with the use of the dark chamber over the scanner, no environmental effects of saturation or luminosity were observed during the entire image acquisition period. We observed that the use of the dark chamber also dismisses with the use of coloured background paper saturated with solution. Based on Otsu method (Otsu, 1979), we found thresholds between 0.75 and 1.10 for the modified *ExR*, considering the nominal amplitude of the new index from -2.4 to 3.0.

After 28 hours of soaking, it was necessary to add solution again to the Petri dishes, due to evaporation. The average increments (%) in the specific area of the seeds in the herbicide solutions are shown in Figure 2. There is a significant ($p < 0.05$) effect of the herbicide concentration in terms of both absorption rate and final gain. The treatments with 0 and 2% reached about 30% of swelling at the end of the 28 hours, with an absorption rate (increase) of 2.78 and 3.56% h^{-1} (Table 1), respectively. Silva et al. (2017) observed a lower value of the absorption rate, around 1.33% h^{-1} in seeds soaking on water only. This difference is believed to be due to the imbibition medium, since the absorption of seeds immersed in solution is expected to be faster than in moist paper substrate. On the other hand, the results found by Bolaji et al. (2017) indicate that the water content of several maize cultivars increased up to 24 to 36 h after the start of soaking, with absorption rates in the first 12 hours ranging from 2.2 to 4.2% h^{-1}. Both works cited corroborate our results in terms of duration of phase I. In addition, Miller et al. (2018) found area increments similar to the ones in the presented here, about 20% after 22 hours of soaking the seeds of several maize genotypes.

From 5% concentration, we observed final increments (28 h) significantly ($p < 0.05$) lower, between 13 and 20% (Figure 2). The treatments 5, 10 and 50% presented no statistical difference ($p > 0.05$) between 10 and 28 hours. There is a significant effect ($p < 0.05$) of the concentration of the herbicide on the absorption rate ($1/k_1$), with a logarithmic effect, changing from 3.56 % h^{-1} at 2% to 1.88 % h^{-1} at 50% concentration (Table 1).

Table 1. Estimates of the Peleg model for water absorption (% intumescence) of maize seeds soaked in solutions of the herbicide *s*-metolachlor + atrazine.

	Herbicide concentration** (%)					
Parameter	0	2	5	10	20	50
k_1	0.3608	0.2840	0.3200	0.3965	0.3924	0.5330
k_2	0.0191	0.0201	0.0525	0.0438	0.0307	0.0382
Absorption rate* (% h^{-1})	2.78	3.56	3.15	2.65	2.60	1.88
R^2	0.99	0.99	0.78	0.86	0.98	0.92

*Significative linear effect of herbicide concentration (F-test p-value = 0.0077). Fitted equation: $y = 3.9267 - 0.5036\times\log(x)$. $R^2 = 0.96$. **The 0% concentration was not used for fitting the regression model.

Reider et al. (1970) found that soybean seeds soaking in atrazine solution absorbed 61% of the amount of herbicide present in the solution after 48 h. The herbicide absorption continued after water absorption ceased, indicating that absorption of herbicides could not be associated with absorption of water except during the first few hours when the seed were rapidly imbibing water.

The absorption of herbicides and herbicide syrup by the seeds depends on both physical and chemical soil attributes, and on characteristics of the seed such as the permeability of the integument. In addition, herbicides can also alter stoma closure (Dayan and Duke, 2014), which may have contributed to the reduced water absorption observed in the present study as the concentrations of atrazine + *s*-metolachlor increased. Studies (Abenavoli et al., 2010; Cheng and Cheng, 2015) have also shown that inhibitions of H^+-ATPase activity and proton pumping function induced by herbicides, such as sorgoleone and juglone, affected the absorption of solute and water in maize, which may represent another explanation for the results we found.

Figure 3 shows the logit model fitted for primary root emission (%) under the effect of concentrations of *s*-metolachlor + atrazine throughout the experiment (114 hours). After 32 hours of soaking in the solution without herbicide, we observed radicle emission, indicating the beginning of phase III. Half of the seeds emitted radicle after 75 hours. And approximately 85% of seeds emitted primary root by the end of the experiment (114 hours).

From 20% concentration on, there was no root protrusion. With 10% concentration in aqueous solution, protrusion is only observed after 80 hours, reaching less than 35% emission at the end of the experiment. For 1% (2.9 g L^{-1} *s*-metolachlor + 3.7 g L^{-1} atrazine) concentration, emission reached 70% after four days. The fitted equation, with an average absolute error of 0.049, indicates that the absolute effect of the herbicide (-0.1285) is 2.3 times greater than the effect of the time (0.0548) of imbibition on the primary root emission.

Galon et al. (2016) observed that the herbicide *s*-metolachlor when applied solely in pre-emergence or ready-mix with atrazine promoted morphological changes in sweet sorghum cultivars, causing tissue swelling and young stem curling, leading to reduced growth and failure in the final stand of the crop. This is probably due to the main effect of *s*-metolachlor at the beginning of plant development, being absorbed in the coleoptile region of grasses and hypocotyl of dicotyledons (Pimentel et al., 2019). Cottingham and Hatzios (1992) observed a slow growth of maize seedlings for prolonging the contact time between the coleoptile and the soil treated with metolachlor. Another study conducted by Moore and Locke (2012) evaluated the phytotoxicity of atrazine and *s*-metolachlor in seeds of *Typha latifolia* L., showing that the exposure to atrazine in combination with *s*-metolachlor significantly reduced the root development. In addition, a stimulating effect for the development of coleoptile was observed at atrazine exposures at higher concentrations.

The phytotoxic effect of *s*-metolachlor on maize seed development is due to its capacity to impair some essential metabolic activities triggering oxidative stress and inhibiting long chain fatty acids synthesis (Panfili et al., 2019), thus affecting germination.

Qi et al. (2017) showed the effects of atrazine on the third stage of imbibition, which is associated with atrazine exposure during the vegetative and reproductive periods, significantly inhibited the germination and radicle growth of *Amaranthus retroflexus* L. The dose applied had significant effect on percent germination, mean germination time, hypocotyl length and radicle length. Higher effects of herbicides like atrazine were noticed on seeds when they are applied during the early reproductive stage rather than the later growth stage. This happens due to the rapid embryo cell division after fertilization followed by a subsequent fall in cell division (Qi et al., 2017).

In order to guarantee the management of weeds with initial applications of pre-planting, pre-emergence and initial post-planting, indicators that respond to short-term biochemical and microbiological criteria regarding sensitivity and herbicide doses in the system are necessary (Chaer and Tótola, 2007; Hang et al., 2007). As herbicide phytotoxicity in maize seeds is related to application attributes such as timing and dose and to soil attributes such as clay content, organic carbon and moisture, our findings suggest that is preferable to manage weeds with preemergence applications rather than with early preplant or preplant incorporated. Soil water content and herbicide sorption should be taken into account. In soil solution, 58 g L^{-1} *s*-metolachlor + 74 g L^{-1} atrazine can be fully restrictive to seed emergence.

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Conflicts of interest

None to declare.

References

AGROFIT (2020). Sistema de agrotóxicos fitossanitários. Ministério da Agricultura. Available at: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons

Abenavoli MR, Lupini A, Oliva S and Sorgona A. (2010) Allelochemical effects on net nitrate uptake and plasma membrane H^+-ATPase activity in maize seedlings. Biol Plant **54**,149–153.

Barnes ER, Knezevic SZ, Lawrence NC, Irmak S, Rodriguez O and Jhala AJ. (2019) Preemergence herbicide delays the critical time of weed removal in popcorn. Weed Technology **33**,785-793.

Bedmar F, Daniel PE, Costa JL and Giménez D. (2011) Sorption of acetochlor, S-metolachlor, and atrazine in surface and subsurface soil horizons of Argentina. Environmental Toxicology and Chemistry **30**,1990-1996.

Boelt B, Shrestha S, Salimi Z, Jørgensen JR, Nicolaisen M and Carstensen JM. (2018) Multispectral imaging – a new tool in seed quality assessment? Seed Science Research **28**,222-228.

Bolaji OT, Awonorin SO, Shittu TA and Sanni LO. (2017) Changes induced by soaking period on the physical properties of maize in the production of Ogi. Cogent Food & Agriculture **3**,1323571.

Carles L, Joly M, Bonnemoy F, Leremboure M, Donnadieu F, Batisson I and Besse-Hoggan P. (2018) Biodegradation and toxicity of a maize herbicide mixture: mesotrione, nicosulfuron and S-metolachlor. Journal of Hazardous Materials **354**,42-53.

Chaer MC and Tótola MR. (2007) Impacto do manejo de resíduos orgânicos durante a reforma de plantios de eucalipto sobre indicadores de qualidade do solo. R. Bras. Ci. Solo **31**(6),1381-1396.

Cheng F and Cheng Z. (2015) Research Progress on the use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy. Front Plant Sci **6**,1020.

Cottingham CK and Hatzios KK. (1992) Basis of differential tolerance of two corn hybrids (*Zea mays*) to metolachlor. Weed Sci **40**,359-363.

Dayan FE and Duke SO. (2014) Natural compounds as next-generation herbicides. Plant Physiol **166**(3),1090-1105.

de Oliveira LA, de Honorio MJ, Grecco KL and Tornisielo VL. (2019) Atrazine movement in corn cultivated soil using HYDRUS-2D: A comparison between real and simulated data. Journal of Environmental Management **248**,109311.

Food and Agriculture organization of the United Nations (2017) Crop Prospects for 2018. Statistics Division – FAOSTAT.

Galon L, Fernandes FF, Andres A, Silva AF and Forte CT. (2016) Selectivity and efficiency of herbicides in weed control on sweet sorghum. Pesquisa Agropecuária Brasileira **46**,123-131.

Gomes MP, Bicalho EM, Smedbol E, Cruz FVDS, Lucotte M and Garcia QS. (2017) Glyphosate can decrease germination of glyphosate-resistant soybeans. Journal of Agricultural and Food Chemistry **65**,2279-2286.

Halcro K, McNabb K, Lockinger A, Socquet-Juglard D, Bett KE and Noble SD. (2020) The BELT and phenoSEED platforms: shape and colour phenotyping of seed samples. Plant Methods **16**,49.

Hang S, Nassetta M, Cañas AI, Rampoldi EA, Fernández-Canigia MV and Díaz-Zorita M. (2007). Changes in the atrazine extractable residues in no-tilled Mollisols. Soil & Tillage Research **96**,243-249.

Heck T, Cinelli R, Polito RA, Ribas JL, Bagnara F, Hahn AM and Nunes AL. (2020). A importância dos herbicidas residuais no controle da tiririca. Brazilian Journal of Development, **6**(9),65147-65163.

ISTA (1985) International Seed Testing Association. Seed Science & Technology **13**,299-355.

Joly P, Bonnemoy F, Charvy JC, Bohatier J and Mallet C. (2013) Toxicity assessment of the maize herbicides S-metolachlor, benoxacor, mesotrione and nicosulfuron, and their corresponding commercial formulations, alone and in mixtures, using the Microtox test. Chemosphere **93**,2444–2450.

Karam D, da Cruz MB and Rizzardi MA. (2006) Manejo de plantas daninhas na cultura do milho. Available at: https://ainfo.cnptia.embrapa.br/digital/bitstream/item/30122/1/Manejo-plantas.pdf.

Lerro CC, Beane Freeman LE, Portengen L, Kang D, Lee K, Blair A, Lynch CF, Bakke B, De Roos AJ and Vermeulen RC. (2017) A longitudinal study of atrazine and 2,4-D exposure and oxidative stress markers among iowa corn farmers. Environmental and molecular mutagenesis, **58**(1),30–38.

Lev J and Blahovec J. (2017) Imbibition of wheat seeds: Application of image analysis, International Agrophysics **31**,475-481.

Lowry DM, Greiner D, Fretheim M, Ubben M and Dhanwada KR. (2013) Mechanism of metolachlor action due to alterations in cell cycle progression. Cell Biol Toxicol **29**,283–291.

Meyers SP, Nelson CJ and Harrocks RD. (1984) Temperature effects on imbibition, germination and respiration of grain sorghum seeds. Field Crops Research **8**,135-142.

Meyer GE, Hindman TW and Lakshmi K. (1999) Machine vision detection parameters for plant species identification. Proc. SPIE 3543, Precision Agriculture and Biological Quality.

Miller ND, Stelpflug SC, Kaeppler SM and Spalding EP. (2018) A machine vision platform for measuring imbibition of maize kernels: quantification of genetic effects and correlations with germination. Plant Methods **14**,115.

Moore MT and Locke MA. (2012) Phytotoxicity of Atrazine, S-Metolachlor, and Permethrin to *Typha latifolia* (Linneaus) Germination and Seedling Growth. Bulletin of Environmental Contamination and Toxicology **89**,292–295.

Mueller CT, Boswell BW, Mueller SS and Steckel LE. (2014) Dissipation of Fomesafen, Saflufenacil, Sulfentrazone, and Flumioxazin from a Tennessee Soil under Field Conditions. Weed Science **62**,664-671.

Noblet A, Leymarie J and Bailly C. (2017) Chilling temperature remodels phospholipidome of *Zea mays* seeds during imbibition. Scientific Reports **7**,8886.

Otsu N. (1979) A threshold selection method from gray-level histograms. IEEE Transactions on Systems, Man, and Cybernetics **9**,62-66.

Panfili I, Bartucca ML, Marrollo G, Povero G and Del Buono D. (2019) Application of a plant biostimulant to improve maize (*Zea mays*) tolerance to metolachlor. J Agric Food Chem. **6;67**(44),12164-12171.

Pau G, Fuchs F, Sklyar O, Boutros M and Huber W. (2010) EBImage - an R package for image processing with applications to cellular phenotypes. Bioinformatics **26**,979-981.

Peleg M. (1988) An empirical-model for the description of the moisture sorption curves. Journal of Food Science **53**,1216–1217.

Pimentel GV, Guimarães DF, Moreira SG, Ávila MOT, Martins IA and Bruzi AT. (2019) Selectivity and effectiveness of herbicides in the grain sorghum crop. Planta Daninha **37**,e019187771.

Qi Y, Yan B, Fu G, Guan X, Du L and Li J. (2017) Germination of seeds and seedling growth of *Amaranthus retroflexus* L. following sublethal exposure of parent plants to herbicides. Sci Rep **7**(1),157.

Reider G, Buchholtz KP and Kust CA. (1970) Uptake of herbicides by soybean seed. Weed Science **18**,101-105.

Silva AR. (2020) Seedwater: models for drying and soaking kinetics of seeds. R package version 2.0. Available at: https://cran.r-project.org/package=seedwater.

Silva AR and Lima RP. (2017) Determination of maximum curvature point with the R package soilphysics. International Journal of Current Research **9**,45241-45245.

Song Y, Fiaz M, Kim DW, Kim J and Kwon CH. (2019) Increasing forage yield and effective weed control of corn-soybean mixed forage for livestock through using by different herbicides. Journal of Animal Science and Technology **61**(4),185-191.

Subedi M, Willenborg CJ and Vandenberg A. (2017) Influence of harvest aid herbicides on seed germination, seedling vigor and milling quality traits of red lentil (*Lens culinaris* L.). Frontiers in Plant Science **8**,311.

Tanabata T, Shibaya T, Hori K, Ebana K and Yano M. (2012) SmartGrain: high-through-put phenotyping software for measuring seed shape through image analysis. Plant Physiology **160**,1871–1880.

Yan X, Wang J, Liu S and Zhang C (2011) Purity identification of maize seed based on color characteristics, pp. 620-628 in Li D., Liu Y. and Chen Y. (Ed.) Computer and Computing Technologies in Agriculture IV. Berlin, Heidelberg, Springer.

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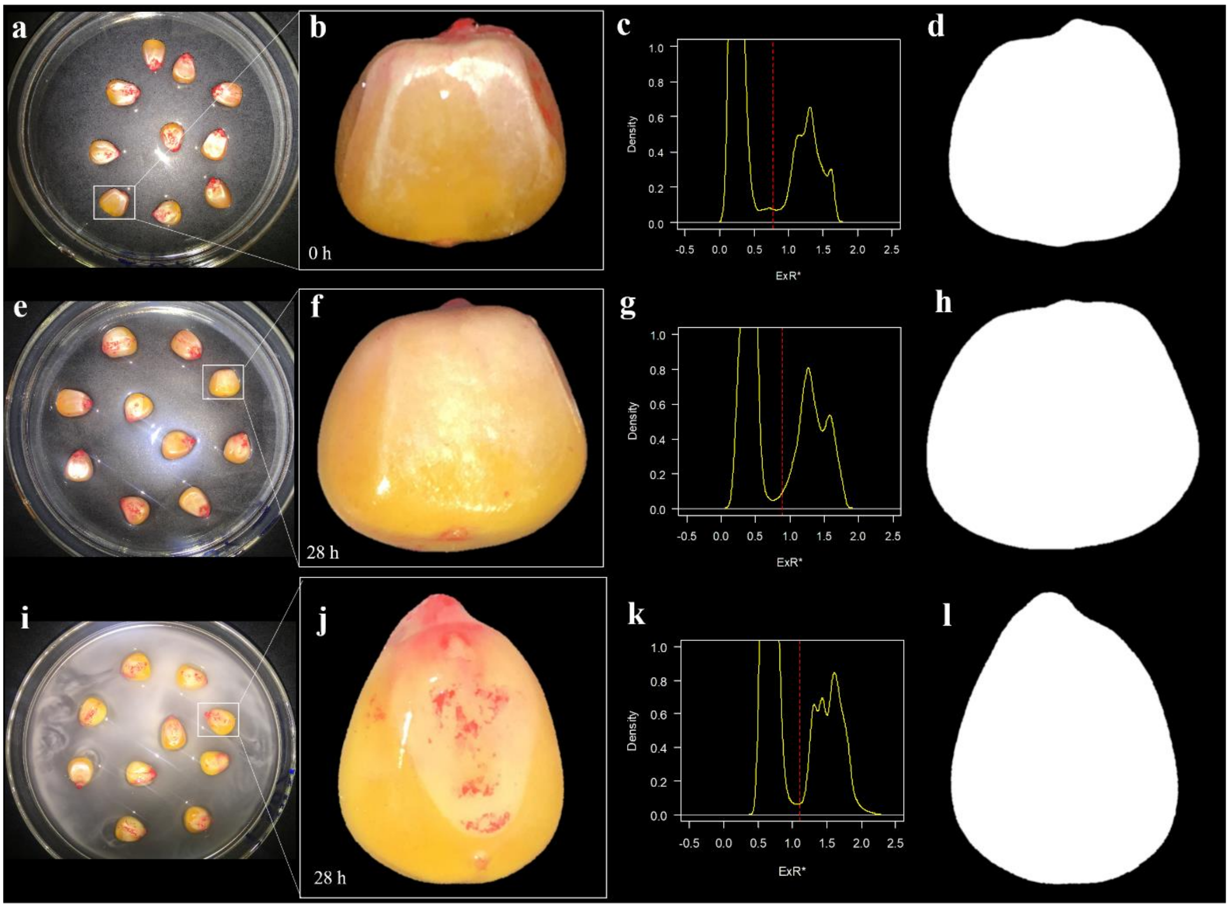

Figure 1. Maize seeds in imbibition process. (**a**) The seeds at the beginning of imbibition (0 h) in 0% solution of the herbicide S-Metolachlor + Atrazine. (**b**) A zoom in a segmented seed. (**c**) The kernel density of the adapted red excess index (*ExR**), with demarcation of the threshold between background (left) and seed (right). (**d**) The result of segmentation. (**e-h**) The analyses of the same seeds after 28 hours of soaking. (**i-l**) The analysis of seeds soaking in 50% herbicide solution after 28 hours.

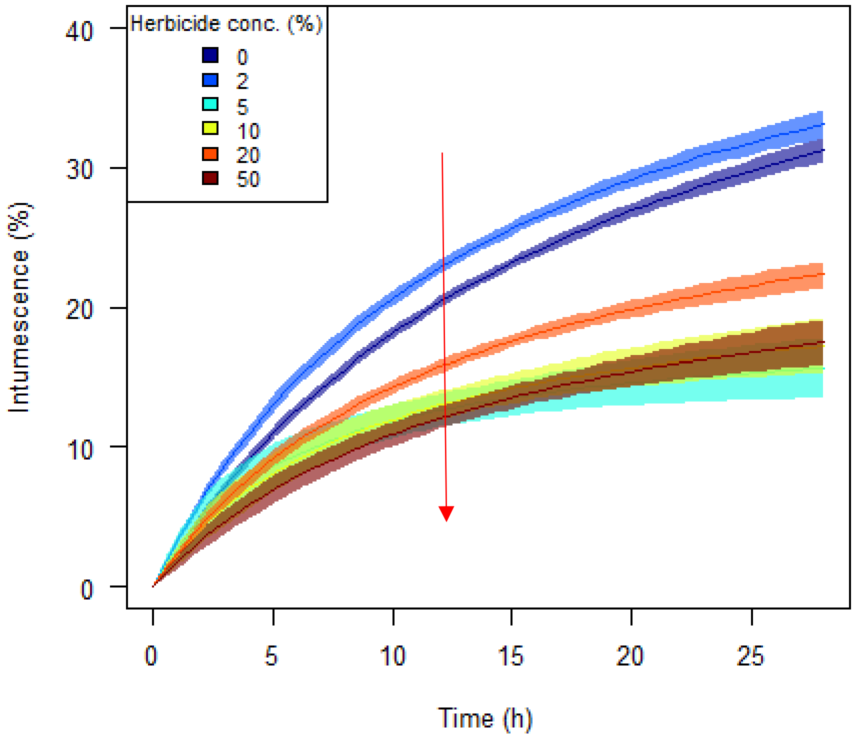

Figure 2. 95% confidence bands for maize seed soaking curves under the effect of *S*-Metolachlor + Atrazine concentrations.

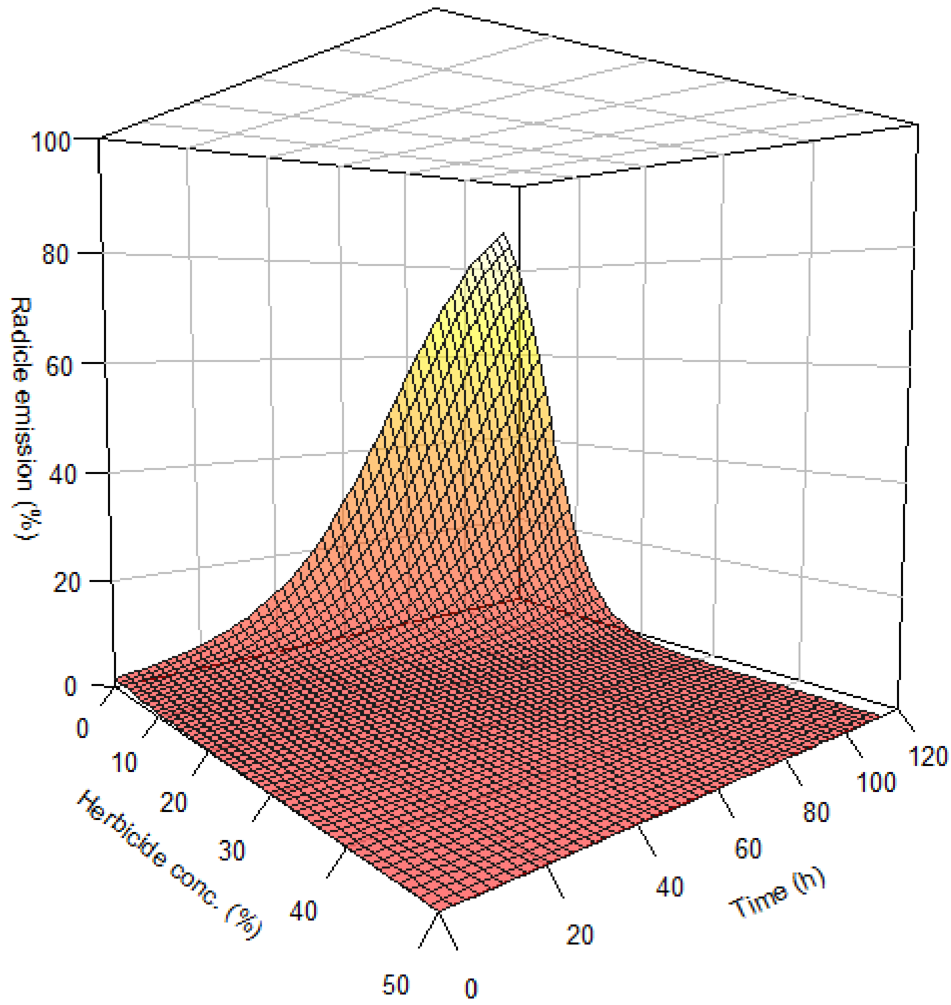

Figure 3. Regression of radicle emission (z, %) as a function of the concentration of the herbicide S-Metolachlor + Atrazine (x, %) and the time (y, hours) of soaking maize seeds. Fitted equation: $z(x, y) = \frac{\exp(-4.1727-0.1285x+0.0548y-0.0013xy)}{1+\exp(-4.1727-0.1285x+0.0548y-0.0013xy)}$.

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Books

Cromarty AS, Ellis RH and Roberts EH (1985) The design of seed storage facilities for genetic conservation. Rome, International Board of Plant Genetic Resources

Leopold AC and Vertucci CW (1986) Physical attributes of desiccated seeds, pp. 22-34 in Leopold, A.C. (Ed.) Membranes, metabolism and dry organisms. Ithaca, NY, Cornell University Press.

Chang CW (1975) Fluorides, pp. 57-95 in Mudd JB; Kowlowski TT (Eds) Responses of plants to air pollution. New York, Academic Press.

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Eira MTS, Walters C and Caldas LS (1999) Critical water content for desiccation damage in coffee seeds: a role for aqueous glasses? p. 105 in Proceedings from the VI international workshop of seed biology, January 1999, Merida, Mexico.

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