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Image-based modelling of the effect of s-metolachlor plus atrazine on the soaking kinetics of maize seeds**

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A b s t r a c t. Pre-emergent herbicides can have negative effects on maize seeds. The objective of this study was to model seed soaking curves through the processing of red-green-blue imagery of maize seeds under the influence of concentrations of *s*-metolachlor + atrazine on both the soaking kinetics and primary root emission. Seeds were placed to soak for 114 h in Petri dishes containing aqueous solutions of a herbicide containing *s*-metolachlor (290 g l^{-1}) + atrazine (370 g l^{-1}) with the following concentrations: 0% (water only), 2, 5, 10, 20 and 50%, based on the recommended dose (4.0 l of the commercial product per hectare). The images were systematically taken from a flatbed scanner with artificial light control. The red excess index was adapted to improve image segmentation. From the binary masks applied, the soaking curves for each herbicide concentration were obtained using estimates of seed intumescence over time. The soaking curves were described by fitting Peleg's model. The herbicide concentration has significant effects on both the absorption rate and primary root emission; the absorption rate was reduced by 50%. A concentration of *s*-metolachlor (290 g l^{-1}) + atrazine (370 g l^{-1}) in aqueous solution that is above 20% can fully inhibit seed germination.

K e y w o r d s: imbibition curve; *Zea mays*; herbicide; image processing.

INTRODUCTION

Maize (*Zea mays* L.) is an important crop on a global scale as it can be used by humans and animals (cattle, poultry and pigs) as a food source. Agricultural practices in the production of maize crops have evolved considerably in recent decades, with the adoption of integrated pest management. This trend triggered the use of herbicides, which are often used in mixtures, in order to improve their efficiency and range concerning weed control (Carles *et al*., 2018). However, this may cause negative effects on crop seeds, altering the kinetics of their absorption of water and/or solutes, a process commonly studied through the imbibition curve.

The imbibition or rehydration process of viable maize seeds takes place, under the appropriate conditions 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, which again requires water absorption for cell division (Noblet *et al*., 2017).

The standard method for determining the imbibition curve consists of systematically weighing seed samples during 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 the 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. That being

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the case, Miller *et al.* (2018) developed a system based on RGB scanner imaging for automating measurements related to the imbibition of corn seeds.

Da Silva *et al.* (2018) presented models for the seed imbibition curve of the different species cultivated, including maize, showing how to calculate certain important parameters such as absorption rates taking into account the presence or absence of the third phase. Modelling the imbibition curve allows for a comparison to be made between seed lots with differences in physiological potential or those subjected to different imbibition conditions.

Environmental factors such as temperature and humidity affect the duration of the imbibition phases and germination itself, thus allowing, for example, ideal planting conditions to be defined. It is also known that phytosanitary products such as herbicides can affect the final stage of imbibition – the emission of the primary root (Subedi *et al.*, 2017). But the explanation for this lies in the 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, thereby promoting H_2O_2 accumulation in the seeds of non-genetically modified soybean and consequently, reducing germination rates. Moore and Locke (2012) found significant reductions in the root growth of *Typha latifolia* (L.) which had been subjected to *s*-metolachlor + atrazine. In sorghum hybrids, reductions of up to 69% in plant stand were caused by the pre-emergence application of *s*-metolachlor + atrazine (Pimentel *et al.*, 2019).

Atrazine is a herbicide of the triazine group (C1) which is used extensively throughout the world for the control of broadleaf plants and also some narrow-leafed species, being used predominantly in the cultivation of maize, given its selectivity for this crop and its broad spectrum of control for different weed plants (Lerro *et al.*, 2017). This herbicide is 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 widely detected herbicides in surface waters (de Oliveira *et al.*, 2019). Its mechanism of action involves the photosystem II, the site of action is the thylakoid membrane, this results in electron transport block and the interruption of the fixation of CO_2 and consequently, the reduction in ATP and $NADH_2$ production; in addition, this herbicide also induces oxidative stress and causes lipid peroxidation (Heck *et al.*, 2020; Lerro *et al.*, 2017).

The herbicide *s*-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide) has also been widely used in maize crops, it is applied in pre-emergence or in pre-planting and incorporated for weed control, being one of the most widely sold herbicides for the 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 having a higher herbicidal activity than the R isomer (Lowry *et al.*, 2013). The use of *s*-metolachlor is noteworthy, mainly with regard to its effectiveness in controlling *Commelia benghalensis* L. (Pimentel *et al.*, 2019). This herbicide can inhibit the synthesis of proteins, chlorophyll and very long chain fatty acids, in addition to interfering with cell division, thereby inhibiting the growth of target plants (Lowry *et al.*, 2013). Moreover, the phytotoxic effect of *s*-metolachlor may be observed after the germination of seedlings, and culminating in the non-opening of the coleoptile and the wrinkling of the definitive leaves, which is caused by the lower growth of the central vein in relation to the growth of the leaf blade.

According to Barnes *et al.* (2019), *s*-metolachlor + atrazine can provide broad-spectrum weed control as compared with either herbicide applied alone. With this perspective and considering the current need for pre-emergent herbicides to control weeds in maize crops, a study of the process of seed soaking in herbicide solutions may reveal potentially harmful effects to the crop (Mueller *et al.* 2014). In addition, increasing the level of understanding of the effect of pre-emergent herbicides on both the absorption of water and the germination rate is essential to carrying out the chemical management of weeds taking into account the edaphic conditions, in particular, the clay and organic matter content, given the sorption of pre-emergent herbicides in these particles of soil. Thus, it is possible to consider, with a reasonable degree of confidence, the feasibility of changing the timing of herbicide application or sowing to guarantee the desired plant stand.

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

MATERIALS AND METHODS

Commercial maize seeds, hybrid Syngenta Status Vip3, were obtained during the 2018/2019 harvest in an experimental area located at the geographic coordinates 18º55'32.9"S and 48º09'50.8"W, Mid-Western Brazil. An industrial phytosanitary treatment of the seeds was carried out using fungicide (100 ml 100 kg^{-1} Metalaxyl-M 20 g l^{-1} + Thiabendazole 150 g l^{-1} + Fludioxonil 25 g l^{-1}) and insecticide (120 ml 60 000 seeds^{-1} of 350 g l^{-1} Thiametoxam).

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

Germination: This was carried out in four replications of 50 seeds selected in a random manner. 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, of the Mangelsdorf type, at a constant temperature of 25°C. The count of normal seedlings was performed 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°C for 24 h. The initial and final weight of the samples was computed using 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) in order to estimate the weight of a thousand seeds.

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, with the aim of simulating 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 (https://agrofit.agricultura.gov.br/). In addition, the concentrations used were based on studies by Oliveira *et al*. (2019), Joly *et al*. (2013) and Moore and Locke (2012).

Three replications of ten seeds each were placed to soak in Petri dishes with 90 mm diameters containing 20 ml of the solution and stored in a germination chamber, of the Mangelsdorf type, at 25°C during the entire imbibition process. RGB images of the dishes were captured using a flatbed scanner, model Epson Perfection V19, with a resolution of 600 pixels per inch and dimensions of 100×100 mm, every hour during the first 6 h, and then at 2 h intervals until a duration of 12 h of imbibition was reached; afterwards, the images were acquired at intervals of 4 h up to a duration of 24 h of imbibition and, finally, at intervals of 6 h until 50% of the seeds of each sample presented the emission of the primary root. In order to control light variation, a box coated with opaque black paper was placed over the scanner platform, thereby covering it completely.

The images were processed as follows:

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

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

ii) seed segmentation through an adaptation (Eq. 2) of the red excess index (Meyer *et al*., 1999) and the 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 the relative increase in area, so that differences in the shape and size of individual seeds could not affect water absorption estimates (Fig. 1). The curves produced by each Petri dish were obtained, that is, for 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).

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

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

where: k_1 (h $\%^{-1}$) and k_2 are the model parameters, representing the kinetic rate of hydration in phase I and the capacity constant, respectively; ε – the residual term. The inverse of k_1 was used to analyse the absorption rate of the treatments. The absorption rate was subjected to regression analysis in order to model the effect of herbicide concentrations by applying the Student's t-test and by evaluating the model goodness-of-fit through the coefficient of determination. 95% confidence bands were imposed as suggested by Da Silva *et al*. (2018) 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 the primary root count. The goodness-of-fit of this model was assessed using the mean absolute error. An analysis of deviance was performed with regard to 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 a water content of 8.5%, a germination rate of 94% and the average weight of a thousand seeds amounted to 355 g. These values are similar to those found by Da Silva *et al*. (2018), 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, no

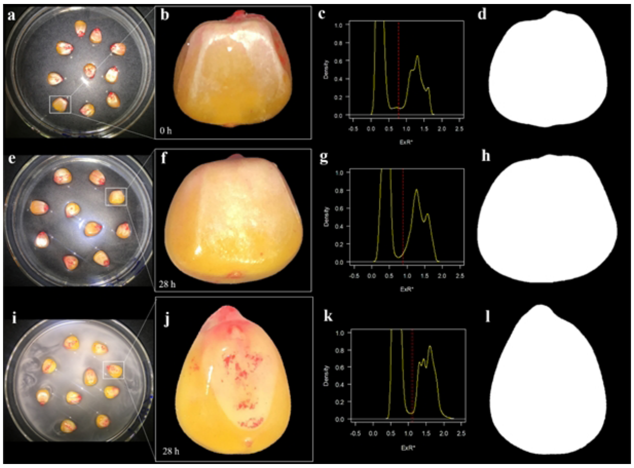

Fig. 1. Maize seeds during the imbibition process. (a) The seeds at the beginning of imbibition (0 h) in 0% solution of the herbicide S-Metolachlor + Atrazine. (b) A zoom in a segmented seed. (c) The kernel density of the adapted red excess index (ExR*), with demarcation of the threshold between the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

loss in segmentation quality was observed (Fig. 1). Some published works have endorsed our findings: Miller *et al.* (2018) achieved excellent results with the segmentation of maize seeds based on the thresholding hue of the histogram using 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 the "Blue" channel and fixed threshold (140/255), Lev and Blahovec (2017) obtained the effective segmentation of wheat seeds during imbibition by maintaining controlled light conditions 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. It was observed that the use of a dark chamber may also be omitted with the application of coloured background paper saturated with solution. Based on the Otsu method (Otsu, 1979), thresholds of between 0.75 and 1.10 were found for the modified *ExR*, with regard to the nominal amplitude of the new index which ranged from –2.4 to 3.0.

After 28 h of soaking, it was necessary to add the solution to the Petri dishes again, due to evaporation. The average increments (%) in the specific area of the seeds in the herbicide solutions are shown in Fig. 2. The significant ($p<0.05$) effect of the herbicide concentration can be observed in terms of both the absorption rate and final gain. Treatments with concentrations of 0 and 2% resulted in a 30% swelling at the end of the 28-h experimental period, with an absorption rate (increase) of 2.78 and 3.56% h^{-1} (Table 1), respectively. Da Silva *et al.* (2018) observed the lower value of the absorption rate, which was around 1.33% h^{-1} in seeds soaking in water only. This difference

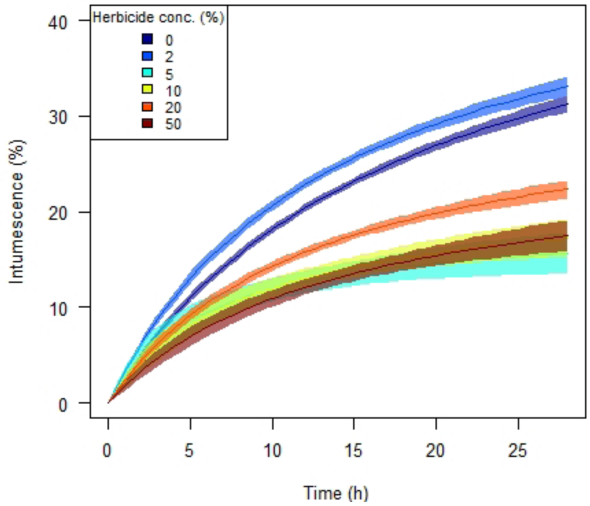

Fig. 2. 95% confidence bands for maize seed soaking curves under the effect of S-Metolachlor + Atrazine concentrations.

is believed to be due to the imbibition medium, since the absorption of seeds immersed in solution is expected to be more rapid than that 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 a duration of 24 to 36 h after the start of the soaking period, with absorption rates in the first 12 h ranging from 2.2 to 4.2% h^{-1}. Both works cited corroborate our results in terms of the duration of phase I. In addition, Miller *et al.* (2018) found area increments similar to the ones being presented here, at about 20% after 22 h of soaking the seeds of several maize genotypes.

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

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. Herbicide absorption

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

Parameter	Herbicide concentration** (%)					
	0	2	5	10	20	50
k_1	0.361	0.284	0.320	0.397	0.392	0.533
k_2	0.019	0.020	0.053	0.044	0.031	0.038
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

*Significant 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.

continued after water absorption ceased, thereby indicating that the absorption of herbicides could not be associated with the absorption of water except during the first few hours of the experiment when the seeds were rapidly imbibing water.

The absorption of herbicides and herbicide syrup by the seeds depends on both the physical and chemical soil attributes, and also on the 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 in H^+-ATPase activity and the proton pumping function induced by herbicides, such as sorgoleone and juglone, are affected by the absorption of solute and water in maize, which may represent another explanation for the results that were 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 h). After 32 h of soaking in a solution without herbicide, radicle emission was observed, thereby indicating the beginning of phase III. Half of the seeds emitted a radicle after 75 h. And approximately 85% of the seeds emitted a primary root by the end of the experiment (114 h).

From a 20% concentration upwards, there was no root protrusion. With a 10% concentration in aqueous solution, the protrusion is only observed after 80 h, and reaching a rate of less than 35% emission at the end of the experiment. For a 1% (2.9 g l^{-1} *s*-metolachlor + 3.7 g l^{-1} atrazine) concentration, emission reached a rate of 70% after four days. The fitted equation, with an average absolute error of 0.049, indicates that the absolute effect of the herbicide (–0.129) is 2.3 times greater than the effect of the time (0.055) of imbibition on the primary root emission.

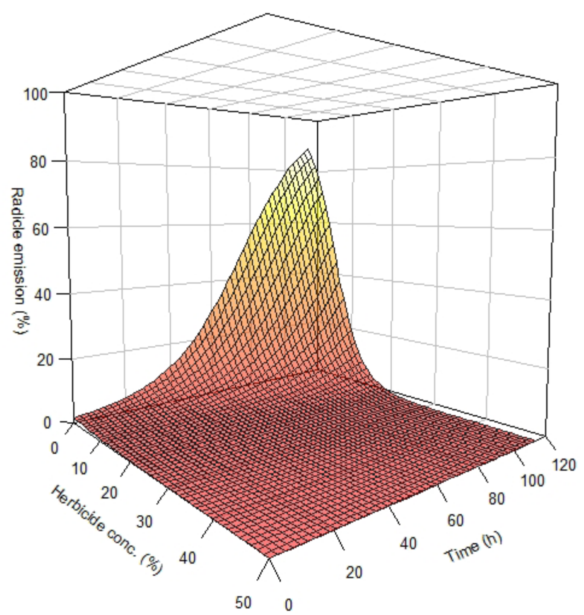

Fig. 3. Regression of radicle emission (z, %) as a function of the concentration of the herbicide S-Metolachlor + Atrazine (x, %) and the time (y, h) of soaking maize seeds. Fitted equation:

$$z\,(x,y) = \frac{\exp\left(-4.1727 - 0.1285x + 0.0548y - 0.0013xy\right)}{1 + \exp\left(-4.1727 - 0.1285x + 0.0548y - 0.0013xy\right)}.$$

Galon *et al*. (2016) observed that the herbicide *s*-metolachlor, when applied solely in a pre-emergence or ready-mix form with atrazine promoted morphological changes in sweet sorghum cultivars, causing tissue swelling and young stem curling, and leading to reduced growth and a failure in the final stand of the crop. This is probably due to the main effect of *s*-metolachlor occurring at the beginning of plant development, as it is absorbed in the coleoptile region of grasses and in the hypocotyl of dicotyledons (Pimentel *et al*., 2019). Cottingham and Hatzios (1992) observed the slow growth of maize seedlings in the case of prolonged 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 exposure to atrazine in combination with *s*-metolachlor significantly reduced root development. In addition, a stimulating effect which stimulated the development of the coleoptile was observed at atrazine exposures with 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 also inhibiting long chain fatty acids synthesis (Panfili *et al*., 2019), thereby affecting germination rates.

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, it significantly inhibited the germination and radicle growth of *Amaranthus retroflexus* L. The dose applied had a significant effect on the germination rate, mean germination time, hypocotyl length and radicle length. It was noted that herbicides like atrazine had more of an effect on seeds when they are applied during the early reproductive stage rather than during the later growth stage. This occurs due to the rapid embryo cell division after fertilization which is followed by a subsequent decrease in cell division rates (Qi *et al*., 2017).

In order to guarantee the management of weeds with initial pre-planting, pre-emergence and initial post-planting applications, indicators that respond to short-term biochemical and microbiological criteria with regard to 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 certain application attributes such as timing and dose and also to soil attributes such as clay content, organic carbon and moisture, our findings suggest that it is preferable to manage weeds with pre-emergence applications rather than with early preplant or preplant incorporation. 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 may be fully restrictive to seed emergence.

CONCLUSIONS

1. With this pioneering study, we have shown how to model the effect of an herbicide on the soaking kinetics of maize seeds through an image-based computation of the imbibition curve.

2. A modified red excess colour index was presented to effectively categorize maize seeds with red-green-blue images taken under a light-controlled environment.

3. The concentration of *s*-metolachlor + atrazine in aqueous solution reaching the maize seeds has significant effects on both the absorption rate and primary root emission. The absorption rate is reduced logarithmically. Concentrations of *s*-metolachlor (290 g l^{-1}) + atrazine (370 g l^{-1}) of over 20% can fully inhibit seed germination.

Conflict of interests: The authors declare no competing financial interests or personal relationships that could have appeared to influence the content of this article.

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