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REGULAR ARTICLE

Eucalyptus and *Ipomoea nil* phytotoxicity after herbicide application

Ilca Puertas de Freitas e Silva[1], Josué Ferreira Silva Junior[1], Caio Antonio Carbonari[2], Edivaldo Domingues Velini[2], Rosilaine Araldi de Castro[3], Leandro Tropaldi[4], João Domingos Rodrigues[2]

[1]Federal University of Triângulo Mineiro – UFTM, Iturama, MG, Brazil.
[2]São Paulo State University – UNESP, Botucatu, SP, Brazil
[3]AgroQuatro-S Agronomic Experimentation, Orlândia, SP, Brazil.
[4]São Paulo State University – UNESP, Dracena, SP, Brazil.

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Abstract

The presence of weeds in forest estates is considered one of the biggest problems in the implantation, maintenance, and renovation of eucalyptus plantations. This research aims to evaluate the phytotoxicity of herbicides on eucalyptus and I. nil. The experiment was installed inside a greenhouse in a completely randomized design with five replications. The treatments consisted of the application of 0.2% (v/v) adjuvant with the herbicides: atrazine (2250 g i.a. ha^{-1}), clomazone (720 g i.a. ha^{-1}), sulfentrazone (600 g i.a. ha^{-1}), glyphosate (1440 g i.a. ha^{-1}), and control. The following variables were analyzed: electron transport rate (ETR), water consumption, and plant phytotoxicity. For I. nil plants treated with atrazine, it was possible to detect phytotoxicity previous to the appearance of symptoms in the visual analysis, due to the inhibition of ETR at 24 hours after application (HAA). The highest levels of phytotoxicity for eucalyptus and *I. nil* were obtained by glyphosate and sulfentrazone, respectively.

Keywords

Photosystem; *Eucalyptus urograndes*; Viola string. Fluorometer.

Introduction

The planted forest area in Brazil reached 9.3 million hectares in 2020, out of which eucalyptus represents 80.2% (Campos, 2021). That is due to its adaptation in various Brazilian soil and climate conditions, fast vegetative development, and wide employability of its wood (Embrapa, 2019). Eucalyptus cultivation in Brazil continues to expand, accounting for 38% of the world's total cultivated area (Ibge, 2019).

Correct weed management is among the silvicultural practices that most interfere with productivity (Schetz et al., 2021), since the initial development of eucalyptus is slow compared to that of weeds, which favours the effects of competition from weed species in relation to eucalyptus culture (Carbonari, 2017).

Among the integrated management tools, the use of herbicides stands out, mainly in large-scale plantations (Carbonari, 2017), however, there are few active ingredients that are registered for the eucalyptus crop when compared to countries that grow eucalyptus in large areas (Pereira; Alves, 2015).

According to the Brazilian Ministry of Agriculture, Livestock, and Development (MAPA), the cultivation of eucalyptus has 173 registered products, out of which 76% correspond to herbicides (Mapa, 2021). There is a portion of EMRnon-selective herbicides that are used in post-emergence (Minogue et al., 2018), a fact that demonstrates the need for research to measure the level of phytotoxicity caused by these herbicides.

In the chemical control of weeds in silvicultural systems, the herbicide glyphosate stands out for being widely used in commercial plantations. Nevertheless, glyphosate is a non-selective herbicide, that is, the application is carried out in a directed way on the weed (Machado et al., 2009). Glyphosate blocks the enzyme EPSPs (5-enolpyruvylshikimate-3-phosphate synthase), leading to the accumulation of shikimate in vacuoles (Fedtke; Duke, 2005) and blocking the synthesis of three aromatic amino acids: tryptophan, phenylalanine, and tyrosine (Zablotowicz; Reddy, 2004).

Another herbicide that is widely used for the eucalyptus crop is sulfentrazone, which can be applied before or after transplanting the seedlings, with the intensity of phytotoxicity dependent on the clone, as verified by Carbonari et al. (2012).

* Corresponding author
E-mail address: ilca_pfs@yahoo.com.br (I.P.F. Silva).

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This herbicide inhibits the activity of the enzyme protoporphyrinogen oxidase (PROTOX) that is present in the biosynthesis of chlorophyll (Freitas et al., 2014).

There are herbicides that act directly or indirectly on chlorophyll fluorescence, according to Dayan; Zacarro (2012), who observed that, in addition to photosynthesis inhibitor herbicides (photosystems I and II), the mechanisms of action that inhibit carotenoid synthesis and lipid peroxidation (PROTOX inhibitors) can also influence chlorophyll fluorescence. In this way, the measurement of chlorophyll fluorescence can be a tool to provide detailed information about the phytotoxicity of herbicides.

The herbicide atrazine blocks the flow of electrons between photosystems (II and I), which generates energy dissipation from photosystem II in the form of fluorescence, when the flow is interrupted (Ventrella et al., 2010). In this way, it is possible to monitor the action of photosystem II inhibitor herbicides a few hours after application on intact leaves, before the appearance of visual symptoms of phytotoxicity (Araldi et al., 2011; Girotto et al., 2011; Araldi et al., 2015, Tropaldi et al., 2015).

Another mechanism of action that influences chlorophyll fluorescence is carotenoid synthesis inhibitor herbicides, such as clomazone. Carotenoids are responsible for dissipating energy that was not used in photosynthesis, when excess energy convert chlorophylls to the triplet state (^{3}Chl), initiating several degradation reactions, including chlorophyll itself, as well as lipid peroxidation (Dayan; Zacarro, 2012).

Water consumption is another way of evaluating the phytotoxicity of herbicides in plants, since the efficiency of water use by plants is directly related to the time of stomatal opening. Thus, the action of herbicides can reduce stomatal conductance and consequently decrease the consumption of water leading plants to death (Brodribb; Holbrook, 2003).

Given the above, this study aimed to evaluate the phytotoxicity of herbicides on eucalyptus and *I. nil*.

Materials and methods

The I. nil plant specimens used in this research were sown and cultivated in a substrate composed of pine bark and vermiculite (Plant Max®) for approximately 30 days. Afterwards, they were carefully removed from the substrate, avoiding possible injuries to the root system. Then, they were placed in 50 ml falcon tubes, filled with 45 ml of water. The opening of the tube was insulated with aluminium foil to avoid evaporation from the system, ensuring that the only way to consume water was through plant transpiration.

Eucalyptus urograndes (clone 219) seedlings with 30 cm in height also had the root substrate removed and placed in disposable cups filled with 500 ml of water and isolated with aluminium foil, as described above. At the same time, flasks with water were kept covered with aluminium foil and opened to assess the efficiency of the method and verify the evaporative demand of the environment. The falcon tubes and disposable cups with the plants in water were left to rest for 24 hours for the restoration of the plants, kept in greenhouse conditions (average temperature 28°C and relative humidity 70%).

The research consisted of five treatments: control (without herbicide application) and the application of four herbicides: atrazine, clomazone, sulfentrazone, and glyphosate, in the respective doses of 2250 g i.a. ha^{-1}, 720 g i.a. ha^{-1}, 600 g i.a. ha^{-1}, and 1440^{-1} with the addition of 0.2% (v/v) post-emergence adjuvant. The experiment was installed in a completely randomized design with five replications.

For the application of herbicides, a stationary sprayer was installed in the laboratory, equipped with a bar containing four XR11002 tips, at a constant pressure of 1.5 bar, pressurized by compressed air, with a spray consumption of 200 L ha^{-1} (Figure 1). The temperature at the time of application was 25°C, with a relative humidity of 70%. After the treatments were applied, the plants were transported again to the greenhouse, where they remained until the end of the experiment.

The analysed variables were as follows: electron transport rate (ETR), water consumption, and plant phytotoxicity. The ETR was obtained by measuring the chlorophyll fluorescence in the middle part of the plant leaves using the Yield protocol of the portable modulated fluorometer Multi-Mode Chlorophyll Fluorometer OS5p (Opti-Science) (Tropaldi et al., 2015). Measurements were performed at intervals of 0, 1, 24, 48, 72, 96, 120, 144, 168, and 192 hours after application (HAA) of the herbicides.

Figure 1: Treatment application using a stationary sprayer (XR11002) equipped with a bar with four tips.

Water consumption was determined by the volume of water transpired per leaf area of the plants, expressed in cm^3 cm^{-2}. The volume of transpired water was verified by daily weighing the water amount from the containers and leaf area using the leaf disc method, as described by Toebe et al. (2010), in which the leaf area is the ratio of the dry mass of a known area and the total leaf dry mass.

Phytotoxicity was visually analysed using the percentage scale, in which zero corresponds to no injury and 100 to plant death (Sbcpd, 1995), in the intervals of 72, 120, and 168 HAA for I. nil plants and at 120, 144, and 168 HAA for eucalyptus.

The ETR data were expressed as a percentage of the control and submitted to analysis of variance using the F test at 5% probability and the comparison of means using the LSD test at 10% probability. The water consumption and phytotoxicity data were submitted to analysis of variance by the F test at 5% probability and the means compared by the Tukey test at 5% probability.

Results and discussion

The herbicides atrazine (Figure 2a), clomazone (Figure 2b), and sulfentrazone (Figure 2c) caused a larger reduction in ETR for *I. nil* when compared to eucalyptus, while glyphosate inhibited ETR for both species in a similar manner (Figure 2d).

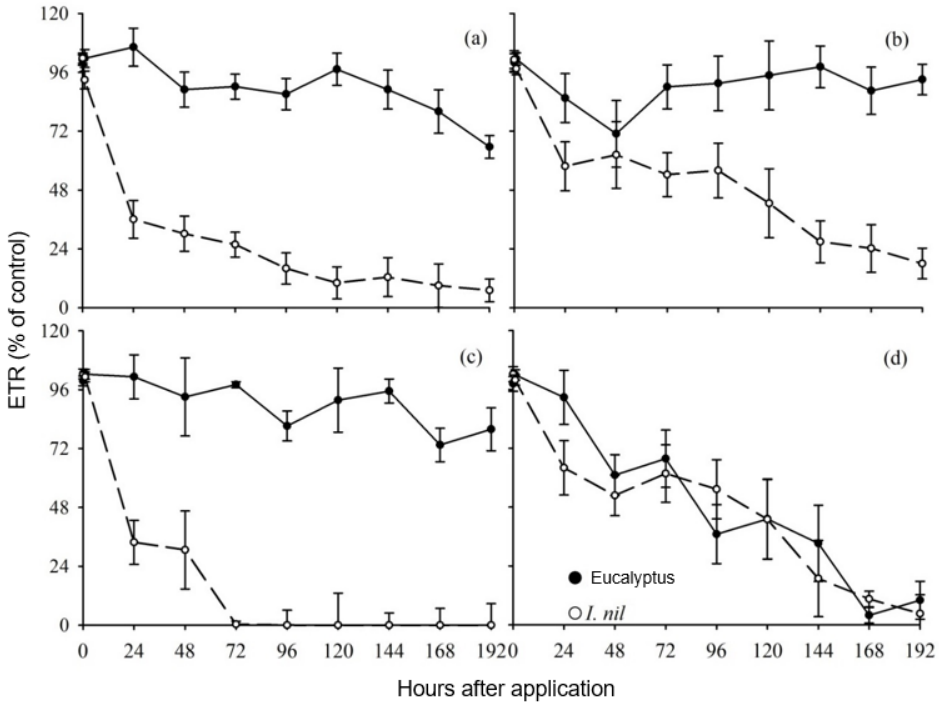

Figure 2: Electron transport rate (ETR), in relation to time, for eucalyptus and *I. nil* after application of atrazine (a), clomazone (b), sulfentrazone (c), and glyphosate (d). Bars indicate the DMS of the evaluated periods.

The application of atrazine effected little toxicity to eucalyptus plants, with reductions of less than 35% of the ETR during the evaluated period. Yet, a gradual reduction can be observed from 120 HAA, possibly due to the antioxidant defence of the plant, such as the increase in the enzyme superoxide dismutase (SOD). According to Alves et al. (2005), when working with the identification of expressed sequences of genes involved in defence mechanisms against environmental stress in a eucalyptus database produced by the FORESTs/FAPESP project, an increase in SOD activity was observed in response to treatment with a herbicide that blocks electron transport, such as atrazine and diuron.

For *I. nil* plants, atrazine rapidly inhibited ETR by more than 50% with 24 HAA. Rapid ETR inhibition was also observed by Dayan et al. (2009), in *Digitaria sanguinalis* and *Abutilon theophrasti plants*, when submitted to the application of amicarbazone and atrazine, in which there was complete inhibition 8 hours after herbicide application. This inhibition is evident because these herbicides act directly on electron flow and can be easily detected by evaluating fluorescence when susceptible plants are exposed to herbicides.

In eucalyptus plants, the ETR reduction was less than 30% when submitted to treatments with the clomazone and sulfentrazone herbicides, which can be explained by the fact that these herbicides do not directly inhibit the flow of electron transport. According to studies developed by Takahashi et al. (2009), the critical dose of the herbicide clomazone was 800 and 1200 mL ha^{-1} in clones VCP1 (*E. grandis*) and VCP2 (*E. urophylla*), respectively. As for the herbicide sulfentrazone, the critical dose was 75 mL ha^{-1} for clone VCP1 and 1200 mL ha^{-1} for clone VCP2, demonstrating that genetic materials can act in significantly different ways. For *I. nil* plants, clomazone inhibited ETR by more than 50% at 120 HAA and 75% at 192 HAA.

Sulfentrazone reduced 70% of ETR at 24 HAA for *I. nil*, having complete inhibition at 72 HAA. According to Silva et al. (2015), a high efficacy of the herbicide sulfentrazone was found to control *I. nil* at doses close to 800 g ha^{-1}.

For glyphosate, with 120 HAA, ETR was inhibited by more than 50% for both species. There are many indirect effects of glyphosate, such as decreased activity levels of the enzyme ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) (Ahsan et al., 2008) and disorganization of the photosynthetic apparatus (María et al., 2005).

Water consumption accumulated by the eucalyptus seedlings (Figure 4a) was not influenced by the action of herbicides. Eucalyptus plants transpiration is correlated with their evaporative demand, having similar oscillations in sunnier and cloudier days (Figure 3).

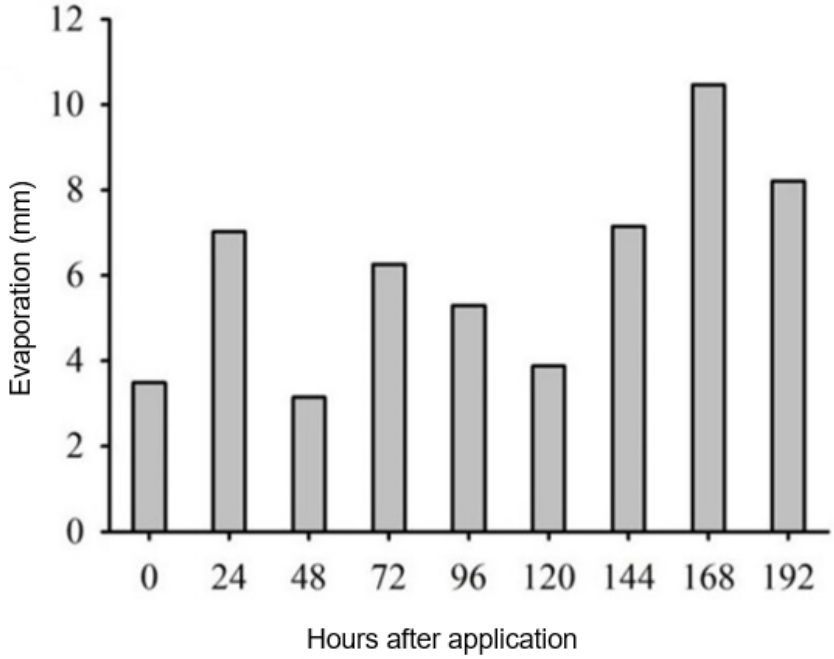

Figure 3: Daily evaporative demand in the greenhouse.

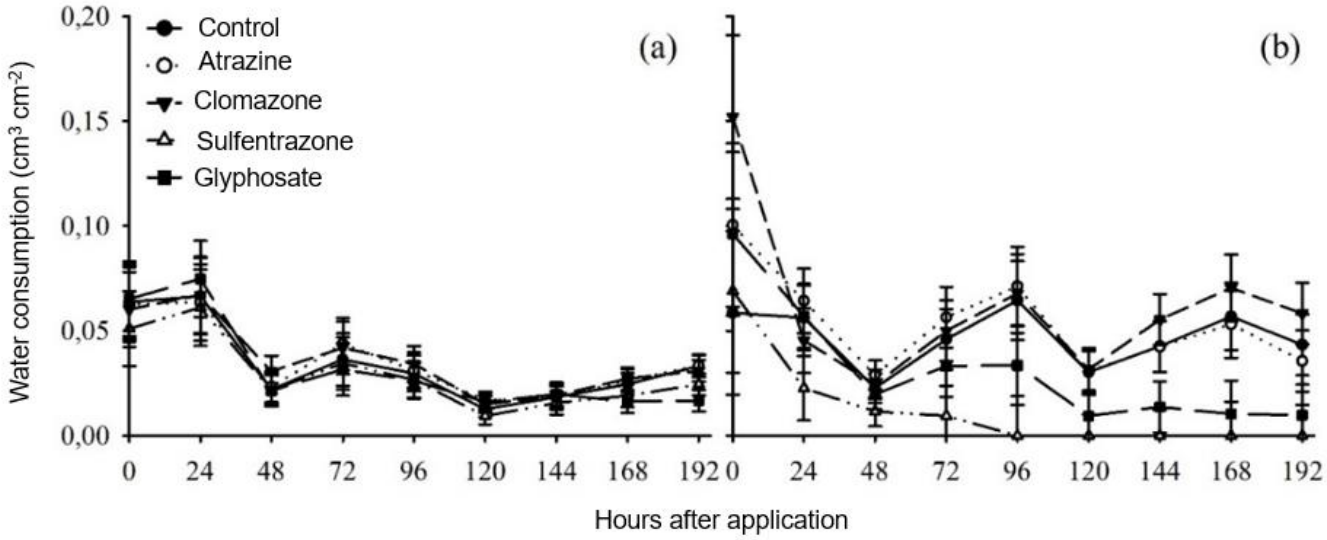

Figure 4: Accumulated water consumption in eucalyptus (a) and *I. nil* (b) seedlings, as a function of time, after application of the herbicides: atrazine, clomazone, sulfentrazone, and glyphosate. Bars indicate the DMS of the evaluated periods.

Water consumption by *I. nil* (Figure 4b) was higher than by eucalyptus, with no significant difference verified between clomazone and atrazine, which had a similar behavior to the control. Araldi et al. (2011), in a study with weed species *Brachiaria decumbens*, *I. grandifolia*, *I. hederifolia*, *Panicum maximum*, and *Digitaria horizintalis*, found that these plants consumed more water per cm^2 than sugarcane, indicating greater competitiveness for water from the invasive plants. Similarly, Dalley et al. (2006), working with corn, observed greater use of water by weeds. Sulfentrazone nullified water consumption at 96 HAA, a similar response to ETR, which showed inhibition of its transport rate at 72 HAA. Glyphosate significantly inhibited more water consumption than electron transport rate.

Glyphosate provided the highest percentage of visual phytotoxicity for eucalyptus throughout the experiment, with 10% at 120 HAA reaching 98% at 168 HAA (Figures 5 and 6). Comparing it with the ETR data, an average of 8% of phytotoxicity was obtained in the first 120 hours, placating the drop in ETR. According to Carvalho et al. (2015), the general consequences of exposure to glyphosate are chlorosis and reduced plant growth, especially in metabolically active tissues such as immature leaves, buds, flower buds, and roots. At high doses, these symptoms are followed by plant death.

According to Tuffi Santos et al. (2009), the first symptoms of phytotoxicity caused by glyphosate were observed four days after application in six eucalyptus clones.

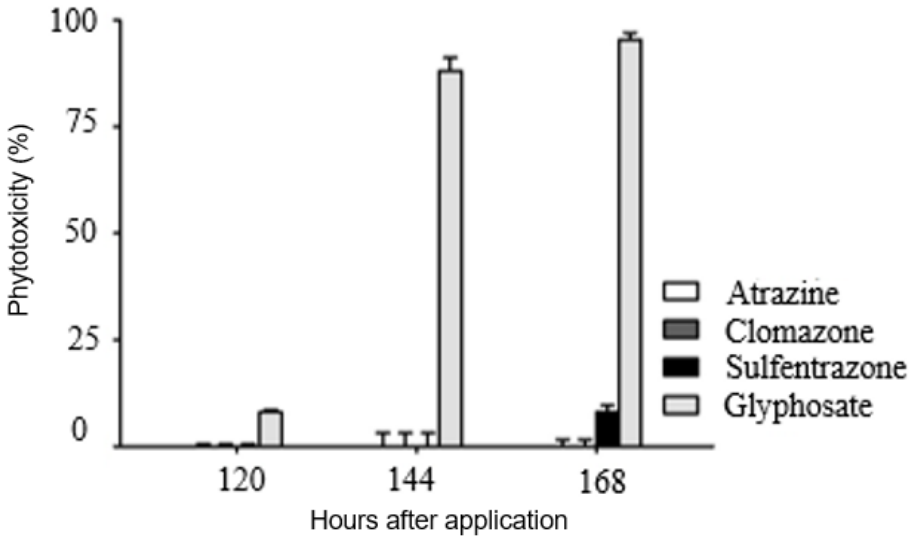

Figure 5: Percentage of visual phytotoxicity in eucalyptus at 120, 144, and 168 hours after application of the herbicides: atrazine, clomazone, sulfentrazone, and glyphosate. Bars indicate the DMS of the evaluated periods.

Sulfentrazone provided a higher percentage of visual phytotoxicity for *I. nil* (Figure 7), in 72 HAA, it presented 85% of phytotoxicity, reconciling with the inhibition of water consumption in 96 HAA and with ETR. Campos et al. (2009), using sulfentrazone, obtained control of *I. triloba* and *Merremia cissoides* in the first evaluations, performed 15 days after application.

Figure 6: Visual symptom of eucalyptus phytotoxicity caused by the herbicide glyphosate.

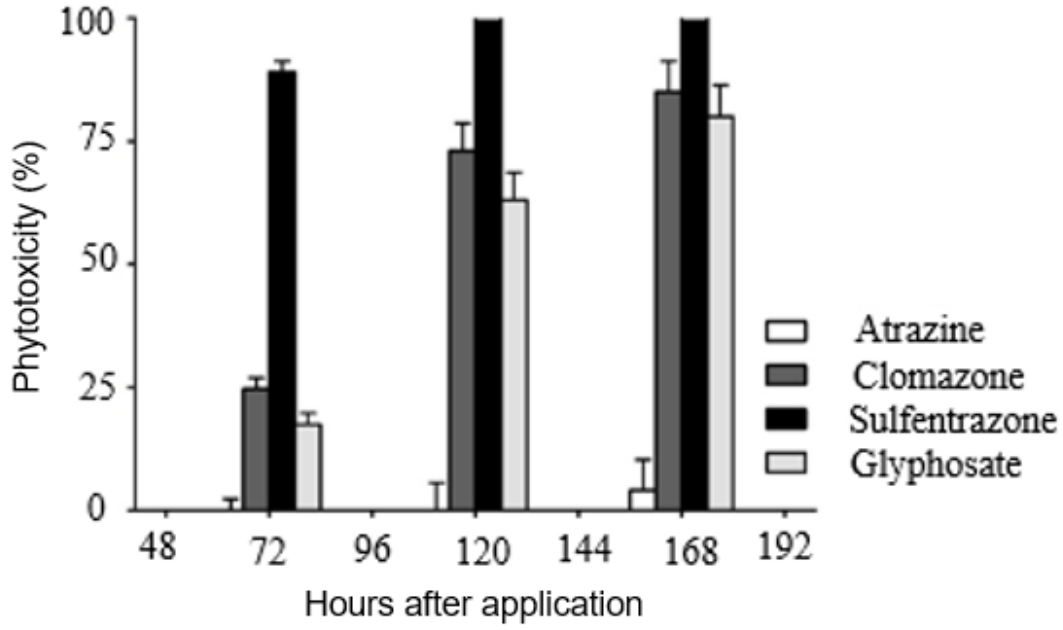

Figure 7: Percentage of visual phytotoxicity in *I. nil* at 120, 144, and 168 hours after application of the herbicides: atrazine, clomazone, sulfentrazone, and glyphosate. Bars indicate the DMS of the evaluated periods.

The visual phytotoxicity of *I. nil* to clomazone was 22% in the 72 HAA, ending in the 168 HAA with 82%, coinciding with the ETR reduction. Glyphosate obtained an increasing phytotoxicity rate, similar to clomazone, ending with an average of 80%, but the ETR value reduced more slowly.

Monquero et al. (2005) obtained efficient control corresponding to 85% of *I. hederifolia* using clomazone. Visual phytotoxicity by atrazine occurred only in 168 HAA, having a different behavior to that of ETR, where a marked reduction was only observed with 24 HAA. Similar results were observed by Girotto et al. (2012) in a study with photosynthetic efficiency after diuron application in *M. cissoides*, *I. grandifolia*, *I. hederifolia*, *Digitaria horizontalis*, *Panicum maximum,* and *Urochloa decumbens*.

Thus, with this work it was possible to identify the phytotoxicity caused by the herbicides before the manifestation of visual symptoms by the plants, through the evaluation of the ETR. Therefore, it is recommended to spray the herbicide glyphosate on weeds in a targeted way. As a means of verifying the selectivity of the herbicides studied on eucalyptus, it is suggested the installation of a field experiment.

Conclusions

The herbicide glyphosate inhibited ETR for both eucalyptus and I. nil plants. Water consumption for I. nil was inhibited by the glyphosate and sulfentrazone herbicides. Sulfentrazone caused the greatest phytotoxicity to I. nil and glyphosate to eucalyptus.

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