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Morphological responses of different eucalypt clones submitted to glyphosate drift

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Abstract

This work aimed to evaluate the effects of simulated glyphosate drift on leaf growth and micromorphology of *Eucalyptus* spp. clones, using subdoses. A factorial scheme consisting of three clones, *Eucalyptus urophylla*, *E. grandis* and the hybrid *E. urophylla* × *E. grandis* (*E. urograndis*) and five sub-rates (0; 43.2; 86.4; 172.8 and 345.6 g e.a. ha^{-1} of glyphosate) were used in a randomized block design, with four repetitions. The herbicide was applied on the plants so as not to reach the superior third, 23 days after seedling planting. At 7 and 15 days after application (DAA), the leaves collected from the first basal branch of the plants were processed according to the conventional methodology used for micromorphological studies. The effects of glyphosate drift were proportional to the rates tested, with *E. urophylla* being more tolerant to the herbicide than *E. grandis* and *E. urograndis*. Glyphosate symptoms were the same for the different clones tested, being characterized by wilting, chlorosis and leaf curling, and, at higher rates, by necrosis, foliar senescence and death of the eucalypt plants. Plants submitted to 172.8 and 345.6 g ha^{-1} of glyphosate had severe injuries in the aerial part, affecting their development, resulting in reduced height, stem diameter and dry mass at 50 DAA. The micromorphological damages occurred prior to the appearance of visible symptoms, with erosion of the epicuticular waxes and fungal hypha infestation in plants exposed to glyphosate drift being observed in the three clones. No marked difference in leaf micromorphology was observed that could explain the differential tolerance among the three clones studied. The results show that further studies on wax and cuticle constitution of *Eucalyptus* spp. are needed for the elucidation of the mechanisms of differential tolerance of eucalypt species and clones to glyphosate.

Keywords: *Eucalyptus* spp.; Scanning electron microscopy; Herbicide; Epicuticular wax; Phytotoxicity

1. Introduction

Forest sustainability and competitivity depend, among other factors, on a forest foundation capable of meeting the increasingly demanding standards of quality and productivity, leading to an effective research investment and adoption of adequate silvicultural practices (Brito, 1995). The use of different weed control methods, alone or in combination, is one of the factors responsible for productivity optimization, aiming at efficiency, low environmental impact and decreased yield costs.

Weed management in reforestation areas is basically performed by applying mechanical and chemical methods during the various stages of its productivity process. The chemical method is the main practice adopted since it is not labor dependent, providing efficient control even of plants with vegetative propagation and during rainy seasons, allowing minimal or no-till, without affecting the root system of the cultures.

Two herbicides are especially important in weed management of eucalypt reforestation: oxyfluorfen and glyphosate.

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However, glyphosate has been used in a large scale since it can be applied during weed post-emergence, facilitating operations in minimally cultivated areas (Toledo et al., 2003) and providing an effective control of a large number of perennial and annual mono- and dicotyledonous weed species, without causing environmental damage (Malik et al., 1989).

Although glyphosate is being frequently used in reforestations, little is known about its effects on eucalypt. In areas where chemical control is used in weed management, it is very common the occurrence of drift, i.e., herbicide injury to off-target organisms nearby or even in the same area, as in the case of direct applications of non-selective herbicides such as glyphosate.

The effect of herbicide drift is directly linked to the amount of active component reaching the crops, which in turn depends on the rates recommended for weed control. According to Rodrigues and Almeida (1998), the glyphosate rates recommended for eucalypt vary greatly, with doses ranging from 0.36 to 2.16 kg of e.a. ha^{-1} being used for the control of annual and perennial species.

Toxicity has been verified in areas where glyphosate has been applied for weed control for eucalypt reforestation, mainly in young seedlings and sprouts, as in the case of eucalypt stump regrowth. Many works on 'simulated drift' are available (Bailey and Kapusta, 1993), aiming to study the effects of different herbicide formulations on a wide range of cultures. However, a great variation is observed in the subrates adopted by the authors to determine the treatments to be utilized in these studies.

Several leaf parameters may be used to qualify and/or quantify the influence of toxic substances on plants, such as leaf lesions and growth (Prado-Filho, 1993; Silva et al., 2000; Soda et al., 2000; Fornasiero, 2001, 2003); foliar micromorphology changes (Chaves et al., 2002); and anatomic and ultra-structural aspects (Silva et al., 2000; Fornasiero, 2001; Chaves et al., 2002; Reig-Armiñana et al., 2004).

In practice, little is known about the behavior of different cultivated genetic materials when exposed to glyphosate drift, and about the likely direct and indirect effects of this herbicide molecule on eucalypt.

This work aimed to evaluate the effects of simulated glyphosate drift on the growth and foliar micromorphology of *Eucalyptus* spp. clones, by providing information characterizing the tolerance of such plants to the herbicide.

2. Materials and methods

The experiment was carried out in an unprotected environment in an area owned by the Department of Plant Science of the Universidade Federal de Viçosa, from April 12 to June 15, 2004. Fig. 1 shows data on maximum temperature (T_{max}), minimum temperature (T_{min}), mean temperature (T_{med}) and relative humidity (RH) regarding the period during which the experiment was conducted.

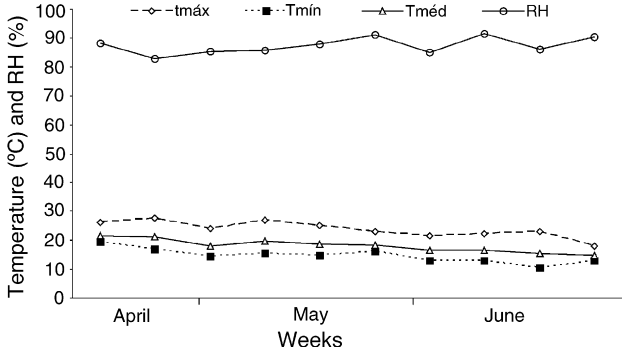

Fig. 1. Week averages of maximum temperature (T_{max}), minimum temperature (T_{min}), mean temperature (T_{med}) and relative humidity (RH) from April 12 to June 15, 2004.

The following clones were used: 15—*Eucalyptus urophylla* S.T. Blake × *E. grandis* W. Hill ex. Maiden (*E. urograndis*, 72—*E. urophylla* S.T. Blake hybride, both provided by Celulose Nipo-Brasileira SA (CENIBRA), and an *E. grandis* W. Hill ex. Maiden clone supplied by the Instituto Estadual de Florestas (Forest State Institute)-(IEF). Three-month-old seedlings, approximately 0.3 m high, were planted in 10 L vases with clayey soil fertilized with 216.6 g of N-P-K (6–30-6) and 12 g of lime in the ratio Ca:Mg = 4:1 equivalent, with 6 g/vase of N-P-K (20-5–20) applied 15 days after planting (DAP) and three applications of 4 g/vase of $(NH_4)_2SO_4$ at 40, 60 and 80 DAP. The experiment was arranged in a randomized block design, with four repetitions, in a factorial scheme (three clones and five treatments), each vase being the experimental plot. The treatments consisted of the rates 0; 43.2; 86.4; 172.8 and 345.6 g e.a. ha^{-1} of glyphosate, corresponding to 0, 3, 6, 12 and 24% of 1.440 g. e.a. ha^{-1} of glyphosate in the formulation isopropylamine salt, applied on the plants so as not to reach the superior third at 23 DAP the eucalypt seedlings, which reached around 0.35 m height. The salt of isopropylamine of *N*-(phosphonomethyl) glycine (glyphosate), used in this experiment, is manufactured as soluble concentrate (SC) by Monsanto do Brasil Ltda under the commercial brand *Roundup CS*.

A backpack sprayer equipped with a handheld boom consisting of two flat fan nozzle TT110.02, spaced 0.5 m apart, 250 kPa pressure and water volume of 200 L ha^{-1} was used. Air relative humidity at application time was 85%, temperature of 21 °C in a covered area for 24 h and remained the rest of the time in an unprotected environment.

Following herbicide application, the aerial part of the plants was monitored daily for possible morphological changes and at 7, 15, 30 and 45 days after application (DAA), intoxication percentage was determined in relation to the control. In this visual analysis the number and size of the necrotic and chlorotic spots were considered using the proportional scale proposed to Frans (1972): 0% corresponding to absence of foliar and stem visible symptoms and 100% to plant death. For the visual characterization of foliar injuries, representative leaf blades were photographed 7 and 15 DAA.

For scanning electron microscopy, samples of the 3 clones at rates approximately of 0 and 6% (84.6 g e.a. ha^{-1} of glyphosate) were collected, measuring approximately 0.5 cm^2 in the median portion of the leaves at the third node of the first basal branch of the eucalypt plants at 7 and 15 DAA. All the glyphosate treatment samples were collected from regions without visible symptoms. The scanning electron microscopy samples were fixed in Karnovisk (glutaraldehyde 2.5% + paraformaldehyde 2.5% in cacodilate buffer 0.05 M, pH 7.2) and post-fixed in osmium tetroxide (1%). After dehydration in ethylic series, the material was dried at the critical point, utilizing CO_2 in Balzers equipments (model CPD 020, Bal-Tec, Balzers, Liechtenstein). The fragments were covered in gold using the catodic spraying process in Sputter Coater equipment (Model FDU 010, Bal-Tec, Balzers, Liechtenstein), according to methodology proposed by Silveira (1989). The observations and photographic documentation were carried out using a scanning electron microscope (model Leo 1430 VP, Zeiss, Cambridge, England).

At 50 DAA, data on plant height (the region between the transition region of the shoot-root axis and the plant apex were recorded, as well as stem diameter at 10 mm from the soil, followed by cutting of the aerial part of the plants in the region of the shoot–root axis, and dried forced air oven (65 ± 3 °C), until constant weight was reached to obtain biomass.

The quantitative data were submitted to analysis of variance by the program Statistical Analysis System (SAS, 1989), with regression equations being fitted, if applicable.

3. Results

3.1. Symptomatology

Plant intoxication varied according to the rates tested ($p < 0.01$) at 7, 15, 30 and 45 DAA), increasing with the increase of the glyphosate rates (Fig. 2).

The first intoxication symptoms were observed at four DAA, on the three eucalypt clones due to wilting, chlorosis and curling of the leaves in the apex of the plants sprayed with glyphosate rates above 86.4 g e.a. ha^{-1}; besides the necroses observed at greater intensity in *E. grandis* and *E. urograndis* plants treated with 172.8 and 345.6 g e.a. ha^{-1} of glyphosate (Fig. 3). *E. grandis* plants sprayed glyphosate rates of 345.6 g e.a. ha^{-1} had necrosis over practically the

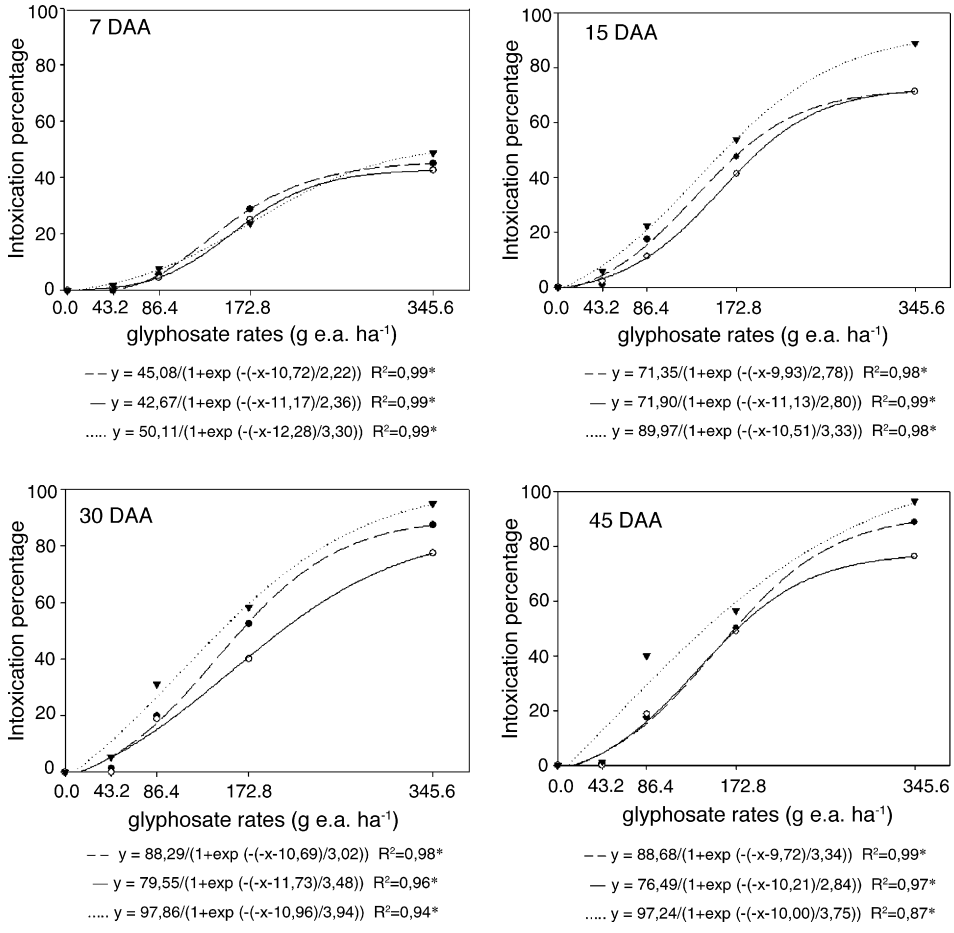

Fig. 2. Intoxication percentage (number and size of foliar and stem visible symptoms) of eucalypt plants submitted to glyphosate drift, at 7, 15, 30 and 45 DAA ((· · ·) *E. grandis*, (- - -) *E. urograndis*, (—) *E. urophylla*).

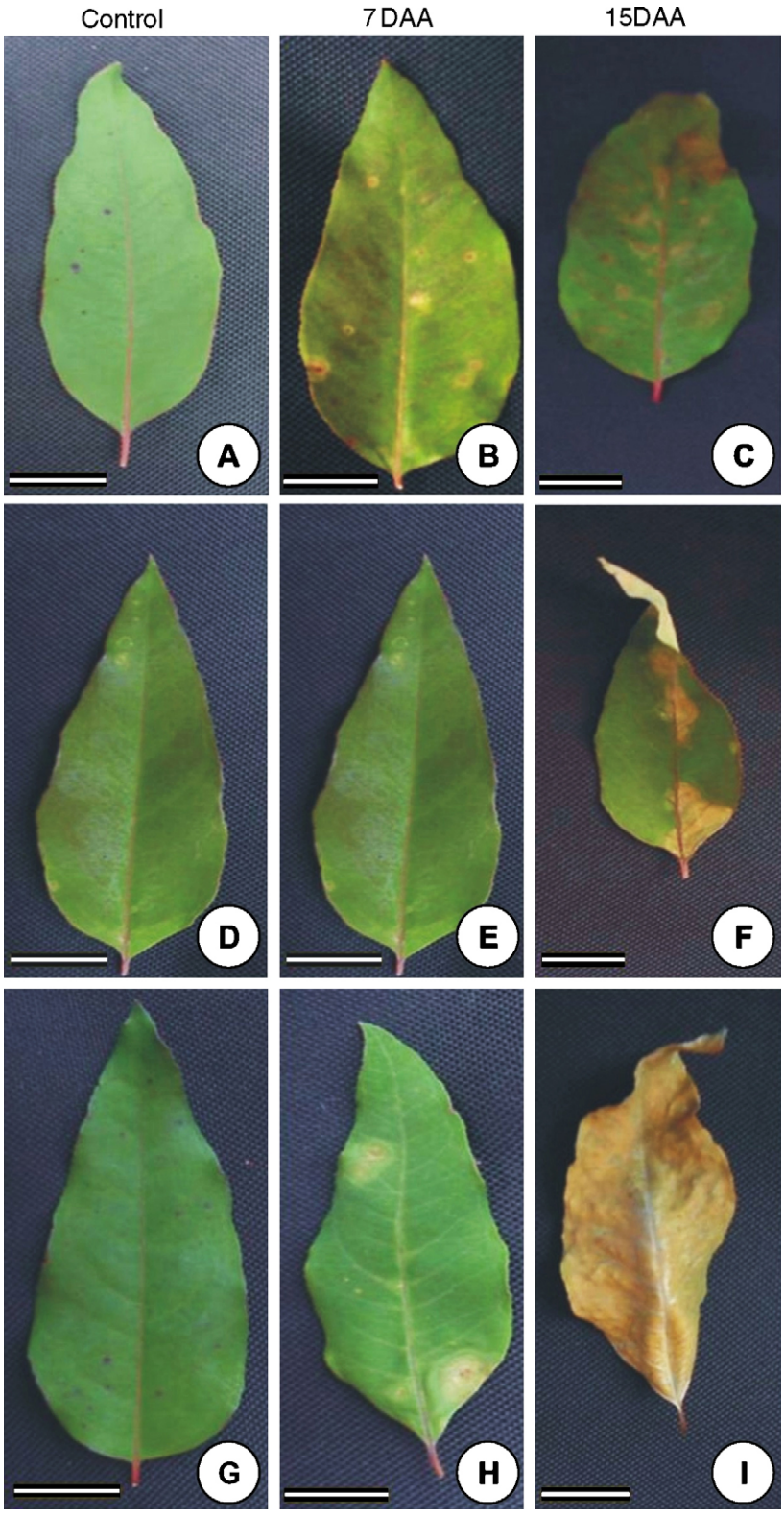

Fig. 3. Aspect of representative leaf blades of eucalypt submitted to glyphosate drift (rate = 345.6 g e.a. ha^{-1}). (A–C) *E. urograndis*, (D–F) *E. urophylla*, (G–I) *E. grandis.* DAA = days after application (bar = 20 mm).

entire foliar area at 15 DAA (Fig. 3I). Around the same time, slight intoxication symptoms became visible on the three clones treated with 43.2 g e.a. ha^{-1} of glyphosate, being more intense on *E. grandis*. However, these symptoms disappeared 45 DAA, indicating a total recovery of the plants treated with 43.2 g e.a. ha^{-1} of glyphosate.

E. grandis was most susceptible to glyphosate, resulting in death of 75% of the plants treated with 345.6 g e.a. ha^{-1} of glyphosate at 30 DAA, while *E. urophylla* and the hybrid *E. urograndis* presented, respectively, 15 and 0% of dead plants. Such difference was quite visible at 45 DAA with glyphosate rate of 345.6 g e.a. ha^{-1} causing 96.3% intoxication in *E. grandis*, compared to 87.5 and 77.5% in *E. urograndis* and *E. urophylla*, respectively (Fig. 2).

Plants submitted to 172.8 g e.a. ha^{-1} of glyphosate had new sprouts, but with intoxication symptoms in *E. urophylla*, and less intensely in *E. urograndis*. When submitted to 345.6 g e.a. ha^{-1} of glyphosate, these two genotypes did not produce new sprouts, the same being verified for *E. grandis* plants treated with 172.8 and 345.6 g e.a. ha^{-1} glyphosate.

Plant height, diameter and biomass differed significantly ($p < 0.01$) among the rates tested (Fig. 4); varying among the clones in plant diameter and dry biomass ($p < 0.01$) but not plant height ($p > 0.05$).

Plants submitted to 172.8 and 345.6 g e.a. ha^{-1} of glyphosate had reduced height, diameter and biomass, while the plants in the other treatments did not differ among themselves in relation to these characteristics. The severe injuries that led to non-growth and senescence of the leaves explain the reduced height, diameter and biomass observed in plants exposed to the higher rates.

3.2. Scanning electron microscopy

No visible damage was verified in plants submitted to glyphosate rates of 86.4 g e.a. ha^{-1}. However, micromorphological samples of these treatments showed changes in the epidermal surface, compared to the control plants in *Eucalyptus urograndis* (Fig. 5), *E. grandis* (Fig. 6) and *E. urophylla* (Fig. 7), indicating the importance of leaf micromorphological studies on characterization of injuries caused by glyphosate drift, prior to visual damage.

All *Eucalyptus* species have amphistomatic leaves. *E. urograndis* presents wax plates in the adaxial (Fig. 5A) and abaxial (Fig. 5D) leaf faces. Plants submitted to 86.4 g e.a. ha^{-1} of glyphosate showed, in the adaxial face epidermis at 7 (Fig. 5B) and 15 (Fig. 5C) DAA of the herbicide, epicuticular wax loss and presence of fungi hyphae, respectively. Epicuticular wax erosion (Fig. 5E) also occurred on the leaf abaxial surface, 7 DAA.

Epidermal cells in *E. grandis* that cover the secretory cavities of the mesophyll ("overlying cells") were observed to present a shape distinct from the others (Fig. 6A). Epicuticular wax loss (Fig. 6D) was observed on the adaxial surface of the leaves submitted to glyphosate 7 DAA, which favors fungus infestation, as observed in the hyphae at 15 DAA

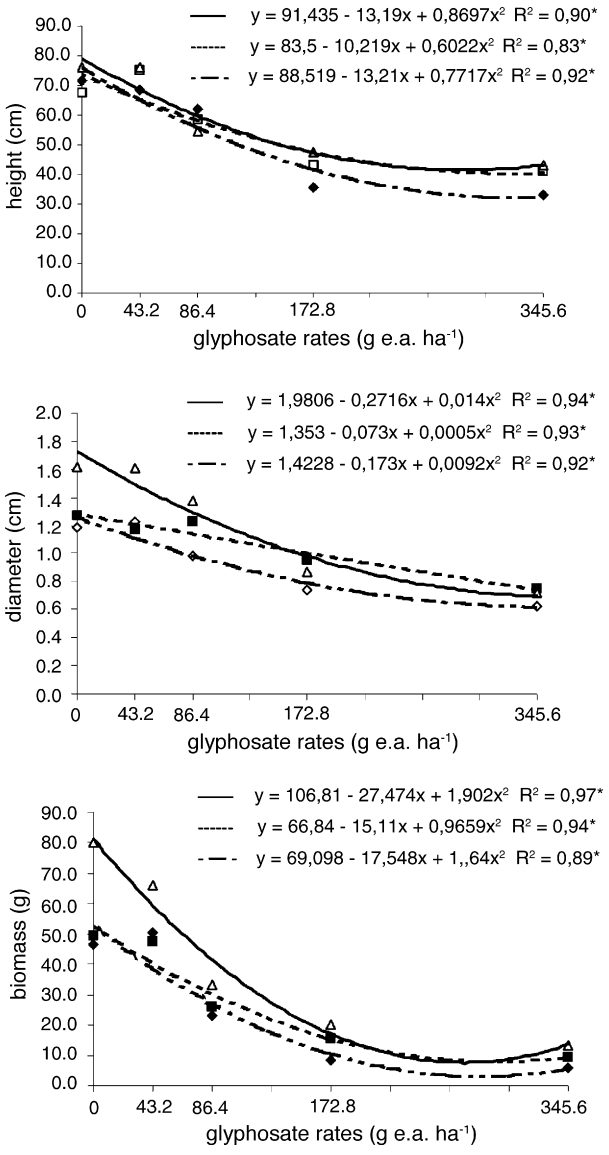

Fig. 4. Height, stem diameter, and biomass of the plants of three eucalypt clones submitted to simulated glyphosate drift at 50 DAA. (—) *E. grandis*, (- - -) *E. urograndis* and (- — -) *E. urophylla*.

(Fig. 6B). Such fungi are responsible for the dissolution in the waxes in the infested regions (Fig. 6B). The adaxial face epidermis in the control treatment is shown in Fig. 6C. Epicuticular wax loss and stomata obliteration by the eroded wax were observed in the samples collected 15 DAA of 86.4 g e.a. ha^{-1} of glyphosate (Fig. 6D).

The adaxial surface in the control treatment of *E. urophylla* shows well-defined relief and cellular delimitation (Fig. 7A). Waxes with amorphous aspect were observed on the adaxial surface of the leaf samples collected 7 DAA glyphosate treatment (Fig. 7B). Epicuticular wax erosion and fungi hyphae infestation in *E. urophylla* were only observed in the samples collected 15 DAA glyphosate treatment (Fig. 7C). The abaxial surface of *E. urophylla* in the control treatment is observed in Fig. 7D. Regions of the abaxial face epidermis colonized

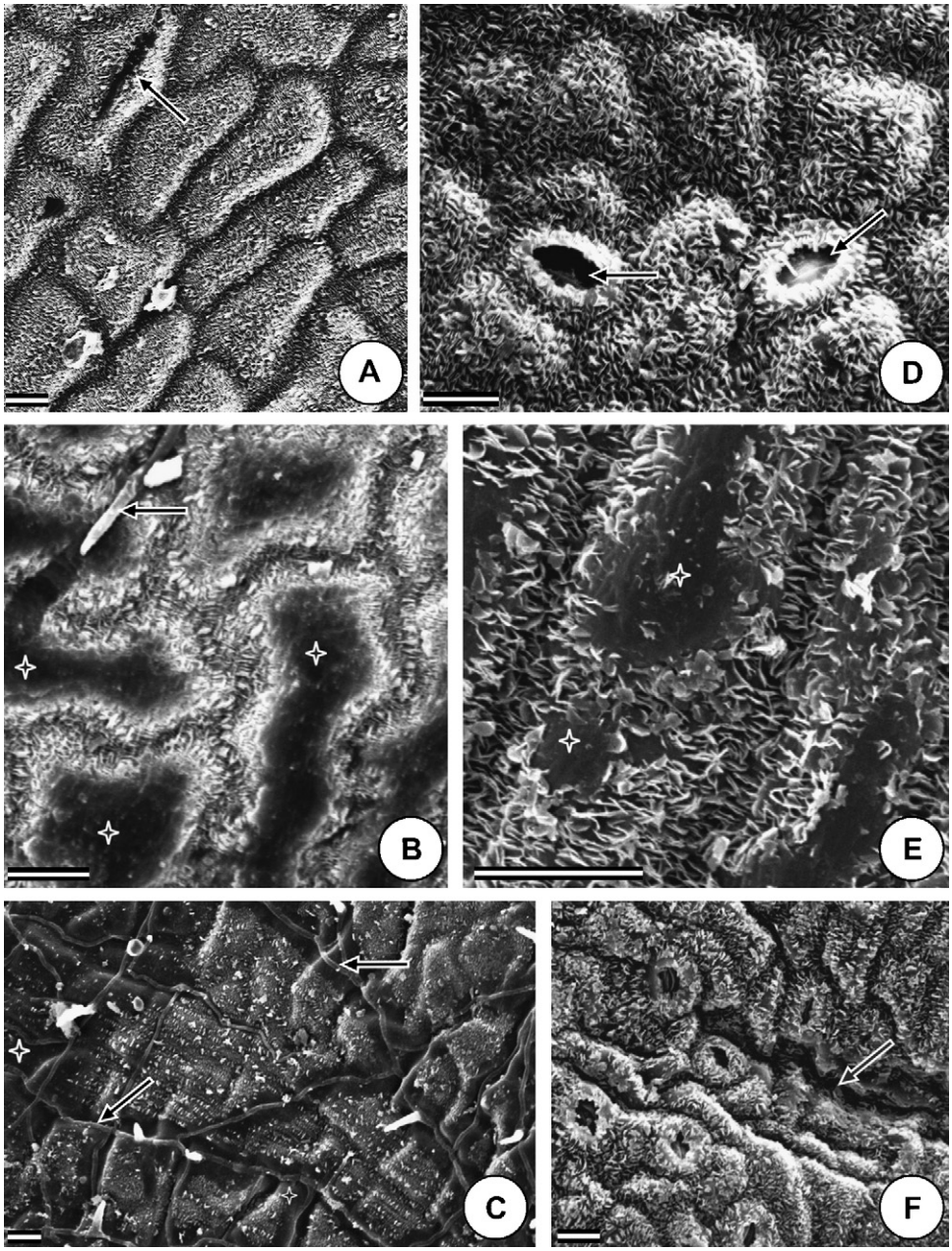

Fig. 5. Epidermis of *E. urograndis* (scanning electron micrographs). (A–C) Adaxial surface. (D–F) Abaxial surface. (A) Arrow indicates stomata in the control treatment. (B) Fungi hypha (arrow) in the region with epicuticular wax loss (stars) 7 days after exposure to herbicide. (C) Area colonized by mass of fungi hyphae (arrows): stars indicate epicuticular wax erosion areas 15 days after glyphosate treatment. (D) Arrows indicating stomata in the control treatment. (E) Epicuticular wax erosion (stars) 7 days after exposure to treatment. (F) Area displaying relief depressions (arrow) 15 days after treatment. Bars = 10 μm.

by a mass of fungi hyphae showed relief depressions 7 DAA (Fig. 7E), as observed in *E. urograndis* (Fig. 5), 15 days after exposure to glyphosate.

4. Discussion

Similar leaf symptoms in plants of *Eucalyptus urograndis*, sprayed with 172.8 and 345.6 g e.a. ha^{-1} of glyphosate were reported by Tuffi Santos et al. (2005). Chlorosis may be the result of chloroplast degeneration (Campbell et al., 1976), and/or chlorophyll formation inhibition (Cole et al., 1983) in glyphosate treated plants. Chlorosis has also been reported to occur in response to other phytotoxic substances such as fluorine, both in mono (Chaves et al., 2002) and dicotyledonous species (Silva et al., 2000). Glyphosate is a non-selective systemic herbicide that acts on shikimate pathway, inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs). The action of this herbicide on the treated plants prevents the formation of the aromatic amino acids tryptophan, tyrosine and phenylalanine (Hess, 1994).

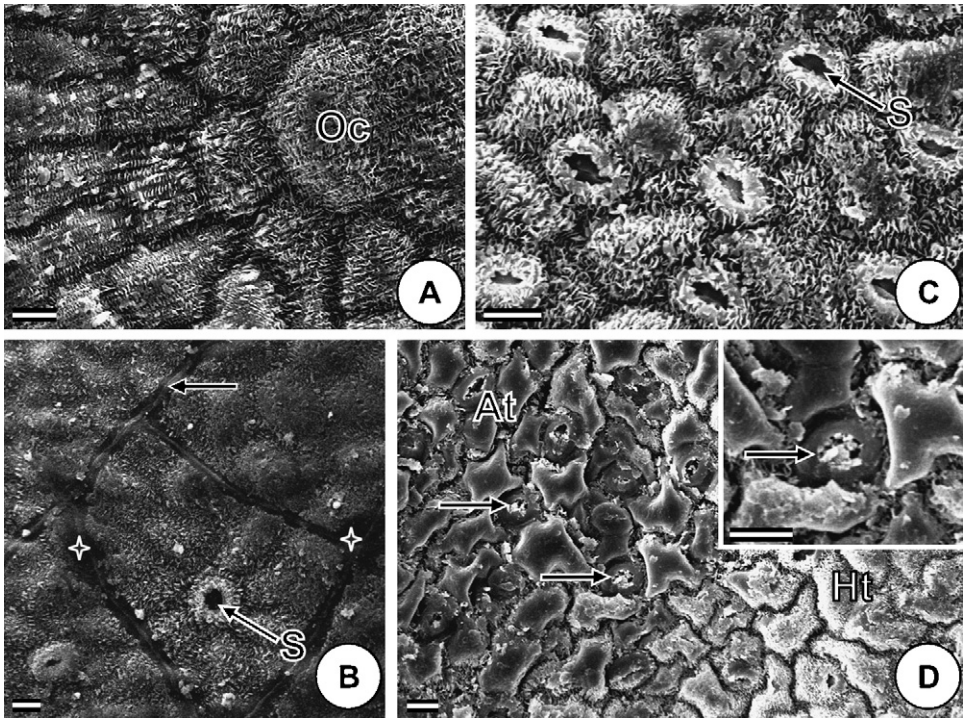

Fig. 6. Epidermis of *E. grandis* (scanning electron micrographs). (A and B) Adaxial surface. (C and D) Abaxial surface. (A) "Overlying cell" in the control treatment. (B) Fungi hyphae (arrows) in the regions with epicuticular wax loss (stars), 15 days after exposure to the herbicide. (C) Control treatment. (D) Epicuticular wax erosion in the obliterated affected region and stomata (arrows), shown in the detail, 7 days after herbicide application. Oc, overlying cell; S, stomata; At, affected tissue; Ht, healthy tissue. Bars = 10 μm.

According to Bromilow and Chamberlain (2000) glyphosate is very mobile in the plant, being rapidly distributed through symplastic route down to the roots and meristematic regions of the plant, during its foliar application. When applied for controlling weed with drift, sub-doses of the product come into contact with non-target plants, with the injuries caused by glyphosate developing from the younger to the older parts of the plant. (Tuffi Santos et al., 2005; Magalhães et al., 2001a,b). Injuries caused by glyphosate drift developing from the younger to the older parts of the eucalypt plant were verified in the present study, corroborating with the literature results and with the characteristics of a systemic product of high mobility in the plant. However, in situations where glyphosate comes into contact with specific parts in the plant, such as only one leaf, or in the case of an overdose, the injury observed may be restricted to that particular organ or develop from it, as observed by Schönherr (2002).

When glyphosate is used to control stump regrowth in different eucalypt clones, the sprouts produced show intoxication symptoms. Tuffi Santos et al. (2005) also described the production of normal sprouts and with intoxication symptoms in eucalypt plants submitted to glyphosate rates of 172.8 and 345.6 g e.a. ha^{-1}, respectively. Magalhães et al. (2001a,b), working with glyphosate drift simulation in corn and sorghum, respectively, observed a drop in yield and necroses in the aerial part of the plants treated with glyphosate rates above 115.2 g e.a. ha^{-1}. The results obtained show greater injury in younger plants (25 days after transplanting) treated with glyphosate than observed by Tuffi Santos et al. (2005) in plants with longer cultivation time (45 days after transplanting) undergoing similar treatments. Thus, the longer the chemical control is postponed after transplanting, the lower are the glyphosate drift risks in the eucalypt culture. However, weed height due to chemical control operational difficulties and culture competition are factors that should be monitored to avoid eucalypt growth losses.

Micromorphological studies are widely used to evaluate damage caused by other phytotoxic substances (Turunen and Huttunen, 1991; Turunen et al., 1995; Chaves et al., 2002; Sant'Anna-Santos et al., in press). This work evaluated general changes on the epidermal surface in response to glyphosate, with sample fixing being considered fundamental for good tissue preservation; however, it should be emphasized that the methodology applied is not recommended for specific epicuticular wax micromorphology studies. However, other authors (Sant'Anna-Santos et al., in press) have been using similar methods to analyze the effect of acid rain on plants, generally mentioning changes in the structure of the epicuticular waxes of the material exposed to the stressing agent, compared to the control treatment, as used in this study.

Flattening of the epidermal surface, observed 15 days after treatment, is likely due to turgidity loss in the epidermal cells of the abaxial face, leading to the formation of depressions, also reported as response to pollutants in *Glycine max* (Azevedo, 1995), *Panicum maximum* and *Chloris gayana* (Chaves et al., 2002).

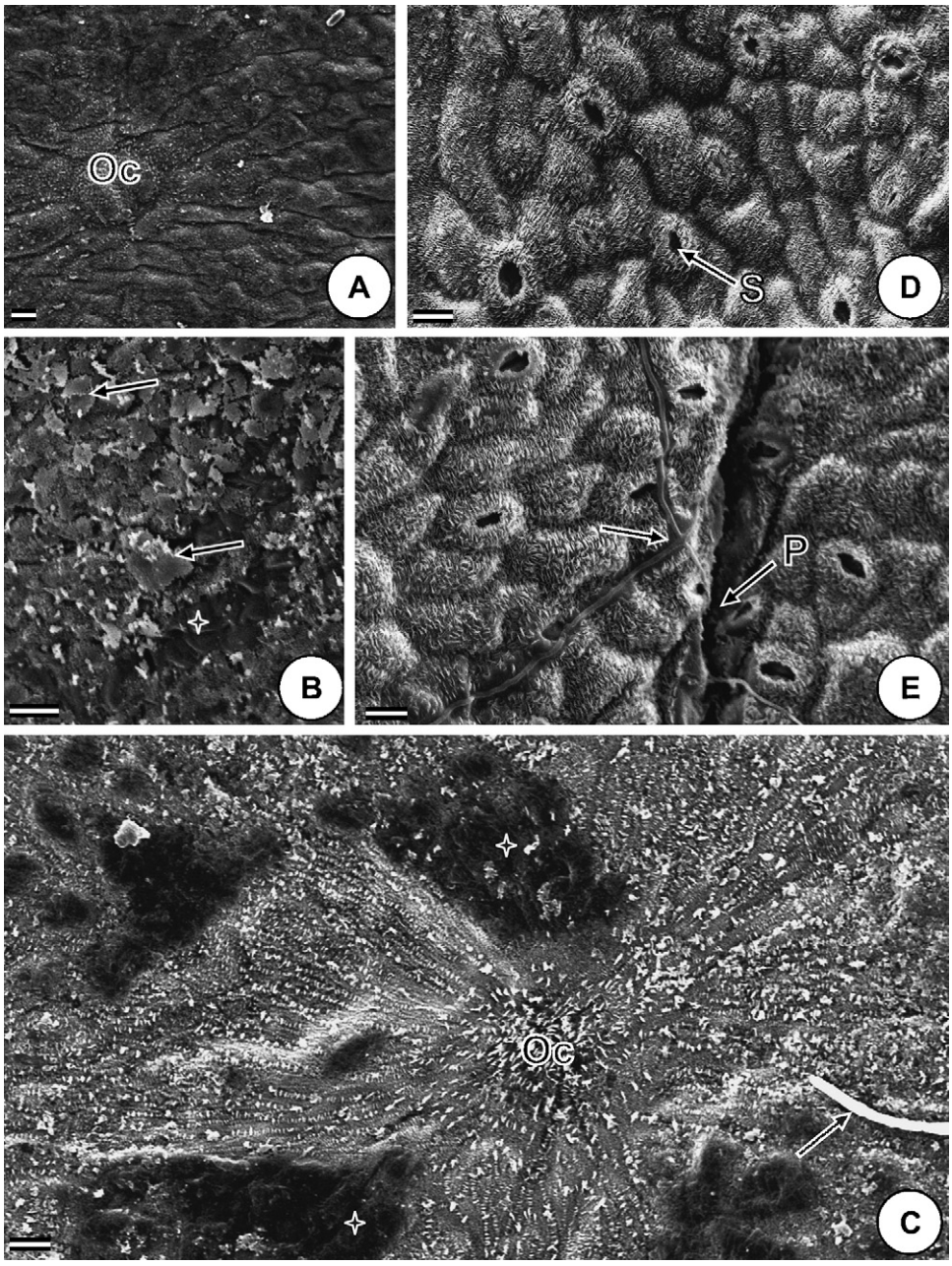

Fig. 7. Epidermis of *E. urophylla* (scanning electron micrographs). (A–C) Adaxial surface. (D and E) Abaxial surface. (A) Control treatment. (B) Region with epicuticular wax loss (star), 7 days after exposure to glyphosate. (C) Fungi hyphae (arrow) next to the regions with epicuticular wax loss (stars), 15 days after exposure to glyphosate. (D) Control treatment. (E) Epidermal cells with turgidity loss and surface colonized by fungi hyphae (arrow), 7 days after exposure to treatment. Oc, overlying cell; S, stomata; P, cells with plasmolized aspect. Bars = 10 μm.

Epicuticular wax erosion by glyphosate followed by fungi attack were also verified through leaf micromorphological studies in *Pinus sylvestris* and *Picea abies* submitted to acid rain (Turunen et al., 1995). Epicuticular wax erosion may be attributed to the presence of adjuvant in the commercial herbicide formulations to enhance penetration of the active ingredient into the plants to be controlled. In the case of glyphosate drift, such adjuvant may maximize the problems of undesired contact of the herbicide with the eucalypt plants, since, besides intoxication, caused by the herbicide molecule, wax removal may facilitate infection by phyto-pathogenic fungi. Wax erosion due to the herbicide formulation used enhances the affinity of the leaf surface with the water. According to Barthlott (1981), wettable surfaces, i.e., surfaces presenting water film, favor colonization by microorganisms. The wax loss observed in *Pinus sylvestris* due to acid rain was caused by direct chemical reaction between the waxes or their precursors and the solution containing the stressing agent (Turunen and Huttunen, 1991).

Eucalypt plants exposed to simulated glyphosate drift often display epidermal cells killed by the action of the herbicide (Tuffi Santos et al., 2005). These authors believe that dead epidermal cells may facilitate the pathogens infection in eucalypt exposed to glyphosate drift. In addition, Rizzardi et al. (2003) report that glyphosate may facilitate the attack of pathogens in plants, since sub- rates of the herbicide may lead to reduced accumulation of both phytoalexins and lignin (Lévesque and Rahe, 1992), considered as physical-physiological plant barriers.

No significant differences were found in the micromorphological observations that might explain the difference of tolerance to glyphosate among the clones studied. Some works in the literature show that plant tolerance to glyphosate is due to a differential penetration or translocation (Sandberg et al., 1980; D'Anieri et al., 1990; Satichivi et al., 2000; Monquero et al., 2004). Leaf herbicide absorption rates and their efficacy are directly related to the types of foliar structures and cuticle permeability (Baker, 1982), which, in turn, depend on the constitution and polarity of the cuticle components.

The cuticle is the main barrier against glyphosate penetration, thus knowing its structure and composition is fundamental in studies on the absorption of the herbicide (Devine, 1990). The use of surfactants has contributed to breaking the superficial tension of the spray on the leaf, resulting in better spreading of the product and also allowing the stomata to play a relevant role in herbicide penetration. Also, the surfactants favor glyphosate penetration into the leaf by allowing it to go through the foliar cuticle and membrane barriers, reaching its site of action. (Feng et al., 1999). According to Schönherr (2002) the presence of water is fundamental for good glyphosate absorption by the leaves, with a large part of the herbicide absorbed passing through the hydrated pores of the cuticle.

Studies show that the stomata can be routes for herbicide penetration, since the cuticle over the guard cells is thinner and more permeable to polar substances due to a lower content of epicuticular wax (Hess and Falk, 1990; Schreiber, 2005). Such fact may favor glyphosate penetration in plants with predominance of stomata on the adaxial face of the foliar epidermis, where the contact with the herbicide spray applied is more intense. Although the three eucalypt clones have amphistomatic leaves, the reduced number of stomata on the adaxial face becomes a limiting factor for good glyphosate absorption through this route.

The herbicide glyphosate has low Kow, with little affinity with lipids (Kirkwood et al., 2000); thus, epicuticular waxes with large amount of apolar compounds may be a barrier against penetration of this herbicide. However, glyphosate diffusion through the cuticle of the five species studied by Subramaniam and Hoggard (1988) was considered low, slightly improving after epicuticular extraction. The authors concluded that for hydrophilic products such as glyphosate, waxes are not the main barriers against herbicide diffusion; rather, the polymer matrix constituting the cutin would be the real barrier against glyphosate diffusion through the cuticle.

Further studies using specific techniques to analyze changes in the structure of epicuticular waxes and cuticle composition combined with ultra-structural studies may provide relevant information leading to a better understanding of the differential tolerance of *E. grandis*, *E. urograndis* and *E. urophylla* to glyphosate and the damages caused by glyphosate drift.

5. Conclusion

The effects of glyphosate drift were proportional to the rates tested, with *E. urophylla* being more tolerant to the herbicide than *E. grandis and E. urograndis*.

Glyphosate intoxication symptoms were the same for the different clones tested and was characterized by wilting, chlorosis and leaf curling and, at higher doses, by necrosis, foliar senescence and death of the terminal twigs and branches. The micromorphological injuries were prior to the appearance of visible symptoms. There wax erosion of the epicuticular waxes and infestation by fungi hyphae in both faces of the epidermis of the three clones and turgidity loss followed by depression formation on the abaxial epidermis of *E. urophylla* and *E. urograndi*s. In *E. urophylla,* the surface of the adaxial face of the leaves exposed to the herbicide presented epicuticular waxes with an amorphous aspect. In *E. grandis,* stomata obliteration was also observed on the abaxial face of the leaves by the eroded wax in the regions affected by the herbicide.

Glyphosate rates of 43.6 and 86.4 g e.a. ha^{-1} caused slight intoxication symptoms in the plants of the three clones, with a gradual and total recovery being observed in plants treated with 43.6 g e.a. ha^{-1}.

Plants submitted to 172.8 and 345.6 g e.a. ha^{-1} of glyphosate had severe injuries in the aerial part, affecting their development and leading to reduced height, stem diameter and biomass.

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