Research Article

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# Carfentrazone-ethyl and glyphosate drift inhibits uredinial formation of *Austropuccinia psidii* on *Eucalyptus grandis* leaves

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## Abstract

**BACKGROUND: The response to infection of *Austropuccinia psidii* in resistant (CLR-383) and susceptible (CLR-384) *Eucalyptus grandis* clones, exposed to herbicide drift of carfentrazone-ethyl, glyphosate and a mixture of these two herbicides, was evaluated at microscopic and physiological levels.**

**RESULTS: Plants of the two clones showed symptoms of phytotoxicity caused by herbicide drift. However, net $CO_2$ assimilation rate, height and shoot dry matter were lower in CLR-384 than in CLR-383. At the ultrastructure level, the leaves of both clones exposed to the herbicides showed thylakoid disorganization and accumulation of starch grains in the chloroplasts. Only plants of CLR-384 were infected by *A. psidii*, but when exposed to herbicide drift, rust severity was lower than in control plants. Six days after inoculation (dai), plants of this clone exposed to the herbicides had smaller uredinia than control plants. At 12 dai, non-herbicide treated plants showed normal uredinia, containing abundant urediniospores. By contrast, plants exposed to the herbicides were less colonized by the fungus, and the uredinia were smaller with reduced production of urediniospores, which were sometimes not even detected.**

**CONCLUSION: Glyphosate and carfentrazone-ethyl herbicide drift reduce infection and uredinial formation of *A. psidii* and to some extent induce basal resistance in a susceptible clone of *E. grandis*.**


*Supporting information may be found in the online version of this article.*

**Keywords:** fungal infection; myrtle rust; photosynthesis; transmission electron microscopy

## 1 INTRODUCTION

Glyphosate is the most successful herbicide used for weed control in the world.[1] In Brazil, it is the most used post-emergent herbicide on eucalyptus plantations. However, in areas with frequent applications of glyphosate, some weed species such as *Conyza* sp.[2–4] and *Commelina* sp.[5,6] have evolved resistance and tolerance to the herbicide molecule, mainly due to the selection pressure.[7] Carfentrazone-ethyl is an alternative herbicide to control glyphosate-resistant weeds, and has been used alone or in combination with glyphosate.[8,9]

Glyphosate and carfentrazone-ethyl are phytotoxic to eucalyptus, so applications should be conducted appropriately, avoiding direct contact between the herbicide and the crop. Despite this caution, during application, herbicide drift may reach eucalyptus plants and induce leaf chlorosis, necrosis and wilt.[10] Recently it was found that glyphosate drift reduces rust severity (*Austropuccinia psidii*) on eucalyptus.[11] Other studies have also reported that several herbicides with the same mode of action (inhibition of protoporphyrinogen oxidase, PPO) as carfentrazone-ethyl may cause changes in plant metabolism related to plant defense mechanisms against pathogen infection,[12–15] and affect the severity of fungal diseases in crops.[16,17]

The critical period of weed competition on eucalyptus is ~3.5 months after planting,[18] but successive application of herbicides for weed management is commonly performed for up to 2 years. Eucalyptus rust affects mainly young shoots and coppice,[19] and may occur on plants of susceptible genotypes at 0.5 and 3.0 m height.[20] Thus, eucalyptus plant stage favorable for

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rust development is the same period for herbicide application for weed management.

Although glyphosate drift reduces the severity of eucalyptus rust,[11] we do not know the response of eucalyptus exposed to carfentrazone-ethyl, or a mixture of glyphosate and carfentrazone-ethyl, or the effects of drift of these herbicides on uredinial formation of eucalyptus rust. Therefore, we studied the interaction at the anatomical, ultrastructural and physiological levels of *A. psidii* infection in two *Eucalyptus grandis* clones, exposed to drift-level doses of these two herbicides alone and in a mixture.

## 2 MATERIAL AND METHODS

### 2.1 Plant material and experimental design

Two assays were carried out between February and April 2015. Rooted cuttings of the first assay were re-conducted for the second assay, and the same methodology was used in both experiments. Cuttings of a resistant (CLR-383) and a susceptible (CLR-384) clone of *Eucalyptus grandis* to *A. psidii* were employed.[21] At 60 days of age, the cuttings were transplanted into 2 L pots containing MecPlant® substrate enriched with 26 g of super simple phosphate and 12 g of Osmocote® (NPK, 19-6-10). During transplanting, each plant per pot received 100 mL of a mono-ammonium phosphate solution (P and N at 52% and 12%, respectively; Vale Fertilizantes S.A., Uberaba, MG, Brazil). Plants were kept in a greenhouse with an average temperature of ∼ 25 °C and natural light (∼ 12:12 h photoperiod) and received the first herbicide drift treatment at 30 days after transplanting.

To simulate field conditions, after finishing the first assay, branches that received the first application of the herbicides were pruned, and rust-infected leaves were removed. After pruning, each plant received 100 mL of Biofert Universal® fertilizer solution (N, P, K, S, Mg, Cl, Zn, Fe, Mn, Cu, B, Co and Mo at 70.2, 46.8, 46.8, 11.7, 5.85, 3.51, 1.17, 1.17, 0.59, 0.59, 0.23, 0.23 and 0.12 g $L^{-1}$ respectively; Biofert, Contagem, MG, Brazil) diluted in water (1:100). Plants remained in the greenhouse for 20 days and because new shoots did not show any symptoms of herbicide phytotoxicity, the second assay was started.

In both assays, a randomized block experimental design was used with 10 replicates in a factorial scheme (2 × 4, two clones and four herbicide drift-level doses including the control). Each experimental block consisted of a plastic pot containing a eucalyptus plant.

### 2.2 Herbicide application

We tested 3 g a.i. $ha^{-1}$ carfentrazone-ethyl, 86.4 g a.i. $ha^{-1}$ glyphosate and a mixture (3 g a.i. $ha^{-1}$ carfentrazone-ethyl + 86.4 g a.i. $ha^{-1}$ glyphosate), corresponding to 6% of the manufacturer's recommended dose of each herbicide.[11] Plants sprayed with water served as the control.

In the first assay, the application was performed in February at 30 days after transplanting when plants were a∼ 50 cm tall. In the second assay, the application was performed in April (20 days after the end of the first assay). Both applications simulated drift in the lower third of the plants, by utilizing a $CO_2$-pressurized pulverizer backpack equipped with a bar containing two nozzles (model TT 110.02), which were calibrated to apply 150 L $ha^{-1}$ of herbicide solution.

To avoid contact between the product and other parts of the plant, the top of the canopy was protected with a plastic bag at the time of application, leaving only the lower portion of the plant (approximately three lower branches) exposed to the herbicide.

### 2.3 Plant inoculation with *Austropuccinia psidii*

In both assays, inoculation was performed 2 days after herbicide application (daa).[11] The UFV-2 isolate of *A. psidii* (race 1) obtained from *E. grandis* plants[22] and multiplied in plants of *Sygyzium jambos* was used. An inoculum suspension ($2 \times 10^4$ urediniospores $mL^{-1}$) was homogenously sprayed on both sides of the leaf blade of plants, using a De Vilbiss® atomizer #15, attached to an electric compressor. To evaluate the efficiency of the inoculation, three *S. jambos* plants were randomly distributed among the eucalyptus plants. After inoculation, plants were incubated in a mist irrigation chamber, in the dark. After 24 h of incubation, plants were maintained in a growth chamber at 22 °C, with a 12 h photoperiod and a light intensity of 130 µmol photons $m^{-2}$ $s^{-1}$,[23] until evaluation of rust severity.

### 2.4 Response of two clones of eucalyptus to the herbicides

The sensitivity of the two eucalyptus clones was evaluated at 23 daa, through of the net $CO_2$ assimilation rate (*A*), plant height and shoot dry matter. The parameter *A* was determined using a portable open-flow gas exchange system (LI-6400XT, LI-COR). Three measurements were performed on of the fully expanded leaf of the apical part of the plant (protected from the spray), in each plant for each treatment. The measurements were performed from 09:00 to 10:30 am (solar time) – during which *A* was at its maximum, under artificial photosynthetically active radiation (PAR); i.e. 1000 µmol photons $m^{-2}$ $s^{-1}$ at the leaf level and 400 µmol $CO_2$ $mol^{-1}$. All measurements were performed at 23 °C, the vapor pressure deficit was maintained at ∼ 1.0 kPa and the amount of blue light was set to 10% PAR to optimize stomatal aperture.

At the end of assay 2, plant height (from the substrate base to the apex) was determined. To determine shoot dry matter, plants were collected near the substrate and stored in paper bags, followed by oven drying with forced ventilation at 65 °C. The paper bags were weighed daily for 5 days until they reached a constant weight.

### 2.5 Disease evaluations

Rust severity was evaluated at 21 days after inoculation (dai) according to the diagrammatic scale.[24] The data obtained were used to calculate the McKinney Index (MI).[25] In each plant, three branches were marked, and two leaves were chosen from each branch.

The two leaves selected from each branch were collected and photographed at a distance of 10 cm. Image Pro-Plus v. 4.5 software to determine the infected leaf area (ILA). After photographing, two disks of each sheet were removed using a 6 mm diameter punch and placed in a 1.5 mL microcentrifuge tube containing distilled water and Tween-20. After homogenization with a magnetic stirrer for 1 min, two samples of the obtained suspension were placed in a Neubauer camera for urediniospore quantification (UQ).

### 2.6 Sample preparation for light, scanning and transmission microscopic analyses

Leaf samples taken from the apical part of each plant were collected at 6, 12 and 21 dai, and fixed in Karnovsky's solution.[26] For better penetration of the fixative solution, samples were desiccated under vacuum for 20 min. This last step was performed five times.



  

**Table 1.** Effects of the drift of glyphosate (G), carfentrazone-ethyl (CE) and a mixture of both herbicides (G + CE) on net $CO_2$ assimilation rate (*A*), plant height and shoot dry matter of *Eucalyptus grandis* plants of CLR-383 and CLR-384 clones, at 23 days after application

| | *A* (μmol $m^{-2}$ $s^{-1}$) | | Plant height (cm) | | Shoot dry matter (g/plant) | |
|---|---|---|---|---|---|---|
| Treatments | CLR-383 | CLR-384 | CLR-383 | CLR-384 | CLR-383 | CLR-384 |
| Control | 14.5 ± 0.5Aa | 13.2 ± 0.6Aa | 71.8 ± 1.1Aa | 69.8 ± 0.8Aa | 30.3 ± 0.9Aa | 27.7 ± 0.7Aa |
| G | 9.9 ± 0.7Ab | 9.6 ± 0.5Ab | 55.4 ± 0.8Ab | 58.5 ± 1.0Ab | 23.4 ± 0.8Ab | 23.1 ± 0.5Ab |
| CE | 12.8 ± 0.5Aa | 6.8 ± 0.4Bc | 68.3 ± 1.0Aa | 47.9 ± 1.5Bc | 28.5 ± 0.9Aa | 19.7 ± 0.5Bc |
| G + CE | 9.4 ± 0.8Ab | 6.5 ± 0.7Bc | 52.5 ± 0.7Ab | 45.1 ± 0.7Bc | 22.7 ± 0.6Ab | 19.3 ± 0.6Bc |

Means (± SEM) followed by the same capital letters in the columns and by the same lowercase letters in the rows did not differ significantly ($P \leq 0.05$) by Duncan's test.

For light microscopy, samples were dehydrated in an increasing ethanol series (30%, 50%, 70%, 90% and 100%; the last wash was repeated three times) and polymerized in acrylic resin-type methacrylate. Cross-sections (7 μm thickness) were obtained using a manual rotary microtome (Leica RM2155), stained in a toluidine blue solution and mounted on a microscope slide containing Entellan® mounting medium. The samples were observed in a light microscope (Zeiss Axioskop 2), containing a digital camera (MRC3) coupled.

For scanning electron microscopy (SEM), samples were post-fixed in 1% osmium tetroxide, dehydrated in an acetone series (30%, 50%, 70%, 90% and 100%; the last wash was repeated three times) and dried to the critical point (Balzers CPD 030). After assembly in stubs using double-sided carbon tape, samples were metalized with gold (model Balzers SCD 050). Afterward, the material was analyzed by a scanning electron microscope (Zeiss LEO 435-VP) to obtain the electromicrographs.

For transmission electron microscopy (TEM), samples were taken from the Karnovsky fixative, washed three times (10 min each) in 0.05 M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide for 1 h. Subsequently, samples were treated with 0.5% uranyl acetate (overnight) and dehydrated in acetone (30%, 50%, 70%, 90% and 100%; the last wash was repeated three times). For pre-infiltration and infiltration, samples were treated with a solution of 100% acetone + Spurr resin (1:1, v/v) for 6 h and in Spurr resin for 12 h, respectively. The samples were then polymerized on Spurr resin at 70 °C for 3 days. After obtaining the blocks, ultrathin sections (70 nm) were obtained in an ultramicrotome (Leica UC6), using glass razors. The cuts were arranged in copper grids (300 mesh) and contrasted with 2% uranyl acetate and 1% lead citrate.[27] The grids were observed under a transmission electron microscope (JEOL JEM 1100, Akishima, Tokyo, Japan).

### 2.7 Data analysis

The data obtained from the two tests as the net $CO_2$ assimilation rate (*A*) and the variables MI, ILA and UQ were combined due to the homogeneity of variances between the assays.[28] The data of *A*, MI, ILA, UQ, plant height and shoot dry matter were submitted to analysis of variance (ANOVA), and the means were compared by Duncan's test ($P \leq 0.05$). The Pearson correlation of parameter *A* with the variables plant height, shoot dry matter, MI, ILA and UQ.

## 3 RESULTS

### 3.1 The sensitivity of two clones of eucalyptus to the herbicides

There was significant clone–herbicide interaction for all variables studied. At 23 daa, plants of clone CLR-384, exposed to all herbicide treatments displayed significantly lower net $CO_2$ assimilation rate (*A*) and plant growth (height and shoot dry matter) compared with control plants (Table 1). However, CLR-383 was affected only by glyphosate drift-level doses and the mixture of the two herbicides, with reductions of 32% and 36% in *A*, of 23% and 27% in plant height and of 23% and 25% in shoot dry matter, compared with control plants (Table 1).

Plants of both clones showed typical phytotoxicity symptoms due to drift doses of the herbicides. Leaves from the basal part of the plants, directly exposed to carfentrazone-ethyl drift, had brown necrotic spots at 1 daa (Fig. S1). Chlorosis on apical leaves protected from the herbicide application began at 10 daa, except for plants of CLR-383 exposed to the drift-level carfentrazone-ethyl dose (Fig. S2). By contrast to CLR 383, at 23 daa, the basal and apical parts of all plants of clone CLR 384 exposed to the drift-level carfentrazone-ethyl dose had chlorosis on the leaf blade (Fig. S3).

### 3.2 Disease evaluation

On plants of CLR-384, the uredinia dried out when exposed to herbicide drift (Fig. 1b,d), especially to carfentrazone-ethyl or a mixture of glyphosate + carfentrazone-ethyl (Fig. 1c,d), compared with control (Fig. 1a). However, for CLR-383, no uredinia were formed regardless of the treatment (Fig. 1e–h). At 21 dai plants of clone CLR-384 showed less rust infection when exposed to the herbicides glyphosate, carfentrazone-ethyl and their mixture (Fig. 2).

### 3.3 Microscopic observations

Chloroplasts of non-herbicide treated plants of both clones had well-organized thylakoids in grana and without starch grains accumulation (Figs. 3a,d and 4a,b). However, plants of both clones exposed to all herbicide treatments had chlorotic leaves, and the thylakoids were disorganized (Fig. 3b). A large number of peroxisomes were observed in leaf cells of CLR-384 clone plants exposed to carfentrazone-ethyl drift (Fig. 3c). These plants also had an accumulation of starch grains in the chloroplasts when exposed to all three herbicide treatments (Figs. 4c,d,e,g,h), except for the plants of the CLR-383 clone exposed to carfentrazone-ethyl drift (Fig. 4f).

At 6 dai, inoculated and non-herbicide treated plants (control) of CLR-384 showed normal uredinial formation (Fig. 5a,b). However, in herbicide-treated plants, especially those treated with glyphosate + carfentrazone-ethyl, there was almost no epidermis elevation and very few, and small uredinia were formed (Fig. 5c–h). Intense cell division was also observed in cell layers at the infection site of plants exposed to herbicides (Fig. 5c,e,g).

At 12 dai, the uredinia were completely formed, causing rupture of the cuticle and consequent release of the urediniospores



  

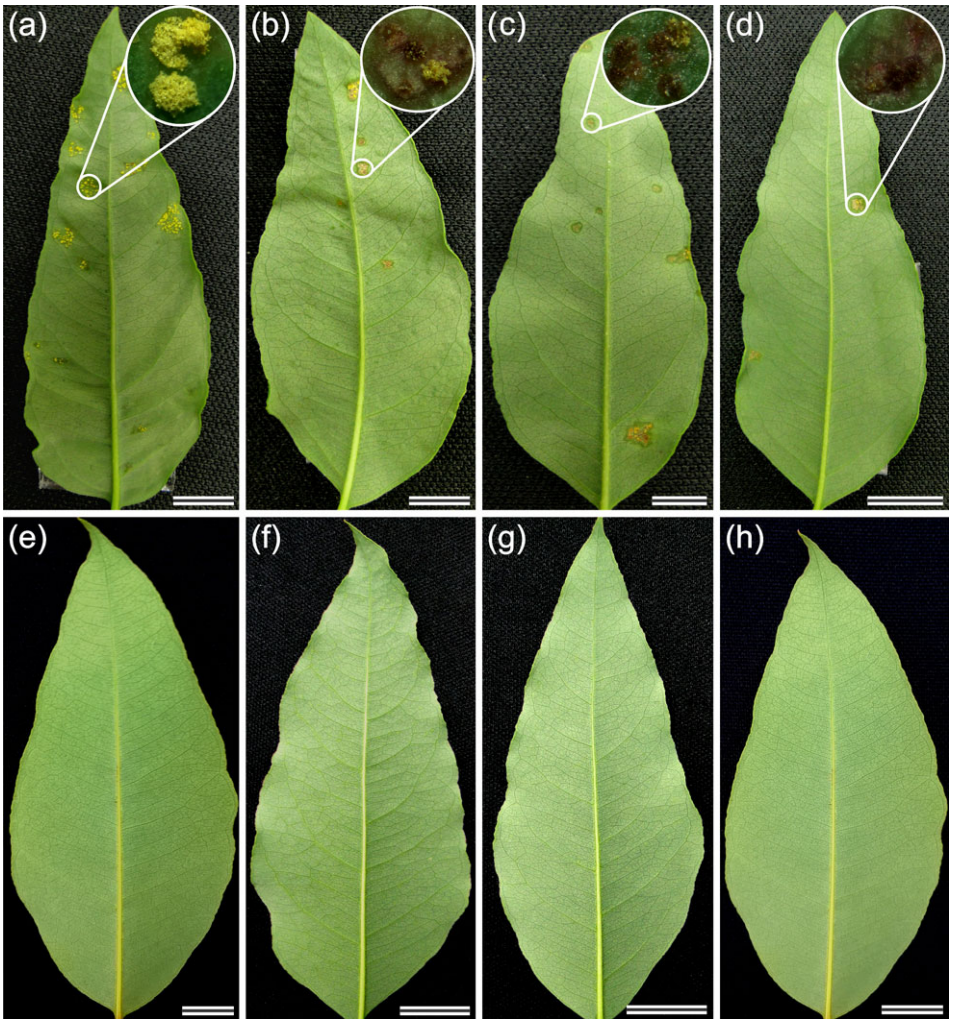


**Figure 1.** Uredinial formation of *Austropuccinia psidii* in the abaxial face of leaves of *Eucalyptus grandis* plants of clones CLR-384 (a–d) and CLR-383 (e–h) exposed to water (control treatment) (a, e), glyphosate (b, f), carfentrazone-ethyl (c, g) and a mixture of glyphosate + carfentrazone-ethyl (d, h) at 21 days after inoculation. (a) Well-developed uredinia with an abundant production of urediniospores. (b, d) Uredinia with malformation and with reduced production of urediniospores. (e–h) Absence of uredinia. Scale bars = 1 cm.

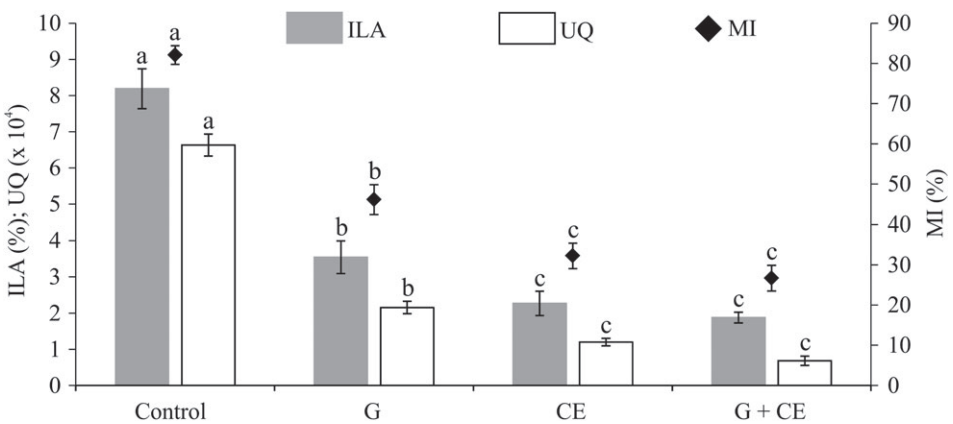


**Figure 2.** Infected leaf area (ILA), urediniospore number (UQ) and McKinney index (MI) on *Eucalyptus grandis* plants of clone CLR-384 sprayed with water (control), glyphosate (G), carfentrazone-ethyl (CE) and a mixture of glyphosate + carfentrazone-ethyl (G + CE) at 21 days after inoculation with *Austropuccinia psidii*. For each variable, means followed by the same letters did not differ significantly ($P \leq 0.05$) by Duncan's test. Bars = mean SE.



  

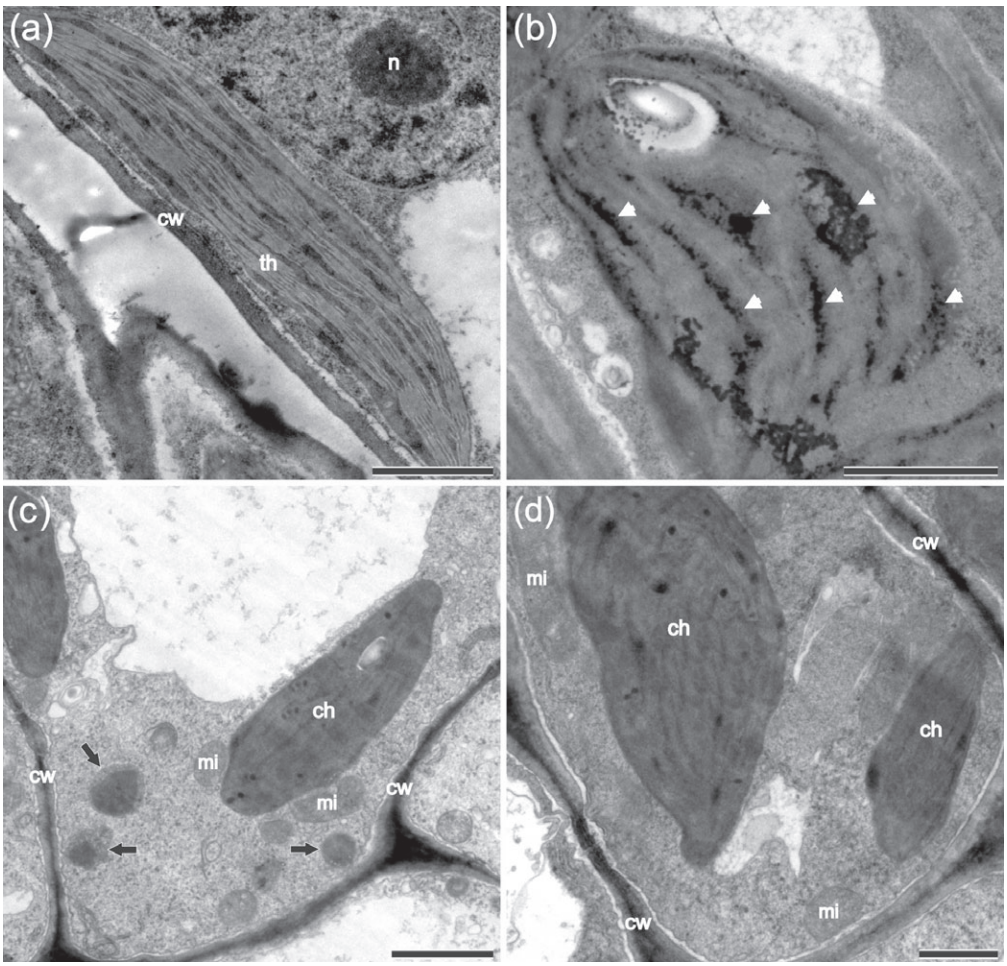


**Figure 3.** Transmission electron micrographs of transverse sections of *Eucalyptus grandis* leaves of clones CLR-383 (a, b) and CLR-384 (c, d) sprayed with water (a, d), glyphosate (b) and carfentrazone-ethyl (c) at 14 days after spraying. (a) Chloroplast with normal appearance and thylakoids organized in grana. (b) Chloroplast with disorganized thylakoids (arrowhead). (c) Presence of peroxisomes (arrows). (d) Absence of peroxisomes on leaf tissue of plants exposed to water. cw, cell wall; mi, mitochondria; n, nucleus; th, thylakoids. Scale bars = 1 μm.

(Fig. 6). In plants exposed to herbicide drift, leaf tissue was less colonized by the fungus, compared with control plants in which uredinia were large and showed abundant urediniospore production (Fig. 6a,b). In plants previously exposed to herbicides, the uredinia were smaller with reduced (Fig. 6c,f) or undetected (Fig. 6g,h) urediniospore production. In addition, cells of the palisade parenchyma showed altered morphology at the infection site (Fig. 6c,e). In plants exposed to the glyphosate + carfentrazone-ethyl mixture, cells were completely degenerate (Fig. 6g).

In transverse sections of leaves exposed to herbicide drift, compartmentalization tissue was formed (Fig. 6c,e,g). In plants sprayed with glyphosate and carfentrazone-ethyl drift-level doses in isolation, the tissue had two layers (Fig. 6c,e), whereas in plants exposed to a drift-level dose of the glyphosate + carfentrazone-ethyl mixture, the compartmentalization tissue was stratified into three or more layers (Fig. 6g).

### 3.4 Pearson correlation

There was a significant ($P \leq 0.01$) and positive correlation between net $CO_2$ assimilation rate, *A*, and plant height ($r = 0.92$) and shoot dry matter ($r = 0.97$) for the CLR-383 clone ($r = 0.96$ and 0.98, respectively, for CLR-384 clone). For the CLR-384 clone, parameter *A* was also significantly ($P \leq 0.01$) correlated with MI, ILA and UQ ($r = 0.95$, 0.94 and 0.93, respectively).

## 4 DISCUSSION

Although there has been a report of reduced rust infection in eucalyptus by glyphosate drift,[11] the current study confirms previous findings and demonstrates microscopic and physiological responses in *E. grandis* treated with glyphosate, carfentrazone-ethyl and a mixture of these two active ingredients.

The two clones tested differed in herbicide sensitivity, expressed by the net $CO_2$ assimilation rate (*A*), plant height and shoot dry matter. Differences in tolerance to glyphosate have been reported in *Eucalyptus* genotypes.[10] The activity of glyphosate varies with its absorption and translocation rate.[29] Other authors working with fomesafen, a herbicide with the same mode of action (inhibition of PPO) as carfentrazone-ethyl, also observed a reduction in the dry mass of shoots from two hybrids of *E. urophylla* × *E. grandis*.[30]

The tested herbicides may have affected $CO_2$ assimilation through their effect on disorganization of the thylakoid membranes. Also, glyphosate indirectly affects photosynthesis by inhibiting the biosynthesis of carotenoids, chlorophylls, fatty acids



  

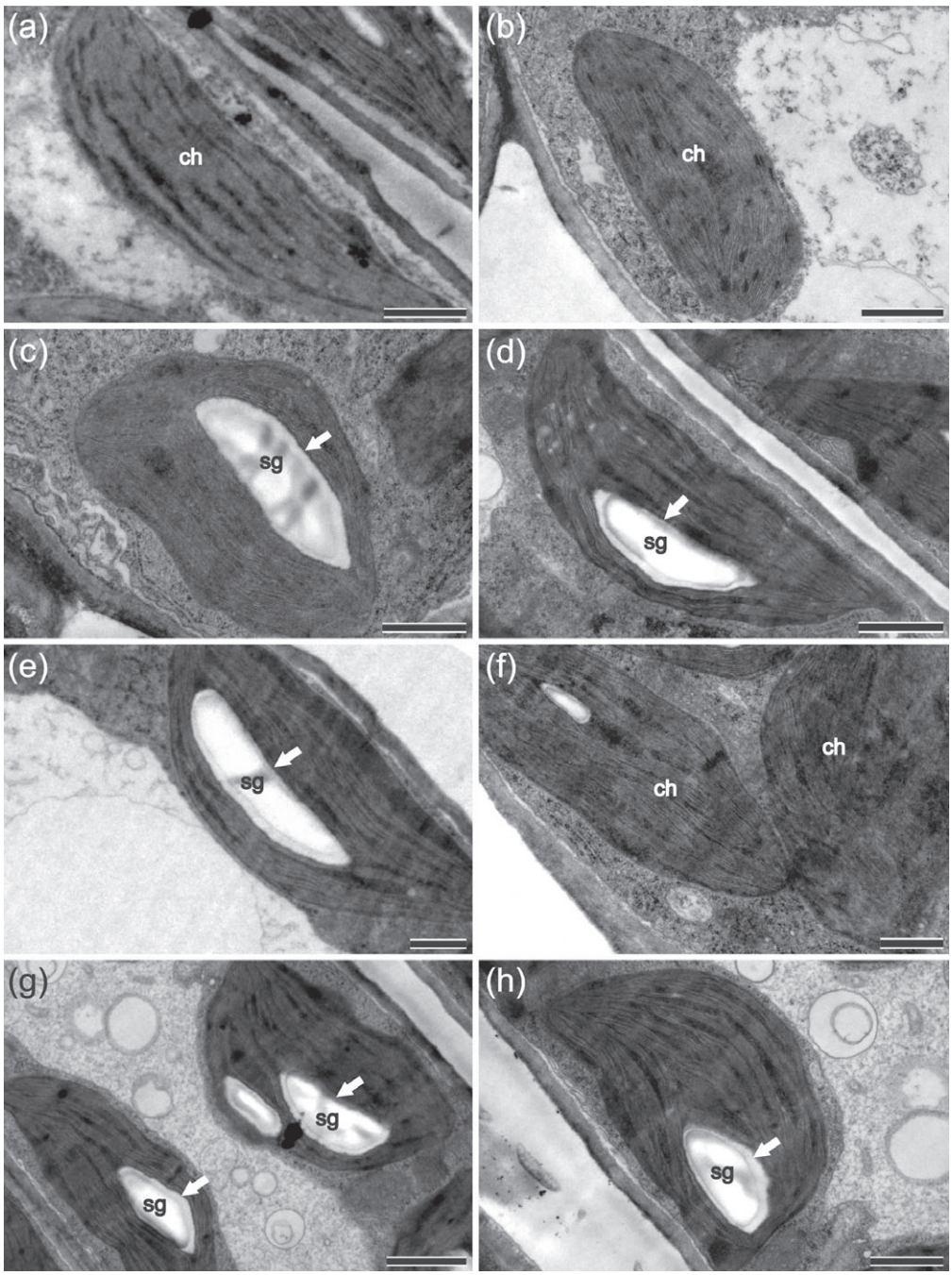


**Figure 4.** Transmission electron micrographs of transverse sections of *Eucalyptus grandis* leaves of clones CLR-384 (a, c, e, g) and CLR-383 (b, d, f, h) exposed to water (a, b) and to the herbicides glyphosate (c, d), carfentrazone-ethyl (e, f) and a mixture of glyphosate + carfentrazone-ethyl (g, h) at 23 days after spraying. (a, b, f) Starch grains were not evident. (c–e, g, h) Presence of very well formed starch grains (white arrows). ch, chloroplast; sg, starch grains. Scale bars = 1 μm.

and amino acids,[31] as well as secondary metabolism products essential for photosynthesis such as quinones.[32] In the current study, there was a reduction in net $CO_2$ assimilation rate (*A*) values for plants of the two *E. grandis* clones exposed to glyphosate, as reported for several plant species exposed to this herbicide.[33–35] Although there are no reports of the effect of carfentrazone-ethyl on photosynthesis in eucalyptus plants, this herbicide inhibits PPO, which is important in chlorophyll biosynthesis.[36] However, most of its effects are due to oxidative stress caused by accumulation the photosensitizing pigment protoprorphyin IX.[37]

Although the respiration rate was not evaluated in this study, the presence of starch grains in the chloroplasts, associated with reduced growth in plants exposed to herbicide drift, indicates a possible reduction in metabolite processes such as respiration rate in these plants. Plant respiration consists of the oxidation of glucose that yields energy, used by plants in their vital processes. During the day, the glucose consumed in respiration comes from photosynthesis, whereas at night it comes from reserves accumulated during the day, usually in the form of starch grains.[38] Because starch grains were observed in leaf samples collected at 08:00, this



  

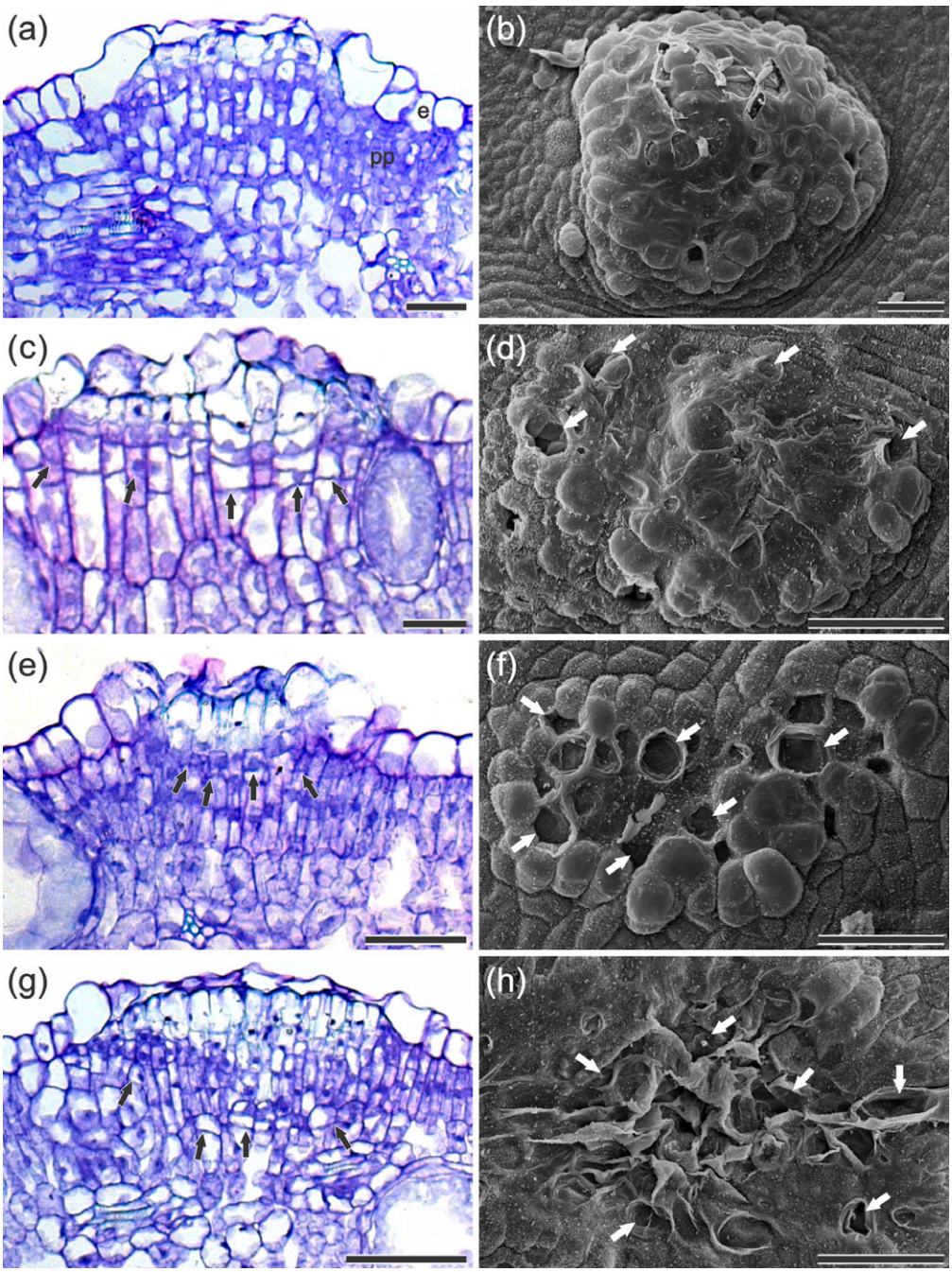


**Figure 5.** Light micrographs of transverse sections (a, c, e, g) and scanning electron micrographs of the abaxial surface (b, d, f, h) of *Eucalyptus grandis* leaves of the clone CLR-384 sprayed with water (a, b), glyphosate (c, d), carfentrazone-ethyl (e, f) and a mixture of glyphosate + carfentrazone-ethyl (g, h) at 6 days after inoculation with *Austropuccinia psidii*. (a, b) Well-developed uredinia. (c, e, g) Cell division at the infection site (black arrows). (d, f, h) Malformed uredinia with (white arrows). e, epidermis; pp, palisade parenchyma. Scale bars = 50 μm.

may indicate that starch was not used by the respiratory system in the previous night. Also, a reduction in the respiration rate is common in plants exposed to herbicide drift.[39]

Glyphosate and carfentrazone-ethyl did not break down the rust resistance of CLR-383 clone, controlled by the *Ppr*-1 locus,[21] but they increased the basal resistance of the susceptible clone (CLR-384). Several studies report *in vitro* glyphosate inhibition of spore germination and hyphal growth of various fungal species,[40,41] including *A. psidii*.[11] This inhibition may occur in the shikimic acid pathway, which is affected by glyphosate in some fungi.[42] This molecule has a negative effect on the development of some fungal diseases, mainly rusts.[43] Previously, studies have shown significant lower rust severity of eucalyptus rust in cuttings of the same clones used in our studies, when these were submitted to glyphosate drift.[11] Similar results were observed for other pathogens in several crops. In herbicide-resistant transgenic soybeans, spraying with glyphosate reduced the severity of soybean Asian rust (*Phakopsora pachyrhizi*),[44,45] powdery mildew (*Erysiphe diffusa*) and leaf spot (*Myrothecium roridum*).[40] In glyphosate-resistant alfalfa cultivation, spraying of this herbicide

  

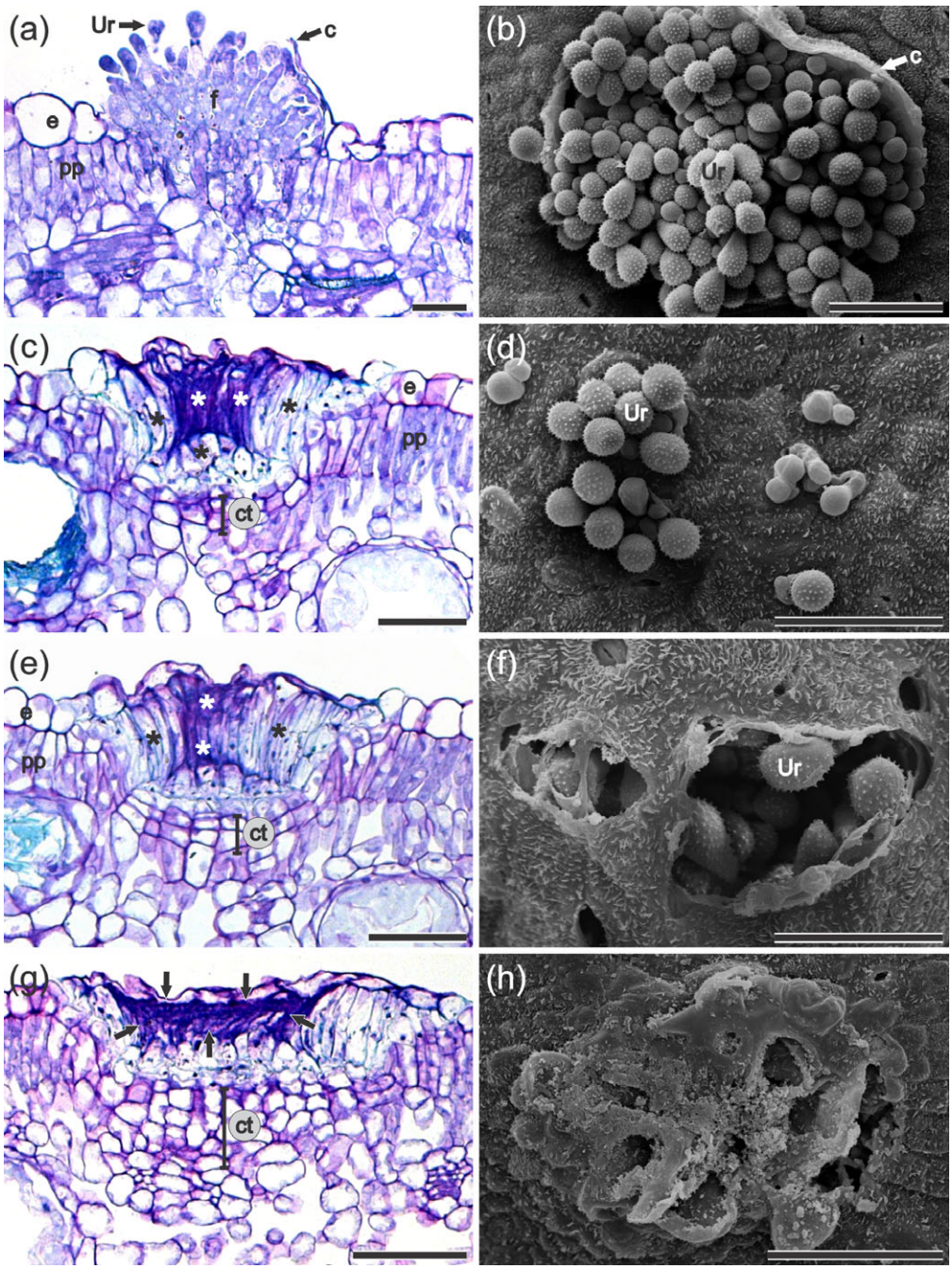


**Figure 6.** Light micrographs of transverse sections (a, c, e, g) and scanning electron micrographs of the abaxial surface (b, d, f, h) of *Eucalyptus grandis* leaves of clone CLR-384 sprayed with water (a, b), glyphosate (c, d), carfentrazone-ethyl (e, f) and a mixture of glyphosate + carfentrazone-ethyl (g, h) at 12 days after inoculation with *Austropuccinia psidii*. (a, b) Well-developed uredinia with abundant production of urediniospores; (c, h) malformed uredinia; (c, e) cells of the palisade parenchyma with altered morphology (asterisks); (d, f) reduced production of urediniospores; (g) cells of the palisade parenchyma degenerated (arrows); and (h) uredinia without evident release of urediniospores. c, cuticule; ct, compartmentalization tissue; e, epidermis; f, fungal hyphae; pp, palisade parenchyma; Ur, urediniospores. Scale bars = 50 μm.

reduced the severity of rust caused by *Uromyces striatus*.[41] In glyphosate-resistant wheat, glyphosate application provided both preventive and curative activities on control of stripe (*Puccinia striiformis* f. sp. *tritici*) and rust (*Puccinia triticina*).[44,46]

Although there are no reports of the effect of carfentrazone-ethyl on the severity of fungal diseases, the abundance of peroxisomes observed in leaves of the rust-susceptible clone (CLR-384), exposed to herbicide drift, may be related to a reduction in the severity by the herbicide. Peroxisomes produce peroxidase, a key enzyme in the defense against fungal pathogens.[47] In addition, studies with other PPO inhibitors reported their induction of pathogen resistance in plants. Soybean plants treated with lactofen and inoculated with *Sclerotinia sclerotiorum* had lower lesion diameters, due mainly to a high glyceollin content in the leaves.[16] In glyphosate-resistant soybean, lactofen also increased phytoalexin production, reduced *Sclerotinia* stem rot lesion diameter and reduced disease severity index.[17] In several crops, the herbicides acifluorfen and lactofen



  

induce the accumulation of some compounds related to plant defense mechanisms against pathogens such as phytoalexins and stress metabolites,[12] camalexin[13] and isoflavone.[14]

By inhibiting the synthesis of the 5-enolpyruvyl-shikimate-3-phosphatase, glyphosate reduces the synthesis of aromatic amino acids such as tryptophan, phenylalanine and tyrosine.[48] Such amino acids are essential for fungal mycelial growth.[40] In additionally, by reducing the net $CO_2$ assimilation rate (*A*), the action of herbicides such as glyphosate also affects the synthesis of sugars, amino acids and proteins.[49] Biotrophic pathogens, such as *A. psidii*, depend on living host tissues.[19] In this study, plants of both eucalyptus clones exposed to a drift-level dose of the herbicides glyphosate and carfentrazone-ethyl had a reduced net $CO_2$ assimilation rate (*A*). The positive and significant Pearson correlation of the parameter *A* with plant height, shoot dry matter, MI, ILA and UQ, in addition to poor uredinial formation, reinforces the deleterious effect of glyphosate and carfentrazone-ethyl herbicide drift on *A* values, as well as their indirect influence on development of rust in plants of clone CLR-384 of *E. grandis*.

The results of this study demonstrate the effects of glyphosate and carfentrazone-ethyl herbicide drift on rust in eucalyptus. Application of these herbicides for weed management is more frequent in the first year after planting,[18] when the plants are more vulnerable to rust infection.[19] It is emphasized that most plant defense compounds against pathogen infection come from secondary metabolism, which may be directly or indirectly affected by the use of herbicides. In this context, there is a need for further studies to determine the effect of herbicides on infection by other important pathogens in eucalyptus plantations for a sustainable integrated phytosanitary management.

## 5 CONCLUSION

The glyphosate and carfentrazone-ethyl herbicide drift does not break down rust resistance, but increases the basal resistance of susceptible eucalyptus clones, as well as inhibiting the uredinial formation of *A. psidii* during infection on eucalyptus leaves.

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## SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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