FOLIAR APPLICATION OF PROCESSED CALCITE PARTICLES IMPROVES LEAF PHOTOSYNTHESIS OF POTTED *VITIS VINIFERA* L. (VAR. 'COT') GROWN UNDER WATER DEFICIT

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Abstract

Aim: To determine the effect of foliar sprays of processed mineral particles (micronized calcite, Megagreen®) on leaf water relations and photosynthesis in grapevines (*Vitis vinifera* L. var. Cot) grown under water deficit.

Methods and results: Potted plants were grown in a glasshouse in summer under natural light (Toulouse, lat. 43°32'14.50''N; long. 1°29'44.25''W; altitude 148 m a.s.l.). Well-watered and drought stressed vines were foliar sprayed with various doses of micronized calcite. Leaf water potential, gas exchange (light-saturated CO_2 assimilation rate, A_{CO2}; stomatal conductance, g_s) and chlorophyll fluorescence (quantum efficiency of PSII photochemistry, ΦPSII) were measured. Water stress affected predawn and midday leaf water potentials, A_{CO2}, g_s and ΦPSII of the 40-day-old (younger fully expanded) leaves. Megagreen® application did not lead to appreciable changes in leaf water potentials but was able to mostly restore A_{CO2} and ΦPSII in water stressed plants to levels of well-watered control plants. This effect was associated with a positive, although less pronounced, effect on g_s. The beneficial effects of processed calcite particles were also observed on younger and older leaves, at low and high atmospheric vapor pressure deficit, and in the morning as well as in the afternoon. In well-watered plants, processed calcite particles had no effect on photosynthesis except under very high evaporative demand. In water stressed plants, A_{CO2} was increased by increasing ambient CO_2 concentration. At elevated CO_2, calcite particles did not increase CO_2 assimilation.

Conclusion: Foliar application of processed calcite particles alleviated most of the adverse effects of water stress on grapevine photosynthesis. This was associated with enhanced g_s in the whole plant canopy.

Significance and impact of the study: In the context of climate change, grapevine will most likely experience long periods of drought during its seasonal cycle. Foliar application of processed mineral particles is widely used to reduce heat stress in perennial fruit crops. Here, the micronized calcite Megagreen® does improve photosynthesis of water stressed grapevines.

Key words: chlorophyll fluorescence, micronized calcite, foliar spray, photosynthesis, stomatal conductance, water potential, stress, *Vitis vinifera* L.

Résumé

Objectif : Quantifier les effets d'applications foliaires de particules micronisées de calcite (Megagreen®) sur l'équilibre hydrique et la photosynthèse chez la vigne (*Vitis vinifera* L. var. Cot) en condition de stress hydrique.

Méthodes et résultats : Des plants, élevés pendant l'été en pot dans une serre sous lumière naturelle (Toulouse, lat. 43°32'14.50''N ; long. 1°29'44.25''W ; altitude 148 m a.s.l.) avec une alimentation en eau optimale ou limitante ont reçu différentes doses foliaires de calcite micronisée. Au niveau des feuilles, il a été mesuré le potentiel hydrique, les échanges gazeux (assimilation du CO_2 sous lumière saturante, A_{CO2} ; conductance stomatique, g_s) et la fluorescence de la chlorophylle (rendement maximal du PSII, ΦPSII). Dans les feuilles matures (âgées de 40 jours), la contrainte hydrique altère le potentiel hydrique en fin de nuit comme à midi, ainsi que A_{CO2}, g_s et ΦPSII. L'application de Megagreen® ne modifie pas le potentiel hydrique foliaire mais rétablit largement A_{CO2} et ΦPSII chez les plantes stressées. Un effet positif, bien que moins prononcé, apparait sur g_s. Les effets bénéfiques des pulvérisations foliaires de calcite se vérifient quel que soit l'âge des feuilles, sous forts ou faibles déficits de pression de vapeur d'eau et le matin comme l'après midi. Chez les plantes bien alimentées en eau, la calcite n'a d'effet que dans des conditions de demande évaporative intense. Sous stress, A_{CO2} augmente avec la concentration en CO_2. Sous fort CO_2, la calcite n'affecte plus la photosynthèse.

Conclusion : L'application foliaire de particules de calcite micronisées atténue fortement l'impact négatif du stress hydrique sur la photosynthèse de la vigne. Cet effet bénéfique est associé à une augmentation de la g_s de la canopée.

Signification et impact de l'étude : Dans le contexte du changement climatique, la vigne va devoir supporter des périodes de sècheresse de plus en plus longues au cours de son cycle de développement. L'application foliaire de particules minérales est communément utilisée pour réduire le stress hydrique et thermique associés à la sécheresse en arboriculture. Il apparait ici que la calcite micronisée Megagreen®, dont la dose d'application est inférieure à celle des autres minéraux, améliore notablement la photosynthèse de la vigne en condition de sécheresse.

Mots clés : fluorescence de la chlorophylle, calcite micronisée, application foliaire, photosynthèse, conductance stomatique, potentiel hydrique, stress, *Vitis vinifera* L.

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INTRODUCTION

Drought stress is one of the most important environmental factors inhibiting plant growth and production (Chaves *et al*., 2002). Severe water stress results in reduced metabolism and low gas exchange rate (Hsiao, 1973). Both stomatal and non-stomatal factors contribute to the effects of drought on photosynthesis and transpiration (Kaiser *et al*., 1981). Under mild drought stress, stomata closure can be the main factor since the photosynthetic apparatus is largely unaffected by water limitation in the whole plant (Cornic *et al*., 1992). However, reduction in net CO_2 assimilation has also been attributed to non-stomatal factors such as carbohydrate accumulation, reduction in ribulose-1,5-biphosphate regeneration caused by inhibition of ATP synthesis, or photoinhibition (Lawlor, 2002; Medrano *et al*., 2003).

Grapevine is generally grown in regions with dry summers and, therefore, must sometimes endure several months of drought during its seasonal cycle (Lovisolo *et al*., 2010). The visible symptoms of excessive water deficit during the vegetative phase are leaf wilting and decreases in plant height, leaf number and canopy leaf area (Cramer *et al*., 2007). Drought effects can also include injury to plasma membranes, disturbances in the water status of different organs, and a decrease in chlorophyll content (Medrano *et al*., 2003). Plants can resist drought stress by reducing their leaf canopy and by closing their stomata (Flexas *et al*., 2002). At the cellular level, drought stress in grapevines affects the transcript level of genes involved in metabolism, transport, and biogenesis (Cramer *et al*., 2007).

In recent years, foliar sprays containing processed mineral particles have been developed in order to repel insects (Glenn *et al*., 1999; Showler, 2002), suppress disease incidence (Glenn and Puterka, 2005), and reduce heat stress and solar injury (Glenn *et al*., 2003; Jifon and Syvertsen, 2003), the latter leading to enhanced net carbon assimilation(Glenn *et al*., 2003). The presence of mineral particles on the leaf surface can reduce the amount of photosynthetically active radiation (*PAR*) reaching the leaf surface by reflecting *PAR*. However, under drought stress conditions, Glenn *et al*. (2001) reported that the application of a reflective coating on plants may provide a net benefit by sufficiently reducing the heat load in order to offset the potential loss in assimilation rate. Megagreen® is a micronized calcite elaborated from finely ground sedimentary limestone rock and activated by tribomechanical process (European Patent No. WO/2000/064586, 2000). Particles are grey in color and non reflective. The application of Megagreen® has been shown to have a beneficial effect on several horticultural crops such as olive tree, maize, strawberry and lettuce under drought conditions (F. Attia, personal correspondence). However, the physiological effects of Megagreen® have yet to be described. Applications of different particles have produced conflicting results on leaf net photosynthesis, stomatal conductance (g_s), transpiration rate and fruit yield depending on species, light intensity, temperature, etc. (Glenn *et al*., 2003; Rosati *et al*., 2006). The purpose of our study was to determine the effects of processed calcite particles applied as a foliar spray on gas exchange, g_s and primary photochemistry in water stressed, glasshouse grown potted grapevines. Since the cultivar 'Cot' is mostly grown in areas where only occasional summer rainfalls occur, this study should add to basic knowledge about the mechanisms of how water stress affects this grape cultivar.

MATERIALS AND METHODS

1. Plant material

Two-month-old *Vitis vinifera* L. (red cultivar 'Cot') grapevines grafted onto 3309C rootstock (1-year-old vines produced in nursery) were grown in individual 10-L plastic pots containing 4 parts peat and 1 part sand by volume (Pozzolana). The experiment was carried out in summer (July and August) in Toulouse (southern France, lat. 43°32'14.50''N; long. 1°29'44.25''W; altitude 148 m a.s.l.), in a glasshouse of the Institut National Polytechnique under natural light. During the experiment, sunrise was at about 0450 h and sunset at about 2030 h. The temperature inside the glasshouse was approximately 30/25°C (day/night) with 40±10% relative humidity (RH) at midday. Vines were irrigated three times a week to full pot capacity with 0.6 L/vine of complete nutrient solution (Ibrahim, 2001).

2. Water stress and Megagreen® treatments

Water stress was imposed by decreasing the irrigation volume from 0.6 L (control) to 0.2 L (stress). Plant water status was monitored every two days from the end of shoot and inflorescence development (E-L number 18; plant height of about 2 m) to the end of the experiment by measuring predawn (ψ_{PD}) and midday (ψ_{MD}) leaf water potential with a pressure chamber as described below, after Scholander *et al.* (1965). ψ_{PD} varied between -0.2 and -0.3 MPa in control vines and between -0.4 and -0.5 MPa in drought stressed vines, based on stress levels suggested by Girona *et al.* (2006).

Control and water stressed plants received two spray applications of Megagreen®: the first at the onset of the drought treatment and the second two weeks later. Megagreen® water suspension (ca 50 mL per plant) was sprayed on foliage (25-30 leaves/vine). Fives Megagreen® application rates – 0.00% (D_0) as a control, 0.33% (D_1), 0.66% (D_2), 1.00% (D_3) and 1.33% (w/v) (D_4) – were prepared by adding the particles to water with no other materials. Most of the results shown are for the D_3 treatment (1.00%) since it is the recommended treatment rate in the field (applications of 1.50 kg in 150 L water per ha).

The experimental design was a completely randomized plan with n = 12 replicates per well-watered and drought stressed treatment for each of the five rates of Megagreen® for a total 120 vines.

3. Plant water relations

Every two days, at predawn (0300 to 0400 h), ψ_{PD} was determined on three 40-day-old (young fully expanded) leaves per treatment by using a pressure chamber (PMS Instruments, Corvallis, OR). At midday (1200 to 1300 h) on the same day, ψ_{MD} was measured on similar leaves (same age) than those previously used for photosynthesis and chlorophyll fluorescence measurements.

4. Chlorophyll fluorescence

Data on leaf chlorophyll fluorescence and gas exchange were collected from 0900 to 1100 h. Chlorophyll fluorescence was measured on attached leaves using an open infra-red gas exchange system with an integrated fluorescence chamber head (LI-6400-40 LCF; LI-COR, Lincoln, NE, USA). For stressed and control plants, six uniform 40-day-old leaves of different plants were measured (two measurements per leaf). At the time of measurement, leaf temperature in the gas exchange chamber was set at glasshouse air temperature (about 30 °C), RH was about 60 %, and chamber air CO_2 concentration was set at 400 μL L^{-1}. The analyses of fluorescence were performed using the light-doubling technique and terminology as described by Khamis *et al*. (1990). The quantum efficiency of PSII photochemistry was estimated as follows: ΦPSII = (Fm' – Fs)/Fm' where Fm' is the pulse-saturated fluorescence yield in the light (when all the PSII traps are closed) and Fs is the steady-state fluorescence yield in the light. Photochemical quenching (qP) of chlorophyll fluorescence was estimated as follows: qP = (Fm' – Fs)/(Fm' – Fo') where Fo' is the lowest fluorescence yield with open PSII centers. The maximal quantum yield of PSII ("Fv/Fm") of dark-adapted sample was calculated as (Fm' – Fo')/Fm'.

5. Net photosynthesis

Single leaf gas exchange measurements were coupled with measurements of leaf chlorophyll fluorescence using the same leaves. Light saturated net CO_2 assimilation rates (A_{CO2}) and stomatal conductance to water vapor (g_s) were measured on six leaves (two measurements per leaf) in each treatment from 0900 to 1,100 h using the Li-6400-40 LCF gas exchange analyzer. Each sample leaf was placed in a cuvette maintained at ambient temperature and humidity (about 30°C and 60% RH) and exposed to saturating photosynthetic photon flux density (1500 μmol m^{-2} s^{-1} *PAR*) at a fixed CO_2 concentration of 400 μL L^{-1} ("ambient" concentration) or 800 μL L^{-1} ("high" concentration). A_{CO2} (μmol m^{-2} s^{-1}) and g_s (mol m^{-2} s^{-1}) were measured after gas exchange rates became stable, usually within 4 min.

6. Data analysis and experimental design

Data were analyzed by analysis of variance (ANOVA) using SigmaStat® 2.03 software. Differences between means were determined using Fisher's Least Significant Difference. A 5% significance level was used throughout. KaleidaGraph〉 was used to determine relationships between parameters by means of regressions.

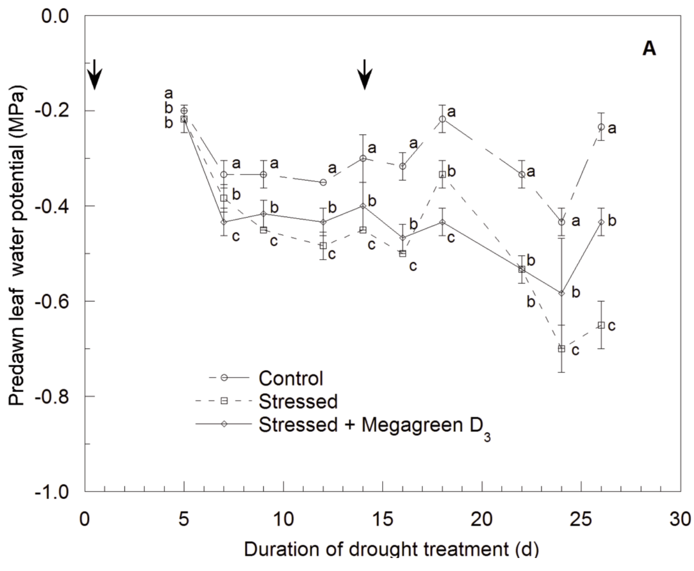

Figure 1. Changes in predawn (ψ_{PD}, A) and midday (ψ_{MD}, B) water potentials of young fully expanded leaves from well-watered control, water stressed and water stressed with Megagreen® application (Stressed + Megagreen® D_3) 'Cot' grapevines grown in a glasshouse.

Arrow denotes when Megagreen® was applied. Each point represents mean (n = 3) ± 1 SD. Different lower case letters denote significantly different means by Fisher's LSD at *P*d"0.05.

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RESULTS

Water potential measured in 40-day-old leaves markedly decreased with increasing evaporative demand during the day, as indicated by the difference between ψ_{PD} (Figure 1a) and ψ_{MD} (Figure 1b). Compared to well-watered plants, water stress (with or without foliar spray of processed calcite particles (Megagreen®)) resulted in a significant decrease in water potentials before dawn and at midday. Under water conditions, Megagreen® application slightly increased ψ_{PD} but generally did not affect ψ_{MD}. However, a positive effect of foliar spray of processed calcite particles was observed on ψ_{MD} at the end of the experiment (day 26, after 4 weeks of treatment).

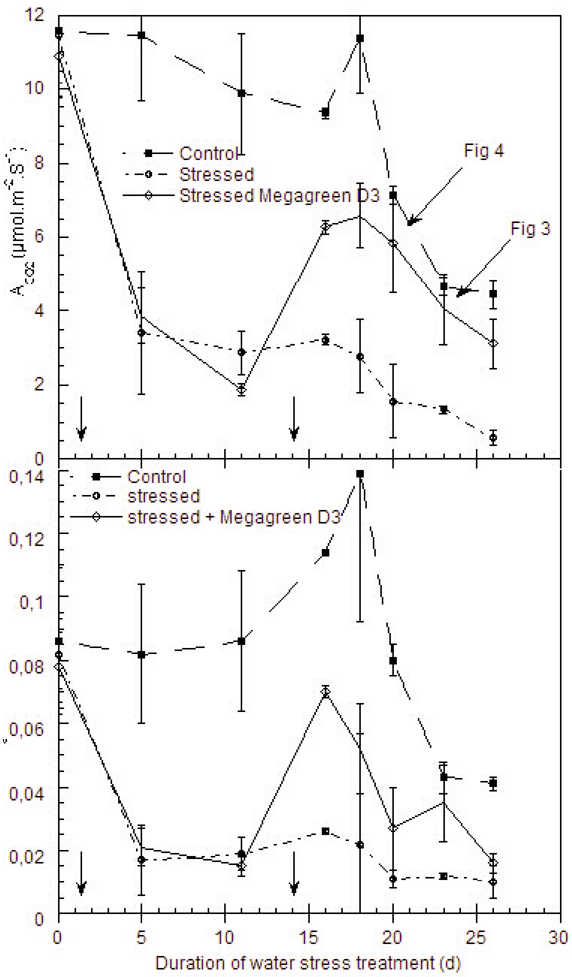

Figure 2. Net CO_2 assimilation rate (A_{CO2}, A) and stomatal conductance (g_s, B) (expressed as percentage of control) under saturating *PAR* of young fully expanded leaves of 'Cot' grape cultivar after Megagreen® treatments.
Arrow denotes when Megagreen® was applied.
Each point represents mean (n = 12) ± 1 SD. * indicates significantly different means at a given day of treatment based on Fisher's LSD at P=0.05.

In 40-day-old leaves, A_{CO2} was already markedly depressed after 5 days of water stress (30% of the controls) and remained on average at similar low values thereafter (Figure 2). The negative effect on A_{CO2} was associated with a parallel effect on g_s. In water stressed plants, the single foliar spray of processed calcite particles did not affect A_{CO2} after 5 and 11 days but enhanced A_{CO2} after the 2nd application at 14 days and thereafter. After 3 weeks, A_{CO2} reached a similar value in the Megagreen®-treated stressed vines as in control vines. The positive effect of foliar spray of processed calcite particles on A_{CO2} was associated with a positive but less pronounced effect on g_s.

As indicated in Figure 3, the effects of drought and foliar spray of processed calcite particles (duration of treatment longer than 2 weeks) were similar in young (less than 35 days) and old (more than 45 days) leaves as compared to those observed on the younger fully expanded leaves. Thus, only the latter were used to further characterize the effects of

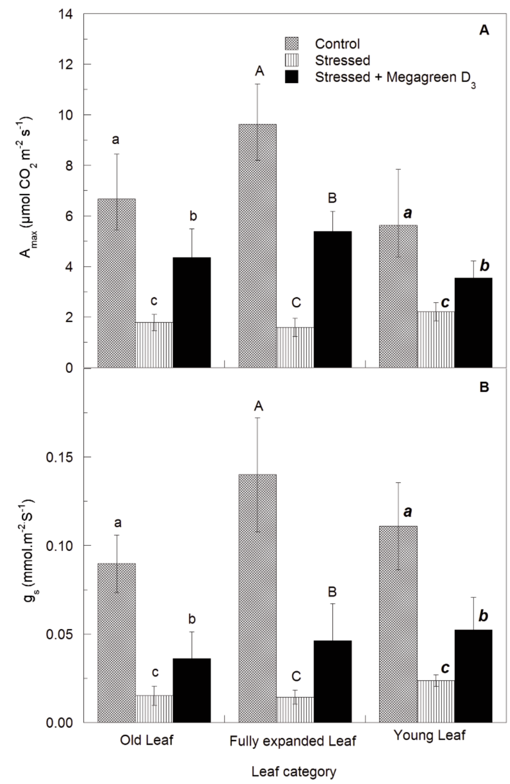

Figure 3. Net CO_2 assimilation rate (A_{CO2}, A) and stomatal conductance (g_s, B) under saturating *PAR* of n = 7 old and recently fully expanded leaves and not fully expanded young leaves of 'Cot' grape cultivar after 25 days of drought stress and Megagreen® treatments.
Different lower case letters for the same leaf category denote significantly different means by Fisher's LSD at P≤0.05.
Vertical lines represent ± 1 SD.

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Megagreen® action. Foliar spray of processed calcite particles increased CO_2 fixation in the morning as well as in the afternoon in comparison with stressed vines (Figure 4); however, A_{CO2} in drought + Megagreen® treatment did not recover to control values.

The steady-state values reached by ΦPSII were low in all (control, water stress and water stress + Megagreen®) plants due to the high irradiances on the leaves (1500 μmol m^{-2} s^{-1} *PAR*). Chlorophyll fluorescence analysis of plants after 2 weeks of water stress revealed that ΦPSII in stressed plants was lower than in the controls (Figure 5). Foliar spray of processed calcite particles partly restored the PSII photochemistry in the water stressed plants. Neither water stress nor Megagreen® had significant effect on qP or Fv/Fm.

For treatment duration > 2 weeks, foliar spray of processed calcite particles did not significantly affect the photosynthetic parameters (A_{CO2}, g_s and ΦPSII) in well-watered plants, whereas the beneficial effect of Megagreen® on photosynthesis, g_s and ΦPSII of water stressed plants was again clearly observed (Figure 6). The recommended treatment rate in the field (1.00% w/v) equivalent to 150 L mixture per ha (D_3) had beneficial effect on these photosynthetic parameters.

CO_2 uptake was slightly decreased by water shortage when the CO_2 concentration around the leaves was 800 μL L^{-1} (high concentration, Figure 7). Interestingly, under high CO_2 concentration, Megagreen® did not stimulate A_{CO2} in stressed plants.

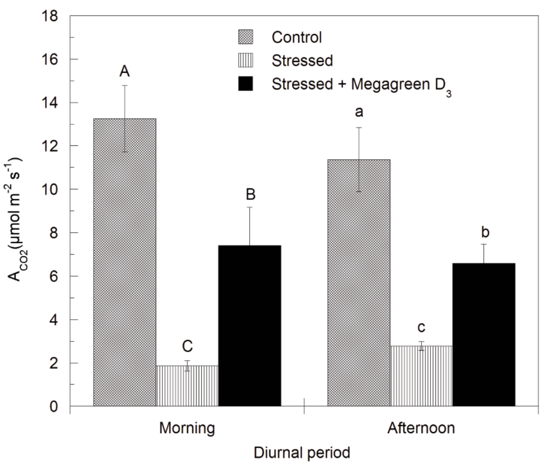

Figure 4. Effects of drought and drought + Megagreen® treatment on morning and afternoon net CO_2 assimilation rate under saturating *PAR* (A_{CO2}) of young fully expanded leaves. Vines were subject to drought treatment durations longer than 2 weeks.
Each bar represents mean (n = 6) ± 1 SD.
Different lower case letters denote significantly different means by Fisher's LSD at $P \leq 0.05$.

DISCUSSION

ψ_{PD} and ψ_{MD} can serve as indicators of water status of plants (Williams and Araujo, 2002). Here, the lowest value of leaf water potential reached during

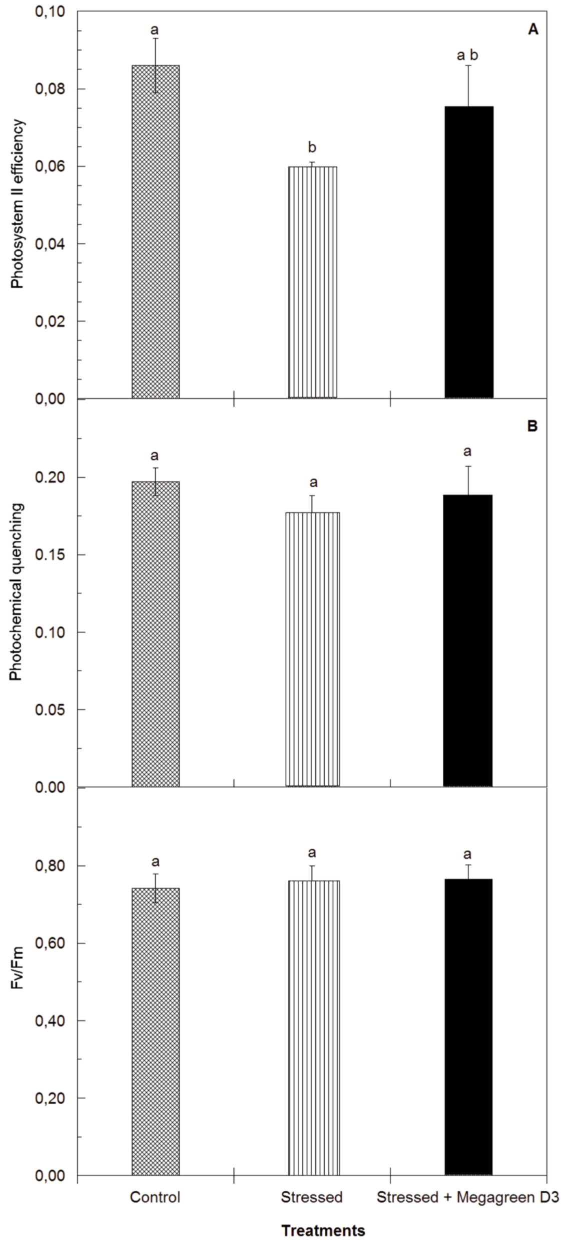

Figure 5. Effects of drought and drought + Megagreen® treatment on Photosystem II efficiency (ΦPSII, A) and photochemical quenching (qP, B) under saturating *PAR*, and maximal Photosystem II efficiency of dark-adapted leaves (Fv/Fm, C) of fully expanded leaves after 25 days of drought stress.
Each bar represents mean (n = 6) ± 1 SD.
Different lower case letters denote significantly different means by Fisher's LSD at $P \leq 0.05$.

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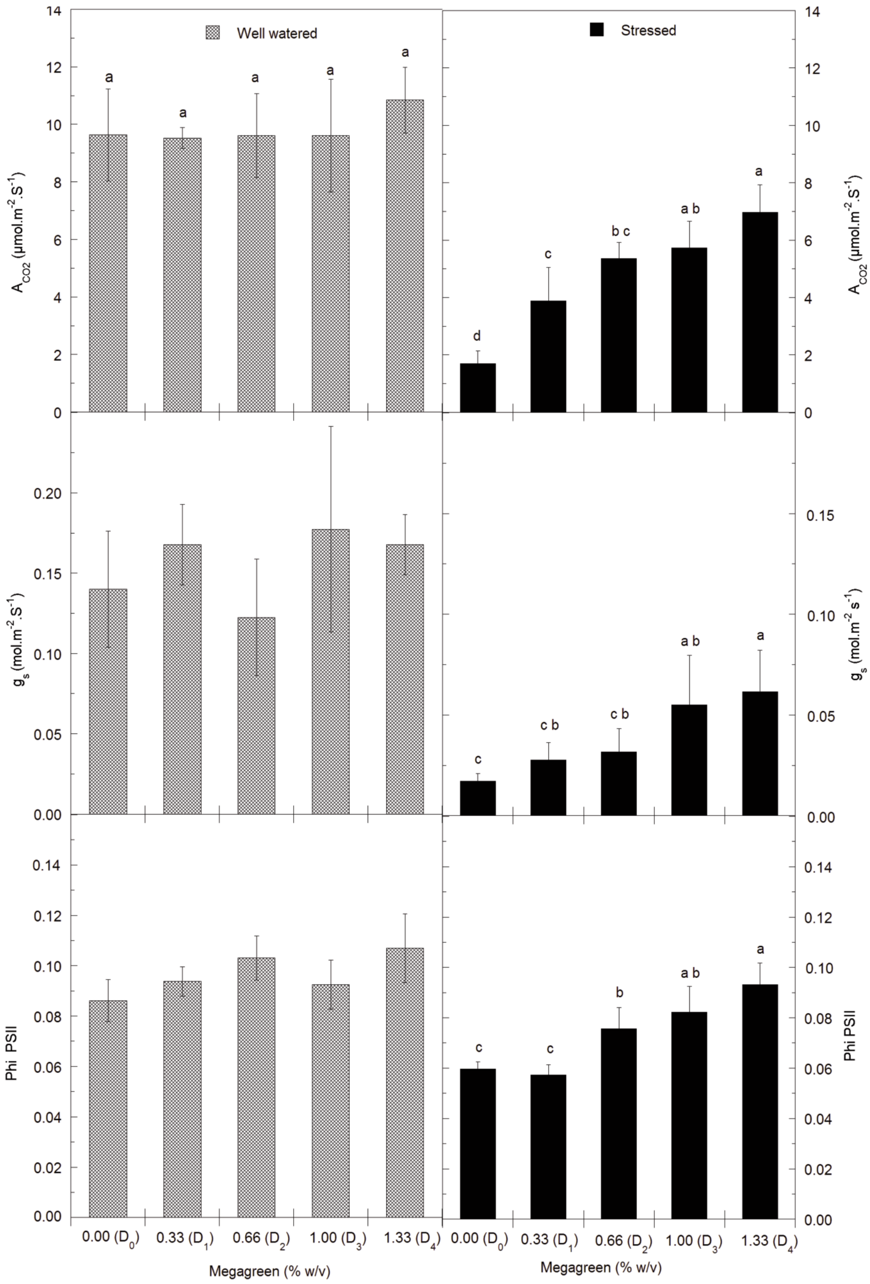

Figure 6. Effect of different doses of Megagreen® on net CO_2 assimilation rate (A_{CO2}), stomatal conductance (g_s) and Photosystem II efficiency under saturating *PAR* (ΦPSII) of recently fully expanded leaves from well-watered and stressed treatments of 'Cot' grape cultivar. Megagreen® water suspension was sprayed on foliage at the onset of the drought treatment and 2 weeks later. Plants were subject to drought treatment duration longer than 2 weeks.

Each bar represents mean (n = 9) ± 1 SD. Different lower case letters denote significantly different means by Fisher's LSD at $P \leq 0.05$. Regression analysis was performed and data were fitted to a linear regression function, r^2 significant at $P<0.001$.

the treatment in stressed vines was -0.7 MPa before dawn and -1.7 MPa at midday (at the very end of the treatment). Although the water limited plants showed visible signs of water stress (e.g., loss of turgor), no defoliation or leaf necrosis occurred. In well-watered plants, ψ_{MD} was lower at the end (end of August) than at the beginning (end of July) of the experiment. This indicates that some water constraints occurred in control plants probably due to a high vapor pressure deficit (VPD) at midday at the end of August. In general, foliar spray of processed calcite particles did not change ψ_{MD} of water stressed plants, suggesting that the effects of Megagreen® application are not the result of any improvements in plant water status. Water stress strongly decreased A_{CO2} and g_s of fully expanded leaves (Fig. 2). Mattii and Storchi (2002)

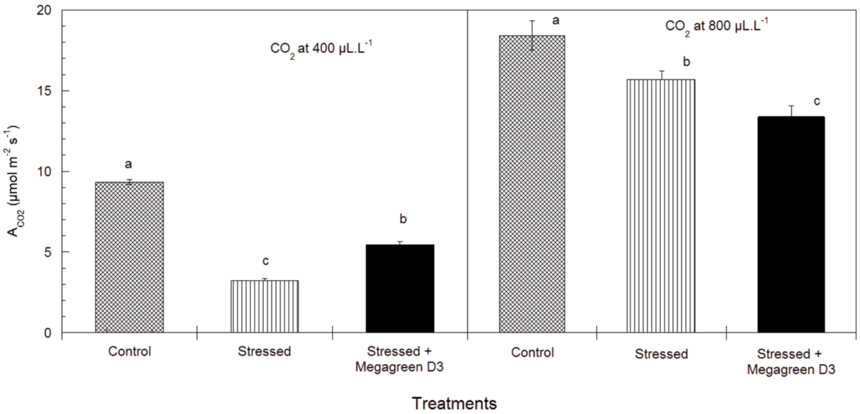

Figure 7. Effects of drought and drought + Megagreen® treatment on net CO_2 assimilation (A_{CO2}) of fully expanded leaves subjected to 25 days of drought treatment at ambient (400 μL L^{-1}) and high (800 μL L^{-1}) CO_2 external concentrations.

Each bar represents mean (n = 6) ± 1 SD.
Different lower case letters denote significantly different means by Fisher's LSD at $P \leq 0.05$.

showed a linear negative correlation between leaf assimilation and ψ_{MD} for 'Sangiovese' grape cultivar, with a 35% reduction in A_{CO2} for leaf water potentials ranging from -1.0 to -1.5 MPa. Schultz (2003) reported that water stress influences gas exchange and water relations in grapevine cultivars in relation to difference in their hydraulic architecture. In the present case, A_{CO2} was mostly restored under elevated CO_2 concentration (800 μL L^{-1}) around the leaf. This was because the substrate of Rubisco was allowed to diffuse to the site of carboxylation, indicating that the low A_{CO2} values in the water stressed plants resulted from stomatal factors rather than damage to the photosynthetic apparatus (Lawlor, 2002). Foliar spray of processed calcite particles led to a clear restoration of A_{CO2} in water stressed plants under natural CO_2 concentration. This positive effect of Megagreen® was associated with a positive but less pronounced effect on g_s. Thus, we suggest that processed calcite particles stimulate photosynthesis in stressed plants mainly via a positive effect on g_s. This hypothesis is strengthened by the fact that Megagreen® had only a minor effect on leaf water potential and did not further increase CO_2 uptake in water limited plants for which photosynthesis was already largely restored by enhancing external CO_2 concentration. Heat stress is often associated with drought stress in summer. Our hypothesis that the prevailing mechanism of Megagreen® action is through stomatal opening (at least under the present water limitation) does not exclude the possibility that foliar spray of processed calcite particles may have also cooled the leaves, reducing the heat stress on photosynthesis. However, (1) we did not observe any noticeable effect of processed calcite particles on leaf temperature (table 1) and (2) leaf temperature was allowed to come to cuvette temperature (ca 30 °C) when measuring gas exchanges and chlorophyll

Talbe 1 - Vapour pressure deficit (VPDleaf) and temperature of young fully expanded leaves from well-watered control, water stressed and water stressed with Megagreen® application 'Cot' grapevines grown in a glasshouse.

Duration of water deficit (stress)	VPD_{leaf} (kPa)			Leaf temperature (°C)		
	Control	Stress	Stress + Megagreen	Control	Stress	Stress + Megagreen
1	3,28	3,26	3,28	29,2	30	30
5	3,03	3,04	3,05	28,3	28,8	28,7
11	3,59	3,65	3,78	29,6	29,8	30
16	3,25	3,02	3,16	29,1	30	29,8
18	3,26	3,48	3,17	30,2	30,5	29,9
20	3,48	2,76	3,26	29,3	30,3	29,9
23	4,34	4,1	3,79	33,3	33,3	32
26	2,93	2,21	2,95	32	32	31,6

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fluorescence. Therefore, the action of Megagreen® appears to be different to that of other particle films including kaolin and calcium carbonate. These agents have been shown to affect photosynthesis by decreasing leaf temperature and increasing light reflection from the leaf (Glenn *et al.*, 2003). However, the amount of mineral sprayed on the leaves is markedly smaller for Megagreen® (the recommended treatment in the field is 1.5 kg per ha) than for other compounds and Megagreen® particles are non reflective.

Photoinhibition might be one of the main factors for A_{CO2} reduction. A decrease in Fv/Fm is considered as an indicator of photoinhibition and structural damage to the PSII reaction center complex. The lack of significant effects of drought on Fv/Fm in the present study implies that there was little effect of stress on PSII and the primary reactions of photosynthesis. Severe drought stress conditions may be required to induce quantifiable reduction in PSII efficiency. This also supports our hypothesis that photosynthesis was affected by drought through stomatal activity.

The positive effect of Megagreen® application on A_{CO2} and g_s occurred in the morning as well as in the afternoon, suggesting it took place throughout the day (Figure 4). This effect was observed under low (middle of the experiment) and high (end of the experiment) VPD (table 1). Most of our results concern the 40-day-old (younger fully expanded) leaves. However, we verified that the major effects observed on these leaves were also observed on both younger and older leaves. It thus may be assumed that the modifications of leaf photosynthesis induced by foliar spray of processed calcite particles involve the whole plant canopy.

We can compare the electron transport rates estimated from fluorescence measurement (Jf) with the theoretical minimum rates for CO_2 assimilation (Jc) calculated from gas exchange measurements (Cornic and Briantais, 1991). Jf was estimated by multiplying the quantum yield of PSII electron transport by irradiance. The result was multiplied by 0.4 because leaves typically have an absorbance of 0.8 and because it was assumed that incident radiation is equally distributed to the photosystems. Jc is based on A_{CO2} (neglecting dark respiration) multiplied by 4 since there is a minimum requirement of 4 electrons per CO_2 molecule fixed in photosynthesis. In the leaves of control and Megagreen® treated water stressed plants, Jf was ca 42 μmol m^{-2} s^{-1} (ΦPSII = 0.08) and Jc was ca 40 μmol m^{-2} s^{-1} (A_{CO2} = 10 μmol m^{-2} s^{-1}). Thus, the rates of electron flow for carbon assimilation were very similar to those calculated from PSII activity. This is consistent with low activity of reactions other than those of the Calvin cycle which consume photosynthetically-generated electrons (Calatayud *et al.*, 2000). By contrast, in water stressed plants not treated with Megagreen®, Jf (ca 30 μmol m^{-2} s^{-1} with ΦPSII = 0.06) exceeded Jc (8 μmol m^{-2} s^{-1} with A_{CO2} = 2 μmol m^{-2} s^{-1}), showing that processes such as photorespiration can be major sinks for photosynthetic electrons. Indeed, water stress did not significantly alter the steady-state values of qP, i.e. the PSII red-ox state. It has also been observed that despite the much lower rates of photosynthesis in nitrogen-deficient plants, qP was not lowered, that is, PSII remained relatively oxidized even when carbon metabolism was limited (Khamis *et al.*, 1990).

Megagreen® is a micronized calcite and (1) calcium may function directly in several aspects of photosynthesis (enzyme activities, oxidation of water by PSII, electron transfer, etc.) and (2) the control of turgor in guard cells is Ca^{2+}-dependent. However, only very small amounts of calcium are required for normal photosynthetic activity. Thus, the molecular mechanisms allowing foliar processed calcite particles to open stomata have not yet been elucidated. Our preliminary results on *Arabidopsis thaliana* and *Nicotiana plumbaginifolia* suspension cells indicate that processed calcite particles induce a cellular signaling process (changes in cytosolic calcium, in polarization of plasma membrane and in the activation of anion channels; data not shown). Further research is needed to understand the mechanisms responsible for the beneficial effect of Megagreen® on plants subjected to drought conditions.

CONCLUSION

Megagreen® (a commercial calcium carbonate particle film material) foliar spray application mostly alleviated the adverse effect of water stress on grapevine photosynthesis. The beneficial effect was associated with enhanced g_s and was observed over the whole day and in the whole plant canopy (young, 25-day-old; mature, 40-day-old; and old, 50-day-old leaves). Megagreen® application may also improve well-watered plant photosynthesis under high leaf to air VPD. These effects on photosynthesis are dose-dependent and the recommended dose gave good results.

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