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Use Of Micronized Calcium Carbonate-Based Sunscreen on Young European Hazelnut (*Corylus Avellana* L.) in Commercial Nursery Under Environmental Stress Conditions During the Summer Period

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Abstract

Chile has become one of the main exporters of European hazelnuts in the world, mainly due to favorable edaphoclimatic conditions. Currently, due to the negative effects of climate change, Chile has been suffering recurrent heat waves and droughts, which are damaging the productive efficiency of the European hazelnut. The present research proposes to evaluate the use of a sunscreen composed of micronized calcium carbonate, pinolene and lignosulfonate on young European hazelnut plants growing in a commercial nursery. In this study, the environmental conditions of heat stress observed during the summer months were evaluated. The results show statistically significant differences between treatments, in favor of plants treated with the sunscreen in at least one of the three months of evaluation. Thus, stomatal conductance (g_s) showed increases of up to 29% compared to the control treatment, transpiration rate (T_r) increased by up to 25% and the net assimilation rate (a_n) increased by up to 18% compared to the control treatment without application. The use of sunscreen would increase the gas exchange of young European hazelnut plants, thus providing greater tolerance to conditions of high environmental temperature, promoting an increase in the production of photo-assimilates compared to untreated plants.

Keywords: European hazelnut, sunscreen, physiological response, micronized calcium carbonate, stress

1. Introduction

Chile is the main producer in the southern hemisphere and ranks fifth worldwide, after countries such as Turkey (63.2%), Italy (13.4%), Azerbaijan (4.7) and the USA (4.2%) (FAOSTAT, 2020). The production areas in Chile are concentrated in the central south and south of the country, characterized by a Mediterranean and temperate climate, respectively (Durán *et al*., 2022). Currently, these areas cover an area of 30,000 hectares (ha) with an annual growth rate of 2,000 to 3,000 ha (ODEPA, 2020, Moya-Elizondo *et al*., 2022). Ninety percent of this production is exported to Italy (Ellena *et al*., 2014), which has allowed it to gain relevant in the world hazelnut market, due to its position as an off-season exporter (Ghisoni *et al*., 2019). In addition, the low commercial risks and socio-political effects affecting the price of hazelnuts, together with a high productive potential (2.0 - 4.0-ton ha^{-1}), even higher than the great productive exponents such as Turkey and Italy (0.85 -1.5-ton ha^{-1}), give Chile a privileged position in the world hazelnut market (Grau and Sandoval, 2009; Bozoglu *et al*., 2019).

The production of fruit trees such as European hazelnut has been strongly affected in Mediterranean climate zones, due to the intensification of droughts events and heat waves generated mainly by the negative effects of climate change (Del Pozo *et al*., 2019). In southern latitudes such as Chile, the ozone layer has also decreased, increasing the intensity of incident solar radiation, especially ultraviolet radiation (UV) (Lizana *et al*., 2009). Like other fruit trees of Mediterranean climate, hazelnut has a saturation point of incident light that is around 50% of the intensity in full sun during the summer, which would decrease its physiological performance during the productive period (Corelli-Grappadelli and Lakso, 2007). Moreover, in the case of European hazel this threshold is even lower (Luciani *et al*., 2020) and exceeding this value would generate damage to the photosynthetic apparatus (PSII), increasing photorespiration and photoinhibition of the plant. This situation is even more aggravated under a scenario of water restriction, due to the increase in temperature in plant tissues (Corelli-Grappadelli and Lakso, 2007; Salazar-Canales *et al*., 2021). This affects plants in different processes of development and organ growth, which would ultimately significantly affect fruit production, so it is essential to have alternatives to overcome the negative effects of climate, providing conditions to minimize the damage caused by high radiation and lack of water in key phenological periods for European hazel.

As an alternative, the use of sunscreens is proposed, being kaolin the most used via foliar application, increasing albedo through the formation of a whitish layer of this mineral on the leaves, which would increase the reflection of photosynthetically active radiation (PAR), UV and infrared (IR) (Brito *et al*., 2019), favoring the reduction of leaf temperature from 1 to 4 °C, thus reducing heat stress. However, under water stress conditions, the decrease in leaf temperature could reach up to 6.9 °C, also decreasing g_s, T_r and a_n (Luciani *et al*., 2020). Foliar applications of micronized calcium carbonate ($CaCO_3$) would also reduce the negative effects of high solar radiation not only by increasing albedo, but also by generating greater efficiency in calcium translocation within plant tissue cells (Deepa *et al*., 2015), which is essential in the synthesis of salicylic acid and chitinase, increasing plan tolerance to biotic and abiotic stress (Pugliese *et al*., 2018). Among the adjuvant compounds used to enhance the effect of sunscreens, we have pinolene, which is a terpene polymer derived from pine resin, which helps limit plant transpiration (Brillante et al., 2016). On the other hand, the lignosulfonate added in the formulation would fulfill the function of a binder (Przywara et al., 2021).

Based on the literature review, the present research aims to evaluate the use of a formulation based on micronized calcium carbonate, pinolene and lignosulfonate as a sunscreen, on the water status and gas exchange in young European hazel plants in a commercial nursery, under environmental stress conditions recorded during the summer period in central Chile.

2. Materials and Methods

2.1 Experimental Site

The study was conducting during the 2020-2021 season in a commercial nursery of young European hazelnut trees (*Corylus avellana* L.) belonging to the company AgriChile S.A., located in Los Niches, Maule Region, Chile (35° 3'13.42" S; 71° 7'48.41" W; 288 m.a.s.l). The orchard corresponds to the cv. Tonda di Giffoni, used for plant propagation (nursery). The Niches area has a Mediterranean climate with a long dry season. The average annual temperature is 14.7 °C, and the average temperature of the warmest month is 21.9 ºC and the average minimum temperature during the coldest month is 8 °C (January and July, respectively). The total average annual precipitation in the last 10 years was 860 mm. The soils of this site are moderately deep of superficial clay loam texture, with slow permeability and flat topography.

2.2 Experimental Design

Two treatments with application of commercial product BLOCKER (micronized calcium carbonate, pinolene and lignosulfonate), and a control (without application) were evaluated. For this, an experimental design of randomized blocks was carried out, with four repetitions per treatment, where each repetition corresponds to a block. Each of the blocks is made up of a total of 20 plants. Three applications were made during the summer season, during the months of highest climatic demand, that is middle January, February and March in the southern hemisphere. Ten days after the application of the products, measurements were made in the field.

2.3 Plant Measurements

To characterize plant water status, midday xylem water potential (Ψ_x; MPa) was measured using the pressure chamber methodology (PMS instrument Co., model 1000, Corvallis, Oregon, USA; Scholander *et al*., 1965). To evaluate the physiological response of the plants, the physiological variables of stomatal conductance (g_s; mol m^{-2} s^{-1}), transpiration rate (T_r; mol m^{-2} s^{-1}) and net assimilation rate (a_n; µmol m^{-2} s^{-1}) were performed using a portable infrared gas analyzer (Li-6800 LI-COR). All measurements were made between 12:00 and 14:00 h. in the afternoon, during the period of highest climatic demand on healthy, medium-sized leaves in full sun.

2.4 Statistical Analysis

For the data information analysis, it was carried out using a generalized linear model (GLM), where the treatment was considered as a fixed variable and the block as a random variable. For the separation of means, a multiple range comparison test was performed using HSD, Tukey, p-value ≤ 0.05.

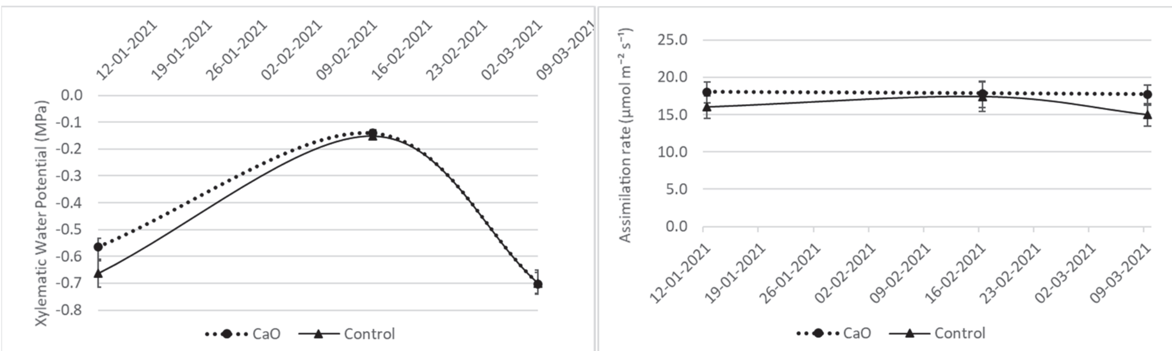
3. Results

Table 1 shows the climatic analysis for the 3 measurement dates during the study season. In this regard, it is observed that on January 12 and March 9, maximum temperatures fluctuated around 30°C, while photosynthetically active radiation (PAR) exceeded 1,500 µmol m^{-2} s^{-1}, a normal situation in the southern hemisphere during the summer season high temperatures and high solar radiation are observed (Del Pozo *et al*., 2019). On the contrary, during February 16, temperatures and PAR were ostensibly lower, due to the high cloudiness of that day (8/8 oktas of cloud).

Table 1. Climatic analysis for the dates of physiological measurements made in the field. Data were obtained from the Agromet weather station, located in San Jorge, los Niches**.**

Date	Average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Solar radiation (µmol m^{-2} s^{-1}).
12-01-2021	16.7	28.6	4.8	1877
16-02-2021	14.5	17.8	8.7	533
09-03-2021	18.6	31.2	7.2	1577

Figure 1 shows the xylem water stress values and the assimilation rate for the evaluated treatments. For xylem water stress, statistical differences were only observed on January 12, in which the plants covered with sunscreen presented a lower water stress with -0.57 MPa. A_n also showed statistically significant differences during the measurements made in January and March, being the plants treated with sunscreen the ones that presented a better performance, increasing these measurements by 13% and 18%, respectively, compared to the control treatment without application.

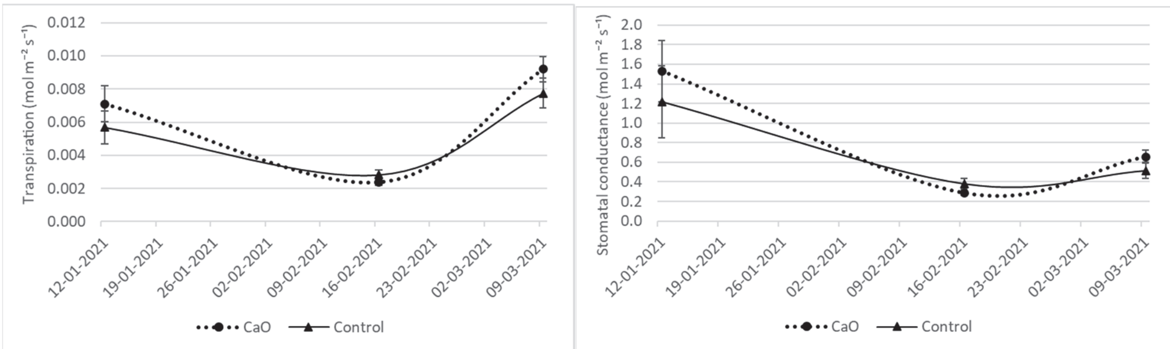


Variable	Xylematic water potential (MPa)			Assimilation rate (µmol m^{-2} s^{-1})		
Treatment	12-01-2021	16-02-2021	09-03-2021	12-01-2021	16-02-2021	09-03-2021
Sunscreen	-0.57 a	-0.14	-0.70	18.04a	17.86	17.73
Control	-0.66 b	-0.15	-0.69	16.01b	17.40	15.00
Valor-p	0.004 (*)	0.15	0.93	0.018 (*)	0.74	0.0014(*)

Figure 1. Evolution of midday stem water potential (Ψx; MPa) and net assimilation rate (an; µmol m-2 s-1) and analysis of variance performed. Different letters indicate significant differences between treatments. * p-value <0.05

On the other hand, in Figure 2, T_r presented statistically significant differences during the three measurement dates. In this case, the plants treated with sunscreen showed increases of 25% and 20% for the measurements made in January and March, respectively. However, this variable also decreased during the month of February by 14%, compared to the control treatment. Finally, g_s increased by 25% and 29% in plants treated with sunscreen during

January and March, respectively. However, the increase in g_s represented statistically significant differences only in March. On the other hand, the measurement of g_s in February showed significant statistical differences in the sunscreen treatment, being 24% lower than the control treatment.



Variable	Transpiration (mol m^{-2} s^{-1})			Stomatal conductance (mol m^{-2} s^{-1})		
Treatment	12-01-2021	16-02-2021	09-03-2021	12-01-2021	16-02-2021	09-03-2021
Sunscreen	0.0071a	0.0024b	0.0092a	1.53	0.29b	0.66a
Control	0.0057b	0.0028a	0.0077b	1.22	0.38a	0.51b
Valor-p	0.02 (*)	0.02 (*)	0.0057 (*)	0.12	0.008 (*)	0.0027 (*)

Figure 2. Evolution of leaf transpiration (T_r; mol m^{-2} s^{-1}), stomatal conductance (g_s; mol m^{-2} s^{-1}) and analysis of variance for each variable studied. Different letters indicate significant differences between treatments. * p-value <0.05.

4. Discussion

The plants evaluated in this trial did not present xylem water stress values, obtaining Ψ_x values lower than -0.7 MPa, remaining below the threshold range proposed by Grau and Sandoval, (2009) and Salazar-Canales *et al*., (2021); corresponding to -0.85 and -0.89 MPa, respectively for European hazel plants cv. *Tonda di Giffoni* L, well irrigated and under climatic conditions like those of the present study. Despite this, statistical differences of Ψ_x were observed during January 12, where the treatment with sunscreen application reduced the water stress of plants, increasing the Ψ_x by 14% compared to control treatment without application. The water stress values observed in the study show that the plants were not subjected to water stress conditions, therefore, the differences observed in the physiological response of the plants would have been generated mainly by thermal and radiative stress to which the plants were subjected during the growing season, especially during the first and third measurement dates.

In the cloudy conditions observed during the February measurement, only significant statistical differences were found in the g_s and T_r variables, with lower values for plants treated with sunscreen. This is explained by the occurrence of low temperatures (below 25 °C) in conjunction with the10% decrease in PAR intensity observed. Under these conditions the whitish film of the sunscreen could generate decreases in gas exchange variables, which was evidenced by lower rates of g_s and T_r (Glenn *et al*., 2001).

The gas exchange values recorded in this research differ from the experiences reported in literature, reaching values 5 times higher for g_s and around two time higher for T_r and a_n under similar climatic and water conditions (Luciani *et al*., 2020; Salazar-Canales *et al*., 2021). The above results could be due to the age difference between the plant material used in this trial (young plants in nursery), this justifies the gap in gaseous exchange values observed in this study. However, data obtained from 2-year-old European hazelnut trees (height 58 ± 5 cm) cv. Tonda Gentile Romana, growing in Rome, in a Mediterranean climate locality in evaluation periods similar to those of the present study (July, equivalent to January in the southern hemisphere) show even more marked differences in gas exchange variables with g_s up to 8 times lower than that reported in this study (8 µmol m^{-2} s^{-1}) and about 2 times lower in T_r and a_n (0.13 and 0.0035 mol m^{-2} s^{-1}, respectively) (Catoni *et al*., 2017). These differences observed in relation to the information reported in literature could be due to measurements performed during a phenological period of active growth in new shoots of young plants growing in nursery.

As for the use of micronized calcium carbonate, pinolene and lignosulfonate, there are no precedents in the literature on their use as a sunscreen for agricultural purposes. Instead, the most widely used and studied sunscreen corresponds to kaolin. According to studies conducted by other authors, this product reduces leaf temperature and increases g_s, thus T_r and an. However, other studies with kaolin show decreases in a_n (Glenn *et al*., 2001). Such is the case of the study of by Luciani *et al*., (2020) where applications of kaolin as a sunscreen were made on European hazelnut, with results coinciding with those obtained by Glenn *et al*., (2001), regarding the decrease in leaf temperature between 2.9 to 6.9 °C, but differing in gas exchange variables, without observing benefits in relation to the control treatment. They also observed an interaction between the application of kaolin and water deficit, which would generate a greater stomatal resistance and therefore a decrease in the net assimilation rate, independently of leaf temperature.

Brito *et al*., 2019 mentions that in both irrigated and rainfed olive trees, benefits are seen from kaolin applications, mainly associated with the decrease in leaf temperature. This differs from what was observed by Rotondi *et al*., 2021, who evaluated kaolin and zeolite in olive trees, carrying out a total of 8 applications between June and September, finding that the use of kaolin reduced gas exchange and photosynthesis evaluated in August and September, compared with witness trees. The zeolite increased the gas exchange, compared to the control. These results can be explained by the large number of applications, which generates an excessive density of lamellar particles, which act as a physical barrier on the upper surface and underside of the leaf, exacerbating stomatal resistance. On the other hand, with zeolite, although the same application pattern was followed, as they are pseudo cubic particles with a smaller specific surface area, they did not generate the limitation caused by kaolin. Another experience that differs from what was expressed by Brito *et al*., 2019, corresponds to that carried out by Cabo *et al*., 2020 in a dry European hazelnut orchard, located in Moimenta da Beira, Portugal, where trees in full production were treated. (the age of the trees is not specified) with kaolin, applying irrigation and the combination of both, obtaining as a result that these applications can increase yield through greater volume and weight of the fruit; but if the trees are irrigated.

The discordance in results obtained when evaluating the same sunscreen can be explained by morphological and physiological characteristics intrinsic to each fruit species. Have the olive tree, which is a species widely studied in its resistance to adverse environmental conditions thanks to its leaf morphology and physiological mechanisms such as osmolyte production. On the other hand, the European hazelnut is a species in which environmental adaptation mechanisms are unknown, but it can be seen that its resilience to environmental stress is less than that of the olive tree, therefore, obtaining favorable results in the olive tree does not ensure it in the hazelnut tree. European. On the other hand, the favorable results in mitigating environmental stress presented in this article, associated with plants treated with sunscreen (micronized calcium carbonate), which we show by the reduction in water stress and increase in gas exchange and photosynthesis, may be explained by the increase in albedo and consequent decrease in leaf temperature. However, as we saw in other research carried out with kaolin, the decrease in temperature does not guarantee the best photosynthetic performance. On the other hand, the formulation of the sunscreen evaluated corresponds to pinolene and lignosulfonate. Pinolene is recognized as a film-forming antiperspirant that in research has contrasted its effect with that of kaolin, highlighting that it does not generate beneficial effects on gas exchange or photosynthesis, which has an impact on quality and performance (Abdallah *et al*., 2019; Brillante *et al*., 2016). For their part, lignosulfonates that have been evaluated as effective bio stimulants capable of promoting metabolic responses, physiological performance, increasing the photosynthetic rate (greater RuBisCO activity) and thereby increasing plant growth evaluated as dry weight (Ertani *et al*., 2019).

On the contrary, the results of the application of diverse sources of calcium as sunscreen allow us to observe statistically significant differences that show a benefit for the plant. On a sunny day, photosynthetically active radiation (PAR) is around 2,000 µmol m^{-2} s^{-1} and European hazelnut leaves exposed to full sun saturate at 40 to 50% of PAR, while shaded leaves of the same species have a lower light saturation point, which is reached at 15 to 25% of PAR on a sunny day, fluctuating between 300 and 500 µmol m^{-2} s^{-1} (Hampson *et al*., 1996). Therefore, the solar protection effect of calcium is based on the increase of plant albedo, reflecting the excess of incident solar radiation as PAR, UV and infrared radiation (IR), allows to reduce heat stress by reducing leaf temperature (Luciani *et al*., 2020). The above practice maintains the photochemical functioning of PSII, respecting temperature thresholds and light intensities, maximizing photosynthesis, which is reflected in an increase in net plant assimilation (Glenn *et al*., 2001; Glenn *et al*., 2003). The use of sunscreen allowed increasing g_s in January and March, which would be related to high plant water availability and high transpiration, even under conditions of high temperature, high light intensity and high vapor pressure deficit (VPD) (Cincera *et al*., 2018). The sunscreen, reduces leaf temperature, decreases VPD between air and leaves. Plants not being under water restriction, would allow maintaining a low plant stomatal resistance, increasing T_r and a_n. Calcium, in addition to generating a benefit as a physical barrier, would also do so through chemical properties, due to reports that indicate that foliar applications of nanoscale calcium, such as micronized calcium carbonate, would allow the transport of calcium through the phloem (Deepa *et al*., 2015). Thus, it is suggested that sunscreen could also increase calcium content within the plant, stimulating the production of salicylic acid and chitinase, fulfilling a SAR role that would increase tolerance to heat and water stress in hazelnut plants (Pugliese *et al*., 2018).

5. Conclusion

Applications of micronized calcium carbonate, pinolene and lignosulfonate on young European hazelnut plants in commercial nursery managed without water stress during summer would improve gas exchange in hazelnut plants. The results obtained in this research stand out from the bibliographic reports of applications of other sunscreens on European hazelnut, being always favorable for plants treated with sunscreen under heat stress conditions, since this product allows maximizing the water efficiency of the plant, decreasing the stomatal resistance of the leaves, increasing their transpiration rate. This would improve the cooling process of plant tissues which, together with the increase in albedo, would help to protect the photochemical efficiency of PSII, favoring the productivity of European hazelnut.

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