

Universidad de Concepción Dirección de Postgrado Facultad de Agronomía Programa de Magíster en Ciencias Agronómicas

# Evaluación de nuevas técnicas para mejorar la eficiencia en el uso de la luz en huertos de avellano europeo (*Corylus avellana* L.)

Tesis para optar al grado de Magíster en Ciencias Agronómicas con Mención en Producción y Protección vegetal

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LA PODA DE VERANO Y UNA PELÍCULA PARTICULADA INCREMENTAN EL RENDIMIENTO Y EL DESEMPEÑO FOTOSINTÉTICO EN AVELLANO EUROPEO

SUMMER PRUNING AND PARTICLE FILM INCREASE YIELD AND PHOTOSYNTHETIC PERFORMANCE IN HAZELNUT

#### RESUMEN

Los huertos de avellano europeo están continuamente expuestos a condiciones de luz solar baja o excesiva, sin embargo, no hay suficiente conocimiento sobre sus efectos en el rendimiento y la respuesta fisiológica de la planta. Esta investigación evaluó el efecto de la exposición del dosel a la luz mediante la poda y la protección del dosel a la luz mediante una película particulada a base de caolinita, sobre el rendimiento (peso del fruto, nuez y de la semilla) y la respuesta fisiológica de la hoja (intercambio gaseoso y fluorescencia de clorofila) en avellano europeo. Avellanos 'Tonda di Gifoni' fueron sometidos a podas de invierno del eje central (WCA), y del eje central más 2 ramas laterales (WCA+2L). Se aplicó además la poda de verano del eje central más 2 ramas laterales (SCA+2L), y combinada con aspersión de partículas de caolinita (SCA+2L+K). Arboles sin podar se incluyeron como control. WCA, WCA+2L, SCA+2L y SCA+2L+K aumentaron en más del 120% la transmisión de luz desde la parte superior hasta la mitad de la zona del dosel. SCA+2L+K aumentó, en la zona media del dosel, la tasa de fotosíntesis neta  $(A_n)$ , el rendimiento, el peso de la nuez y el peso del grano en un 100%, 90%, 4% y 5%, respectivamente. El tratamiento SCA+2L+K mostró una relación significativa entre rendimiento y la transmisión de luz ( $R^2$  aj= 0.68\*) y entre  $A_n$  y la transmisión de luz ( $R^2$  aj= 0.58\*\*). Los resultados sugieren que la poda de verano combinada con la película

particulada aumenta el rendimiento y el desempeño fotosintético en avellano europeo.

#### Summary

Hazelnut orchards are continuously exposed to low and excess sunlight conditions, but there is not enough knowledge about their effects on yield and plant physiological responses. This research evaluated the effect of light-canopy exposition by pruning and light-canopy protection by kaolin-based particle film on yield (fruit, nut, and kernel weight) and leaf physiological (gas exchange and chlorophyll fluorescence) responses in hazelnut. 'Tonda di Gifoni' hazelnut trees were subjected to winter pruning of the central axis (WCA), and of the central axis plus 2 lateral branches (WCA+2L). Summer pruning was applied to the central axis plus 2 lateral branches (SCA+2L), and combined with kaolin particle spraying (SCA+2L+K). Unpruned trees were included as a control. WCA, WCA+2L, SCA+2L, and SCA+2L+K increased more than 120% the light transmission from the top to the middle of the canopy zone. SCA+2L+K increased, at the middle zone canopy level, the net photosynthetic rate  $(A_n)$ , yield, nut weight, and kernel weight by 100%, 90%, 4%, and 5%, respectively. SCA+2L+K treatment showed a significant relationship between yield and light transmission (R<sup>2</sup> aj= 0.68\*) and between  $A_n$  and light transmission (R2 aj= 0.58<sup>\*\*</sup>). Results suggest that summer pruning combined with particle film increase yield and photosynthetic performance in hazelnut.

# **CAPÍTULO 1**

#### INTRODUCCION GENERAL

El manejo de la luz solar en avellano europeo (*Corylus Avellana* L.) es una práctica clave, pero que presenta una dualidad. Debido al hábito de crecimiento de esta especie, la transmisión de luz al interior del dosel resulta ser insuficiente, lo que trae como consecuencia una baja tasa de asimilación de CO<sub>2</sub>, pobre desarrollo floral, disminución del rendimiento y menor calidad de fruta (Hampson et al., 1996; Farinelli et al., 2005; Pannico et al., 2017). Por otra parte, un exceso de luz solar incidente en las hojas de esta especie provoca una disminución de la capacidad de fotosíntesis, al reducir la conductancia estomática y promover inhibición del proceso de transporte de energía a nivel del PS-II (Luciani et al., 2020).

En los árboles frutales, es deseable maximizar la intercepción de la luz solar junto con una distribución apropiada dentro del dosel de la planta (Lauri et al., 2009; Cherbiy-Hoffmann et al., 2012). Esto permite la máxima eficiencia del uso de la radiación solar en los procesos de la fotosíntesis, desarrollo de yemas florales, crecimiento y la calidad de los frutos (Cherbiy-Hoffmann et al., 2012). La poda es una práctica usada habitualmente para mejorar la transmisión de luz solar al interior del dosel de los árboles frutales, logrando una producción estable en cantidad y calidad de frutos (Pescie et al., 2011). En avellano europeo la poda se realiza durante el invierno y se orienta a la eliminación de brotes excesivamente vigorosos como "chupones" y madera vieja, enferma o dañada (Cristofori et al., 2009; Silvestri et al., 2021). En esta especie la práctica de poda permitió incrementar el rendimiento de la planta al cabo de 3 a 5 años de producción y como consecuencia de una mayor formación de brotes mixtos con una alta cantidad de yemas fértiles (Lauri et al., 2009, Roversi et al., 2009).

Por otra parte, la poda puede generar daños en las hojas, brotes y frutos por sobre exposición de estos a una alta intensidad de luz solar (Racsko and Schrader, 2012; Yu et al., 2014). En avellano europeo se ha demostrado que las altas temperaturas y la alta luz solar incidente, a menudo inducen el cierre estomático, reduciendo la transpiración que es la principal vía de disipación de calor de las hojas. Al incrementar la temperatura del follaje se disminuye la fotosíntesis neta (Luciani et al., 2020). En manzano (Malus domestica Borkh.) se ha reportado que estas condiciones de estrés por exceso de luz y altas temperaturas pueden producir una inhibición de la fotosíntesis a nivel del PS-II o activación de radicales libres que originan daño foto oxidativo en hojas o frutos por su sobre exposición al sol (Olivares-Soto y Bastías, 2021). El uso de partículas reflectantes como la caolinita ha sido efectivo para mitigar el nivel excesivo de temperatura y radiación solar en cultivos como el pomelo (Citrus paradisi L.) (Jifon y Syvertsen, 2003), vid (Vitis vinífera L.) (Dinis et al., 2018), nogal (Juglans regia L.) (Gharaghani et al., 2018), olivo (Olea europea L.) (Brito et al., 2021) y avellano europeo (Luciani et al., 2020). La aplicación de esta partícula en frutos, forma una película protectora de color blanco en la superficie de la epidermis permitiendo reducir la absorción de luz, y particularmente de la radiación UV-B, la que es reflejada desde la superficie (Glenn et al., 2002; Sharma et al., 2015). La aplicación de caolinita ha presentado buenos resultados en cuanto a protección de cultivos frutales ante el estrés de verano. Por ejemplo, en vid, disminuyó la temperatura de la hoja aminorando la limitación estomática y, por ende, incrementando los niveles de intercambio gaseoso de la hoja (Dinis et al., 2018). En nogal, redujo el daño por sol en frutos y hojas e incrementó la conductancia estomática, asimilación neta de CO<sub>2</sub>, y el tamaño y calidad de la fruta producida (Gharaghani et al., 2018). Por otra parte, en mandarino (Citrus unshiu Marc.) se determinó que el uso de caolinita fue efectivo en el control de daño por golpe de sol sin alterar la calidad organoléptica de la fruta (Chabbal et al., 2014). En avellano europeo, el uso de caolinita redujo la temperatura de la

hoja, mejorando la fotosíntesis neta (Luciani et al., 2020). Este estudio propone que la práctica de poda combinada con el uso de caolinita mejora el potencial fotosintético y productivo del avellano europeo.

# **HIPOTESIS**

La práctica de poda combinada con el uso de caolinita mejora el potencial fotosintético y productivo del avellano europeo.

# **OBJETIVO GENERAL**

Evaluar el efecto de la poda combinada con el uso de caolinita sobre parámetros de rendimiento fotosintético y productivo en avellano europeo.

# **OBJETIVOS ESPECIFICOS**

- Evaluar el efecto de diferentes tratamientos de poda de invierno y verano combinada con caolinita sobre la transmisión de luz fotosintética al interior del dosel de los árboles.
- Cuantificar el efecto de los tratamientos sobre variables de intercambio gaseoso y fluorescencia de clorofila de la hoja.
- Determinar el efecto de los tratamientos sobre los componentes de rendimiento del árbol a nivel de frutos y de desarrollo floral.
- Relacionar variables de intercambio gaseoso y de rendimiento con la transmisión de luz al interior del dosel.

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# **CAPÍTULO 2**

# Summer pruning and particle film increase yield and photosynthetic performance in hazeInut

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#### Abstract

Hazelnut orchards are continuously exposed to low and excess sunlight conditions, but there is not enough knowledge about their effects on yield and plant physiological responses. This research evaluated the effect of light-canopy exposition by pruning and light-canopy protection by kaolin-based particle film on yield (fruit, nut, and kernel weight) and leaf physiological (gas exchange and chlorophyll fluorescence) responses in hazelnut. 'Tonda di Gifoni' hazelnut trees were subjected to winter pruning of the central axis (WCA), and of the central axis plus 2 lateral branches (WCA+2L). Summer pruning was applied to the central axis plus 2 lateral branches (SCA+2L), and combined with kaolin particle spraying (SCA+2L+K). Unpruned trees were included as a control. WCA, WCA+2L, SCA+2L, and SCA+2L+K increased more than 120% the light transmission from the top to the middle of the canopy zone. SCA+2L+K increased, at the middle zone canopy level, the net photosynthetic rate  $(A_n)$ , yield, nut weight, and kernel weight by 100%, 90%, 4%, and 5%, respectively. SCA+2L+K treatment showed a significant relationship between yield and light transmission ( $R^2$  aj= 0.68\*) and between  $A_n$  and light transmission (R2 aj= 0.58\*\*). Results suggest that summer pruning combined with particle film increase yield and photosynthetic performance in hazelnut.

**Keywords:** *Corylus avellana* L.; sunlight management, leaf gas exchange, sustainable production, climate change.

#### 1. Introduction

Effective sunlight management is of outmost importance in European hazelnut (*Corylus Avellana* L.). Due to the growth habit of this species, light transmission tends to be limited to canopies, resulting in low CO<sub>2</sub> assimilation rate, poor floral development, decreased yield and reduced fruit quality [1-3]. Furthermore, an excess of incident radiation on the leaves causes a decrease in photosynthetic capacity by reducing stomatal conductance and leading to PS-II inhibition [4].

To maximize sunlight interception and distribution within the canopy is desirable in tree fruit crops [5,6], because it allows maximum efficiency of solar radiation in photosynthesis, flower bud development, and fruit growth and quality [6]. Pruning is a commonly used practice to enhance yield and fruit quality by improving sunlight transmission into the canopy [7]. In hazelnut, pruning is done during winter in order to remove excessively vigorous shoots, such as suckers, as well as old, dead, and damaged wood [8,9]. In fact, there is evidence that pruning resulted in increased yield after 3 to 5 years of production due to a greater formation of mixed shoots with a high number of fertile buds [5,10].

Overexposure to intense sunlight can damage leaves, shoots and fruit [11,12]. In hazelnut, high temperatures and high incident radiation often induce stomatal closure, reducing transpiration, which is the main route of heat dissipation from leaves, increasing foliage temperature, and decreasing net photosynthesis [4]. In apples (*Malus domestica* Borkh.), it has been reported that stress conditions due to excess light and high temperatures can produce inhibition of photosystem II or activation of free radicals, causing photo-oxidative damage in leaves or fruits induced by sun overexposure [13]. In this sense, the use of reflective particles such as kaolin has been suggested as a strategy to reduce the negative effects derived from excessive heat and solar radiation in crops such as grapefruit (*Citrus paradisi* L.) [14], grape (*Vitis vinífera* L.) [15], walnut (*Juglans* 

regia L.) [16], olive (Olea europea L.) [17], and European hazelnut [4]. Kaolin particles form a white protective film on the surface of the epidermis, reducing light absorption and particularly UV-B radiation, which is reflected out the surface [18,19]. The application of kaolin has proven effective in protecting fruit crops from heat stress in the summer. For example, it decreased leaf temperature in grapes, reducing stomatal limitation and, therefore, increasing leaf gas exchange levels [15]. In walnut, it reduced sun damage on fruits and leaves and increased stomatal conductance, net CO<sub>2</sub> assimilation, and fruit size, while it also improved fruit quality [16]. Furthermore, there is evidence that the foliar spray of kaolin reduced sun damage in mandarin, without altering the organoleptic quality of the fruit [20]. In European hazelnut, the use of kaolin reduced leaf temperature, improving net photosynthesis [4]. This study proposes that pruning combined with the use of kaolin improves the photosynthetic and productive potential of European hazelnut. Accordingly, the objective of this work was to evaluate the effect of pruning and kaolin application on light transmission, leaf physiological (gas exchange, chlorophyll fluorescence) and fruit yield components (yield, nut, and kernel weight) parameters.

#### 2. Materials and Methods

#### 2.1. Plant material

The study was conducted during the 2019-20 and 2020-21 seasons in a 4year-old commercial orchard of European hazelnut cv. 'Tonda di Giffoni', located in Pinto, Ñuble Region, Chile (36°39'21" S 71°59'29" W). Minimum, mean and maximum air temperatures reach 3, 14, and 28 °C, respectively, with a maximum solar radiation level of 980 W m<sup>-2</sup> in the summer. In terms of relative humidity, minimum, mean and maximum values reach 48%, 72%, and 96%, respectively (Agromet, 2022). Spacing was 3 m apart between trees and 5 m apart between rows, with a north-south orientation. The trees were trained as a single-axis system, which consists of a single trunk from which 4-5 branches are born, inserted at a height of 80-90 cm from ground level. Irrigation was provided by two drip lines per plant; emitters were spaced 0.5 m apart and with a flow rate of 3 L per hour. Pruning, nutrition, and phytosanitary management were conducted according to standard procedures for a young European hazelnut orchard.

#### 2.2. Treatments and experimental design

Two trials consisting of different pruning treatments were conducted (winter and summer). Winter pruning was conducted on August 24, 2019, and included: i) a control treatment without winter pruning (*WC*); ii) winter pruning of the central axis (*WCA*); and iii) winter pruning of the central axis plus 2 lateral branches (*WCA+2L*). Summer pruning was conducted on January 14, 2020, and included: i) a control treatment without summer pruning (*SC*); ii) summer pruning of the central axis plus 2 lateral branches (*SCA+2L*); and iii) summer pruning of the central axis plus 2 lateral branches, and kaolin application (*SCA+2L+K*). Kaolin (Screen duo ®) (Crop Microclimate Management, North Carolina, USA) was applied at 1.25 %, using an SR 200 motorized sprayer (STILH, Dieburg, Germany). The experiment was established in a completely randomized block design with 4 replicates and 2 plants per treatment as the experimental unit.

#### 2.3. Light Transmission Measurements

Light transmission measurements were made the last week of January 2020, using an AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, USA). The measurements were made on sunny days at three time points throughout the day: i) 2-3 hours before solar noon; ii) at solar noon; and iii) 2-3 hours after solar noon [21]. The amount of photosynthetically active photon flux (*PPFD*, µmol m<sup>-2</sup> s<sup>-1</sup>) was estimated in the top (1.6 m above ground level), middle (1.1 m above ground level) and bottom (0.6 m above ground level) canopy zones, using the methodology proposed by Hampson et al. [1] and Pannico et al. [3] (Figure 1). The amount of *PPFD* was determined by measuring from the center of one row to the center of the next row and at a distance of 0.4 m between each

measurement point, for a total of 13 points in each level [22] (Figure 1), using an SQ-100X-SS quantum sensor (Apogee Instruments, Logan, Utah, United States) located in full sun and at a height of 1.2 m. With the information obtained, the *PPFD* transmitted to each point of the plant (LT, %) was calculated according to the following relationship:

#### $LT = (PPFD below/PPFD above) \times 100.$

where *PPFD below* is the photosynthetically active photon flux density measured below the canopy and *PPFD above* is the photosynthetically active photon flux density measured above the canopy.

#### 2.4. Leaf gas exchange and chlorophyll fluorescence

The influence of the treatments on the incidence of photosynthetically active photons (*PPFD*, µmol m<sup>-2</sup> s<sup>-1</sup>), net CO<sub>2</sub> assimilation (*A<sub>n</sub>*, µmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), stomatal conductance ( $g_s$ , µmol m<sup>-2</sup> s<sup>-1</sup>), transpiration rate (*E*, µmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), and leaf intercellular CO<sub>2</sub> concentration (*C<sub>i</sub>*, µmol mol<sup>-1</sup>) was determined using a TARGAS-1 infrared gas analyzer (PP Systems, Massachusetts, USA). Measurements were made the first week of February 2020, on a sunny day at noon, and on healthy mature leaves from one-year-old shoots located in the central point (0.0) of the top, middle, and bottom canopy zones (Figure 1). Simultaneously, maximum (F<sub>m</sub>) and minimum (F<sub>0</sub>) leaf chlorophyll fluorescence measurements were made in the same zones used for gas exchange determinations using an OS-30p portable fluorometer (Opti-Sciences, Hudson, USA), after dark-adaptation of the leaf for 30 min [23]. Maximum photochemical efficiency of PSII (*F<sub>w</sub>/F<sub>m</sub>*) was determined by the relationship F<sub>v</sub>/F<sub>m</sub> = (F<sub>m</sub>-F<sub>0</sub>)/F<sub>m</sub> proposed by Maxwell and Johnson [24], which reflects the photochemical efficiency of PSII.



**Figure 1.** Vertical and horizontal division of the canopy of European hazelnut. Source: Own elaboration.

#### 2.5 Fruit yield components

Harvest was conducted in the first week of March 2021. Fruits were manually harvested from the top, middle, and bottom canopy zones [2] (Figure 1), while yield (kg) was determined using a digital scale, model PCE-PM 6 T (PCE Instruments, Alicante, Spain). Subsequently, a sample of 20 fruits was randomly selected per canopy zone (Figure 1), and both nut (g) and kernel (g) were weighed using a digital desktop scale, model A6702231 (Veto, Santiago, Chile). Kernel yield was calculated as the relationship between kernel weight and fruit weight with the shell.

#### 2.6. Additional information

During the flowering stage, the total number of catkins and glomeruli from two complete branches per plant was estimated for the, top, middle and bottom canopy zones (Figure 1) according to the methodology described by Tombesi and Farinelli [25]. The number of 1-year shoots per branch was determined, while shoot length (cm) was measured in order to evaluate the effect of pruning on vegetative growth.

Additionally, the incidence of species of genera *Botrytis*, *Fusarium*, *Alternaria*, *Cladosporium*, *Penicilium*, *Trichothecium*, and other saprophytic fungi was evaluated in order to determine the possible influence of the different treatments on disease incidence, and their possible relationship with yield. Samples of 5 catkins per each canopy zone were taken for each treatment (Figure 1) and moistened with distilled water at a temperature of 20 °C for 7 days to induce mycelial growth, then the fungi were identified by optical microscope (BA310E Motic, Hong Kong, China).

#### 2.7 Statistical analysis

Data of light transmission were subjected to a non-parametric Friedman test at a significance level of 95%. For determinations of gas exchange, chlorophyll fluorescence, yield and fruit quality, data were subjected to an analysis of variance at the same significance level, and a Tukey's mean comparison test. In order to comply with the variance analysis assumptions, the 'fruit filling' variable was transformed using the 'normal scores' formula; the floral return variables were transformed to their natural logarithm; and the variables corresponding to the percentage of incidence of pathogens were transformed with the equation  $y = \sqrt{(x + 0.5)}$ , where x = percentage value. Finally, a quadratic regression analysis was performed using the  $ax^2 + bx + c$  model to determine the relationships between i) net photosynthetic rate  $(A_n)$  and stomatal conductance  $(g_s)$ , ii) instantaneous carboxylation efficiency  $(A_n/C_i)$  and stomatal conductance  $(g_s)$ , iii)

net photosynthetic rate ( $A_n$ ) and light transmission (%), and iv) yield and light transmission (%) at a significance level of 95%. Data were analyzed using the statistical software INFOSTAT [26].

#### 3. Results

#### 3.1 Light transmission

In the top canopy zone (Figure 2A) and in central part of the tree (vertical division), light transmission increased by 232% and 226% in the *WCA* and *WCA+2L* treatments, respectively, with respect to *WC*, showing a significant effect (p<0.001). In the midpoint of the tree, *WCA* increased light transmission significantly (p=0.042) by 85% compared to *WC*. A similar pattern, although to a lesser extent, was observed in the middle canopy zone (Figure 2B). In the central part of the tree, a significant increase (p=0.031) in light transmission (66%) was observed in *WCA+2L*, whereas significant increases (p=0.0018) of 247% and 463% were also observed in the mid-point of the tree in *WCA* and *WCA+2L*, respectively. Finally, the pruning treatments did not significantly affect light transmission in the lower part of the tree (Figure 2C) (p>0,05).



**Figure 2.** Light transmission pattern of photosynthetically active photon flux (PPFD) measured in top (A), middle (B), and bottom (C) canopy zones of European hazelnut for the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*) and winter pruning of the central axis plus 2 lateral branches (*WCA+2L*). Asterisks indicate level of significance according to Friedman test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Bars in the same point indicate standard error for n= 12. Source: Own elaboration.

In the top canopy zone (Figure 3A) and in the central part of the tree (vertical division), SCA+2L and SCA+2L+K significantly increased (p=0.002) light transmission by 137% and 123% % compared to SC, respectively. In the mid-

point of the tree, SCA+2L+K showed a significant effect by increasing the amount of light transmitted by 63% (p=0.02) (Figure 3A). This was also observed in the middle canopy zone (Figure 3B) since SCA+2L resulted in a significant increase (p=0.007) of 190% in light transmission in the central part of the tree. On the other hand, SCA+2L and SCA+2L+K in the mid-point of the tree increased light transmission significantly (p=0.002) by 474% and 378%, respectively (Figure 3B). In the bottom canopy zone, the treatments did not significantly affect light transmission (p>0.05) (Figure 3C).



**Figure 3.** Light transmission pattern of photosynthetically active photon flux (PPFD) measured in top (A), middle (B), and bottom (C) canopy zones of European hazelnut for

the control treatments without summer pruning (*SC*), summer pruning of the central axis plus 2 lateral branches (*SCA+2L*), and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA+2L+K*). Asterisks indicate level of significance according to Friedman test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Bars in the same point indicate standard error for n= 12. Source: Own elaboration.

#### 3.2. Gas exchange and chlorophyll fluorescence

*WCA* and *WCA+2L* had no significant effect on PPFD,  $A_n$ ,  $g_s$ , and *E* compared to *WC* (p>0.05) (Figure 4) and did not significantly affect the  $F_v/F_m$  ratio (p>0.05) (Figure 6A).

In the middle canopy zone, SCA+2L+K resulted in a 13% increase (p=0.0458) in PPFD compared to the control treatment without pruning (Figure 5A). In addition,  $A_n$  was significantly higher (p=0.011) in SCA+2L and SCA+2L+K, with increases of 117% and 100%, respectively, compared to SC (Figure 5B). In addition,  $g_s$  and E were not significantly affected by the summer pruning treatments (p>0.05) (Figure 5). Finally, no treatment significantly affected  $F_v/F_m$  in the different canopy zones (p>0.05) (Figure 6B).



**Figure 4.** Photosynthetically active photon flux, *PPFD* (A), net photosynthetic rate,  $A_n$  (B), stomatal conductance,  $g_s$  (C) and transpiration rate, E (D) of leaves from the top, middle and bottom canopy zones of European hazelnut for the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*) and winter pruning of the central axis plus 2 lateral branches (*WCA+2L*). Different letters indicate significant differences according to Tukey's test (p≤0.05). Bars in the same point indicate standard error for n= 4. Source: Own elaboration.



**Figure 5.** Photosynthetically active photon flux, *PPFD* (A), net photosynthetic rate,  $A_n$  (B), stomatal conductance,  $g_s$  (C) and transpiration rate, E (D) of leaves from the top, middle and bottom canopy zones of European hazelnut for the control treatments without summer pruning (*SC*), summer pruning of the central axis plus 2 lateral branches (*SCA+2L*), and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA+2L+K*). Different letters indicate significant differences according to Tukey's test (p≤0.05). Bars in the same point indicate standard error for n= 4. Source: Own elaboration.



**Figure 6.** Photochemical efficiency of PS-II ( $F_v/F_m$ ) measured in leaves of the top, middle and bottom canopy zones of European hazelnut for the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*), winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) (A); control treatments without summer pruning (SC), summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) and summer pruning of the axis central plus 2 lateral branches and kaolin application (*SCA+2L+K*) (B). Different letters indicate significant differences according to Tukey's test (p≤0.05). Bars in the same point indicate standard error for n= 4. Source: Own elaboration.

#### 3.3. Fruit yield components

Winter pruning did not significantly (p>0.05) affect yield in any of the canopy zones Table 1). *WCA* resulted in a significant increase (p=0.015) of 5% in nut weight in the bottom canopy zone (Table 1). In both winter pruning treatments,

kernel weight increased significantly (p=0.002), recording a 5% increase in the lower part of the plant. For WCA+2L, the same parament recorded an increase of 4% (p=0.012) in the top canopy zone. In the bottom canopy zone, WCA+2L increased kernel yield by 3% compared to WC (p=0.02) (Table 1).

Regarding summer pruning, yield had a significant increase (p=0.022) of 90% in the middle canopy zone for SCA+2L+K compared to SC (Table 2). The same treatment recorded significant increases of 4% (p=0.013) and 5% (p<0.001) in nut and kernel weight, respectively. Finally, SCA+2L+K increased kernel yield by 2% in the bottom (p=0.019) and top (p=0.05) canopy zones (Table 2).

#### 3.4. Vegetative growth, floral return, and incidence of fungal pathogens

In the top canopy zone, WCA+2L showed a significant increase (p=0.015) of 59% in the number of shoots with respect to the control without pruning (WC), decreasing (p=0.038) total shoot length by 45% (Table 3). In the middle canopy zone, the same treatment increased mean shoot length by 61% compared to WC (p=0.047) (Table 3). SCA+2L and SCA+2L+K had no significant effect (p>0.05) on the number, total length, and mean length of one-year shoots in any of the canopy zones (Table 4).

**Table 1.** Influence of the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*) and winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) on yield, nut weight, kernel weight and kernel yield for the top, middle and bottom canopy zones of European hazelnut. Different letters indicate significant differences between treatments according to Tukey's test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

Devementer	Treetmente	Canopy Zone			
Parameter	Treatments	Bottom	Middle	Тор	
	WC	242.25 ± 81.1	733.50 ± 162.3	1443.00 ± 228.0	
Yield (a plant <sup>-1</sup> )	WCA	240.25 ± 55.4	716.00 ± 110.6	1206.75 ± 191.7	
	WCA+2L	244.00 ± 48.0	778.25 ± 65.4	1501.25 ± 243.7	
	p value	0.99 <sup>ns</sup>	0.931 <sup>ns</sup>	0.627 <sup>ns</sup>	
	WC	3.10 ± 0.03 b	3.32 0 ± 0.04	3.33 ± 0.03	
Nut weight (g)	WCA	3.26 ± 0.04 a	3.31 ± 0.03	$3.40 \pm 0.03$	
Nut weight (g)	WCA+2L	3.19 ± 0.03 ab	$3.30 \pm 0.04$	$3.43 \pm 0.03$	
	p value	0.015 *	0.748 <sup>ns</sup>	0.073 <sup>ns</sup>	
	WC	1.47 ± 0.02 b	1.56 ± 0.02	1.58 ± 0.02 b	
Kernel weight (g)	WCA	1.55 ± 0.02 a	1.56 ± 0.02	1.59 ± 0.02 b	
Remei weight (g)	WCA+2L	1.55 ± 0.02 a	1.56 ± 0.02	1.64 ± 0.02 a	
	p value	0.002 **	0.876 <sup>ns</sup>	0.012 *	
	WC	47.32 ± 0.4 b	47.07 ± 0.3	47.49 ± 0.3 ab	
Kernel yield (%)	WCA	47.67 ± 0.3 ab	47.15 ± 0.2	46.64 ± 0.3 b	
	WCA+2L	48.65 ± 0.3 a	47.16 ± 0.3	47.88 ± 0.3 a	
	p value	0.020 *	0.837 <sup>ns</sup>	0.008 **	

**Table 2.** Influence of the control treatments without summer pruning (*SC*), summer pruning of the central axis plus 2 lateral branches (*SCA*+2*L*) and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA*+2*L*+*K*) on yield, nut weight, kernel weight and kernel yield for the top, middle and bottom canopy zones of

European hazelnut. Different letters indicate significant differences between treatments according to Tukey's test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

Baramotor	Troatmonte	Canopy Zone			
Falametel	reatments	Bottom	Middle	Тор	
	SC	172.50 ± 73.5	460.00 ± 55.4 b	1238.00 ± 138.0	
Viold (a plant-1)	SCA+2L	263.50 ± 33.0	734.50 ± 94.7 ab	1384.75 ± 224.2	
	SCA+2L+K	315.50 ± 62.9	876.25 ± 100.8 a	1529.25 ± 192.4	
	p value	0.273 <sup>ns</sup>	0.022 *	0.570 <sup>ns</sup>	
	SC	3.21 ± 0.03	3.28 ± 0.03 b	$3.39 \pm 0.03$	
Nutwoight (g)	SCA+2L	3.18 ± 0.03	3.34 ± 0.03 ab	$3.40 \pm 0.03$	
Nut weight (g)	SCA+2L+K	$3.20 \pm 0.03$	3.40 ± 0.03 a	$3.41 \pm 0.03$	
	p value	0.992 <sup>ns</sup>	0.008 **	0.839 <sup>ns</sup>	
	SC	1.50 ± 0.02	1.54 ± 0.02 b	1.59 ± 0.02	
Korpol woight (g)	SCA+2L	1.50 ± 0.01	1.58 ± 0.01 ab	1.60 ± 0.01	
Kernel weight (g)	SCA+2L+K	1.54 ± 0.02	1.62 ± 0.01 a	1.63 ± 0.02	
	p value	0.167 <sup>ns</sup>	0.0003 ***	0.167 <sup>ns</sup>	
	SC	47.30 ± 0.4 ab	46.90 ± 0.3	46.87 ± 0.3 b	
Kernel yield (%)	SCA+2L	47.18 ± 0.3 b	47.29 ± 0.3	47.20 ± 0.3 ab	
	SCA+2L+K	48.29 ± 0.3 a	47.82 ± 0.3	47.77 ± 0.3 a	
	p value	0.019 *	0.063 <sup>ns</sup>	0.050 *	

**Table 3.** Influence of the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*) and winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) on the number, total length, and mean length of one-year shoots (cm) for the top, middle and bottom canopy zone of European hazelnut. Different letters indicate significant differences between treatments according to Tukey's test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

Parameter	Canopy Zone			
		Bottom	Middle	Тор
	WC	56.63 ± 9.5	79.63 ± 13.7	29.13 ± 4.5 a
Number of objects	WCA	39.38 ± 5.2	71.00 ± 13.0	22.38 ± 4.5 ab
Number of shoots	WCA+2L	57.63 ± 6.4	41.63 ± 6.6	12.00 ± 1.7 b
	p value	0.160 <sup>ns</sup>	0.073 <sup>ns</sup>	0.015 *
	WC	177.63 ± 35.4	398.50 ± 60.0	498.00 ± 53.8 a
Total shoot length (cm)	WCA	102.25 ± 20.2	387.00 ± 50.9	372.38 ± 72.5 ab
	WCA+2L	163.88 ± 22.8	325.75 ± 36.8	274.63 ± 40.4 b
	p value	0.134 <sup>ns</sup>	0.554 <sup>ns</sup>	0.038 *
	WC	3.13 ± 0.3	5.37 ± 0.7 b	18.66 ± 2.1
Mean shoot length (cm)	WCA	$2.49 \pm 0.2$	6.28 ± 0.9 ab	17.65 ± 1.8
	WCA+2L	$2.82 \pm 0.2$	8.65 ± 1.0 a	23.21 ± 2.0
	p value	0.196 <sup>ns</sup>	0.047 *	0.123 <sup>ns</sup>

**Table 4.** Influence of the control treatments without summer pruning (*SC*), summer pruning of the central axis plus two lateral branches (*SCA*+2*L*) and summer pruning of the central axis plus 2 lateral branches and kaolin (*SCA*+2*L*+*K*) on the number, total length, and mean length of one-year shoots (cm) for the top, middle and bottom canopy

zones of E	uropean ha	zelnut.	Different	lett	ers indic	ate	significar	nt dif	ferences	betw	een
treatments	according	to Tuk	ey's test	(*:	p≤0.05,	**.	p≤0.01,	***.	p≤0.001,	ns:	not
significant).	. Source: Ov	vn elab	oration.								

	_		Canop	y Zone
Parameter	Treatments	Bottom	Middle	Тор
	SC	43.63 ± 8.5	72.38 ± 19.7	15.17 ± 3.1
Novel en ef els este	SCA+2L	41.38 ± 9.7	54.75 ± 11.2	18.00 ± 3.9
	SCA+2L+K	43.43 ± 6.6	38.38 ± 6.7	14.25 ± 3.3
	p value	0.978 <sup>ns</sup>	0.234 <sup>ns</sup>	0.727 <sup>ns</sup>
	SC	173.25 ± 44.2	343.13 ± 39.6	315.50 ± 50.7
Total shoot length (cm)	SCA+2L	138.13 ± 38.0	326.00 ± 34.8	353.88 ± 56.6
	SCA+2L+K	151.14 ± 28.4	303.88 ± 52.1	277.88 ± 42.2
	p value	0.800 <sup>ns</sup>	0.811 <sup>ns</sup>	0.572 <sup>ns</sup>
	SC	3.77 ± 0.5	8.49 ± 2.6	23.83 ± 2.7
Mean shoot length (cm)	SCA+2L	$3.26 \pm 0.2$	7.46 ± 1.3	21.46 ± 1.9
	SCA+2L+K	3.37 ± 0.2	9.44 ± 1.8	22.38 ± 2.2
	p value	0.579 <sup>ns</sup>	0.777 <sup>ns</sup>	0.763 <sup>ns</sup>

Both WCA and WCA+2L as well as SCA+2L and SCA+2L+K did not significantly affect (p>0.05) floral return in terms of number and density of catkins and glomeruli (Tables 5 and 6).

Despite the high diversity of pathogens observed in male flowers (Figure 7), associated with genera such as *Botrytis*, *Fusarium*, *Alternaria*, *Cladosporium*, *Penicilium*, and *Trichothecium*, neither winter (Figure 7A) nor summer (Figure 7B)

pruning affected the incidence of these fungi, except for species of *Fusarium*, which recorded a 9% increase in incidence in *WCA* (p=0.0125) (Figure 7A). However, it is important to note that the pruning treatments did not significantly affect pathogen incidence in any of the three assessed canopy zones (data not shown).

**Table 5.** Influence of the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*) and winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) on the number and density of male (catkins) and female (glomeruli) flowers for the bottom, middle and top canopy zones of European hazelnut. Different letters indicate significant differences between treatments according to Tukey's test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

	<b>-</b>		Canopy Zone		
Parameter	Ireatments	Bottom	Middle	Тор	
	WC	4.50 ± 1.6	16.63 ± 5.0	26.38 ± 7.1	
Number of catkins	WCA	3.75 ± 1.0	14.38 ± 4.1	17.50 ± 7.5	
Number of Catkins	WCA+2L	$9.00 \pm 3.2$	12.25 ± 5.1	19.88 ± 10.5	
	p value	0.564 <sup>ns</sup>	0.809 <sup>ns</sup>	0.533 <sup>ns</sup>	
	WC	22.50 ± 4.2	58.00 ± 14.8	65.13 ± 9.1	
Number of glomeruli	WCA	12.25 ± 2.8	43.88 ± 8.3	53.63 ± 12.0	
	WCA+2L	21.38 ± 4.9	38.13 ± 4.6	40.50 ± 9.2	
	p value	0.514 <sup>ns</sup>	0.670 <sup>ns</sup>	0.178 <sup>ns</sup>	
	WC	$0.022 \pm 0.007$	$0.038 \pm 0.011$	$0.051 \pm 0.014$	
Catkins density (n° cm <sup>-1</sup> )	WCA	0.056 ± 0.022	0.039 ± 0.013	0.035 ± 0.012	
	WCA+2L	0.054 ± 0.017	0.037 ± 0.014	0.087 ± 0.026	

	p value	0.345 <sup>ns</sup>	0.631 <sup>ns</sup>	0.565 <sup>ns</sup>
	WC	0.21 ± 0.08	0.21 ± 0.07	0.14 ± 0.03
Glomeruli density (n° cm <sup>-1</sup> )	WCA	$0.13 \pm 0.03$	$0.13 \pm 0.03$	$0.16 \pm 0.03$
	WCA+2L	$0.13 \pm 0.03$	$0.13 \pm 0.02$	0.16 ± 0.03
	p value	0.960 <sup>ns</sup>	0.905 <sup>ns</sup>	0.955 <sup>ns</sup>

**Table 6.** Influence of the control treatments without summer pruning (*SC*)), summer pruning of the central axis plus two lateral branches (*SCA*+2*L*), and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA*+2*L*+*K*) on the number and density of male (catkins) and female (glomeruli) flowers for the bottom, middle and upper canopy of European hazelnut. Different letters indicate significant differences between treatments according to Tukey's test (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

			y Zone	
Parameter	Treatments			
		Bottom	Middle	Тор
		0.40.4.0	40.00 4.4	
	SC	$9.43 \pm 4.0$	$18.63 \pm 4.4$	$20.88 \pm 9.2$
Number of catkins	SCA+2L	$5.00 \pm 0.8$	14.00 ± 5.5	15.88 ± 5.4
	SCA+2L+K	4.63 ± 1.7	11.38 ± 2.4	20.88 ± 9.6
	p value	0.295 <sup>ns</sup>	0,483 <sup>ns</sup>	0.778 <sup>ns</sup>
	SC	$19.88 \pm 5.4$	41.88 ± 8.3	52.63 ± 11.9
Number of glomeruli	SCA+2L	21.00 ± 3.2	40.88 ± 6.7	51.75 ± 10.1
	SCA+2L+K	22.75 ± 5.5	39.25 ± 6.7	38.38 ± 7.7
	p value	0.840 <sup>ns</sup>	0.973 <sup>ns</sup>	0.467 <sup>ns</sup>
Catkins density (n° cm <sup>-1</sup> )	SC	0.052 ± 0.013	0.055 ± 0.015	0.081 ± 0.036

	SCA+2L	0.048 ± 0.010	$0.037 \pm 0.011$	$0.040 \pm 0.011$
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#### SCA+2L+K 0.042 ± 0.017 0.037 ± 0.005 0.057 ± 0.024

	p value	0.363 <sup>ns</sup>	0.450 <sup>ns</sup>	0.724 <sup>ns</sup>
	SC	0.27 ± 0.12	0.13 ± 0.03	$0.22 \pm 0.06$
Glomeruli density (n° cm <sup>-1</sup> )	SCA+2L	0.26 ± 0.08	0.13 ± 0.02	0.21 ± 0.07
	SCA+2L+K	0.19 ± 0.05	0.14 ± 0.02	0.14 ± 0.03
	p value	0.797 <sup>ns</sup>	0.868 <sup>ns</sup>	0.897 <sup>ns</sup>



**Figure 7.** Influence of the control treatments without winter pruning (*WC*), winter pruning of the central axis (*WCA*), winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) (A); and control treatments without summer pruning (*SC*), summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) and summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) and summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) and summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA+2L+K*) (B) on the incidence (%) of different fungal pathogenic agents in male flowers (catkins) of European hazelnut.

Different letters indicate significant differences according to Tukey's test ( $p \le 0.05$ ). Bars in the same point indicate standard error for n=12.

#### 3.5. Regression analysis

*WCA+2L*, *SCA+2L* and *SCA+2L+K* showed a statistically significant relationship between  $A_n$  and  $g_s$  (p=0.0001; 0.039 and 0.0144, respectively) (Table 7). However, no significant relationship was found in *SC* for these variables (p>0.05). For all the pruning treatments, the variation in  $A_n$  was explained in at least 40% by the effect of the variation in  $g_s$  (Table 7). All the treatments showed a negative polynomial curve, except for *SC* (Table 7; Figure 8). In addition, a statistically significant relationship was found between  $A_n/C_i$  and  $g_s$  for *WCA+2L* and *SCA+2L+K* (p=0.0004 and 0.0417, respectively), but not for *SC* or *SCA+2L* (p>0.05) (Table 7). However, *WCA+2L* had a positive polynomial curve pattern ( $\beta_2$ =0.00000029). In addition, *WCA+2L* recorded the highest R<sup>2</sup> values (0.78 and 0.83), i.e., about 80% of the variation in  $A_n$  and  $A_n/C_i$  was explained by the variation in  $g_s$  (Table 7; Figure 8).

A significant relationship was found between yield and light transmission for *SCA+2L* and *SCA+2L+K* (p=0.0023 and 0.0025, respectively), being yield variation mostly explained (68%) by the effect of the variation in the transmitted light (Table 8; Figure 9). No statistically significant relationship was observed between *yield* and light transmission for *SC* and *WCA+2L* (p>0.05) (Table 8). A statistically significant relationship between  $A_n$  and light transmission was found in *SC*, *SCA+2L* and *SCA+2L+K* (p=0.0086 and 0.0078, respectively), while light transmission explained 41%, 57% and 58% of  $A_n$  variations, respectively. Conversely, the *WCA+2L* treatment did not present a significant relationship (p>0.05) (Table 8). In both cases, the curves corresponding to *SCA+2L* and *SCA+2L+K* presented a negative polynomial pattern (Table 8; Figure 9). It is important to note that *SCA+2L+K* showed  $\beta$ 2 values higher than those observed with *SCA+2L* in all the relationships studied (Tables 7 and 8). **Table 7.** Regression coefficients for the relationship between net photosynthetic rate ( $A_n$ ) and stomatal conductance ( $g_s$ ), and between instantaneous carboxylation efficiency ( $A_n/C_i$ ) and stomatal conductance ( $g_s$ ) affected by the control treatments without summer pruning, winter pruning of the central axis plus 2 lateral branches (WCA+2L), summer pruning of the central axis plus 2 lateral branches (SCA+2L) and summer pruning of the central axis plus 2 lateral branches (SCA+2L). Asterisks indicate level of significance (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

Regression coefficients	Pruning treatments						
	SC	WCA+2L	SCA+2L	SCA+2L+K			
β₀	-4.37 <sup>ns</sup>	-3.73 <sup>ns</sup>	-18.28 <sup>ns</sup>	6.84 <sup>ns</sup>			
β <sub>1</sub>	0.10 <sup>ns</sup>	0.09 <sup>ns</sup>	0.47 <sup>ns</sup>	0.22 <sup>ns</sup>			
β <sub>2</sub>	0.000001 <sup>ns</sup>	-0.000064 <sup>ns</sup>	-0.002 <sup>ns</sup>	-0.00087 <sup>ns</sup>			
R² Aj	0.35 <sup>ns</sup>	0.83 ***	0.40 *	0.52 *			
$A_n/C_i$							
β <sub>0</sub>	-0.03 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.11 <sup>ns</sup>	-0.03 <sup>ns</sup>			
β 1	0.0006 <sup>ns</sup>	0.00024 <sup>ns</sup>	0.0029 <sup>ns</sup>	0.00097 <sup>ns</sup>			
β <sub>2</sub>	-0.0000016 <sup>ns</sup>	0.00000029 <sup>ns</sup>	-0000013 <sup>ns</sup>	-0.0000043 <sup>ns</sup>			
R² Aj	0.25 <sup>ns</sup>	0.78 **	0.23 <sup>ns</sup>	0.40 *			

**Table 8.** Regression coefficients for the relationship between *yield* and light transmission, and between net photosynthetic rate ( $A_n$ ) and light transmission affected by the control treatments without summer pruning (*SC*), winter pruning of the central axis plus 2 branches (*WCA+2L*), summer pruning of the central axis plus 2 lateral branches

(*SCA*+2*L*) and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA*+2*L*+*K*). Asterisks indicate level of significance (\*:  $p \le 0.05$ , \*\*:  $p \le 0.01$ , \*\*\*:  $p \le 0.001$ , ns: not significant). Source: Own elaboration.

Regression coefficients —	Pruning treatments						
	SC	WCA+2L	SCA+2L	SCA+2L+K			
Yield							
βο	3135.23 <sup>ns</sup>	-2219.75 <sup>ns</sup>	-28415.75 *	-8109.13 <sup>ns</sup>			
β <sub>1</sub>	-141.52 <sup>ns</sup>	56.63 <sup>ns</sup>	971.89 *	252.68 <sup>ns</sup>			
β2	1.77 <sup>ns</sup>	-0.09 <sup>ns</sup>	-7.93 *	-1.58 <sup>ns</sup>			
R² Aj	0.29 <sup>ns</sup>	0.28 <sup>ns</sup>	0.68 **	0.68 *			
An							
βο	-46.36 <sup>ns</sup>	-10.42 <sup>ns</sup>	-205.1 *	-162.00 **			
β <sub>1</sub>	1.53 <sup>ns</sup>	0.22 <sup>ns</sup>	7.07 *	5.69 **			
β2	-0.01 <sup>ns</sup>	0.00029 <sup>ns</sup>	-0.06 *	-0.05 *			
R <sup>2</sup> Aj	0.41*	0.20 <sup>ns</sup>	0.57 **	0.58 **			



**Figure 8.** Relationship between net photosynthetic rate ( $A_n$ ) and stomatal conductance ( $g_s$ ) (A, B, C, D), and between instantaneous carboxylation efficiency ( $A_n/C_i$ ) and stomatal conductance ( $g_s$ ) (E, F, G, H) affected by the control treatments without summer pruning (*SC*) (A, E), winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) (B, F), summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) (C, G) and summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) (C, G) and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA+2L+K*) (D, H). Source: Own elaboration.



**Figure 9.** Relationship between net photosynthetic rate ( $A_n$ ) and light transmission (%) (A, B, C, D), and between *yield* and light transmission (%) (E, F, G, H) affected by the control treatments without summer pruning (*SC*) (A, E), winter pruning of the central axis plus 2 lateral branches (*WCA+2L*) (B, F), summer pruning of the central axis plus 2 lateral branches (*SCA+2L*) (C, G) and summer pruning of the central axis plus 2 lateral branches and kaolin application (*SCA+2L+K*) (D, H). Source: Own elaboration.

#### 4. Discussion

Light transmission into the inner canopy was greater in the top zone, decreasing towards the bottom zone (Figures 2 and 3). This agrees with studies carried out in crops such as blueberries [27], mango [28], apple [29], olive [30], kiwi [31], and European hazelnut [3]. In fact, light transmission into the inner canopy increased in WCA+2L and SCA+2L+K (Figures 2 and 3), which coincide with the results obtained in european hazelnut by Farinelli et al. [2] and Cristofori et al. [8]. Several studies have reported that as the amount of light available to the leaves increases, production increases [32-34]. This has been related to variables such as net CO<sub>2</sub> assimilation, stomatal conductance, floral development, yield, and fruit quality (30, 31, 34, 35). In this sense, SCA+2L+K was the treatment that resulted in the highest increase in light transmission. This effect could be attributed the use of kaolin since, being a white substance, it has the capacity to increase the reflected light [15], increasing the light transmitted into the inner canopy [18, 36]. In the present study, the greater light availability into the inner canopy was associated with an increase in  $A_n$ ,  $g_s$  and E (Figures 4 and 5), which agrees with the results reported in previous studies on crops such as apple [33], blueberry [35], olive [30], mango [28], and European hazelnut [3].

It should be noted that winter pruning did not have a significant effect on  $A_n$ ,  $g_s$  and E despite the increase in light transmission levels in the upper and middle parts of the plant. When analyzing the relationships between  $A_n$  and  $g_s$ , and between  $A_n/C_i$  and  $g_s$ , it was observed that the variation in  $A_n$  and  $A_n/C_i$  is largely explained by the variation in  $g_s$ , particularly in WCA+2L (Table 7). In addition, no significant relationship was found between  $A_n$  and light transmission for WCA+2L (Table 8). These results suggest the existence of non-stomatal limitations in the leaves of plants pruned in winter, such as a lower electron transport rate [37, 38], which would prevent an increase in net CO<sub>2</sub> assimilation by increasing light inside the canopy.

Summer pruning had a significant effect on the net CO<sub>2</sub> assimilation rate in the middle canopy zone, resulting in an increased  $A_n$  in both SCA+2L and SCA+2L+K (Figure 5) for that zone. This effect has also been observed in apple trees [29], mainly due to the increase in light transmission into the inner canopy, and in turn to a greater availability of radiant energy for photosynthesis [29]. However, the relationship between light transmission and  $A_n$  had a negative polynomial pattern (Figure 9), which could be explained by low acclimatization of the leaves as they were suddenly exposed to high radiation. Several studies have shown lower photosynthetic capacity in leaves under shaded conditions due to changes in anatomy and morphology such as lower development of palisade tissue, greater intercellular space in the mesophyll tissues, reduced leaf specific weight, and lower stomatal density [37, 38]. However, when analyzing the relationship between  $g_s$  and  $A_n$ , and that between  $g_s$  and  $A_n/C_i$ , higher  $\beta_2$  values were observed in SCA+2L+K compared to SCA+2L, indicating that the values of  $A_n$  y  $A_n/Ci$  remained stable as  $g_s$  increased in plants with kaolin, but not in SCA+2L (Table 7; Figure 8).

Several studies have described that kaolin can increase gas exchange capacity and photochemical efficiency of PS-II in leaves, particularly under high temperature conditions, excessive radiation, or severe water stress [4, 15 - 17]. In many cases, this occurs due to an increase in reflected light and thus to a reduction in the potential damage caused by high levels of visible and ultraviolet light on leaves [15, 18], a decrease in leaf temperature, which reduces heat stress [16, 18], or greater tolerance to water stress [15, 36]. In this sense, these finding are confirmed by the higher levels of  $A_n$  and  $g_s$  found in the pruning treatments that included kaolin application (Figure 5).

The positive effect that winter and summer pruning had on fruit weight, kernel weight and fruit filling rate (Tables 4 and 5) is consistent with previous studies on European hazelnut [8]. Previous findings have shown that pruning results in increases in yield (after 3 years) and kernel/fruit ratio compared to low intensity of pruning or no pruning. This positive effect of both winter and summer pruning would be mainly due to an increase in light into the inner canopy (Figures 2 and 3), as previously reported in apple [29] and European hazelnut [8]. A study carried out on apple trees [39] showed that the lack of sunlight affects and delays of assimilate partitioning, so that the winter and summer pruning treatments could have increased fruit weight, kernel weight and fruit filling by facilitating carbon movement towards the fruit due to improved light conditions inside the plant.

The yield increase observed with SCA+2L+K in the present study could also be explained by increased availability of radiant energy, which in turn led to an increase in the net CO<sub>2</sub> assimilation rate (Figure 5), and therefore to a greater amount of available carbohydrates for fruit growth. However, the same result was not observed with WCA+2L or SCA+2L, suggesting that the application of kaolin has an effect.

When analyzing the relationship between *yield* and light transmission (Figure 8), it was observed that yield remained stable in SCA+2L+K as the amount of transmitted light increased. However, this did not occur in SCA+2L ( $\beta_{2=}$  - 1.58 vs  $\beta_{2=}$  -7.93; Table 8), suggesting that the increase in luminosity was more productively efficient in the plants in which kaolin was applied. Various studies have shown the effect of kaolin application on fruit yield and quality. Similarly, Dinis et al. [15] reported an increased yield after kaolin application in grapevines, while Gharaghani et al. [16] reported an increase in yield and fruit quality in walnut plants treated with this reflective substance. In addition, the positive results of kaolin application on yield were associated with an increase in net CO<sub>2</sub> assimilation resulting from reduced stress by high temperatures, favoring a greater amount of available carbohydrates for fruit development. Glenn et al. [36] conducted a study on apples and found a significant relationship between weight increase by the application of kaolin, temperature, and vapor pressure deficit,

reporting a greater increase in weight under higher temperatures and greater vapor pressure deficit, which confirms that kaolin is more effective when the plant is subjected to greater stress. In our study, even though there was no significant effect of summer pruning on the photochemical efficiency of PS-II measured through the  $F_v/F_m$  ratio, the values of this parameter were lower in *SCA+2L* and increased with the application of kaolin (Figure 6), demonstrating the positive effect of kaolin on the decrease in photo-inhibition of leaves overexposed to light due to summer pruning.

In terms of vegetative growth, the reduction in shoot number and total shoot length in the top canopy zone by WCA+2L as well as the increase of these parameters in the middle zone (Table 3) would be explained by higher light transmission into the inner canopy (Figure 2), generating a lower growth of shoots compared to the control. It has been proven that, under low light availability, the plant would prioritize shoot growth by reducing carbohydrate supply to the fruit [40]. This would also explain the higher weight and fruit filling observed in WCA+2L (Table 1). Conversely, summer pruning did not exert a significant effect on shoot growth, probably because this practice was carried out in the least active period of shoot growth induced by paradormancy (January in the southern hemisphere), in which carbohydrate movement was mainly required for fruit filling and differentiation of flower primordia rather than shoot growth [41 - 43].

Although pruning affected light transmission, vegetative growth, and photosynthesis, no changes were observed in floral return, either catkins or glomeruli (Tables 5 and 6). This finding agrees with Farinelli et al. [2], who reported that pruning did not significantly affect the number of glomeruli and catkins in 'Tonda di Giffoni' hazelnut. In our study, the presence of *Fusarium* spp. and *Botrytis* spp. in male flowers (Figure 7) could explain the fact that winter and summer pruning did not trigger any response in floral return, since these pathogenic agents can affect the health of flower buds [44, 45]. Nevertheless, the

presence of different fungal genera associated with kernel mold [44, 45] in male flowers was observed, they did not show an increase in fungal incidence by this management cultural practices.

#### 5. Conclusions

In conclusion, summer pruning and kaolin-based particle film application can increase yield, nut, and kernel weight in hazelnut by greater photosynthetic light availability into the canopy, but also by improving leaf net photosynthesis rate. Although winter pruning also increased light transmission into the inner canopy, this was not reflected in changes in yield components and leaf photosynthesis. The results obtained in this study suggest that the adoption of pruning in hazelnut to form a training system with greater light transmission during summer and combined with the use of technologies, such as reflectance particles, to mitigate excess light could help to reach a more sustainable yield in this horticultural crop, especially in orchard conditions with excessive exposition to solar radiation and high temperatures.

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