Drivers of Flammability of *Eucalyptus globulus* Labill Leaves: Terpenes, Essential Oils, and Moisture Content

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1. Introduction

In general, *Eucalyptus globulus* (*E. globulus*) plantations have contributed to the development of the global economy, and have been proposed as novel ecosystems with resources that can support different biological communities [1]. Nevertheless, this species has been associated with environmental externalities and ecosystem disturbance, as well as loss of biodiversity and human welfare [2,3]. In Chile, *E. globulus* is the second most planted...
species in the country—behind Pinus radiata (P. radiata)—and its principal application is the production of wood pulp (7,353,000 m$^3$) and wood chips (6,972,000 m$^3$), with a surface area of 583,514 ha, equivalent to 18.23% of the national forest extension [4]. Furthermore, information on the distribution of E. globulus is available at different scales [4–6]; there are no official quantifications of the areas where this forest species is naturalized or invasive, nor its productivity, vigor, or phytosanitary status, despite evidence of its impacts.

In Chile, E. globulus plantations have disturbed the frequency and intensity of soil chemistry, nutrient and water cycling, hydrology, microclimate, and forest fires [7,8]. For example, strong erosion caused by Eucalyptus plantations has been reported compared to an adjacent natural forest, based on erosion indicators and soil profiles in south-central Chile [9]. In contrast to native forests, Eucalyptus plantations can substantially decrease water supplies because of the high demands for their growth [10–12]. These changes have led to a loss of biodiversity in Chile’s native forests, homogenization of the landscape, and the fragmentation of native forests, increasing the vulnerability of the land to fire in both rural and urban forest areas [13–17].

From an environmental perspective, the central zone of Chile, according to its climatic characteristics, is a region with important concentrations of endemic species worldwide which is experiencing an important loss of habitat [18]. A major contributor to this phenomenon is forest fires, which have been increasing in both frequency and magnitude in recent decades [19]. Moreover, according to climate change projections for the region, there will be greater difficulty for the survival of plant species (native and exotic), increasing their vulnerability to the occurrence of fires [20]. During the past two decades, fire seasons in the region have been intense and destructive. In 2008, for example, 8000 fires were responsible for the burning of 130,000 ha in Central Chile [13], while in January 2017, a catastrophic fire storm consumed an area of 500,000 ha, 44% more than the average for the period 1978–2018, events in which E. globulus played a leading role [21].

Central Chile is experiencing the most drastic and prolonged drought in the past millennium [22,23] which, in turn, constitutes an important pressure and stress factor for vegetated ecosystems. This produces a series of alterations in the physicochemical characteristics that are related to their vulnerability to ignition when faced with natural and/or anthropogenic factors [24]. For example, it has been recorded that some secondary metabolites—such as volatile terpenes—are drivers of flammability, and their production within plants increases with abiotic stress [25]. These and other chemical compounds that can have influence on the combustion of leaves, which are among the first tree structures to ignite [26], and constitute a significant share of the fuel consumed during forest fires [27]. In turn, the same physiological, structural, and/or chemical characteristics have been defined as parameters that define resource management (advantages/disadvantages) and their interactions with the ecosystem, and consequently how resource-usage strategies drive flammability parameters and the probability of fires [28,29]. Therefore, flammability studies based on plant properties generate scientific knowledge that could improve models that predict fire regimes and ecosystem processes [30], and thus improve the design of resilient and low-fire-risk landscapes in the context of national climate change. However, there are still few studies documenting variations in these compounds associated with changes in climate in different areas of the planet, considering that leaves play a crucial role in the spread of fire, as they allow the vertical rise and horizontal spread of fire [31,32]. In Chile, efforts to relate natural factors to the flammability of vegetation are scarce. In fact, there is very little information on the flammability of the main exotic tree species that cover Chilean landscapes—especially in the central-southern zone, where most of the population centers are located—and which are heavily used in the forestry industry, such as E. globulus.

Given the current scenario in Chile, the vulnerability to forest fires, the growing challenge of water scarcity, the invasive condition of E. globulus plantations, and the scarce characterization of exotic forest fuels frequently affected by forest fires, it is important to conduct studies to determine the thermochemical (e.g., flammability, flashpoint, and heating value) and chemical (e.g., moisture content, essential oils, volatile terpenes, and
other chemical molecules) properties of *E. globulus* leaves, and to compare them with the properties of other exotic species predominant in the Valparaíso region. This information is essential, as it could make it possible to estimate the risk and potential damage related to the fire behavior of these species, along with the local effects of climate change on vegetation, as the basis for fire prevention and suppression strategies [33,34].

In this context, the aim of this study was to evaluate the flammability, flashpoints, and heating values of fresh leaves of predominant exotic species (i.e., *E. globulus*, *P. radiata*, *Acacia dealbata*, and *Acacia melanoxylon*) from the Valparaíso region, and to relate these thermochemical parameters to chemical properties (i.e., chemical compounds, essential oils, and moisture content) called fire drivers, in order to determine the risk and potential damage posed by *E. globulus* species in forest fires, compared to other exotic species that dominate the forest formations in south-central Chile.

2. Materials and Methods

2.1. Study Area and Leaf Sampling

The exotic species leaf sampling was conducted in the Peñuelas Lake National Reserve (PLNR, Figure 1A), during the summer period of 2020–2021, from the 4th to the 8th of January. This reserve is located in the Valparaíso region, and constitutes a closed visual basin, with an extension of 17 km, altitudes ranging from 337 to 613 m above sea level, and a surface area of 9262.3 ha. The area has a temperate Mediterranean climate with an average annual temperature of 13.5 °C, an average maximum temperature of 17.1 °C, and an average minimum temperature of 9.4 °C [35]. The phytosociology of the zone indicates that the woody vegetation is composed of open hawthorn and mixed sclerophyllous scrub. Herbaceous plantations are distributed in dry grasslands, wet grasslands, cattails, and cypress and aquatic communities in the lake [36]. *E. globulus* is the most abundant species in the Valparaíso region, and also within the PLNR, accounting for 1,712.2 ha, while plantations of other exotic species are distributed as follows: 499.9 ha for *P. radiata*, 79.3 ha for *A. dealbata*, 32.5 ha for *E. globulus–P. radiata*, and 4.8 ha for *A. melanoxylon–A. dealbata* [34].

The PLNR has been affected by several forest fires in recent decades. Between the 2011–2012 and 2018–2019 seasons alone, a total of 53 fires occurred, with 131.93 ha of burnt surface [34]. Recently, there was a forest fire that affected the reserve and its surroundings. The fire, called “La Engorda-Reserva Nacional Lago Peñuelas”, consumed an area of 3900 ha of grassland, scrubland, eucalyptus, and native trees. In addition, in the city of “Ciudad del Sol”, it destroyed six houses and caused other damage, affecting 11 people [37].

During the sampling period, real-time measurements of climatic parameters were obtained using a handheld weather meter (Kestrel 5500, Nielsen-Kellerman Co., Pennsylvania USA) to approximate the forest microclimate of the sampled species [38]. Figure 1B–D show the temperature, relative moisture, and pressure, with averages of 17.3 ± 7.8 °C, 67.6 ± 25.2%, and 976.1 ± 8.1 mbar, respectively.

Three individuals per species were selected, and leaves were collected according to the methodology described by Guerrero et al. in 2020 [33] and in 2021 [34]. Six hundred leaves per individual were collected from three different sections of the tree according to the sun exposure (200 leaves from each canopy stratum; sun, sun/shade and shade/shadow), collecting a total of 1800 leaves per species. Leaves were stored and labelled in hermetically sealed bags to prevent them from getting wet. In order to keep the leaves fresh, they were stored in a container with cooling gel before further analysis in the laboratory.
2.2. Flammability Measurements

There are various methods to characterize the flammability of vegetation [39]. In this study, a 500 W epiradiator (model 534 RC2, Quartz Alliance, Villemer France) was used. This method is widely used for the study of plant fuels, as it allows the establishment of standard temperature conditions for combustion tests [25,40,41]. Regarding the flammability tests, 50 successive combustion runs of 1.0 ± 0.1 g of leaves per test for each individual exotic species were performed. These samples were placed on the surface of the epiradiator, where the radiating surface had a temperature of 440 ± 9 °C (2% coefficient of variation). Above the radiating surface, a pilot flame was located at a height of 4 cm to ignite the mixture of volatile compounds resulting from the thermal degradation of the leaves. The
flammability parameters measured were as follows: (1) ignition time (IT), which is defined as the time elapsed from the contact between the leaf sample and the radiating surface of the epiradiator until the ignition of the plant material; (2) flame duration (FD), which corresponds to the time elapsed from the ignition of the flame until its total disappearance; and (3) burning time (BT), which is defined as the time necessary to consume each particle of the sample until the disappearance of small embers. An arithmetic average of the 50 tests was obtained for each individual. Tests were considered positive when ignition occurred in less than 1 min. If successive ignitions occurred, and the duration of the first flame was equal to or less than 10 s, only the time at which the second ignition occurred was considered for test validation [42]. The ignition frequency (Fr) was calculated for each species as the fraction of positive tests with respect to the total number of tests ($n = 50$). By using the IT and Fr parameters, the Valette flammability index (FI) [42] was obtained. Table 1 shows the flammability index (FI) as a function of the IT and Fr parameters for further analysis.

Table 1. Flammability index$^1$ (FI) as a function of ignition frequency (Fr) and mean ignition time (IT).

<table>
<thead>
<tr>
<th>IT (s)</th>
<th>Fr (%)</th>
<th>100–95</th>
<th>94–90</th>
<th>89–85</th>
<th>84–80</th>
<th>&lt;50</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12.5</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>12.5–17.5</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>17.5–22.5</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>22.5–27.5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>27.5–32.5</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>&gt;32.5</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Valette’s classification [37]: minimally flammable (FI = 0), slightly flammable (FI = 1), moderately flammable (FI = 2), flammable (FI = 3), very flammable (FI = 4), and extremely flammable (FI = 5).

2.3. Heat of Combustion

The heating value was assessed to determine the potential energy release of the leaves. It is defined as the amount of heat generated by the complete combustion of one mass unit of the fuel under standard conditions (1 atm and 25 °C) when burned in a bomb calorimeter [43,44]. The heating value is classified as higher heating value (HHV) and lower heating value (LHV), where HHV considers the heat of vaporization of water produced in combustion, while LHV does not. Both parameters were determined according to ASTM 240 [45]. Initially, the samples were treated on a dry basis, where they were dried in a thermostatic oven at 110 ± 5 °C for 24 h and then stored in a desiccator until room temperature was reached. For HHV, 0.5 g of dry leaf was burned in a bomb calorimeter (Parr 1261) operating under an isoperibolic process [45]. HHV was obtained according to Equation (1):

$$HHV = \frac{K \times \Delta T}{m} - Q$$

where $HHV$ is the higher heating value (MJ kg$^{-1}$), $K$ is the heat capacity of the calorimeter (MJ °C$^{-1}$), $\Delta T$ is the temperature rise in the cooling water (°C), $m$ is the mass of dry leaves (g), and $Q$ is the total heat given up by the heating wire (MJ kg$^{-1}$) plus the heat contributed by the formation of acidic chemical compounds. To determine LHV, 1.0 g of dry leaf was weighed and burned in a Junkers calorimeter under adiabatic conditions [45]. To obtain the heat of vaporization, a calcium chloride trap was used to adsorb the vapor from the products and then identify them by mass difference. The heat of vaporization was calculated based on Equation (2):

$$C_v = \frac{m_c \times h_f g}{m}$$
where $C_V$ is the heat of vaporization (MJ kg$^{-1}$), $m_c$ is the mass of condensed water in the calcium chloride trap (kg), $h_{fg}$ is the enthalpy of vaporization (MJ kg$^{-1}$), and $m$ is the mass of burnt fuel (kg). Finally, $LHV$ was obtained according to Equation (3):

$$LHV = HHV - C_v$$ (3)

2.4. Flashpoint

The flashpoint (FP) is defined as the lowest temperature of a substance, corrected to 101.3 kPa, at which under the specified test conditions the vapor of the sample ignites in the presence of a source of ignition, and the flame spreads across the surface of the sample [46]. The flashpoint is used as a criterion to indicate the danger of flammable and fuel materials. Flashpoint measurements were determined in accordance with ASTM D-92-72, using an open-cup Cleveland device consisting of a hot plate, a thermometer (6 to 400 °C), and an ignition source [47]. A beaker filled with leaves was placed on the heating plate, and the thermometer was located inside it at a distance of 6.6 mm from the bottom of the beaker. The heating rate was 15 °C min$^{-1}$. Finally, the flashpoint was recorded when a flash appeared in the flame inside the cup.

2.5. Essential Oils

The essential oils (EOs) in the leaves under study were identified by hydrodistillation using the Clevenger apparatus. A total of 100 g of fresh leaves of each species was collected, brought to the laboratory, and subsequently left to dry at room temperature and without environmental disturbances. After the drying time, leaves were manually cut and fractionated with a mortar in order to obtain small particles. The dry mass was recorded, and the leaves were added to a 250 mL glass ball. Distilled water was added until the leaves were completely moistened. After this process, the sample was heated in a water bath with glycerine to a temperature of 130 °C to maintain the constant boiling process. The extraction time was three hours, according to the ISO 65-71:8 standard [48–50]. The Clevenger apparatus includes a condenser to ensure condensation of the EOs and to separate them from the aqueous phase. At the end of the distillation process, two phases were observed: an aqueous phase (hydrolate/aromatic water), and an organic phase (essential oils), which was less dense than water. EOs were collected in small vials, dried with magnesium sulfate and, finally, filtered.

EOs extraction yields were obtained by summing the polar and nonpolar phases. EOs yield is expressed as the percentage of the essential oil weight according to Equation (4):

$$EOs(\%w/w) = \frac{m_{EOs}}{m_{LV}} \cdot 100$$ (4)

where $m_{EOs}$ corresponds to the mass of EOs collected, and $m_{LV}$ is the initial mass of fresh leaves.

2.6. Chemical Extraction and Analysis

Fresh leaves were collected from the different canopy strata (sun, sun/shadow, and shadow) of the tree. Immediately, in a field laboratory, a composite sample was taken, and 10 g of plant material was weighed and placed in an extraction bottle with 80 mL of hexane. After shaking, filtration was carried out, and magnesium sulfate was added to the extract in order to remove water, followed by filtration. The extract was then stored at −20 °C in a portable freezer (50 L, 12V/220V, Evercool-EV-BD-50, Ningbo Jiayuan Electronic Co. Ltd., Yuyao, Zhejiang, China).

Extracts were analyzed qualitatively and quantitatively by gas chromatography coupled with mass spectrometry (GC–MS), using a GC/MS-QP2010 Ultra (Shimadzu, Kyoto, Japan) equipped with a fused silica capillary column coated with the stationary phase (RTX-5MS nonpolar, 30 m × 0.32 mm DI, 0.25 µm thick; Restek, Bellefonte, PA, USA). The carrier gas was helium (He), at a flow rate of 1.2 mL min$^{-1}$. The oven was programmed to
increase from 60 °C (2 min duration) to 300 °C at 10 °C min\(^{-1}\) (15 min duration), where the total operating time was 41 min. The injection was carried out in split mode (1:50), and the injector temperature was 200 °C. The mass spectrometer was operated in full scan mode (scan range 35–500 m/z; scan frequency 0.3 s/scan) at 70 eV. Compounds were identified by comparing their mass spectra and retention ratios \([51]\) with those reported in databases (NIST11 for MS; Waterman, 1996 \([52]\) for retention ratios). For quantification purposes, a simple calibration curve of six increasing concentrations (0.3, 1.0, 3.0, 10.0, 30.0, and 100 ng mL\(^{-1}\)) of the standard ethyl decanoate ≥99.9% (Cat.: 14,897-0, Sigma-Aldrich, St. Louis, MO, USA) was performed.

2.7. Moisture Content

The moisture content (MC) of the leaves was determined through the following process: 10 g of fresh leaves was weighed on an analytical balance (AS 220.R2, Radwag, Radom, Poland) and placed in a thermostatic drying oven (digital oven, model JK-DO-9030A, Shanghai Jingke Scientific Instrument Co. Ltd., Shanghai, China) at 60 °C for 48 h to guarantee total dehydration. The samples were then weighed, and MC—which was determined as the weight of water versus dry weight—was determined using Equation (5) \([33,53–57]\):

\[
MC(\%) = \frac{m_{LV} - m_{DR}}{m_{DR}} \times 100
\]

where \(m_{DR}\) corresponds to the dry weight of the leaf sample.

2.8. Data Analysis

The results derived from the flammability tests were analyzed by using the Kolmogorov–Smirnov normality test in order to assess the normal distribution resulting from the tests, with 150 data points. As mentioned in Section 2.5, EOs yields were presented as mass concentrations (% \(w/w\)), while the identification of chemical compounds was determined in µg g\(^{-1}\) by analyzing the percentage of the relative area in a chromatogram. In order to assess the relationship between thermochemical parameters (i.e., IT, FD, BT, HHV, LHV, and FP; dependent variables: \(y\)) and natural factors (i.e., MC, EOs, and chemical compounds) of \(E.\) \textit{globulus} leaves and exotic forest species, a simple linear regression analysis was performed to establish whether or not flammability and thermochemical properties could be estimated from natural factors present in tree leaves. All of the results from our investigations are expressed as the mean ± standard deviation with the coefficient of variation. All statistical tests were performed using STATGRAPHICS Centurion XV software. A multiple comparisons test was used to detect statistically significant differences between the means of each group, with the following parameters: \(P:\) \(p\)-value; \(R:\) coefficient of correlation; \(R^2:\) coefficient of determination; confidence level: 95%. Fisher’s least significant difference (LSD) procedure was used to discriminate between the mean values.

3. Results

3.1. Leaf Flammability: Ignition Time, Flame Duration, and Burning Time

The results of the Kolmogorov–Smirnov test showed that the ignition time for all exotic forest species was normally distributed, with a 95% confidence level. In particular, the following \(p\)-values were obtained: \(E.\) \textit{globulus} (0.5), \(P.\) \textit{radiata} (0.52), \(A.\) \textit{dealbata} (0.6), and \(A.\) \textit{melanoxylon} (0.54). The lowest IT was obtained for \(A.\) \textit{melanoxylon}, followed by \(E.\) \textit{globulus}, \(A.\) \textit{dealbata}, and \(P.\) \textit{radiata} (Figure 2A), with coefficients of variation of 25.23%, 33.20%, 35.40%, and 23.62%, respectively. Based on ANOVA, the differences between the IT means were shown to be statistically significant, as the obtained \(p\)-value was 0.00. Moreover, a multiple comparisons test was performed using Fisher’s least significant difference (LSD) procedure (see Table S1, Supplementary Materials), which determined that the mean values for \(E.\) \textit{globulus} and \(A.\) \textit{melanoxylon} showed statistically significant differences when compared to other exotic species. In contrast, \(A.\) \textit{dealbata} and \(P.\) \textit{radiata}
showed no statistically significant differences between their mean IT values at a 95% confidence level.

![Box-whisker plots for the flammability parameters of exotic species analyzed during summer 2020–2021: (A) ignition time (IT); (B) flame duration (FD); and (C) burning time (BT). Each box is defined by the lower and upper quartiles, while the caps at the end of the box represent the range within 2.7 standard deviations considering 1.5 scale factor in IQR Method for outlier detection. The continuous horizontal line in the box is the median, open circle corresponds to the mean of the data samples, and the diamond points represent outlier samples in the data group.](image)

**Figure 2.** Box-whisker plots for the flammability parameters of exotic species analyzed during summer 2020–2021: (A) ignition time (IT); (B) flame duration (FD); and (C) burning time (BT). Each box is defined by the lower and upper quartiles, while the caps at the end of the box represent the range within 2.7 standard deviations considering 1.5 scale factor in IQR Method for outlier detection. The continuous horizontal line in the box is the median, open circle corresponds to the mean of the data samples, and the diamond points represent outlier samples in the data group.

Regarding the FD values, these were found to follow a normal distribution with a 95% confidence level for all analyzed forest species, with p-values of 0.12, 0.40, 0.73, and 0.30 for *E. globulus*, *P. radiata*, *A. dealbata*, and *A. melanoxylon*, respectively. *E. globulus*
presented the lowest FD value, followed by *P. radiata*, *A. melanoxylon*, and *A. dealbata* (Figure 2B), with coefficient of variation values between 33.61% and 56.97%. Based on ANOVA, statistically significant differences between the means of the FD variable were found among all forest species, with a confidence level of 95%. Additionally, Fisher’s LSD test (see Table S1, Supplementary Materials) indicated that *E. globulus* showed statistically significant differences when their mean FD values were compared to those of the other exotic species. In contrast, *A. melanoxylon–P. radiata* pairs showed no differences between their mean FD values.

It was determined that the BT values were normally distributed with a confidence level of 95% for all analyzed species, and the following p-values were found: 0.30 (*E. globulus*), 0.28 (*P. radiata*), 0.24 (*A. dealbata*), and 0.28 (*A. melanoxylon*), with high coefficients of variation (18.54% to 27.38%). Furthermore, *E. globulus* presented the lowest BT, followed by *A. melanoxylon*, *P. radiata*, and *A. dealbata* (Figure 2C). Nevertheless, when applying Fisher’s LSD test (see Table S1, Supplementary Materials), it was determined that the pairs formed between *E. globulus, A. melanoxylon*, and *P. radiata* did not present statistically significant differences between their mean BT values. On the other hand, *A. dealbata* was the species that obtained the most significant differences, with the pairs *A. dealbata–E. globulus, A. dealbata–P. radiata, and A. dealbata–A. melanoxylon*.

### 3.2. Flammability Index

The leaves of the exotic species analyzed were classified according to the flammability index (FI) proposed by Valette [42]. All species obtained an Fr of 100% in the 50 tests. *A. melanoxylon* and *E. globulus* obtained an FI of 5, and were therefore classified as extremely flammable, while *A. dealbata* and *P. radiata* both obtained an FI of 4, classifying them as very flammable species, as shown in Table 2.

#### Table 2. Classification of the flammability of exotic species according to the flammability index (FI), based on the average ignition time (IT) and ignition frequency (Fr).

<table>
<thead>
<tr>
<th>Exotic Species</th>
<th>IT (s) (x ± s)</th>
<th>Fr (%)</th>
<th>FI (-)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. melanoxylon</em></td>
<td>9.86 ± 2.48</td>
<td>100</td>
<td>5</td>
<td>Extremely flammable</td>
</tr>
<tr>
<td><em>E. globulus</em></td>
<td>11.19 ± 3.71</td>
<td>100</td>
<td>5</td>
<td>Extremely flammable</td>
</tr>
<tr>
<td><em>A. dealbata</em></td>
<td>17.27 ± 5.92</td>
<td>100</td>
<td>4</td>
<td>Very flammable</td>
</tr>
<tr>
<td><em>P. radiata</em></td>
<td>17.47 ± 4.12</td>
<td>100</td>
<td>4</td>
<td>Very flammable</td>
</tr>
</tbody>
</table>

### 3.3. Heat of Combustion and Flashpoint

The results showed a normal distribution of the data with a 95% confidence level, with statistically significant differences between the mean values of HHV (F = 21.19, p-value = 0.0004) and LHV (F = 16.20, p-value = 0.0009) at a 95% confidence level. Each HHV and LHV test was performed in triplicate, where the coefficients of variation were found to be less than 2%.

*A. melanoxylon* was the species with the highest HHV, followed by *E. globulus, A. dealbata, and P. radiata* (Figure 3A). Fisher’s LSD test (see Table S1, Supplementary Materials) found no statistical differences between the mean values of *A. melanoxylon–E. globulus, A. melanoxylon–A. dealbata, and E. globulus–A. dealbata* pairs. On the other hand, *A. dealbata* had the highest LHV, followed by *A. melanoxylon, E. globulus, and P. radiata* (Figure 3A). Fisher’s LSD test (see Table S1, Supplementary Materials) found no statistical differences between the means of the *E. globulus–A. melanoxylon, E. globulus–A. dealbata, and A. melanoxylon–A. dealbata* pairs.
when comparing their mean FP values. A. melanoxylon (MT), triterpenes (TP), aliphatic hydrocarbons (AH), aldehydes (AL), steroids (STD), and higher heating value (HHV) and lower heating value (LHV); and (B) flashpoint (FP). Horizontal bars and solid diamond points represent mean values, while the error bounds correspond to the standard deviation of the data set.

The obtained FP values were adjusted to a normal distribution, with a confidence interval of 95%, with p-values of 0.98, 0.99, 0.97, and 0.98 for E. globulus, P. radiata, A. dealbata, and A. melanoxylon, respectively. A. dealbata species the lowest FP, followed by E. globulus, A. melanoxylon, and P. radiata (Figure 3B), with coefficients of variation lower than 10%. Fisher’s LSD test (see Table S1, Supplementary Materials) found no statistical differences between the means of the A. melanoxylon–E. globulus and A. melanoxylon–P. radiata pairs. In contrast, A. dealbata showed statistically significant differences from the other exotic species when comparing their mean FP values.

3.4. Identification and Quantification of Chemical Compounds and Essential Oils in Fresh Leaves

In terms of the EOs’ yields obtained from the exotic species leaves, E. globulus presented the highest concentration (3.07 ± 0.55% w/w), being 43, 121, and 960 times higher than the concentrations of P. radiata (0.07 ± 0.03% w/w), A. dealbata (0.03 ± 0.01% w/w), and A. melanoxylon (0.008 ± 0.004% w/w), respectively. Therefore, the mean values of EOs for E. globulus showed statistically significant differences from the mean values of the other forest species (Fisher’s LSD test; see Table S1, Supplementary Materials).

In total, 54 chemical compounds—comprising sesquiterpenes (ST), monoterpenes (MT), triterpenes (TP), aliphatic hydrocarbons (AH), aldehydes (AL), steroids (STD), and ketones (KO)—were identified and quantified, as shown in Figure 4. E. globulus and P. radiata were the only species containing semi-volatile terpenes (MT and ST), with the MT subcategory predominating, corresponding to 77 ± 5% (E. globulus) and 54 ± 23% (P. radiata) of the total analyzed chemical compounds. On the other hand, for the species A. melanoxylon and A. dealbata, differences in the classes of chemical compounds were analyzed, with KO predominating (62 ± 9% of the total) in A. dealbata and AHs (47 ± 7% of the total) in A. melanoxylon. It should be noted that for these forest species, TPs were determined to be distributed between 21 ± 2% and 24 ± 7% of the total analyzed chemical compounds.
3.5. Moisture Content

Results showed a normal distribution of the data with a 95% confidence level, with p-values of 0.50, 0.94, 0.98, and 0.95 for *E. globulus*, *P. radiata*, *A. dealbata*, and *A. melanoxylon*, respectively, and with statistically significant differences between the means (F = 5.15, p-value = 0.0051) at a 95% confidence level.

The species with the lowest moisture content was *A. melanoxylon*, followed by *A. dealbata*, *E. globulus*, and *P. radiata*, as shown in Figure 5. Fisher’s LSD test (see Table S1, Supplementary Materials) found no statistical differences between the MC mean values for the *E. globulus*–*P. radiata* and *A. dealbata–A. melanoxylon* pairs.

3.6. Relationships between Flammability, Thermochemical Properties, and Chemical Compounds

Figures 6–8 show the main relationships found between the flammability parameters (IT, FD, and BT), thermochemical properties (FP, HHV, and LHV), and the concentrations of EOs, MC, and chemical compounds found in the fresh leaves.
3.6. Relationships between Flammability, Thermochemical Properties, and Chemical Compounds

The species that contained volatile terpenes (MT and ST) were *E. globulus* and *P. radiata*. Therefore, when the relationship between the flammability parameters and the volatile terpenes of these species was considered, both as a subgroup and independently, it was found that volatile terpenes (F = 4.45; p-value = 0.0383) and MT (7.88; p-value = 0.0077) had a statistically significant relationship with the IT parameter, at a 95% confidence level. When a linear regression was performed, the sum of the concentration of volatile terpenes (R = 0.24) and the concentration of MT (R = 0.41) showed a relatively weak positive correlation with the IT parameter (R = 0.24), i.e., as the concentration of volatile terpenes increases, there is a slight increase in the IT value. This relationship was more evident for the *E. globulus* species, where there was a statistically significant correlation between the MT and IT parameters (8.11; p-value = 0.0103), with a relatively strong positive correlation (R = 0.54) (Figure 6).

![Figure 5](image-url)  
**Figure 5.** Results of moisture content of exotic species analyzed during summer 2020–2021. Solid bars represent mean mean values.

![Figure 6](image-url)  
**Figure 6.** Statistically significant correlation between monoterpenes concentration (x-axis) and ignition time (IT, y-axis) of *Eucalyptus globulus* samples. Empty black squares represents data points, while blue straight line represents the least squares regression fit. Red lines (inner limits) represent the confidence interval, while green lines (external limits) represent the prediction limits, both at 95% confidence level.

The species that contained volatile terpenes (MT and ST) were *E. globulus* and *P. radiata*. Therefore, when the relationship between the flammability parameters and the volatile terpenes of these species was considered, both as a subgroup and independently, it was found that volatile terpenes (F = 4.45; p-value = 0.0383) and MT (7.88; p-value = 0.0077) had a statistically significant relationship with the IT parameter, at a 95% confidence level. When a linear regression was performed, the sum of the concentration of volatile terpenes (R = 0.24) and the concentration of MT (R = 0.41) showed a relatively weak positive correlation with the IT parameter (R = 0.24), i.e., as the concentration of volatile terpenes increases, there is a slight increase in the IT value. This relationship was more evident for the *E. globulus* species, where there was a statistically significant correlation between the MT and IT parameters (8.11; p-value = 0.0103), with a relatively strong positive correlation (R = 0.54) (Figure 6).
Figure 7. Statistically significant correlation between the flashpoints (FP) of *Eucalyptus globulus* and *Pinus radiata* samples with respect to their concentrations of (A) essential oils (EOs), (B) terpenes, and (C) limonene. Empty black squares represent data points, while blue straight line represents the least squares regression fit. Red lines (inner limits) represent the confidence interval, while green lines (external limits) represent the prediction limits, both at 95% confidence level.

Moreover, considering that both *E. globulus* and *P. radiata* contained higher concentrations of EOs and low FP volatile terpenes, a statistically significant relationship was found between the parameters of EOs (F = 15.80; p-value = 0.0165)/volatile terpenes (F = 30.04; p-value = 0.0054)/limonene (F = 13.79; p-value = 0.0206) and FP. When applying a linear model, the concentrations of EOs (R = −0.89, Figure 7A), volatile terpenes (R = −0.93, Figure 7B), and limonene (R = −0.88, Figure 7C) showed a relatively strong negative correlation with the FP. This means that the higher the concentration of these parameters, the lower the FP of the fresh leaves of *E. globulus* and *P. radiata*.
4. Discussion

4.1. Variation in the Flammability of Exotic Species

The IT results showed that *A. melanoxylon* and *E. globulus* showed a greater predisposition to foliage ignition when exposed to a heat source, and were classified as extremely flammable species according to the flammability index proposed by Valette [42], so it is possible that these species are more likely to start crown fires. These results are similar to those reported by Guerrero et al. [34], with the difference being that in this study, *A. melanoxylon*, rather than *E. globulus*, was the species with the lowest IT. This means that special care is also required for other exotic species that may increase the flammability of forests, since a lower IT may indicate a greater susceptibility to initial combustion in a crown fire. In contrast, *A. dealbata* and *P. radiata* were less prone to ignition, despite being classified as flammable forest species.

The FD parameter showed that *E. globulus* had the shortest flame duration compared to the other species under study, representing a reduction of 40% and 27% compared to the FD of *A. dealbata* and *A. melanoxylon*, respectively. However, despite being the species...
with the third highest heat of combustion, the energy content of *E. globulus* was still high for the exotic species analyzed, showing a coefficient of variation with respect to the heat of combustion of *Acacia* species of 0.66% and 0.73% for HHV and LHV, respectively. Therefore, considering the relationship between FD and heat of combustion, results showed that *E. globulus* presented the highest rate of heat release of all the species under study, which could be a characteristic that favors the spread of fire when considering the high intensity of the flame phase during the combustion of fresh leaves of *E. globulus* compared to the *Acacia* species under study.

Regarding the BT parameter, the analyzed species that showed the shortest time for which the leaves remained incandescent were *E. globulus*, *P. radiata*, and *A. melanoxylon* (similar BT without statistical difference), which could represent a lower risk of new fires. Nevertheless, considering that *A. dealbata* presented the highest BT and a significant difference from *E. globulus*, *A. dealbata* is more likely to favor secondary fires.

The main contribution of this study was to empirically demonstrate that not only *E. globulus* but also other exotic species present in the forest formations of the Mediterranean climate in central-south Chile contribute to fire risk. Major differences were found in the main chemical classes of compounds between *E. globulus*–*P. radiata* and *A. melanoxylon*–*A. dealbata*. Furthermore, results show that *A. melanoxylon*, where this species is reported to be extremely flammable, and together with an ignition source of low heat flux, can ignite faster and favor the spread of crown fires. Moreover, the flammability parameters (IT, FD, and BT) of the species under study contribute to new scientific knowledge in the area of forest fires, as according to the research by Popović et al. [58], based on a synthesis of the flammability of tree species based on plant characteristics, there is little experimental evidence associated with the exotic species studied using the epiradiator technique and burning of fresh leaves. Table 1, presented in [58], shows the analyzed species, plant characteristics associated with flammability, and relevant scientific articles, highlighting that the species *A. melanoxylon* and *A. dealbata* are not on this list. Furthermore, we found studies regarding *E. globulus* [59–61] that were focused on litter flammability, and on *P. radiata*, where studies [57,62–65] were focused on relating flammability to the chemistry and moisture content of the foliage.

It should be noted that the results were obtained at the laboratory scale and cannot be directly scaled to natural landscape dimensions. These flammability results serve to study and identify intrinsic properties and natural factors that have influence over the fire behavior of fresh leaf samples. Therefore, these results enable the understanding and evaluation of natural factors in ideal conditions for standardized tests, without biases that modify the analysis of the flammability parameters.

### 4.2. Variations of Heating Values and Flashpoints

The energy content (HHV and LHV) of all forest fuels showed that the associated results for HHV and LHV are consistent with those determined by Guerrero et al. [33,34]. The results associated with HHV from our research were used as an energy indicator of vegetation through the risk classification designed by Nuñez-Regueira et al. [62,66], which was developed for different forest species (*E. globulus*, *P. radiata*, and others) from the north coast of Galicia (Spain) sampled during each season of the year, obtaining the following classification based on HHV: HHV < 18.5 MJ kg⁻¹, Class 1 (no apparent risk); 18.5 ≤ HHV < 19.5 MJkg⁻¹, Class 2 (little risk); 19.5 ≤ HHV < 20.5 MJkg⁻¹, Class 3 (moderate risk); 20.5 ≤ HHV < 21.5 MJkg⁻¹, Class 4 (high risk); and HHV > 21.5 MJ kg⁻¹, Class 5 (extremely high risk). According to this classification system, our results show that the species *A. dealbata*, *E. globulus*, and *A. melanoxylon* were classified as Class 4, meaning that these species are high risk, because it is possible that they release high amounts of energy in the combustion process, generating high-intensity fires. In contrast, *P. radiata* was classified as Class 3—a moderate-risk species. Regarding the LHV tests, our results show high values for *A. dealbata*, *A. melanoxylon*, and *E. globulus*; therefore, it is expected that these species sustain a high-intensity fire in the combustion process, due to the emitted heat
without vapor condensation in the combustion products [44]. Additionally, A. dealbata, E. globulus, and A. melanoxylon species had the lowest flashpoints (268 to 327 °C), which may be due to the presence of MT, ST, AL, KO, and AH, which were identified and quantified in these species (see Table S2, Supplementary Materials), and which are low-to-moderate FP chemical compounds.

4.3. Variations in Terpene Content

Chemical factors in plant leaves and litter have been studied as fire drivers for their positive effects on flammability parameters, such as terpenes, where a positive correlation between terpene concentration and flammability has been determined in Mediterranean plants [53,67]. For these reasons, in our study, E. globulus showed the presence of volatile terpene compounds corresponding to MT and ST, and these results are comparable to those found in the literature [68,69]. P. radiata was the other species that presented similar chemical compounds also identified in previous works [70,71], where high amounts of MT (67.7%) and ST (27.7%) were reported.

The terpene content of E. globulus showed high intraspecific variation between individuals, although spatial distances between individuals were short, and individuals grown on the same soil belonged to the same geographical area [72]. Changes in plant secondary metabolite concentrations may vary both qualitatively and quantitatively due to differences in leaf age and ontogenetic stage [73,74]. The main identified and quantified compound was the terpene 1,8-cineol, also called eucalyptol, which is the most common monoterpene found in E. globulus individuals compared to those found in other research [68,69,75], where the relative abundance of this compound varied between 44% and 84%. The other main compounds coexisting were α-pinene (MT) and aromadendrene (ST), in accordance with the findings of Luis et al. [69], where the main compounds were eucalyptol (63.81%), α-pinene (16.06%), and aromadendrene (3.68%).

As is well documented, there is interest in preclinical studies, pharmaceuticals, agriculture, cosmetics, and food, among others, in the use of the genus Eucalyptus for the secondary metabolites that are mainly stored in its leaves. In fact, eucalyptol has been studied, as this compound shows excellent potential for the treatment and management of respiratory disorders, COVID-19, pain, oral healthcare, infectious diseases, and cancer, among others [75–77]. Nevertheless, secondary metabolites have diverse ecological functions, such as interactions at the tritrophic level that promote the inflammability of vegetation [53,78]. MTs and STs have low FP, e.g., eucalyptol is a volatile MT that is emitted at high rates and has a flashpoint of 49 °C, which implies that it can generate a flammable atmosphere, and can contribute to the formation of forest fires [34,53,67].

High concentrations of TP were found for A. dealbata and A. melanoxylon, such as lupeol in both species, which has a moderate FP (216.9 ± 12.4 °C). However, there is no evidence from other studies on the relationship between TP and flammability parameters.

4.4. Effects of Drivers on Leaf Flammability

The results showed that flammability drivers had positive and negative effects on flammability parameters, heat of combustion, and FP. Regarding volatile terpenes (MT and ST), it was possible to compare E. globulus and P. radiata, since both species presented similar chemical compounds (MT and ST). This can be reflected in the positive effects of the volatile terpenes and MT of both species on the IT parameter, which were stronger with E. globulus. This means that as we increased the concentrations of volatile terpenes and MT in leaves, there was an increase in the IT, which implies that volatile terpenes and MT contribute to a decrease in flammability. Nevertheless, these results do not seem logical, mainly since the reported MTs are all highly flammable chemical compounds, with flashpoints ranging from 32.2 to 90 °C. This characteristic makes them highly flammable and means that if the leaves are subjected to an ignition source, they must ignite at relatively low temperatures and concentrations, increasing their flammability [67,79,80]. One possible explanation may be that the emissions of volatile terpenes are closely related to
temperature [81], since there are studies that confirm that there is a higher emission of terpenes at specific temperature; for instance, the specific temperature of Rosmarinus officinalis is about 175 °C [82], while that for the MT α-pinene is 200 °C in Pinus sylvestris [83], and in this case, when the temperature is above the specific temperature (450 °C for the epiradiator), the emission of volatile terpenes could decrease due to thermal degradation and/or polymerization of the terpene compounds. Furthermore, the effect of MT content on flammability variables is difficult to understand, as MTs are highly volatile molecules, meaning that they volatilize before ignition occurs, and would contribute to the formation of a characteristic flammable environment composition [81]. In this case, it is suggested that there would be higher emissions from species rich in these volatile compounds, such as E. globulus, generating a flammable atmosphere in the fire environment, and that they may burn violently, consequently favoring the spread of fire [84]. On the other hand, water could also be an explanation, as it plays an important role in interacting with leaf terpenes, as in reported in [85,86], and some suggest that water may influence the terpene transport in the preheating phase, which could contribute to different flammability results [87].

Regarding the MC of E. globulus leaves, it was similar to that reported by other authors [55,57], where for Mediterranean species it ranged from 68% to 253%. It is generally accepted that MC is a flammability determinant, and it was shown that MC delays the IT, as the water molecule slows down the heat transfer to the fuel and is reflected in an increase in the IT variable [57]. However, our results showed an absence of significant relationships in MC with flammability parameters. In general, and in the case of STs, further research is needed to explain the storage system associated with terpenes in E. globulus leaves, since species such as E. globulus have the ability to store volatile terpenes in their leaves in liquid reservoirs and specialized compartments [88], and the lower ST loss found in pines, where known, may be due to the deeper and more protected terpenoid storage sites (resin ducts). In pines, this could reduce the volatilization of heavier ST molecules.

Considering that E. globulus and P. radiata were the species with the highest concentrations of EOs, chemical compounds of the same nature, and similar MC, our results showed a strong negative influence of the EOs, volatile terpenes, and limonene concentration with the FP. This means that increasing the EOs content, volatile terpenes, and limonene concentration in the leaves should lead to a decrease in FP, i.e., the mixture of gases emitted by the fresh leaves ignited at lower temperatures. These results are expected since, considering the results reported in [75,89], the chemical composition revealed that the main chemical compound present in eucalyptus EOs is 1,8-cineol, which constitutes more than 70% (v/v) of the total oil, followed by limonene and α-terpineol, which have low flashpoint MTs; thus, a lower FP is expected due to the higher concentration of volatile terpenes in the EOs. In this case, the positive relationship between these parameters could be related to the fact that the procedure for obtaining FP is a gradual increase in the temperature to which the leaves are subjected, which could affect the ways in which volatile terpenes are emitted into the environment. Despite these results, the species that obtained the lowest FP was A. melanoxylon, followed by E. globulus, considering that A. melanoxylon obtained a very low content of EOs; however, other chemical compounds, such as AH and KO, were identified as potentially influencing the FP.

Another interesting relationship between E. globulus and P. radiata was found between the concentrations of EOs and the heat of combustion (HHV and LHV), which indicated that the heating value varies according to the energy value of the essential oils—a higher content of EOs increases the heating value (HHV and LHV). However, this relationship was only for the pair E. globulus and P. radiata. This may be as mentioned previously, due to the chemical compounds of the same nature, since A. melanoxylon and A. dealbata presented similar HHV and LHV values when compared to E. globulus, but also possessed other types of compounds. This indicates that there are other compounds that influence the energy content (HHV and LHV)—such as cellulosic materials—which could also vary depending on the MC of the leaves [90].
4.5. The Ecological Role of Terpenes: Promoting E. globulus Flammability

Secondary metabolites (flavonoids, phenolic glycosides, MTs, and STs, among others) contained in eucalyptus play an essential role in different ecological interactions; for example, formylated phloroglucinol compounds are antifeedants against marsupial herbivores [91,92], and phenolics can influence litter decomposition [93], but we found very limited information on the involvement of secondary metabolites (mainly terpenes) as fire drivers and spreaders, considering the current climate change scenario (increasing droughts and heat waves), which causes an increase in the frequency and intensity of forest fires. Therefore, it is a priority to determine the contents of secondary metabolites, and how these compounds behave in E. globulus in ecosystems subjected to important abiotic stress factors (water, heat, etc.). Recent studies [25] have shown that the concentration of organic metabolites (AH) contained in Quercus coccifera litter (the main shrub species of the Mediterranean basis), when subjected to aggravated drought was associated with increased flammability, suggested that the flammability of the species may increase in a drought scenario in the Mediterranean area. Furthermore, McKiernan et al. [94] studied the effect on secondary metabolites contained in E. globulus and E. viminalis leaves, when the species were subjected to different water supplies (high, moderate, and low). The results showed that most of the terpene compounds, formylated phloroglucinol, and condensed tannins were not affected by decreasing water availability, concluding that water limitation had little impact on the total concentrations of secondary metabolites contained in leaves. In this context, the results presented in this work—where forest species were recurrently subjected to drought and heat wave events in the PLNR—shows that volatile terpenes had relevant influence on flammability parameters (e.g., IT, FP) in E. globulus and P. radiata—species with a high concentration of MTs and STs. Furthermore, a significant relationship was found between EOs, HHV, and LHV.

Secondary metabolites are not produced randomly, as they are shaped and optimized during evolution [95]. An important function of secondary metabolite compounds is to act as allelopathic agents, i.e., to intervene in the chemical defense of plants. Environmental conditions influence the synthesis of compounds with potential allelopathic activity, but this potential is again affected by the interaction with the environment, and can be modified by different environmental conditions [96]. All of these compounds increase in concentration when the plant is subjected to different weather conditions. Generally, phenol synthesis varies, and is induced by ecological factors such as UV radiation, water stress [97], or ozone [98]. In this sense, in addition to influencing the synthesis of allelochemicals, they can also enhance their activity [99]. Another function attributed to them is the ability to protect the plant from UV radiation [100]. The effect of UV radiation on the plant is important; high amounts of UV radiation reduce plant biomass and increase the quantity of flavonoids [100], which can increase the concentrations of metabolites both in cells and on the plant surface [101].

In this scenario, however, it is not yet known whether the production of these secondary metabolites will increase or decrease due to climate projections (water scarcity and temperature increase) in Central Chile. This information would be relevant as, if it can be shown that the secondary metabolites of E. globulus species increase under conditions of drought and heat waves, it is possible that it would generate forest fires of greater severity, affecting both native and endemic vegetation that is immersed in the forests next to this species (mixed forests), as well as in interurban forest zones.

5. Conclusions

This research shows that E. globulus is extremely flammable, due to the high concentration of EOs contained in its leaves, and a higher concentration of MTs (mainly eucalyptol, α-pinene, and d-limonene) and ST (mainly aromadendrene, α-gurjunene, and ledene), which can generate a flammable atmosphere due to their low FP. Therefore, EOS and volatile terpenes can be categorized as fire drivers in E. globulus due to their negative correlation with the FP of the fresh leaves. Additionally, this species can generate high-intensity
fires because of its high concentrations of these essential oils (positive correlation with heat of combustion).

In this context, it is necessary to incorporate EOs, volatile terpenes, and/or MTs into physical models of forest fire prediction, and to spread them as natural factors that can drive fire in *E. globulus* to improve fire risk indices.

In general, the forest species under study (*E. globulus*, *A. dealbata*, *A. melanoxylon*, and *P. radiata*) may be prone to crown fires due to their having thermochemical properties (flammability, heat of combustion, and FP) that favor the predisposition to ignite in the presence of a heat source, and which release high amounts of energy (HHV, LHV, and FD) in the combustion process, which contributes to the risk of fire formation and spread in a forest fire.

Finally, this information on flammability associated with secondary metabolites can potentially be considered as a key parameter for the generation and design of resilient and low-flammability landscapes, in order to cope with climate projections in Central Chile. Further studies should generate this type of information for the most dominant forest species in native forests and plantations of exotic species, which is necessary for fire risk reduction in the future.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/f13060908/s1: Table S1: Multiple range test through the LSD test of ignition time (IT), flame duration (FD), burning time (BT), flashpoint (FP), higher heating value (HHV), lower heating value (LHV), moisture content (MC), and essential oils (EOs), with the confidence interval at 95%; * denotes a statistically significant difference. Table S2: Contents of the different chemical compounds. MT: monoterpenes; ST: sesquiterpenes; TP: triterpenes; AL: aldehydes; AH: aliphatic hydrocarbons; KO: ketones; STD: steroids; GLV: green leaf volatiles; I: individual; ND: not detected; %: proportion of the total content; FP: flashpoint.

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