Bioindicators for the short-term response of *Pinus laricio* needles to thermal pruning

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Abstract

Fire is a dominant ecological factor in Mediterranean-type ecosystems. Management strategies have recently begun to include prescribed burning and thermal pruning. Pruning with fire consists in using an intensity of fire greater than during usual prescribed burning. This tool was used on *Pinus nigra* ssp *laricio* var. *corsicana*. It is an endemic pine of Corsica of great ecological and economic importance. Many parameters were analysed on pigments, carbon isotope and phenolic compounds of needles and oleoresin exudation flow discrimination in order to understand its biochemical responses to thermal pruning at short-term. This study was carried out at two stations: a thermal pruning was conducted in April 2006 at the first station while no treatment was applied to the second one. Needles were collected on pines at two heights (2m and 6m above ground level), before the thermal pruning, then 1 and 3months following the thermal pruning. Low contents of pigments were observed 1 month after the burning at 6 m height while phenolic compounds increased at 2 m height. After 3 months, usual values were observed at both heights. Flow resin increased up to 50 % at the burned station, 1 month after the burning. These parameters seem to constitute short-term bioindicators for *Pinus laricio* when managed with thermal pruning.

Introduction

Fire is a constant and periodic threat in Mediterranean forests. Mediterranean region is submitted to 50 000 fires/year, which burn 600 000 ha of forest annually (Quézel and Médail 2003). These fires constitute a real ecological hazard, but also a threat for people. Management strategies have recently begun to include many preventives tools such as prescribed burning (Rigolot 2003). The thermal pruning is a prescribed burning which consists in using an intensity of fire greater than during usual prescribed burning, allowing the pruning of low branches in order to limit the propagation of flame to the upper crow in case of fire.

The prescribed burning is a tool which is more and more used, in particular in France (Rigolot 2003), within the framework of the Defence of the Forest against the Fires, in Portugal (Fernandes and Botelho 2004) and still in the United States (Feeney and others 1998) and Canada (Weber and Taylor 1992). In some forests, prescribed burning are implemented in an attempt to restore fire to the ecosystem, recreate natural disturbance dynamics, reduce fuel loadings and fire risks (Weber and Taylor 1992). Fire use with thermal pruning or prescribed burning is often debated and studies are necessary to bring out answers.

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Numerous studies examined the effects of prescribed burning or fire on the physiological functioning of different species. Fleck and others (1996) show lower carbon isotope discrimination ($\delta^{13}C$) and higher photosynthesis in resprouting leaves in *Quercus* sp. after a fire. Carbon isotope discrimination provides an integrated measure of plant response to changes in the environment, such as water availability (Meinzer and others 1990). Phenolic compounds and pigment contents vary on *Pinus pinaster* Ait after heat damage to stems and crowns (Alonso and others 2002). Prescribed burning is effective in increasing tree defense against bark beetles through increased resin production (Feeney and others 1998). Secondary metabolites, such as phenolic compounds and resin are a particularly interesting because of their involvement in the response of the plant to environmental stress, such as temperature variations (Alonso and others 2002) or beetles attacks (Santoro and others 2001; Lombardero and others 2006). Some authors (Alonso and others 2002; Ferrat and others 2003; Pasqualini and others 2003) demonstrated the potentialities of primary and secondary metabolites as bioindicators of environmental stress.

The effects of prescribed burning on *Pinus nigra* ssp *laricio* (Poir.) Maire var. *corsicana* (Loud.) Hyl. were preliminary studied on morphology (Cannac and others 2006) but no study was realized on the effects of thermal pruning on the physiological functioning of this species. Nevertheless, the thermal pruning and the prescribed burning are more and more used on these stands in Corsica (France; *Fig.1*), because of the important fire risks on this Mediterranean island. *Pinus nigra* (European black pine) is native to Europe and Asia. The species is divided into five subspecies, among them *Pinus nigra* ssp *laricio* is widespread in Calabria and Sicily (Italy) and Corsica (France; 15). In Corsica, *Pinus nigra* ssp *laricio* var. *corsicana* (*Pinus laricio*) is a tree up to 50 m high with a straight trunk and grey to dark brown bark growing on mountainous terrain at altitudes of 900–1800 m (Gamisans and Marzocchi 1996). *Pinus laricio* constitutes high forests covering 21 000 ha (Schabaver 2001). This tree is exploited for timber production under the control of the *Office National des Forêts* (*ONF*).

In this paper, we studied primary and secondary metabolisms to understand the effect of thermal pruning on the vitality and the functioning of *Pinus laricio* and to see if these responses could be used as bioindicators. Our investigations will allow us to determine physiological responses of *Pinus laricio* as a function of height, and to understand the possible short-term physiological changes.
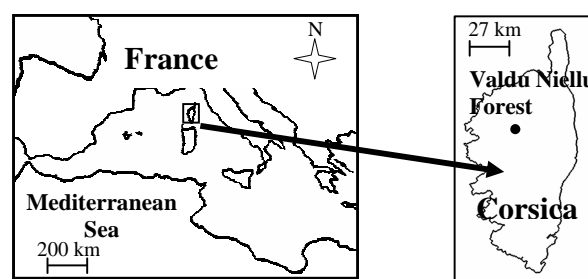

Figure 1—Location of the study site.

Experimental Procedure

Study sites and sampling protocol

The study site is located in Valdu Niellu Forest (42°17'03 N, 8°55'22 E) in the Northwest of Corsica (*fig. 1*). The experiment was conducted on natural regeneration of *Pinus laricio*. The trees average is included between 10 and 12 years (dbh 13 cm, height 6.6 m, and competition index 2.9; Lorimer, 1983). The basal area corresponded to 21.3 m².ha^{-1}. The site is located at 1070 m elevation and 5-10 % slope. The substrate is granitic, and the station mesophyll. The vegetation mixed heather and fern with 60 % of cover. The mean annual rainfall is of 1090 mm and the mean annual temperature of 11.5 °C.

For this study, two stations of 0.2 ha each were selected similar in structure and composition. On the first station, a thermal pruning was conducted at April 24, 2006 (burned station) by *Office National des Forêts*, and the second is the control station (unburned station). 1-year-old needles (their growth began in spring 2005) were collected on six dominant trees in April 19, 2006, before the thermal pruning and in May 24 and July 20, 2006, 1 and 3 months after the treatment. Needles were collected on pines at two heights: 6 m and 2 m above the ground level. Resin was collected in June 30, 2006. A total of 72 needle samples were collected for physiological parameters (pigments, carbon isotope and total phenolic compounds) and 12 samples for oleoresin exudation flow.

Temperature and heat flux measurements

A measurement device was developed in order to provide information on the temperature along the trees height and on the heat flux impinging at the bottom of the trunk during the thermal pruning. Three series of measurements were done moving the measurement device across the vegetation plot during fire spread. The temperature was measured with a set of thermocouples located under and above the ground surface. An insulated supporting rod (1.3 m high), was prepared to hold ten thermocouples and two more were located on the tree canopy. The temperature measurement heights were 0, 1, 2 cm and 5, 10, 20, 40, 60, 80, 100, 200, 300 cm in the soil and the air, respectively. K-type thermocouples with 50 µm wire diameter were used. The total heat flux emitted by the flame front during fire spread was measured using Gardon gauge. The sensor was horizontally oriented and was located 5 cm above the ground surface. The heat flux gage was water-cooled thanks to a pump and a reservoir located near the data logger, about 4 m from the measuring device. The sampling rate was 1 measurement per second per sensor.

Pigments

The method of pigments extraction was based on the work of Hiscox and Israelstam (1979). Chlorophylls *a, b* and carotenoids were extracted using dimethyl sulfoxyde (DMSO). Needles (50 to 80 mg) were placed in a vial containing 5 mL DMSO and were frozen. Pigments were extracted at 60°C during 8 hr. Spectrophotometrical readings were made at 480, 649.1 and 665.1 nm (Wellburn 1994).

Carbon isotope

Needle samples of some milligram were collected to measure the ^{13}C isotopic composition. The carbon isotope ratios were determined on CO_2 after combustion of needle tissue, in an isotope ratio mass spectrophotometer, by CNRS Central

Analytical Departement at Vernaison (France). The ^{13}C content is expressed in delta notation as δ^{13}C (‰) with a precision of the analysis of 0.30 ‰.

Total Phenolic compounds

The method of extraction of the phenolic compounds was based on the work of Penuelas and others 1996. One-half gram (dry weight) of needles per sample was extracted with 20 mL of a 70% aqueous methanol solution (v/v) acidified by a few drops of 1 M HCl. The mixture was left at ambient temperature for 1.5 h, and then filtered. Quantification of the total phenolic compounds was done by colorimetric reaction using Folin-Ciocalteu reagent. After 1 h, the reaction was completed and measured at 720 mm on a spectrophotometer. The quantitative results are expressed with reference to gallic acid.

Oleoresin exudation flow

Oleoresin flow was measured with standard techniques (Lorio and others 1990). Samples of oleoresin of *Pinus laricio* were obtained by tapping the bark of individual trees approximately 1.30m above ground level on the west side (Saracino and others 2002). Oleoresin flowing from the wound was directed into a collection vial. Oleoresin samples were collected after 24 h. Oleoresin volume were measured and weighed.

Statistical analysis

Analyses of variances (Anova) were used when the conditions of application were satisfied (variance homogeneity and normality of stand). Otherwise, non-parametric tests of median comparisons were made (Kruskal-Wallis). To know the correlation between two variables, the test of Pearson was used. The "Statgraphics for Windows" software of Statistical Graphics Corporation ® was used for these various tests.

Results

Temperature and heat flux measurements

The experiments conducted, across a 0.5 kg/m² bed of pine needles, under *Pinus laricio* showed variations of temperature and heat flux (*fig. 2*) during fire spread. Subsurface temperature (1 and 2 cm deep) increases above ambient values were 47 and 11°C, respectively. Strongest variations were observed at 5 cm above the ground surface. At this location temperature and heat flux peaks were 1050 °C and 47 kW/m², respectively. The total heat flux was significant during a greater delay (about 200 s) than temperature. Indeed, the flame residence time measured at the trunk base was 90 s. At 100 cm above the ground surface, highest temperature measured during fire spread was 210°C. Temperature measured at 200 and 300 cm above the ground did not showed significant variations. The average temperatures during flame residence time exhibit values superior to 100°C for measurement heights in the range of 0 – 40 cm. Furthermore, temperature-time curves and visual observations confirmed that the average flame height was about 25 cm. The average rate of fire spread and Byram intensity were 0.3 cm/s and 35 kW/m, respectively.
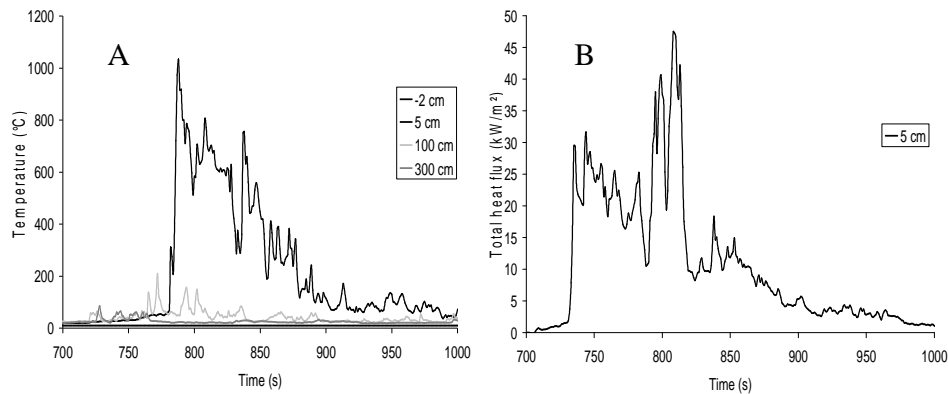
Figure 2 —Temperature-time curves recorded above and under the ground surface (A) and flux-time curves recorded at 5 cm (B) during fire spread.
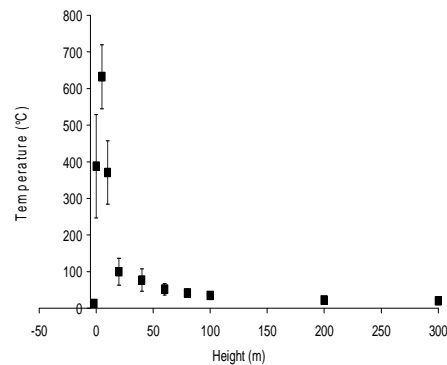

Figure 3 —Average temperature versus height recorded during 3 experiments (℃± confidence interval) at 2 cm in the soil and at 0, 5, 10, 20, 40, 60, 80, 100, 200 and 300 cm above ground.

Pigments

Before the thermal pruning, stations are similar for pigment contents (chlorophyll a, b, and carotenoids; fig. 4). Indeed, at 2 m and 6 m height, contents in chlorophyll a, b and carotenoids are not significantly different between both stations (Anova, $p>0.05$). There is not difference between both heights (Anova, $p>0.05$).

1 month after the thermal pruning, at 6 m, chlorophyll contents increase at unburned station (Anova, $p<0.05$), while contents decrease at burned station (Anova, $p<0.05$, *fig. 4*) from April to May. No difference was found at 2 m (Anova, $p>0.05$). The weakest contents were observed at 6 m height at burned station. Carotenoid contents decrease at both heights at burned stations (Anova, $p<0.05$, *fig. 4*).

After 3 months, chlorophyll *a*, *b* and carotenoids contents are in the same order at both stations and heights (Kruskal-Wallis, $p>0.05$; *fig. 4*). Pigment contents increase at both stations and heights from May to July.
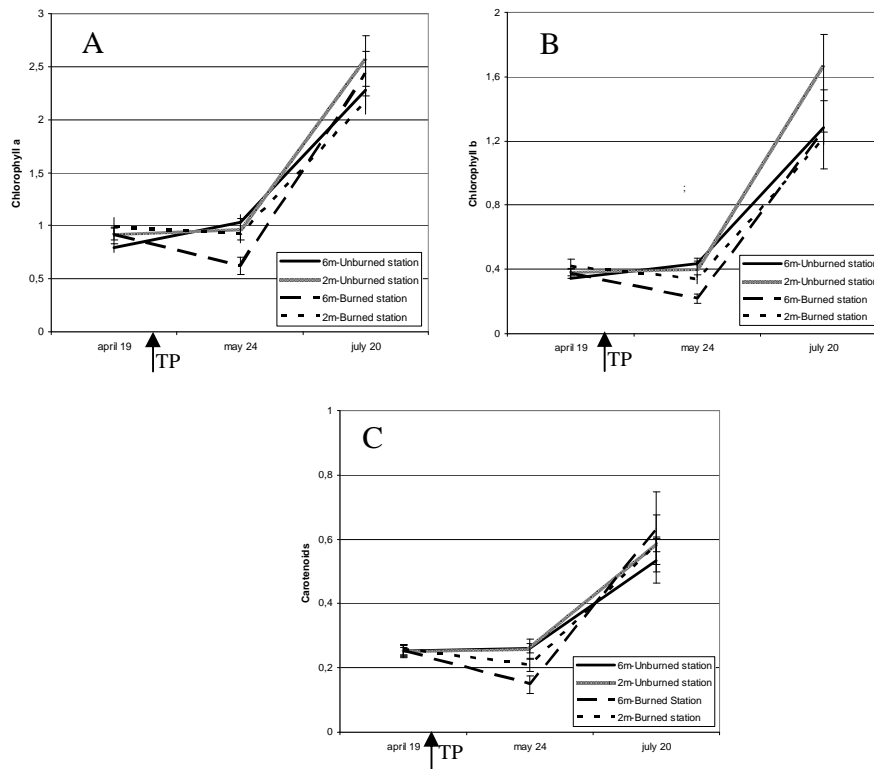
Figure 4—Chlorophyll *a*(A), *b*(B), carotenoids (C) (mg/g dry weight ± confidence interval) in needles of *Pinus laricio* before and after (1 and 3 months) a thermal pruning (TP) at burned and unburned stations, at 6 m and 2 m height.

Carbon isotope

Before the thermal pruning, stations are similar for $\delta^{13}C$ contents as a function of height (*fig. 5*; Anova, p>0.05) and treatment (Anova, p>0.05).

1 month after the thermal pruning, contents are not significantly different between both stations and heights (*fig. 5;* Anova, p>0.05). Strongest contents were observed at burned station at 6 m height. From April to May, there is no difference between stations.

After 3 months, δ13C contents are not significantly different between stations and height (fig. 5; Anova, p>0.05). The strongest contents were still observed at 6 m height at burned station. From May to July, δ13C contents does not differ significantly between stations (Anova, p>0.05).

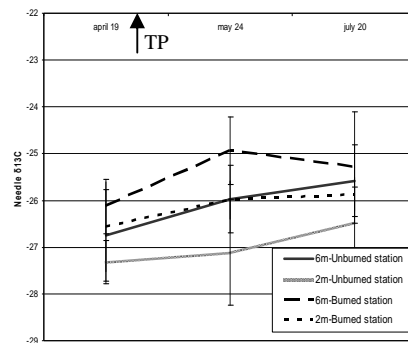**Figure 5**—Needle δ^{13}C contents (‰± confidence interval) in needles of *Pinus laricio*
before and after (1 and 3 months) a thermal pruning (TP) at station burned and
unburned station, at 6 m and 2 m height.

Total Phenolic compounds

Before the thermal pruning, total phenolic compounds contents are more
important at unburned station than at station which will burn (*fig. 6*; Anova, p>0.05).

1 month after the thermal pruning, at 2 m height, phenolic compounds contents
increase from April to May at burned station (Anova, p<0.05; *fig. 6*). There is no
difference at unburned station (Anova, p>0.05).

After 3 months, phenolic compounds contents are more important at burned
station at 2 m height. (Anova, p<0.05; *fig. 6*). Contents increase from May to April at
burned station (Anova, p<0.05). At 2 m height, at unburned station, contents increase
(Anova, p<0.05), while at 6 m height, there is no significant difference from May to
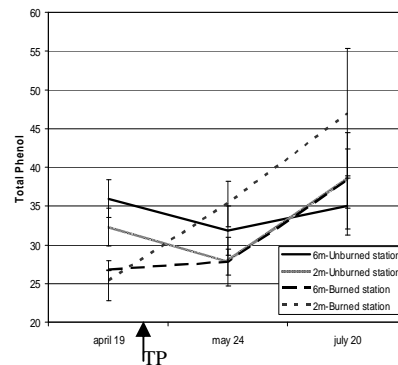July (Anova, p>0.05).

Figure 6—Total phenolic compounds contents (mg of gallic acid/g dry weight ±
confidence interval) in needles of *Pinus laricio* before and after (1 and 3 months) a
thermal pruning (TP) at station burned and unburned station, at 6 m and 2 m height.

Oleoresin exudation flow

Oleoresin flow and weight increased in trees submitted to the thermal pruning
relative to unburned ones (Anova, p > 0.05; *table 1*). There is about 2.5 times more
resin produced at burned station than at unburned one.

Table 1— *Oleoresin resin flow (mL ± confidence interval) and weight (g ± confidence
interval) in Pinus laricio. Different letters indicate significant differences between
treatments (Anova, p<0.05).*

	Volume (mL)	Weight (g)
Burned station	3.07 ± 0.40 (a)	1.59 ± 0.34 (a)
Unburned station	1.27 ± 0.31 (b)	0.64 ± 0.11(b)

Discussion and Conclusion

In order to characterise the burning and to compare burning intensity during our
thermal pruning to data of the literature (Feeney and others 1998 who indicated only
maximal values in their works), we also extracted maximal values from our
experiment. Nevertheless, it must be underlined that the average data over flame
residence time are more representative than maximum values (which may occur
during only 1 or 2 seconds) to characterize a burning and should be preferentially
used in the future. During a prescribed burning on *Pinus ponderosa* Dougl. Ex Laws.,
flame length averaged about 15 cm, surface soil temperature peaked in excess of
204°C, while subsurface temperature (2-10 cm) exceeded 65°C (Feeney and others
1998). In the present work, the average flame length and the surface and subsurface
temperature increases were 25 cm, 758 °C and 11°C, respectively. Our thermal
pruning, in particular, flame length and surface temperature, was more important than
this prescribed burning.

On the whole period of study, chlorophyll *a* and *b* in *Pinus laricio* are in the
same order as those found in others species such *Pinus pinaster* (Alonso and others
2002). Pigments contents are about twice more high in July (growth period) than in
April and May. In unburned station, our results show that pigment contents are not
significantly different between the heights. Feeney and others (1998) suggests that as
a function of height, these differences could be highlighted with leaf area-based net
photosynthetic rate, which is correlated to water potential. Water potential decrease
as a function of tree height due to gravity, and a greater hydraulic resistance to water
flux in the xylem with increasing transport distance (Ryan and Yoder 1997 in Feeney
and others 1998). These authors worked on pine stands aged from 115 to 423 years
with 72 cm dbh in average, while our study was realised on young pines with less
important height (13 cm dbh). The fact that there is no difference as a function of
height in pigment contents (which are correlated with photosynthesis ; Rama and
others 1997 in Alonso and others 2002), could be due to the weaker height of our
trees.

1 month after thermal pruning, chlorophyll *a* and *b* contents decrease at 6 m
height and for carotenoids at both heights. A photosynthetic decline was also
observed 1 month after a controlled heating applied on 100% of the base trunk
circumference on *Pinus halepensis* Mill (Ducrey and others 1996). This decrease
could be explained by the action of phenolic compounds (Rama and others 1997 in
Alonso and others 2002) which increase at the same date. Alonso and others (2002)
show an increase in chlorophyll contents 2 months after fire treatment to restore the
photosynthetic activity that was diminished by crown scorching. We could suppose
that there is an increase 2 months after, like Alonso and others (2002), to restore the
photosynthetic activity that was diminished by thermal pruning 1 month after. In our

study, this effect disappeared 3 months after fire treatment, like Alonso and others (2002), while this decrease continue for Ducrey and others (1996) for trees which die some months after. No variations were found on chlorophyll *a/b* ratio (data not show) 3 months after the burning like Alonso and others (2002). However, this physiological parameter increases 8 months after fire-induced on trunk and crown (Alonso and others 2002).

In Q*uercus ilex* L. resprouts 1 year after fire, Fleck and others (1996) show higher net photosynthetic activity which is highly correlated with foliar nitrogen content. Several studies showed that the concentration of mineral N (NH_4^+ and NO_3^-) increases in the soil surface immediately after burning (Covington and Sackett 1986; Monleon and others 1997). This is due to the increase (i) in ashes in soil surface, which are a major source of soil mineral N and (ii) of microbial mineralization (Covington and Sackett 1986).This nutrient increase was correlated with morphological parameters increase (e.g. needle length, needle number) the year following prescribed burning on *Pinus laricio* (Cannac and others 2006). 3 months after thermal pruning, no difference was found on morphological parameters in *Pinus laricio* (data not show). As in pine stand, net nitrogen mineralization and nitrification increase for a period of months or years after prescribed burning (Covington and Sackett 1986; Covington and Sackett 1992). We can suggest that soil nitrogen is not yet mineralized and nitrified, and that we may observe, in some months, an increase in pine growth when N will be mineralized and nitrified (pigment contents, net photosynthesis and morphological parameters increase).

C3 plant isotopic composition may vary between -22 and -32 ‰ (Troughton 1979), like our study. Leaf $\delta^{13}C$ can vary due to factors that change leaf internal CO_2 concentration, such as a change in the balance between uptake via photosynthesis and supply via stomatal conductance (Pate 2001 in Zausen and others 2005). The plant tissue $\delta^{13}C$ is more and more used in ecological research as an indicator of water use efficiency (Damesin and others 1997). In our study, leaf $\delta^{13}C$ does not vary between treatments and height. Zausen and others 2005 show less $\delta^{13}C$ content in thinned and burned station than in unburned station. Lower $\delta^{13}C$ was correlated with the increase in water availability in trees (Bush and Smith 1993; Fleck and others 1996), resulting from decreased tree competition in thinned stands (Zausen and others 2005). Thermal pruning does not seem to provoke any increase, as only the under ground vegetation was burned. Lower $\delta^{13}C$ was also coherent with the higher leaf nitrogen content (Bush and Smith 1993; Fleck and others 1996). As pigment contents, $\delta^{13}C$ depends on leaf nitrogen content. 3 months after thermal pruning, there is no difference in $\delta^{13}C$, but variations could be observed in some months.

Secondary metabolisms could be activated to produce defence compounds, such as phenolics and resin, to play a defensive role against insects (Santoro and others 2001; Lombardero and others 2006) or following a prescribed burning (Alonso and others 2002). We can observe an increase in secondary metabolism: total phenolic compounds and oleoresin flow, respectively 1 month and 2 months after thermal pruning.

Total phenolic compounds contents are in the same order of magnitude as those found with the data available in the literature (Alonso and others 2002, Pasqualini and others 2003). We can observe an increase only at 2 m height. Phenolic compounds contents were correlated (r = 0.97) with temperature during the thermal pruning as a function of height. Fire impact on trunk and crown was less important at 6 m than at 2 m, where an increase in phenolic compounds was observed.

The carbon/nutrient balance hypothesis argues that under conditions of nutrient limitation, plants accumulate large carbohydrates reserves (Bryant and others 1983). These stores may then be used in the production of carbon-based allelochemicals (Bryant and others 1983). Variation in phenolic concentrations has been documented in plants as a by-product of the internal resource balances (Bryant and others 1983) and in response to nutrient stress (Bryant and others 1983). So, when nitrogen is present in correct quantities, carbon is allocated for the growth and not for secondary metabolite production (Coley and others 1985). As we observed an increase in total phenolic compounds contents, the increase in nutrient contents in soil after prescribed burning (Monleon and others 1997) would not allow explaining directly the increase in total phenolic compounds contents. Another explanation for the phenolic compound variations could be due to phenylalanine ammonia-lyase (PAL) activity. PAL is considered to be the principal enzyme of the phenylpropanoid patway, catalysing the transformation of phenylalanine to trans-cinnamic acid, which is the prime intermediary in the biosynthesis of phenolic compounds (Rösler and others 1997). PAL is a key enzyme in the synthetic pathway of phenolic compounds and increases under the influence of temperature (Rivero and others 2001). Rivero and others (2001) show a correlation between PAL and phenolic compounds increase in tomato (*Lycopersicon esculentum*) following a thermal stress. So, the increase in phenolic compounds could be correlated with PAL increase when *Pinus laricio* undergoes a thermal stress, such as a thermal pruning.

Alonso and others (2002) found when damage exceeds a determined level, the tree does not react to activate the production of defence compounds and their levels diminish. Total phenolic compounds increase in our study; thermal pruning can be considered as having less impact than a fire induced on crown and trunk.

Oleoresin, a mixture of monoterpene olefins and diterpene resin acids, is the primary defence of pines against insect pests (Phillips and Croteau 1999 in Santoro and others 2001). Zausen and others 2005 show a higher resin production on sites that underwent a thinning and a burning, compared to sites with only a thinning. Some authors (Santoro and others 2001; Lombardero and others 2006) show an increase in resin production after an experimental fire. In pine species, trees respond to light burning with increased resin flow (Feeney and others 1998) or with more severe burns, with reduced resin flow or bark terpene contents (Jakubas and others 1994). A simple mechanism invokes increased biosynthesis of oleoresin in the existing epithelial cells that line vertical resin ducts, followed by secretion into the vertical resin ducts where resin could be conducted via lateral ducts to new points of wounding (Lorio 1993). Thermal pruning can provoke trunk wounding, which provoke oleoresin flow increase. Burning can stimulate resin production because of heat damage to cambium, phloem, or xylem tissues (Feeney and others 1998). Oleoresin flow increases following a fire may be an evolved response of pine to the increased probability of bark beetle attack (Feeney and others 1998).

This preliminary analyse allows us to verify that 1 month after a thermal pruning, the tree favours the synthesis of defence molecules (phenolic compounds) rather than its growth (pigments contents). Pigments and phenolic compounds could be used as bioindicators for the short-term response of *Pinus laricio* needles to thermal pruning. After 3 months, usual values were observed at both heights. Carbone balance seemed to be restored 3 months after. A long term study will allow us to better understand the chronology of the strategies of carbone allocation in *Pinus laricio* after a thermal stress.

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