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Variability of specific needle area in *Pinus pinea* L. with environment resources availability: light, water and nutrients

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Abstract. Specific Leaf Area (Specific Needle Area, SNA in "needle shape" leaves) is a measure of leaf thickness. It is used for scaling physiological processes measured at the leaf and shoot scale to the whole-tree foliage, being closely related with species strategy to acquire and use resources. The main objective of this study was to gather a database of SNA values for stone pine collected in different time periods for Portugal and Spain and analyse the relationships between SNA and environmental conditions during needles growth. We analysed the variability of SNA within the tree and between trees from different ecological regions and studied the effect of: (i) hydrological years, (ii) light growing conditions within the crown and (iii) the combined effects of water and nutrients. Mean SNA values under natural conditions, taking all sites together, was 32.9 ± 1.2 cm^2/g. Higher SNA values were found in needles developed during dry years, in needles growing under low light conditions and under higher nutrients and water availability. We conclude that water availability during needle development is an important driver of SNA variability within and between trees. In order to establish accurate leaf area to dry mass ratios for stone pine and to minimize the errors of scalling-up processes from the leaf to the stand level, more studies are required for other regions and environment conditions.

Keywords. Stone pine – Leaf area index – Needle age – Irradiance – Fertilization – Irrigation – SNA.

I – Introduction

Plant leaf area and leaf spatial distribution are important morphological traits involved in CO_2 assimilation, transpiration, light interception and consequently in plant productivity. The diversity of leaf structure and morphology, either within or between species, has been shown to be a good indicator of species adaptation to the environmental conditions and to establishment success (Kellomaki and Oker-Blom, 1981; Xiao and Ceulemans, 2004).

The Specific Needle Area (SNA) is the ratio of fresh needle surface area to unit dry foliage mass and it is a measure of leaf thickness. The variation in leaf thickness is largely due to the formation and organization of palisade parenchyma cells, where the photosynthetic structures responsible for light absorption are (Lambers *et al*., 1998). Among other morphological and biochemical characteristics, SNA is correlated with leaf photosynthetic capacity and foliar nutrition (Bond-Lamberty *et al*., 2002). Therefore, the knowledge of SNA is important in productivity studies.

The specific leaf (or needle) area is frequently used for scaling physiological processes measured at the leaf and shoot scale to the whole-tree level. It is also an important variable in process-based ecosystem models to estimate leaf area index and the partitioning of carbohydrates produced by leaves to the other plant components.

The SNA is a species-specific ratio. According with our literature review on pines, the values range from 27 and 91 cm^2/g. No studies were found for stone pine. The primary sources of variation in needle morphology are related with the seasonal phenology and the environmental conditions during needles formation, especially light intensity and the degree of shading reaching the crowns (Maseyk *et al*., 2008). However, SNA within a stand is influenced by a number of other environmental factors including water and nutrients availability (Eimil-Fraga *et al*., 2015), stand age (Weiskittel *et al*., 2008) and competition (Shi *et al*., 2013). It is important to understand the sources of SNA variability in the tree, within and between stands under contrasting ecological gradients in order to provide accurate leaf area to dry mass ratios estimates that can be used in a broad scale (Breda, 2003). Taking into account the Mediterranean origin of the species, we hypothesise that water availability may be one of the driving factors of SNA variability in stone pine.

The aim of the present study was to establish some of the sources of variation in stone pine (*Pinus pinea* L.) specific needle area between stone pine trees from contrasting ecological regions. The specific objectives were to study the effects of hydrological years, light conditions in the crown and nutrient and water availability on needle growth and morphology. Ultimately we intend to understand if leaf-related attributes can be used as complementary indicators of productive stone pine trees and sites.

II – Materials and methods

1. Experimental sites

The data used in this study gathers SNA values in stone pine needles sampled in different years and sites located in Portugal (PT) and Spain (SP) representative of the natural distribution of stone pine in Iberian Peninsula (Table 1). The samples were taken in young pure *Pinus pinea* stands trees with less than 20 years old with no intraspecific competition.

For inter-site comparisons we used needles from trees growing under natural conditions. The combined effects of nutrients and water on needles SNA was evaluated by sampling the needles in site PT1, which correspond to a fertirrigation trial installed in Portugal in 2014. In this site the amount of nutrients and water, provided between June and October through a drip irrigation system, varied according with the amount and timing of site precipitation each year, in order to compensate site evapotranspiration and provide tree hydric comfort. Two levels of fertirrigation were considered, with the 2nd level using the double of water and nutrients applied to the 1st level.

Current year meteorological data was obtained from local meteorological stations and for long term climatological series we used data from national stations nearby. For consistency, we will use the specific needle area (SNA) terminology, as this species has "needle" shape leaves.

Table 1. Site and tree/stand characteristics: Annual precipitation in mm (PP_{annual}), average site temperature in ºC (T_{annual}), average tree height in m (h), number of trees per ha in the stands where the sampling occurred (N), number of trees sampled in each site (n). PT1 is the fertilization and irrigation trial (FR trial) with 3 trees sampled per treatment (Control, 1st level, 2nd level)

Site	Lat/Long	Years sampled	PP_{annual}	T_{annual}	Age	h	N	n
Canha PT1 (FR trial)	38º44'19"N;8º32'22"W	2014-2015	709	15.9	5	3.1	408	9
Coruche PT2	38º57'34"N;8º25'45"W	2014-2015	642	16	8	3.8	208	8
Madrid SP	40º27'32"N;3º45'14"W	2004-2005	440	14.2	14	5.0	520-1100	9

2. Sampling

Fully expanded needles were sampled from healthy trees without visible insect or pests damages and in trees representative of the population in the stand.

To study the effect of the hydrological years in needle morphology we collected needles from different age cohorts developed in wet (2005 and 2014) and dry years (2004 and 2015) from Portugal and Spain. Note that stone pine trees can have needles with more than 3 years old. In Spain (SP) the dry year of 2004 had only 40% of long term annual site precipitation. The year of 2015 was one of the driest in Portugal in the last 20 years with half the precipitation than the long term average. It was also the second hottest year since records began in 1931 with an anomaly of 1.35 ºC above the average.

To study the effect of light conditions on SNA, we collected needles from the same cohort age both in the upper third part of the canopy exposed to light and in the lower third part of the canopy in shaded conditions.

To study the effect of of artificial water and nutrients availability during growth we compared the control and treatment plots from PT1 trial by sampling needles from different age cohorts and crown positions.

A minimum of 12 needles per tree and age class/treatment were collected in the field and kept in refrigerated conditions while transported to the laboratory. The needles were scanned in a flatbed scanner and the length and projected area were calculated using the WinSeedle package software (Regent Instruments, Sainte-Foy, Quebec, Canada). Samples were oven-dried at 60ºC during 2 days. SNA was calculated as the ratio between fresh needles projected area (cm^2) and the dry weight (g).

3. Data analysis

Due to the unbalanced design, the database compiled was analysed in an exploratory way. The least squares regression approach was used to describe the statistical relationship between two variables and the Tukey test was used to compare statistical differences between the means. Statistical analyses were carried out using SigmaPlot (SigmaPlot for windows V 13, Dundas Software, Germany). All relationships were considered significant at $p < 0.05$.

III – Results and discussion

Average values of SNA were 31.1 ± 1.2 cm^2/g in PT1, 31.6 ± 0.7 cm^2/g in PT2 and 36.1 ± 1.7 cm^2/g in ES (Fig.1) and are within the range reported by other studies on Pines: 27.3 cm^2/g in *Pinus ponderosa* stands in Oregon, USA (Weiskittel *et al*., 2008), 29-35 cm^2/g in *Pinus pinaster* in Galicia, Spain (Eimil-Fraga *et al*., 2015), 44 cm^2/g in *Pinus sylvestris* in Antwerpen, Belgium (Xiao *et al*., 2006), 30-46 cm^2/g in *Pinus contorta* in British Columbia, Canada (Goudie *et al*., 2016) and 91 cm^2/g in a mature stands of *Pinus pinaster* in France (Porté, 1999). The apparent low variability in SNA of *Pinus pinea* from Portugal and Spain, together with the deviation of SNA values by comparison with other pines, suggests it is a species-specific variable. Especial attention should be taken in modelling leaf-related variables and upscaling exercises based on data from other pines.

The anatomy of growing needles adapts according to environmental stimuli (Poorter *et al*., 2009). We observed that during dry years, the needles were, in general, shorter and lighter, consistent with what was observed in other studies (Cinnirella *et al*., 2002; Grill *et al*., 2004). We observed a 83% reduction in both needles area and dry weights in the Spanish site (SP) during the dry year of 2005 (Fig 1, a). A similar, but much less pronounced, reduction in needles areas and mass was observed in the Portuguese sites in the dry year of 2014. The SNA in all sites increased in dry years because needles mass decreased more than area. The reasons behind this result may be related with the lower structural carbohydrates investment, lignin and other non-structural compounds in needles growing during drought, possibly translocated to other plant parts, including productive needles from other age cohorts. Droughts can reduce substantially total tree needle photosynthetic capacity and apparently this effect is more pronounced in more arid sites. More data are required to quantify the extent of droughts impacts on canopy needle area. Particularly the consequences of this reduction in flowering and cone growth and mortality should be addressed in future studies.

Figure 1b shows the results on average SNA values in needles sampleed in different canopy light conditions. We observed a consistently high SNA in needles sampled under low light conditions, that is in the crown base, similar with other studies in Pines (Goudie *et al*., 2016; Weiskittel *et al*., 2008; Xiao *et al*., 2006). Needles area were relatively stable but a 32% decrease on average was observed in low light canopy needles as compared with the top. This is probably the result of a differential carbon investment in the higher canopy with more available light and opportunities to maximize photosynthesis (Correia, 2015).

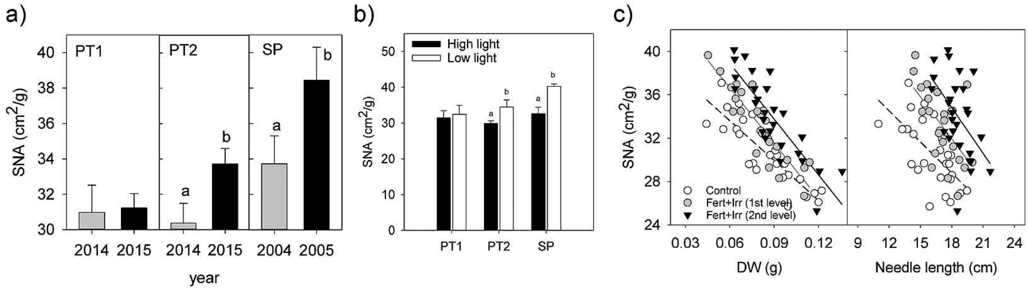

Fig. 1. (a) Average SNA in normal (grey bars) and dry hydrological years (black bars); (b) Average SNA (cm^2/g) for needles growing in low light (white bars) and high light conditions (black bars) in the crown; (c) coorelation between SNA (cm^2/g) and needle dry weight (DW, g) (left) and needle length (cm) (right) in the water and fertilization trial PT1. Different letters represent significant differences between years within the site.

We used data from PT1 trial with 2 levels of water and fertilization to study the impact of artificial water and nutrients availability in needle morphology (Fig. 1, c). We observed a significant and positive effect of both fertilization and irrigation in needle dry-weight and length in line with other studies (Cinnirella *et al*., 2002; Grill *et al*., 2004). SNA was higher with increasing levels of water and nutrients from 31.1 ± 0.8 in the Control, 33.2 ± 0.7 in the Fert+Irr (1^{st} level) and 34.5 ± 0,8 cm^2/g, in the Fert+Irr (2^{st} level) plots. This result was mostly driven by needle length and area rather than by needle dry weight. A potential increase in the total tree photosynthetic capacity is expected but also in canopy water loss due to the increase in transpiring surface area. Future studies should address how nutrients and water availability may interact with stone pine water, light and nutrients use efficiency and how it may impact cone production.

IV – Conclusions

Increasing cone production in stone pine stands can only be possible when investigation reaches an understanding on how environmental factors impact tree physiological processes and functioning and how it interacts with the reproductive cycle of the species. Morphological attributes, namely leaf-related variables, can provide important clues regarding stands nutrient and water status which can potentiate photosynthetic capacity and overall tree adaptation and growth. Whether if leaf-related attributes can be used as complementary indicators of productive stone pine trees and sites remains a question. This study provides the first results of SNA variability trends with environment conditions in a small set of locations. More studies are needed to refine the SNA estimates in the Mediterranean distribution area of *Pinus pinea* and under different conditions and treatments.

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