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Mediterranean pine nuts from forests and plantations

Edited by:
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Foreword

Mediterranean pine nuts kernels, the seeds of *Pinus pinea*, are among the world's most expensive nuts, with a value chain of several hundred million euros annually. Cones are still mainly wild collected from 0.7 million hectares stone pine forests in the Mediterranean area. In the last twenty years, its cultivation as nut crop has been increasing, approximating 0.3 million hectares of new plantations in its home range, and incipiently in New Zealand, Australia and Chile. Domestication is advancing and first registered clones with outstanding cone production have been released for graft scions.

In this panorama, after its first edition held in 2011 in Valladolid, Spain, the 2nd International Meeting on Mediterranean Stone Pine for Agroforestry - AgroPine2016 took place on 18th-20th May 2016 in Oeiras, Portugal. The meeting brought together more than 80 experts, researchers, public and private forest managers and land owners, as well as pine cone processing enterprises from Portugal, Spain, Tunisia and Turkey, with some participants from France, Italy, Lebanon, and Australia. The conference was organised by the Portuguese hosts INIAV (Instituto Nacional de Investigação Agrária e Veterinária), UNAC (União da Floresta Mediterrânica) and ISA/CEF (Instituto Superior de Agronomia / Centro de Estudos Florestais, U. Lisbon), by IAMZ-CIHEAM (Mediterranean Agronomic Institute of Zaragoza / International Centre for Advanced Mediterranean Agronomic Studies) and INIA (National Institute for Agricultural and Food Research and Technology) from Spain, by the FAO-CIHEAM Network on Nuts, the IUFRO (International Union of Forest Research Organizations, RG1.08.00 on Silviculture for production of edible fruits), and FAO Silva Mediterranea[1].

The five topics discussed during the meeting were: Management for cone production in forests and agroforestry; Growth and yield; Genetic improvement; Biotic risks and their impact on stone pine products; and Pine nut value chain. The complete AgroPine2016 Meeting Book of Abstracts is available on the event website http://agropine2016.iniav.pt/; the present issue of *Options Méditerranéennes Series A* comprehends the proceedings of the meeting.

One important conclusion from the **AgroPine** 2016 meeting regarding **Management for cone production in forests and agroforestry** was the recognition that rational reference values are required for fertilization application in stone pine stands, targeting higher **cone harvestings** at younger ages and **lower inter-annual variability** (masting).

Regarding **Growth and yield**, advances in comprehension and quantification of biological processes involved in growth and cone yield were presented, marking as key factors mainly regional differences, as well as constraining rainfall deficits at characteristic moments for cone induction and ripening. Future predictive empirical or process based models **need to respond to the main questions, demands and concerns** of stakeholders and end-users. For a comprehensive understanding of tree **responses to biotic and abiotic stresses,** as well as of the **ecological drivers** for individual and regional cone production and masting, the maintenance of existing permanent plot networks that **monitor cone yield and quality** are essential.

In relation to **Genetic improvement of stone pine**, genetic gains achievable by selection have been estimated in 20 to 40% for cone yield by selection of the 10% most productive clones. Catalogued basic materials for producing certified forest reproductive materials (seeds, grafting scions, plants) have been already registered in Portugal and Spain, allowing for establishing productive plantations.

Considering **Biotic risks and their impact on stone pine products**, there are now strong experimental evidences from different countries that the Western Conifer Seed Bug, ***Leptoglossus occidentalis*** **can considerably reduce the cone yield and quality** in stone pine. The exotic insect has been expanding its area since introduction in 1999 to most European countries, as well as to Tunisia and Western Asia. The finding of *L. occidentalis* as main causal agent of the ***Dry Cone Syndrome***, generalised in the last years in the main stone pine growing areas, force the sector to face the challenge of **developing an integrated pest management system**. Along with the seed bug, endemic cone-feeding insects like *Dioryctria mendacella* and *Pissodes validirostris* can also cause significant damages to pine nut production. A **new pheromone** will be available to monitor and control *D. mendacella*, but no similar options are yet available for *L. occidentalis* or *P. validirostris*.

In relation to **Pine nut industry and markets**, Portugal has instituted a **regulatory system for stone pine supply chain,** similar to already existing regional traceability systems in several Spanish Autonomous Regions. One important outcome of these regulations is the **knowledge and feasible data** regarding regional annual cone production, agents/operators and circuits within the supply chain, in fulfilment of **mandatory European Food Safety Regulation (EC) 178/2002**. Currently, theft, lack of quality standards regarding adequate harvest seasons cone storing and processing, incorrect commercial labelling of different Asiatic pine nut species, and cone price speculation are considered important constrains to the competitiveness of Mediterranean pine nuts in global markets. Due diligence and transparency in the cone and pine nut traceability from harvesting until final destination do not only enforce legality and hinder theft and black market, but will allow for building consumers' awareness of disparate pine nut botanic species, origins and quality grades.

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[1] Links:

Agropine 2011: International Meeting on Mediterranean Stone Pine for Agroforestry (Valladolid, Spain, 17-19 November 2011) http://networks.iamz.ciheam.org/agropine2011/

INIA: http://www.iniav.pt/

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FAO CIHEAM Network on Nuts: http://networks.iamz.ciheam.org/nuts/

IUFRO http://www.iufro.org/science/divisions/division-1/10000/10800/

FAO Silva Mediterranea (RG1.08.00): http://www.fao.org/forestry/silvamed/en/

Session 1

Management

Spatial and temporal changes of stone pine forests in Turkey: A case study in Ayvalik forest planning unit

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Abstract. Timber and pine nuts are considered main products from the stone pine (*Pinus pinea*) forests in Turkey. Stone pine woodlands are approximately 89,000 ha and pine cone and nut production is about 3,500 tons/year and 280 tons/year, respectively. The forests covered by stone pine also present other services and products such as hunting, carbon sequestration, resin, bark and wood. The production and marketing of pine nut in particular has grown dramatically over the last two decades. The great social and economic importance of stone pine in non-wood forest production (NWFP) distinguishes it from other pines, generating employment and supplementary incomes for forest villagers who live in and near the forests. Additionally, villagers prefer incomes from NWFP, because pine nut production is more profitable than timber production. Understanding the landscape dynamics as a historical legacy of disturbances is necessary for sustainable management of NWFP to help to better design the future forest management practices and policies. In this study, spatial and temporal changes in forest cover types in Ayvalık Forest Planning Unit, were analyzed with GIS and Patch Analyst. The analysis was conducted with forest cover type maps from 1989 and 2002. Results indicate that there are marked changes in the temporal and spatial dynamics of land use and forest cover in the study area. Between 1989 and 2002, we observed an increase of 25% in total forested areas with an increase of 43% in stone pine areas. On the other hand, the analysis of canopy cover changes and development stages over 13 years find out an increase of total forest area and stone pine area with medium canopy cover and with trees in "a-regenerated" stage of development.

Keywords. Stone pine (*Pinus pinea*) – NWFP – GIS – Forest dynamics – Land use.

I – Introduction

Pinus pinea is one of the most important non-wood forest products (NWFPs) in the Mediterranean area due to the economic importance of tree seeds and cones. While the cones have been used as wood based panel (Ayrilmiş *et al*., 2009), the nuts are highly valued for food industry (Mutke *et al*., 2012).Other ecosystem services such as soil protection, recreational use and carbon sequestration make stone pine one of the most valued Mediterranean pine species.

The cones and nuts provided by stone pine forests, generates employment and supplementary incomes for forest villagers living in and near the forests. The incomes from NWFP, namely pine nuts, are more profitable than timber production and therefore preferred for villagers. Turkey is one of the largest producers of pine nut in the world and the marketing of pine nut from stone pine in Turkey has grown dramatically over the last two decades. Total annual production of pine nut kernel from Stone pine in Turkey increased from about 166 ton to 1,000 tons and estimated pine nut income changed from 2 million dollars to 38 million dollars based on 2000-2015 exportation statistics (TUIK, 2015). In order to maintain competitiveness and guarantee sustainable incomes from stone pine forests, a diversification of products such as timber and nuts may be required in future management plannings for avoiding excessive cone production.

Understanding historical forest dynamics in terms of spatial and temporal scales helps to better design the future forest management practices and policies. Geographic Information Systems (GIS) are common and effective tool to monitor changes in forest area and other variables in a regional scale. Many studies use remote sensing and geographic information systems to monitor forest development stages, cover types and canopy cover as a surrogate of stand quality for the Turkish forests over time (Mumcu *et al*., 2008; Kadıoĝulları, 2005) but none specifically addresses the spatial and temporal changes of NWFP.

The objective of this study was to analyze the spatial and temporal changes of landscape structure in Ayvalık Forest Planning Unit by using stand cover type maps from forest management plans between 1989 and 2002. Temporal changes were examined in terms of land use, developing stage and canopy cover with a detailed analysis on stone pine areas. In this way, susceptibility of forest to further abrupt changes in the future would be examined, as well as sustainability of forest resources and resilience of the ecosystem mainly for biodiversity for stone pine or all forest areas.

II – Materials and methods

The study was conducted in Ayvalık forest planning unit in western part of Turkey, province of Balıkesir, covering about 60,883 ha of which 34% is forested areas. The study area is covered by even aged pure stands of stone pine, black pine and red pine (*Pinus pinea, Pinus nigra* and *Pinus brutia*, respectively). The elevation ranges from 0 m to 1200 m above sea level. Mean annual temperature of study area is 17 °C and mean annual precipitation is 643 mm, based on the average 1975-2005 (DMI, 2008). To analyze the spatial and temporal structure of stone pine forest areas, stand type maps obtained by interpretation of aerial photographs for forest management plans from 1989 and 2002 (OGM, 1989; 2002) were digitized and a spatial databases were built with Arc/Info GIS. Changes of forest structure such as forest species, stand development, canopy cover as well as changes in land use were analyzed with some functions of Arc/GIS such as Query and Analyzing. The spatial configuration, that is the physical distribution in space and spatial character of elements in forest, was evaluated with Patch Analyst. The Patch Analyst tool of Arc/GIS was used to analyse land use changes between 1989 and 2002. In this analysis the "*patch*" is the basic "element" or "unit" of the landscape and "*number of patches (NP)*" is the amount of elements in each classes. It is an important indicator for forest fragmentation, and the increasing in NP indicates increased fragmentation with more patches of smaller size. "*Class area (CA)*" is the sum of areas of all patches belonging to a given class. *"Mean patch size (MPS)"* is the average patch size of all classes. *"Edge Density (ED)"* is the ratio of perimeter to area ratio for each patch and the unit is metres/hectare therefore it is useful for comparing landscapes of varying size. "*Area Weighted Shape Index (AWMSI)*" is the average perimeter-to-area ratio for a class, weighted by the size of its patches. Patchiness in forested area has special importance because it serves as an important indicator of natural habitat fragmentation (Kammerbauer and Ardon, 1999). Hence these parameters are regarded as an important indicator for habitat fragmentation due to their effect on species. The description of used classes in land use, canopy cower and development stages types are explained in Table 1 and Table 2.

Table 1. Land use/land cover classes descriptions

Land Use/Land Cover Classes	Description
Non forest	Agriculture, settlement, dune and moss areas
Private áreas	Non state forest areas
Degraded stone pine	Sparsely distributed stone pine forest 0-10% cover
Open áreas	Treeless and open areas
Degraded forest	Sparsely distributed forest 0-10% cover
Stone pine	Pure *Pinus pinea* stands
Black pine	Pure *Pinus nigra* stands
Red pine	Pure *Pinus brutia* stands

Table 2. Classification of canopy cover and development stage types

Canopy cover type	Criteria (% cover)	Development stage	Criteria (average dbh)
0 regenerated	no crown cover yet	a (regenerated)	< 8 cm
1 (low coverage)	11%-40%	b (young)	8-19.9 cm
2 (medium coverage)	41%-70%	c (mature)	20-35.9 cm
3 (full coverage)	>71%	d (overmature)	> 36 cm
Degraded forest	0-10%		

III – Results and discussion

1. Temporal change in land use/land cover classes

The change of land use/land cover classes in Ayvalık planning unit between 1989 and 2002 were mapped using forest cover type maps. The results showed that while open areas decreased about 2,845 ha, stone pine, black pine, red pine, Degraded and Non-forest areas increased about 670, 58, 581, 1276 and 366 ha, respectively between 1989-2002 years (Table 3). One reason for such increase relates with afforestation activities. There was also a change of species composition from red pine to stone pine: according to the transition table, about 16% of red pine (532 ha), 2.5% of forest open areas (247 ha), 5.2% of degraded areas (274 ha) and 0.7% of non forest areas (292 ha) changed to stone pine or degraded stone pine areas (Table 4).

Table 3. Changes of land use/ land cover classes in Ayvalık (based on 1989-2002 forest type cover maps)

Land Use/Land Cover Classes	1989		2002		Difference (+ -)	
	Area (ha)	%	Area (ha)	(%)	Area (ha)	(%)
Stone pine	1,463	2.4	2,133	3.5	670	1.1
Black pine	390	0.6	448	0.7	58	0.1
Red pine	3,228	5.3	3,809	6.3	581	0.4
Degraded	4,885	8.1	6,161	10.1	1,276	2.1
Degreaded stone pine	255	0.4	321	0.5	-66	0.1
Open spaces	10,458	17.2	7,614	12.5	-2,845	-4.7
Private forest	161	0.3	156	0.3	-5	-0.0
Non forest	39,876	65.7	40,241	66.1	366	0.4
Total	**60,717**	**100**	**60,883**	**100**		

Table 4. Transitions between land use classes from 1989 to 2002, expressed as percentage [%] of original LU class (based on forest management plans)

1989 \ 2002	Degraded	Degraded stone pine	Stone pine	Black pine	Red pine	Private forest	Oak	Non-forest	Open spaces
Degraded	49	2	3	1	14	0	0	23	8
Degraded stone pine	0	16	48	0	1	0	0	36	0
Stone pine	10	8	53	0	10	0	0	14	5
Black pine	1	0	0	80	5	0	0	9	6
Red pine	9	0	16	0	55	0	0	15	5
Private forest	4	0	0	0	3	88	0	3	2
Non-forest	5	0	1	0	1	0	0	90	4
Open spaces	12	0	2	1	6	0	0	31	49

2. Temporal changes in canopy cover

Changes in forest structure were also analyzed in terms of canopy cover classes (Table 5). Between 1989 and 2002, there was a net decrease in regenerated areas (40 ha), stands with low coverage cover (869 ha) and open spaces (2,845 ha). However, there was a net increase in stands with medium (776 ha) and full coverage (1,441 ha), as well as in degraded forest areas (1,342 ha). The results indicate that in some stands, canopy cover changed in favor of more dense areas, in other to degradation.

For stone pine forests, the changes between 1989 and 2002 in terms of canopy cover, revealed an increase in regenerated areas, and to a medium and full canopy cover stands (Table 5), possibly explained by reforestation activities and with stand management for cone production, respectively.

Table 5. Changes in canopy cover classes, according to forest cover type maps between 1989-2002

	All forest area			Stone pine forest		
Canopy cover (Criteria, % cover)	**1989**	**2002**	**Difference (+ -)**	**1989**	**2002**	**Difference (+ -)**
	Area (ha)	**Area (ha)**	**Area (ha)**	**Area (ha)**	**Area (ha)**	**Area (ha)**
0 (Regenerated areas)	1,375	1,336	-40	635	1,023	388
1 (low coverage, 11%-40%)	2,060	1,191	-869	718	367	-351
2 (medium coverage, 41%-70%)	1,533	2,309	776	110	612	503
3 (full coverage, >71%)	113	1,554	1441	0	130	130
Degraded forest (0-10%)	5,141	6,482	1342	255	321	66
Open spaces	10,458	7,614	-2845	–	–	–
Private Forest	161	156	-5	–	–	–
Non-Forest	39,876	40,241	366	–	–	–
Total	**60,717**	**60,883**		**1,718**	**2,454**	**736**

3. Temporal changes in development stages

Stands in mature and over mature development stages correspond to 1,941 ha and 1,753 ha, respectively in 1989, the forests were mostly clumped into regenerated and mature stands in 2002 year (Table 6). The changes between 1989 and 2002 indicate an increase in regenerated, young and mature stands with 850 ha, 753 ha and 40 ha, respectively. Over-mature stands decreasing about 335 ha were in early regeneration phase concomitantly with a promotion of regenerating activities in the forest opening areas or degraded areas.

Table 6. Changes in development stages according to forest cover type maps between 1989-2002

	All forest area			Stone pine forest		
Development Stages (criteria, average dbh)	**1989**	**2002**	**Difference (+ -)**	**1989**	**2002**	**Difference (+ -)**
	Area (ha)	**Area (ha)**	**Area (ha)**	**Area (ha)**	**Area (ha)**	**Area (ha)**
a (regenerated, <)	1,375	2,225	850	635	1,266	631
b (young, 8 –)	42	794	753	0	14	14
c (mature, 20 –)	1,941	1,981	40	521	160	-361
d (overmature, >)	1,724	1,389	-335	307	694	387
Open spaces	10,458	7,614	-1,845	–	–	–
Private forest	161	156	-5	–	–	–
Degraded forest	5,140	6,482	1,343	255	321	66
Non-Forest	39,876	40,241	410	–	–	–
Total	**60,717**	**60,883**	**–**	**1,718**	**2,454**	**736**

When the changes of development stages between 1989 and 2002 were analyzed for only stone pine forest, we observed that regenerated areas increased prominently (630.47 ha) and about 300 ha mature stone pine stands moved to over mature developmental stages (Table 6).

4. Spatial analysis of changes in forest structure

The results indicated that the total number of patches (NP) increased from 380 to 705 between 1989 and 2002, when all land use/land cover classes were taken into account. The mean patch size (MPS) decreased from 986 ha to 703 ha. While it is decreasing in all land use classes, it is increased for black pine. Similarly Edge Density (ED) and Area Weighted Shape Index (AWMSI) values in almost all classes increased and total values changed from 34 to 45 and 26 to 30, respectively. Specifically in the stone pine and degraded stone pine stand classes, the NP increased from 13 to 41 and from 9 to 20, respectively. The MPS's of stone pine and degraded stone pine stands decreased from 113 ha to 52 ha and from 28 ha to 16 ha, respectively. Also ED and AWMSI values in stone pine areas increased from 1 to 3 and from 3 to 4, respectively. Similarly in degraded stone pine areas these values changed from 0.5 to 0.8 and 2 to 2 (Table 7). The results indicate an increase in forest area fragmentation which can increase further in the future, becoming a threat to conservation as well as the sustainable use of all forest values.

Table 7. Changes of landscape pattern in Ayvalık (1982-2002 forest cover type maps)

Land use	CA (ha)		NP(#)		MPS (ha)		ED(#)		AWMSI(#)	
	1989	2002	1989	2002	1989	2002	1989	2002	1989	2002
Non forest	39,876	40,241	79	187	505	215	10	15	5	7
Red pine	3,228	3,809	76	95	42	40	5	6	3	4
Open space	10,458	7,614	121	166	86	46	10	10	5	3
Degraded	4,885	6,161	74	192	66	32	6	10	4	5
Stone pine	*1,463*	*2,133*	*13*	*41*	*113*	*52*	*1*	*3*	*3*	*4*
Private	161	156	2	2	81	78	0.1	0.1	1	1
Degr. stone pine	*255*	*321*	*9*	*20*	*28*	*16*	*0.5*	*0.8*	*2*	*2*
Black pine	390	448	6	2	65	224	0.6	0.5	3	4
Total	**60,717**	**60,883**	**380**	**705**	**986**	**703**	**34**	**45**	**26**	**30**

IV – Conclusions

This study evaluated the spatial changes of forest structure between 1989 and 2002, including canopy cover, development stages and land use/land cover classes. The results were also specifically analyzed for stone pine areas. The total forested areas decreased slightly from 34.3% in 1989 to 33.9% in 2002. Total stone pine areas (Pure stone pine and degraded stone pine) showed a net increase of 43% (736 ha). Regeneration and low canopy cover areas decrease about 909 ha, while medium and full canopy cover areas increased about 2,217 ha in total. Most forests were in *a young* regeneration development stage.

The results on spatial structure of stone pine areas shows a more fragmented structure. Such changes of forest areas provide a more susceptible forests for further abrupt changes in the future. These parameters analyzed in this study are known to be relatively good indicators for the sustainability of forest resources and the resilience of the ecosystem mainly for biodiversity for stone pine or all forest areas.

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Effect of fertilization on the mineral composition of stone pine needles

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Abstract. Leaf analysis is a suitable technique to evaluate the nutritional status of forest stands since it reflects both the availability of nutrients in the soil and the ability of the trees to absorb them. This study aims to assess the effect of some nutrients on the needle mineral composition of *Pinus pinea* L. one year after their application to the soil. The experiment was established on a stone pine planted in 2009, located in the Portuguese Region of Ribatejo on a soil with acid reaction, medium levels of organic matter and extractable potassium, and low levels of extractable phosphorus, manganese, zinc, copper and boron. Eight experimental treatments consisting of different combinations of nitrogen, phosphorus, potassium, micronutrients, and limestone were considered. The needles were collected in March from the middle part of the growths of the former year. Fertilization induced significant differences ($p \leq 0.05$) on the needle levels of nitrogen, phosphorus, sulphur, manganese, zinc and boron. The application of phosphorus and boron increased the foliar levels of these nutrients, but liming induced lower levels of manganese and zinc. Concerning nitrogen, the levels of soil organic matter may be responsible for the appropriate nitrogen levels in the needles. The study will continue for further years in order to confirm the obtained results.

Keywords. Leaf analysis – Mineral nutrition – *Pinus pinea* L. – Soil analysis.

I – Introduction

In Portugal, stone pine (*Pinus pinea* L.) stands occupy about 176,000 ha, corresponding to 6% of the total forest area of the country (ICNF, 2013). This species assumes a great social and economic importance because of the commercial value of pine nuts, a seed with high quality and currently the main product of this forestry sector.

There are, however, strong annual variations regarding the production of pine cone, which worries producers, being necessary to study the factors that determine these fluctuations, namely genetic aspects, pests and diseases, and water and nutrients availability of the stands. It is also necessary to know the effect of agronomic practices, such as irrigation and fertilization, on the productivity and quality of pine cone and pine nut, bearing in mind the preservation of natural resources and biodiversity.

In what concerns fertilization, if it is performed in a rational way, it will improve the soil fertility and consequently the nutritional status of stone pine stands, which will help to increase their resistance to biotic and abiotic stress factors leading, probably, to higher and more regular yields of pine cone and pine nut. Therefore studies on nutrition and fertilization of stone pine stands must be carried in order to establish fertilizer recommendations to these stands. The required experimental work shall be based on the response of the trees to nutrient applications involving different situations (climatic, soil and management, among others) and conducted over an extended period of time in order to obtain representative results, since they depend on the covered experimental conditions (Calama *et al*., 2007; Piqué and Martín, 2007).

The evaluation of the nutritional status of stone pine stands should be performed through leaf analysis because the mineral composition of the leaves reflects both the nutrient availability in the soil and the ability of the trees to use these nutrients (Brockley, 2001). With that purpose, a fertilizer trial was installed in a young even-aged plantation, as a first step to establish the response of stone pine to the application of several nutrients to the soil. This paper reports the first experimental results of the above referred experiment, concerning the effect of the application of some nutrients on the mineral composition of the needles, one year after their application to the soil.

II – Materials and methods

1. Location and soil characteristics

The field experiment was established in a stone pine planted in 2009 with a 8 m x 6 m layout (156 trees per hectare) with plants from a nursery of the region. It is located at Vale Porquinho, in the Portuguese Region of Ribatejo. The trees were not grafted.

On average, annual mean temperature and rainfall are 16.6 ºC and 722 mm, respectively, the last occurring mainly in autumn and winter.

Soil samples taken before the establishment of the trial, in 2008, show, on average, on the surface layer (0 to 0.2 m) sandy loam texture (Bouyoucos hydrometer), acid reaction (pH_{H2O} = 5.0, potentiometric determination in a 1:2.5 soil-to-water (v/v) suspension), medium levels of organic matter (29 g/kg, organic carbon x 1.724, wet oxidation) and extractable potassium (78 mg/kg K_2O, Egner-Riehm method), very low levels of extractable phosphorus (<23 mg/kg P_2O_5, Egner-Riehm method), manganese (2 mg/kg Mn, AAAc-EDTA method), zinc (0.5 mg/kg Zn, AAAc-EDTA method), and copper (0.25 mg/kg Cu, AAAc-EDTA method), low levels of extractable boron (0.22 mg/kg B, hot water extraction) and very low potential exchange capacity (4.29 cmol(+)/kg, ammonium acetate method).

2. Experimental design and treatments

The experiment was arranged into complete randomized blocks with three replications, assigned to plots with eight trees each.

The experimental treatments were as follows: T1 - control (without fertilization); T2 - N; T3 - NP; T4 - NPK; T5 - NPK + limestone; T6 - NPK + MnZnCuB; T7 - NPK + MnZnCuB + limestone; T8 - NPK + B + limestone. Before planting, in spring 2008, phosphorus, potassium and limestone were applied to the soil. In March 2014, all the treatments were implemented. The nutrient levels used are: N - 40 kg/ha; P - 87 kg/ha; K - 100 kg/ha; Mn - 4 kg/ha; Zn - 2 kg/ha; Cu - 1,5 kg/ha; B - 2 kg/ha; Limestone - 8000 kg/ha (2008) and dolomite limestone - 4340 kg/ha (2014). All the fertilizers were applied to the soil. Nitrogen was applied as ammonium nitrate (20.5% N), phosphorous as calcium superphosphate (18% P_2O_5), potassium as potassium chloride (60% K_2O), manganese, zinc and copper as sulphate (27% Mn, 22% Zn and 25% Cu) and boron as Borax (11% B).

3. Leaf sampling and statistical analysis

Needle samples were collected in each one of the 24 plots of the experimental site in March 2015. A composite sample was prepared by mixing the needles of the eight trees of each plot. The needles were taken from the top third of the crown, from the middle part of the fully expanded growths of the former year.

The needle nutrients were determined as follow: nitrogen and sulphur through catalytic pyrolisis with elemental analyzer (Leco NS2000) and phosphorus, potassium, calcium, magnesium, sulphur, iron, manganese, zinc, cooper and boron through radial and simultaneous ICP-OES in the solution obtained by the uptake in HCl of dry ashes 500ºC.

Statistical analysis was performed through ANOVA in order to evaluate the effect of the experimental treatments on the mineral composition of the needles. Differences among means were established by Duncan multiple range test ($\alpha = 95\%$).

III – Results and discussion

The fertilization showed a significant mean effect on the needle levels of nitrogen, phosphorus, sulphur, manganese ($F_{[7;14]} = 3.05$, $F_{[7;14]} = 3.53$, $F_{[7;14]} = 2.91$ and $F_{[7;12]} = 4.19$, $p \leq 0.05$, respectively), zinc ($F_{[7;14]}=4.29$, $p \leq 0.01$) and boron ($F_{[7;14]}=12.09$, $p \leq 0.001$) and did not affect ($p > 0.05$) the others nutrients: potassium, calcium, magnesium, iron and cooper (Table 1).

Table 1. Mineral composition of needles of *Pinus pinea* L. collected in March

Experimental treatments	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
	g/kg						mg/kg				
T1 - Control	12.3 ab	0.9 bc	5.4	1.9	1.4	1.6 ab	43	33 bc	24 a	3.5	4.6 c
T2 - N	12.0 ab	0.8 c	5.7	1.9	1.2	1.4 b	40	25 bc	23 ab	3.6	4.6 c
T3 - NP	11.0 b	1.7 a	6.1	1.8	1.2	1.5 ab	42	38 b	21 abc	3.6	5.0 c
T4 - NPK	13.0 a	1.5 ab	5.3	2.5	1.5	1.7 ab	41	42 b	24 a	3.7	4.1 c
T5 - NPK + Limestone	11.7 ab	1.4 abc	6.5	2.1	1.2	1.9 a	44	10 c	18 bc	3.4	4.6 c
T6 - NPK + MnZnCuB	13.0 a	1.9 a	5.8	2.4	1.3	1.8 a	41	72 a	25 a	3.7	21.0 a
T7 - NPK + MnZnCuB + Limestone	11.3 b	1.5 abc	5.9	2.3	1.2	1.6 ab	42	14 bc	16 c	3.2	14.3 b
T8 - NPK + B + Limestone	12.3 ab	1.5 abc	6.3	2.4	1.3	1.8 a	42	17 bc	18 c	3.2	15.6 ab

Mean values followed by different letters are significantly different ($p = 0.05$).

The application of phosphorus increased the needle levels of this nutrient, which may be explained by the low levels of extractable phosphorus in soil. This is in accordance with the results reported by Prietzel and Stetter (2010) in a long-term study with *Pinus sylvestris*, who also observed an increase of phosphorus levels in foliage during some years after the fertilization with phosphorus.

The needle levels of boron also increased with the application of the nutrient, being the higher value obtained in the experimental treatment that received macro and micronutrients (T6). The experimental treatments without boron showed low needle levels of this nutrient. In a plantation of *Pinus pinea* with symptoms of boron deficiency, Vale *et al*. (1999) also reported an increase on boron needle concentrations after six months of the application of this nutrient to the soil. Higher doses of the fertilizer resulted in higher boron needle levels, although decreasing over time.

Regarding the manganese needle concentrations, the higher levels were obtained with its application without liming (also in T6). The limestone induced the lowest levels of manganese as well as of zinc, according with their lower availability when soil pH rises (Tisdale *et al*., 1985). Conversely, cooper and iron needle levels were not affected by the applied fertilization.

In what concerns the needle levels of calcium and magnesium, they were not influenced by fertilization, despite the low levels in the soil.

The medium levels of extractable potassium in the soil may explain the absence of fertilization response to the application of this nutrient. Concerning the nitrogen, were not observed significant differences between the treatment without fertilization (T1) and others that are fertilized with nitrogen. The medium levels of the soil organic matter may be responsible for this result and the appropriate nitrogen levels in the needles.

IV – Conclusions

The results showed that the applied fertilization affected the mineral composition of the needles, and therefore the nutritional status of the trees.

This study is preliminary and should continue for further years in order to evaluate the effect of experimental fertilizations on pine nut production. Moreover, other fertilizer experiments (factorial) are necessary to study the most suitable fertilization of stone pine based on soil and leaf analysis.

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Grafted stone pine plantations for cone production: trials on *Pinus pinea* and *Pinus halepensis* rootstocks from Tunisia and Spain

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Abstract. Grafted *Pinus pinea* plantations for early and abundant cone production are a good chance to restore and promote rural Mediterranean areas generating both economical (pine nut production) and ecological (soil protection) benefits. Grafting *P. pinea* on *P. halepensis* rootstocks can result in even more interesting outcomes because they can be used in degraded, dry and calcareous sites, which can hardly support any other relevant forest production. In the framework of a project supported by the Spanish Agency for International Development and Cooperation (AECID), implemented in Tunisia and Spain, we produced grafted plants using two types of stock (*P. pinea* and *P. halepensis*) from known provenances in Spain and Tunisia. During autumn-winter 2011-2012, field trials were established in both countries with the aim of studying the adaptation, development and cone production of the grafted plantations. In Spain and Tunisia, there are respectively five and six grafted field trials, using scions of four Spanish elite clones and nine root-stock provenances (five from Tunisia, four from Spain) of *P. pinea* and *P. halepensis*.In these plantations the basal tree diameter, total tree height, height until grafting point, graft diameter, success of grafting and cone production were measured. Factors influencing the grafting success, trees development and cone production were studied, namely stock species, stock provenance, grafting type and field site conditions**.**

Keywords. Aleppo pine – Mediterranean stone pine – Grafting success – Rootstock adaptation – Cone production.

I – Introduction

Pinus pinea is a multifunctional tree native to the Mediterranean basin. Among the products and services provided (timber production, soil and biodiversity protection, landscape values) the most remarkable one is the production of edible pine nuts. This product is highly appreciated worldwide and represents a major income in adequately managed stands. Spain is the country with the largest area of distribution of these species (470,000 ha) while in Tunisia it occupies 35,000 ha (Sghaier *et al.*, 2012), which represents more than the half of its total area worldwide (Loewe-Muñoz *et al.*, 2016). On the other hand, *Pinus halepensis* is a remarkably widespread species in the Mediterranean basin, with an area of distribution covering 3.5 million ha (Ne'eman and Trabaud, 2000). This species is characterized by its remarkable tolerance to aridity and calcareous soils, being the dominating species in the driest and warmest Mediterranean forest ecosystems.

A very useful practice to improve stone pine cone productivity is grafting with scions from selected highly productive trees (Prada *et al.*, 1997; Mutke *et al.*, 2012). Grafted plantations are therefore a good chance for restoring degraded areas and generating income in rural communities (Piqué *et al*., 2013). Moreover, grafting *Pinus pinea* on *Pinus halepensi*s root-stocks can result in even more interesting outcomes because it allows producing stone pine nuts even in harsh sites limited by drought and calcareous soils.

Grafting can be performed in the nursery or in the field. The first option has as main advantages the high rate of grafts per worker and day, the control of post-grafting conditions and the possibility to plant on the field only successfully grafted trees. The second option consists on grafting 1-2 m high vigorous trees which are already acclimated to the site, being less prone to vegetative or browsing problems than newly planted trees and having a much larger leaf area to sustain the scion (Guadaño and Mutke, 2016).

For studying the adaptation, development and cone production of grafted plantations, we implemented in Tunisia and Spain a reciprocal trial using two types of rootstocks (*P. pinea* and *P. halepensis*) from known provenances in Spain and Tunisia. The main objective was to study field-grafting success and to evaluate the performance of young plantations in relation to rootstock species and provenance, type of grafting and site conditions. We hypothesized that: (i) graft on *Pinus halepensis* rootstocks would have high survival rate and the same or higher cone production than graft on *Pinus pinea* rootstocks in poorest site conditions; (ii) the rootstock provenances might have an effect on the tree survival and cone production; and (iii) the grafting success for both, *Pinus halepensis* and *Pinus pinea* rootstocks, would be higher when grafted in nursery.

II – Materials and methods

1. Vegetative materials

We utilized the following rootstock species and provenances:

- 2 *Pinus halepensis* rootstock provenances from Spain (Palausator, Sallent).
- 2 *Pinus halepensis* rootstock provenances from Tunisia (Kef, Thibar).
- 2 *Pinus pinea* rootstock provenances from Spain (Aiguafreda, Malavella).
- 3 *Pinus pinea* rootstock provenances from Tunisia (Rimel, Ouechtata, Mhibes).

The scions were obtained from four Spanish elite clones from North-east Spain.

Figure 1 shows the location of the different provenances in Spain and Tunisia.

2. Field trials

Five field trials were established in NE Spain during autumn-winter 2011 and four trials in NE Tunisia during autumn 2012), in a range of different soil and climatic conditions, using both nursery grafting and on-field grafting in Spain, only nursery grafting in Tunisia (Tables 1 and 2).

Spanish trials were designed as unbalanced Randomized Complete Block Design (RCBD), as the number of trees from the different rootstock species and provenances was variable. In each trial we identified 3 blocks (except for “Monestir”, with 4), and distributed evenly the number of trees from each type throughout the blocks, therefore each block contained approximately one third (fourth in “Monestir”) of the available trees of each type (Table 3). Tunisian trials, however, were organized as a Randomized Complete Block Design (RCBD) in “Dar Chichou” and “Mellègue” (4 blocks each), and as a fully randomized design in “El Azib” and “Sers” (Table 4). In spring 2011, the nursery grafting for the Spanish trials was performed on 1-year-old seedlings.. In autumn 2011, those succeeded graftings and other ungrafted plants were planted in the field. In spring 2012, on-

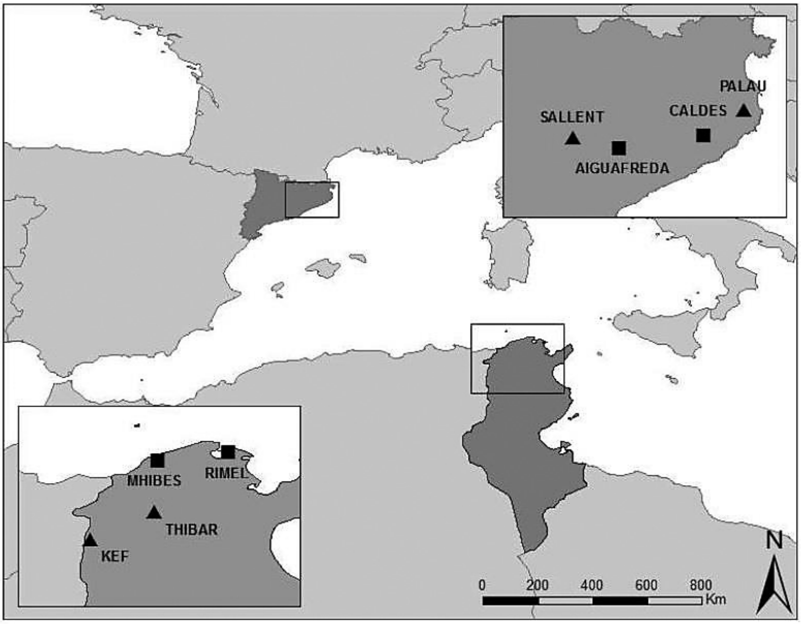

**Fig. 1. Location of the different rootstock provenances in Spain and Tunisia. Triangles refer to *P. halepensis* while squares refer to *P. pinea*.
Source: Bono *et al*. (2013).**

Table 1. Site conditions of Spanish field trials (area: 0.4-0.6 ha/trial, planting grid: 5 x 5 m)

Site/field trail name	Noguera county, Lleida province		Priorat county, Tarragona Province		
	Freixes	**Monestir**	**Marçà**	**Serra d'Almos**	**Capçanes**
Stock species	*Pinus halepensis* and *Pinus pinea*				
Geographical coordinates	0º42'15.71E 41º54'21.66N	0º45'14.78E 41º52'32.38N	0º48'18.87E 41º7'19.71N	0º45'47.36E 41º4'33.40N	0º47'10.80E 41º5'57.31N
Site type	Farmland	Farmland	Farmland	Farmland	Riverbank
Mean annual temperature	12.5-13.0 °C		14.0-16.0 °C		
Annual rainfall	600-650 mm		525-575 mm		
Climate type	Mesomediterranean (subhumid)		Mesomediterranean (subhumid)		

Table 2. Site conditions of Tunisian field trials (area: 0.1-0.15 ha/trial, planting grid: 5 x 6 m and 8 x 6 m)

Site/field trial name	Dar Chichou	El Azib	Sers	Mellègue
Rootstock species	*P. pinea*	*P. halepensis*	*P. halepensis*	*P. halepensis*
Geographical coordinates	10°59'19.63E 36°57'54.48N	9°59'19.15E 37°12'22.06N	8°59'15.78E 36° 4'22.56N	8°42'25.93E 36°19'0.36N
Site type	Afforestation	Afforestation	Farmland	Afforestation
Mean annual temperature	19.0 °C	18.0 °C	17.0 °C	18.0 °C
Annual rainfall	500 mm	500 mm	450 mm	500 mm
Climate type	Thermomediterranean (Semiarid)			

Table 3. Factors and number of blocks and trees per field trial in Spain

Factor 1: stock species	Factor 2: stock country of origin	Factor 3: stock site of origin	Factor 4: grafting type	Freixes	Monestir	Marçà	Serra d'Almos	Capçanes
P. halepensis	Spain	Palau	On-field	8	12	12	14	1
			Nursery	6	6	–	–	–
		Sallent	On-field	13	10	14	14	5
			Nursery	9	8	–	–	–
	Tunisia	Kef	On-field	9	9	12	13	3
			Nursery	1	1	–	–	–
		Thibar	On-field	8	7	9	13	5
			Nursery	6	6	–	–	–
P. pinea	Spain	Aiguafreda	On-field	13	9	16	25	9
			Nursery	11	11	–	–	–
			Ungrafted	5	3	15	14	9
		Caldes	On-field	9	9	20	23	12
			Nursery	9	8	–	–	–
			Ungrafted	5	3	10	15	8
	Tunisia	Rimel	On-field	25	12	2	0	1
			Nursery	3	3	–	–	–
			Ungrafted	8	12	–	3	1
	Trees per treatment and block			0-8	0-3	0-7	0-9	0-4
	Blocks per field trial			3	4	3	3	3
	Trees per field trial			148	129	110	134	54

Table 4. Factors and number of blocks and trees per field trial in Tunisia

Factor 1: stock species	Factor 2: stock country of origin	Factor 3: stock site of origin	Dar Chichou	El Azib	Sers	Mellègue
P. halepensis	Spain	Palau	–	10	8	28
		Sallent	–	10	8	28
	Tunisia	Kef	–	10	8	28
		Thibar	–	10	8	28
P. pinea	Spain	Aiguafreda	40	–	–	–
		Caldes	40	–	–	–
	Tunisia	Rimel	40	–	–	–
		Mhibes	40	–	–	–
		Ouechtata	40	–	–	–
	Trees per treatment and block		10	(fully randomized design)	(fully randomized design)	7
	Blocks per field trial		4			4
	Trees per field trial		200	40	32	112

field, grafting was performed, on those trees that had not been grafted in the nursery, Therefore, this last group of plants were 2 years old when they were grafted. Finally, further *P pinea* trees planted as control and those grafted *P pinea* trees whose scion died were considered as "ungrafted trees". In Tunisian field trials all planted trees had been grafted in the nursery in spring 2012.

3. Data collection and statistical analyses

In May 2012, February 2014 and March 2016 (Spanish trials) and in March 2016 (Tunisian trials) the main variables related to tree and scion status and growth and presence of cones were evaluated: Tree survival (alive vs. dead), Scion status (viable vs. unsuccessful, considering only alive grafted trees), Basal tree diameter, Diameter at graft point, Total tree height, Height of graft point, Crown diameter, Number of 1 year-old conelets (only in 2016), Number of 2 year-old and of ripe 3 year-old cones.

In the case of *P halepensis*, the number of conelets and cones were evaluated only when the scion was alive.

Statistical analyses of tree survival, scion viability after successful nursery grafting, on-field grafting success and number of cones were descriptive: mean number of trees in each category. Dasometric data (diameters and heights) were analyzed with analysis of variance (Anova) using SPSS software, considering that two treatments were different when $p < 0.05$.

III – Results and discussion

In general, tree survival was high, 76% (60-98%) in Spain and 80% (74-82%) in Tunisia, except in the poorest sites, El Azib, Tunisia (42%) and Capçanes, Spain (47%), the latter affected also by damage from wildlife browsing. No evident effect of rootstsock species or provenances was observed.

More than 90% of alive trees grafted in nursery kept the scion alive trials after three and four vegetative seasons in Tunisia and Spain (Freixes and Monestir) trials.

In the Spanish trials, on-field grafting success was lower for *Pinus halepensis* (15%) than for *Pinus pinea* rootstocks (64%), as shown in Fig 2. Grafting success was proportional to the suitability of the rootstock to the site: the sites that were more adequate for *Pinus pinea* (Freixes, Monestir) showed, for this species the best rootsock performance, while the sites more suitable for *Pinus halepensis* (Marçà, Serra d'Almos) had the highest grafting success rates for this species. Capçanes trial, with very poor quality and suffering from wildlife browsing, showed the poorest results of all sites. No clear pattern of rootstsock provenance or scion clone was observed. The poorer performance of *Pinus halepensis* rootstocks compared to *P. pinea* ones was also observed, for the same vegetative materials, during nursery grafting (Piqué *et al.*, 2013), which was probably linked to the excessive slenderness of the former.

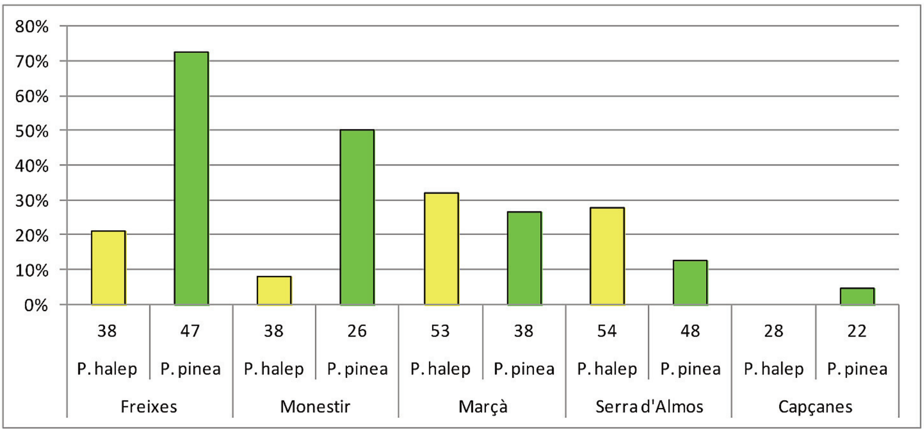

Fig. 2. On-field grafting success in Spanish field trials, four years after grafting, for each rootsock species. The number below each bar indicates the number of grafts performed.

With regard to the tree basal diameter and total height, we found no consistent significant differences between rootstock species, provenances or treatments. However, in the Spanish field trials "Monestir" and "Freixes", a slight trend of Tunisian rootstocks growing more than Spanish ones was observed, while nursery-grafted scions tended to grow more than on-field grafted ones. Overall mean basal diameter of *Pinus pinea* and *Pinus halepensis* was 36 and 40 mm, respectively, average total height 98 and 138 cm, respectively, considering all field trials together.

The percentage of successfully grafted trees with cones was higher on *P halepensis* rootstocks (29-83%) than on *P pinea* (10-40%), while no major effect of provenances or grafting treatment (Spanish field trials) was observed (Fig. 2). Ungrafted *Pinus pinea* trees (i.e. those that were not grafted and those whose grafting was unsuccessful) presented no cones. The higher growth and earlier cone production of *Pinus halepensis* rootstocks compared to *P. pinea* ones contrasts with findings by Gordo *et al*. (2013) who observed the opposite trend, though with quite different soil conditions (arensols) in central Spain where Aleppo pine is not native.

Considering the number of conelets, it seems that this number had increased over time (Fig. 3). Spanish plots (4 years-old) showed similar cone production as Tunisian plots (3 years-old), when correcting the age difference (i.e. number of cones 2 years after grafting). The only exception was in Monestir site, which showed a notably higher production than the rest of the other trials. In Spain, the effect of provenance and grafting treatment (nursery vs on-field) on the cone production was not consistent, but it seems that in general *P. halepensis* rootstocks tended to induce more cones.

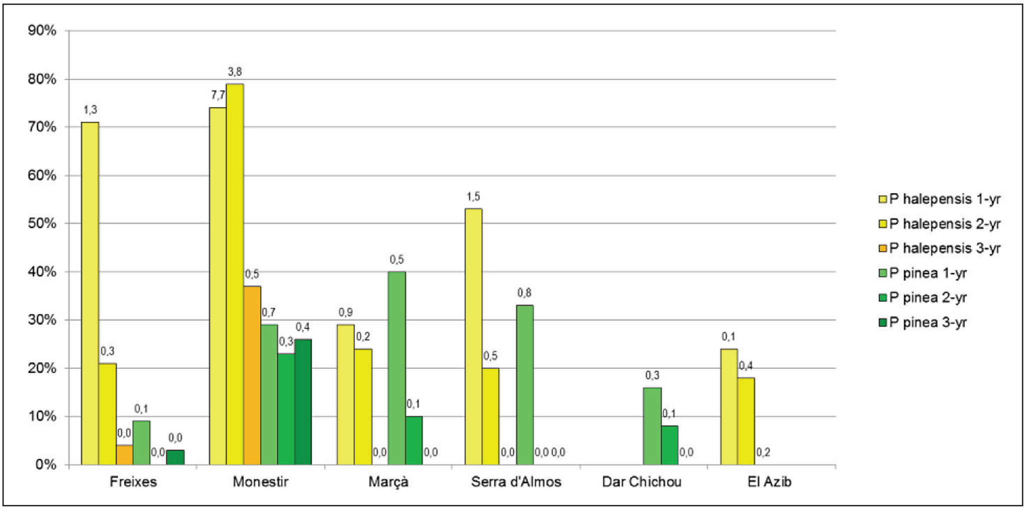

Fig. 3. Percentage of successfully grafted trees with cones of each cohort. The number above each bar indicates the average number of cones/coneletsof the given age on each tree. Spanish trials were 4 years old while Tunisian ones were 3 years old.

IV – Conclusions

Survival of trees and scions depended on site quality, especially climatic conditions, and on the avoidance of browsing damage. This emphasizes the importance of an adequate choice of the planting area and of the consideration of tree protection. In general, grafted *Pinus pinea* plantations in Spain and Tunisia showed high survival and promising growth rates, both with *P. pinea* and *P. halepensis* rootstocks, on medium quality sites. Grafting clearly advances cone production compared to ungrafted trees, which after 3-4 years did not form any cone.

On-field grafting success was lower for *P. halepensis* than for *P. pinea* rootstocks, being particularly lower in the hardest site conditions.

Most trees grafted in nursery kept the scion alive after field plantation. In the case of on-field grafting, the success rate was adequate (>50%) in *P. pinea* on medium quality sites, while it was very poor in poor quality sites subject to severe drought. On-field grafting on *P. halepensis* led to poor results, particularly in the sites where this species was less adapted.

There were no significant effect of the rootstock species or provenance on tree growth.

Regarding cone and conelets production, the results from Spanish and Tunisian field trials showed a generally similar trend, with *Pinus halepensis* rootstocks leading to higher cone production than *Pinus pinea* ones, although no major effect could be attributed to the different provenances. In the two field trials where both nursery and on-field grafting were performed, we found no major difference in cone production.

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Session 2

Models

Which models are needed for *Pinus pinea* forests? A review on current state and potential use

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Abstract. We aim to make a critical review of the current state of the art of modelling in Mediterranean stone pine forests, focusing on the stakeholders and end-users criticisms and points of views. To do this we first present an exhaustive review and analysis of the currently available literature on the topic, in order to detect gaps in knowledge. In a second part of the study, we analyze whether the stakeholders involved in stone pine managements make use of the existing models. We also analyze which are the characteristics and requirements that the potential end users demand to the models. Our results show an extraordinary development in the modelling activity for the species, identifying more than 109 scientific references, of whom 72 are published in JCR® journals, although some gaps are observed. Despite this large availability of models, potential end-users of the models currently don´t make an in-depth use of these tools, since in many occasion their demands are not met by the expected outputs.

Keywords. Type of models – End-users – Simulation tools.

I – Introduction

A forest model is an abstraction, or a simplified representation, of some aspect of forest dynamics and functioning, or of any of the components and relations defining the system (Weiskittel *et al.*, 2011). Forest modelling activity started in Central Europe by the end of the 19th century, with the construction of the growth and yields tables based on normal forest principles, and since then they have been considered basic tools for supporting forest management at different scales. The interest of foresters in models for predicting, explaining and describing forest systems, together with the advances in statistics and computation, have resulted in a recent increasing effort in the modelling activity worldwide.

The Mediterranean stone pine, *Pinus pinea* L. can be considered a paradigmatic example of this evolution. In the last 25 years stone pine has evolved from being a species with a scarce knowledge concerning growth and yield dynamics to being nowadays a well-known species in the Mediterranean forests ecosystems (e.g. Mutke *et al.*, 2012, 2013). This widening of the scientific knowledge was the necessary basis for the considerable effort in constructing models to explain and predict ecologic processes and yield of stone pine forests.

Currently there are models available for the species working at different spatial, temporal and functional scales, with geographical validity in different countries and regions within countries. Classical empirical models for predicting growth and yield (Castellani, 1989; Calama *et al.,* 2007a) now coexist with climate-driven models (Calama *et al*., 2014a), physiological based models predicting photosynthetic activity (Calama *et al*., 2013), models attempting to predict different dynamic processes, as natural regeneration or decay (Manso *et al*., 2014), or large scale process-based models (Pardos *et al.*, 2015). Apart from timber and fuelwood production, existing models for stone pine aim to simulate cone production (e.g. Gonçalves and Pommerening, 2012), nut quality and content (Morales, 2009), and the provision of other ecosystem services, as CO_2 fixation (Correia *et al*., 2010). Temporal scale of different models ranges from the second (Calama *et al*., 2015) to the multiannual scale (Mutke *et al*., 2005), while spatial scales extent from the leaf (Correia and Freire, 2014) to the region (Nanos *et al*., 2003). Moreover, the existing models aim to cover the wide range of stand conditions and forest management objectives, including high-cone producer grafted plantations (Mutke *et al*., 2005c; Carrasquinho and Gonçalves, 2012), naturalized afforestations focusing on protection (Calama *et al*., 2009), or mixed stands oriented to recreational uses (Madrigal, 2014) or agroforestry uses (Palma *et al.,* 2007). Finally, the modelling activity is going on, with new models and approaches being currently under construction in different countries (Sghaier *et al*., 2013; Loewe *et al*., 2015).

All this modelling effort necessarily relies on good quality datasets. In this sense, specific nets of permanent plots and experimental trials have been installed in different countries to analyze growth and yield dynamics for the species. Among those are noteworthy to mention the nets of permanent plots for timber and cone production and thinning trials (covering wide areas of Spain and Portugal), natural regeneration essays (Valladolid) or the irrigation and fertilization trials installed in Portugal. A specific issue concerning the species is the need to obtain sound information on cone production, which can only be afforded in detail by collection the cones directly from the trees.

This extraordinary evolution is more remarkable taken into account that, unlike other timber focused species, the main production from stone pine stands is the pine nut, extracted from cones collected from standing trees. Modelling cone production deserves a real challenge due to some issues: (i) large interannual variability in the production (masting) at tree, stand and regional scales, (ii) abundance of zeroes in some regions (e.g. in Valladolid province more than 50% of trees present null crops), (iii) patterns of spatial dependence, (iv) asymmetric and skewed distribution, with the main part of the production located in a few trees, and (v) lack of physiological knowledge of the flowering-fruiting process.

Despite the wide offer of modelling tools nowadays available, existing models seem not to be perceived as fully useful to answer many of the questions, demands and concerns that stone pine forest managers, forest owners, policy makers and industrials are facing with. Our models are often criticized for being oversimplifications leading to unrealistic results; at the same time, they show complex formulations where the demanded inputs are not easily available. Spatiotemporal scales usually do not match with those required by the users, and outputs from the models are far away from those expected. Meanwhile, some basic questions seem not to be adequately answered by existing models. Many topics remain uncovered by model predictions, such as: cone and timber production in the next decades; how to manage stone pine forests under an uncertain climate; how to optimize cone production for a given stand; how to make a small property profitable; what is the expected impact of an extreme drought event; what to do with the mixed stands… and many others.

In the present study we aim to make a critical review regarding the current state of the art of modelling in Mediterranean stone pine forests, focusing on the stakeholders and end-users criticisms and points of views. To do this we first present an exhaustive review and analysis on the currently available literature focusing on the topic, in order to detect gaps in knowledge. In a second part of the work, we analyse whether the stakeholders involved in stone pine management make use of the existing models. We also analyse which are the characteristics and requirements that the potential end users demand to the models, and focus on identifying which could be the best type of model for each end user.

II – State of the art on modelling for *Pinus pinea* forests

1. Methods

To carry out our review on currently available models for *Pinus pinea*, it was necessary to define first which would be our objective population. We focused uniquely on tools that fulfill the following conditions:

- constructed with the aim of describing, explaining and/or predicting some aspect of forest dynamics and functioning, or of any of the components and subjacent relations of the system;
- the attributes of the system are mainly described by numerical values;
- the dynamics and relationships are expressed by means of mathematical functions;
- specifically constructed for the species *Pinus pinea* L.

We orientated our search towards two different groups: (i) models already published in journals included in JCR®, and (ii) models published on non-JCR® journals, technical reports, academic dissertations and conference proceedings. JCR® query was carried out by means of a Boolean search in Web of Science (www.webofknowledge.com) using as topic keywords the following:

[“Pinus pinea” or “stone pine”] + [“model” or “dendrochronology” or “growth” or “cone”]

In a second step we made a subjective filtering over the whole database in order to match the previously defined conditions. Additionally, to those limiting conditions, we deliberately skipped out all the references related with modelling *Volatile Organic Compounds*, a discipline largely developed by the end of the 90’s of the last century, which on many occasions used *Pinus pinea* as a case species, but which falls far from the scope of the interest topics for our review.

Search on non-JCR® literature was based on consulting books of proceedings of different scientific meetings (e.g. 1st Agropine, Spanish and Portuguese National Forest conferences, MEDPINE...), non-JCR® journals (Montes, Options Méditerranéennes, Cuadernos SECF…), phD & MSc thesis and others sources. Criteria for selection matched those previously presented. In the case of tied references –e.g. a preliminary version of the model presented in a proceeding and thereafter published in a JCR journal– we just included the later one into the database.

2. Results and discussion

Our query resulted in 109 references, of which 72 correspond to models published in JCR journals, while 37 were found in other scientific and technical literature (see Annex I for the complete reference list). Due to the nature of non-JCR literature, it is obvious that we have missed several references from this group, especially from national technical reports, national meetings and others.

A. Temporal analysis

First analysis over the database will focus on the temporal evolution of the effort on modelling for *Pinus pinea* forests (Fig. 1). A clear increasing trend is detected, with only ten references (only two in JCR) covering the 34-year period elapsed between the first reference (Pita, 1966) and 2000 while in a single year (2015), twelve references on modelling in *Pinus pinea* forests were published (eleven in JCR). Although this is a common issue for all the forest species (see Weskittel *et al.,* 2012), we must mention some peculiarities in the case of *Pinus pinea*.

The first of all is a clear delay with respect to other species, even within the same Mediterranean region. Except for the seminal works by Pita (1966, 1967) focusing on site index curves and volume equations, no effort was carried out up to the end of the decade of 1980’s. By that time, on the

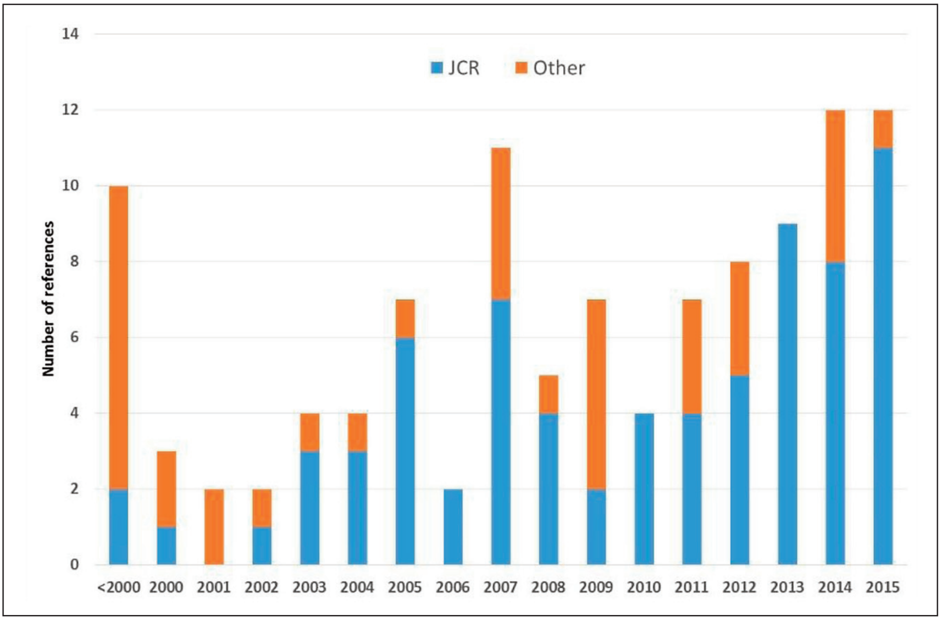

Fig. 1. Temporal evolution in the number of references focusing on "modelling + *Pinus pinea*" in JCR and other literature.

exhaustive revision of the existing growth and yield tables for the Spanish forests species presented in Madrigal *et al.* (1999) the unique main-species non represented was *Pinus pinea*. This situation was similar in other countries with available tools for other species, as Portugal, France or Italy.

Main reasons for this delay can be related with the lack of timber productive interest of *Pinus pinea* forests if compared to cone production, while classical growth and yield tables uniquely focused on wood-biomass production. Related with this, in order to promote cone production *Pinus pinea* forests resulted in low stocking density of the stands, which refutes the basic principle of normal complete stocking basic for the construction of traditional growth and yield tables. The lack of knowledge on the fruiting process in *Pinus pinea* also prevented the inclusion of cone production into classical tables. Due to this the first growth and yield tables –including cone production– for the species were only published in 1989 in Italy (Castellani, 1989).

As mentioned before, the study of the species has progressed over the last 20 years to make *Pinus pinea* one of the best known species in the Mediterranean ecosystems. A factor triggering this was the interest that the species arose in the FAO meetings at the end of the 1980's, which resulted in activities such as the installation of a net of permanent plots for studying cone and timber production in the Spanish forests. This net was installed and maintained since 1992 by the INIA-CIFOR in cooperation with the Regional Forest Services of Castilla y León, Andalusia, Madrid and Catalonia (https://sites.google.com/site/regeneracionnatural/proyecto-rta2013-00011-c02-00/difusion-y-transferencia). A result directly derived from this net was the construction of the first diameter-distribution and tree level models for the species (García-Güemes, 1999; Cañadas, 2000), the first interregional models with validity in Spain (Calama *et al.,* 2003), the integrated model PINEA2 (Calama *et al.,* 2007a, b) together with its associated stand-level simulator. Joint use of this net together with annual recordings of cone production at forest scale permitted the construction of spatial (Calama *et al.,* 2008a), temporal (Mutke *et al.,* 2005) and spatiotemporal (Calama *et al.*, 2011) models for cone production. In Portugal, a similar evolution resulted in the publication of the first integrated tree-level model for the species (Freire, 2009), also incorporating a cone production model (Rodrigues *et al.*, 2014).

Finally, in the last years the modelling effort for the species has been oriented to new topics such as heterogeneous stands (de-Dios-García *et al.*, 2015), natural regeneration processes (Manso *et al.*, 2014a), physiological traits (Mayoral *et al.*, 2015a, Calama *et al.,* 2015), dendrocronological models (Natallini *et al.*, 2014) or the calibration of process-based models for the species (Pardos *et al.*, 2015).

B. Geographical analysis

With respect to the geographical distribution of the modelling activity in *Pinus pinea* there is a clear dominance of the references focusing on works developed in Spanish forests, amounting more than 70% of the total (77 out of 109 records). Portugal and Italy accounts for 9% and 7% of the total, with Portugal showing a recent effort in developing growth and yield models for the species (Freire, 2009; Correia *et al.*, 2010), while in Italy, where the first yield tables were constructed, the modelling activity for the species focuses nowadays on dendrochronology (Piraino *et al.,* 2007). It is noteworthy to mention the recent research carried out in Tunisia (Sghaier *et al.*, 2012). These results contrasts with the lack of models –up to the knowledge of the authors– in countries with such a large modelling tradition, as France, or in two of the countries with larger potential for cone and nut production, as Lebanon or Turkey. Once more it is necessary to mention the lack of information concerning non-JCR in many of the countries, especially in other languages different than English, which surely affects these results.

C. Model objective

Modellers tend to present different classifications of models, according to degree of empiricism, spatiotemporal scale of application, minimal unit of simulation. In this study we adopted a purpose-oriented classification, according to the objectives to achieve. In this sense we classified the selected models into:

- Growth and yield: models focusing on the evolution / growth/ allometry / production of a given forest unit or each of their components.
- Dynamic processes: models focusing on other dynamic processes apart from growth, e.g., regeneration, mortality, competition.
- Dendrochronology: models focusing on climate-growth relations and sensitivity.
- Physiological: models focusing on specific physiological traits, e.g. stomatal conductance, net assimilation.
- Optimization: models aiming to optimize forest management in terms of a given output.
- Genetics: models devoted to identify best genotypes.
- Niche: models identifying optimal sites for species establishment, growth and performance.
- Wood quality: models focusing on the prediction of wood traits (stem rot, mechanical attributes).

Focusing on the 109 models for *Pinus pinea* identified (Fig. 2), almost 50% (53) were classified as growth and yield models, which will be presented in detail later on. Concerning models devoted to other dynamics processes, they account for 19% of the total, mainly orientated to describe and predict the different phases involved in natural regeneration (seed dispersal, germination and survival) under different climate and management scenarios (Manso *et al.,* 2012, 2013a; Carnicer *et al.,* 2014). On the contrary, we detected a clear gap on models describing and predicting mortality for adult trees.

Twelve dendrochronological models were identified, covering different regions from Portugal, Spain, Italy, France, Tunisia and Turkey, aiming to describe climate-growth relationships and identify key climate factors driving secondary growth at a regional scale (Akkemik, 2000; Campelo *et al.*, 2007; Cutini *et al.,* 2013; Natalini *et al.*, 2015). Finally, the fourth main group is that of physiological models (8%), with special attention to photosynthesis and gas exchange processes (Evrendilek *et al.,* 2005; Mayoral *et al.*, 2015a).

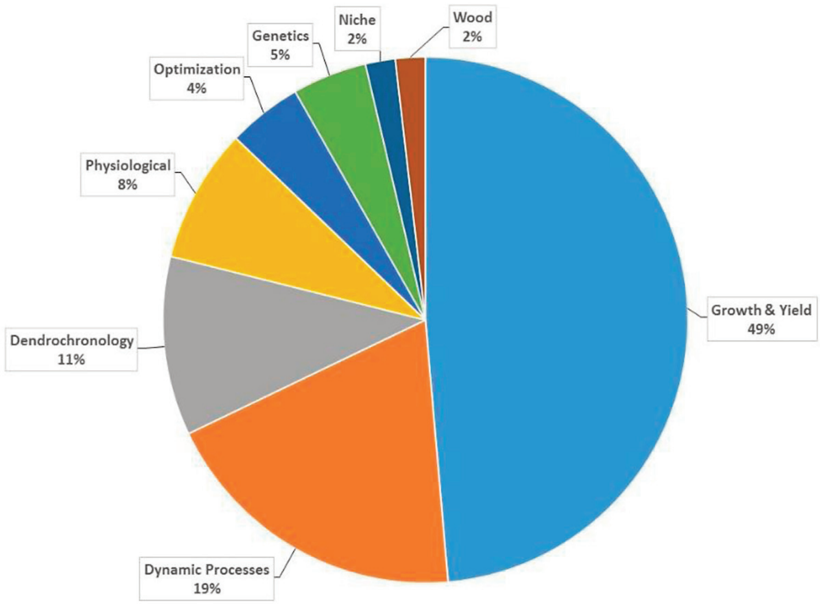

Fig. 2. Percentage of type of models for *Pinus pinea* according to their main objective.

We proposed a subdivision of growth and yield models into different categories, once again with a purpose-oriented aim (Fig. 3). The main group (15 references) was the one devoted to models for cone and nut production with validity on different regions of Spain, Portugal and Tunisia. These models include pure spatial models describing spatial correlation at different scales (Nanos *et al.*, 2003, Gonçalves and Pommerening, 2012), empirical functions predicting cone production at tree level using stand, tree and climate attributes as predictors (Calama *et al.*, 2008a, 2011), and regional scale models (Mutke *et al.*, 2005). We also found twelve references presenting allometric relationships for the species, including volume, stem taper (Calama and Montero, 2006) and biomass equations (Ruiz-Peinado *et al.*, 2011; Correia *et al.*, 2008), height-diameter functions and crown equations (Cañadas *et al.*, 2001). Diameter increment functions, with special attention to the effect of intra and interspecific competition accounted for other six references (Ledo *et al.*, 2014). Additionally, six site index curves with validity for different regions and countries have been published (Calama *et al.*, 2003; Bravo-Oviedo *et al.,* 2005; Sghaier *et al.*, 2012).

Some of these functions were included in the integrated stand-level models, yield tables and tree level models which represent other twelve references. In an independent way, these complete models also include cone production, site curves, growth and/or allometric functions not previously published. These complete models have been either implemented as yield tables, stand density management diagrams, as well as on stand level simulators. The most complete models are the tree-level model PINEA2 (Calama *et al.,* 2007a, b), with validity in different regions in Spain, originally fitted for pure-even aged stands, and currently extended to uneven-aged stands and afforestations, and the stand-level model ORGEST_Pinea (Piqué *et al.*, 2011, 2015), with validity on Catalonia.

A main drawback of the aforementioned growth and yield models for *Pinus pinea* is that they are not climate-sensitive. In this regard, some effort in annualizing estimates by including climate drivers (Calama *et al.,* 2014) are currently under development.

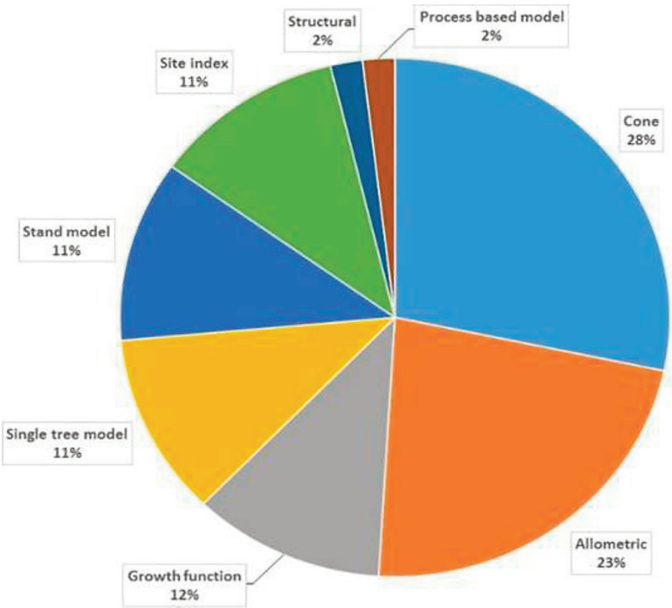

Fig. 3. Percentage of type of growth and yield models for *Pinus pinea* according to their main objective.

D. Empiricism vs *process based models*

The majority of the 109 models and function analyzed must be defined as empirical models, since they rely on statistically fitting mathematical functions over observed data, where predictors are actually variables acting at different spatio-temporal scales (climate drivers, stand and/or tree level attributes, competition, provenance…). The combination of these functions and variables do not represent basic chemical or physical processes at the basic organisational levels of the individuals, but they rather aim at describing phenomenological responses to the environment. In this sense even though many physiological process (e.g. photosynthesis, gas –exchange parameters, etc.) are modelled, these models rely on an empirical formulation.

We only found three exceptions to this general trend, with the first being the crown development model by Mutke *et al.* (2005b) which entirely falls within the category of structural models, defined as those aiming to describe plant growth based on the development of the different organs. Pardos *et al.* (2015) succeeded in calibrating and validating the model PICUS v1.41, a model combining elements of a 3D patch model and a process-based forest production model, for *Pinus pinea* in the Spanish Northern plateau, incorporating the empirical spatiotemporal model for cone production by Calama *et al.* (2011). The model provides estimates of timber and cone production, as well as vulnerability for the species under different climate scenarios and management alternatives. Finally, Calama *et al.* (2015) propose a hybrid model for predicting seedling survival using as predictors daily rate of net assimilation and water status, derived from specific physiological based models.

III – Current use of the identified models

Goodness of fit, statistical correctness and predictive accuracy of a model do not mean success from a practical point of view. As we should expect that not all the models are useful for all the potential users, cooperation among model builders and model users is required. Model building means an iterative process where modellers and potential end users should define the main objective in building the model, design the model structure and agree the expected uses and outputs of the model.

Models are constructed with the aim of describing, explaining and predicting. While in essence all the models are constructed focusing on these topics, the reality indicates that when focusing on the main use of the model, one is commonly dominant. In this sense, we can classify models from the end-use point of view as:

- Descriptive: the main aim of the model is to describe the state of the system and identify relationships among attributes.
- Predictive: the model is used to forecast information required to help in any decision related with management of the forest at different scales.
- Explanatory: the model is used to contrast hypothesis about causal relationships, thus mainly an academic / knowledge building use.

An overview over our 109 models and functions reveals that 14% of the models show a main descriptive use, 35% can be classified as explanatory models, 42% as predictive models and the remaining 9% are proposed for a joint predictive / explanatory use. According to the different categories, growth and yield models are mainly constructed for a predictive or an explanatory/predictive use, while dendrochronological models, physiological models and models describing processes as regeneration aims to an explanatory use. According to previous figures, at least 51% of the models were constructed focusing on a predictive use in order to help management of the forests at different scales, and we would expect that they were nowadays used for potential end users. The main aim of this section is to evaluate the current state of use of the available models.

1. Methods

A short discussion was carried out with a small number of stakeholders (at least one per group) related with *Pinus pinea* forests representing five different groups of potential end users of the model: forest managers, forest planners, policy makers, forest owners and nut and timber industrials. Questions focused on general knowledge of the existence of models for the species, current use of models, need of models in their task, expected outputs, spatio-temporal scale and interface.

In a second phase we selected five different models for the species, representative of the different model types but oriented to predictive or explanatory / predictive use, in order to check whether these models will cover the required demands presented by different end-users group. Selected models were the growth and yield tables by Montero *et al.* (2004), ORGEST_Pinea (Piqué *et al.*, 2015), PINEA2 (Calama *et al.,* 2007a), natural regeneration multi-stage model (Manso *et al.*, 2014a) and hybrid model PICUS_PINEA (Pardos *et al.,* 2015).

2. Results and discussion

Table 1 shows the current state of use and demands from potential end-users of the models for *Pinus pinea*. Forest managers and policy makers reported to make little use of the models, even if they declared to be aware of their state of development. Forest planners, owners and industry usually neglect existing models. Concerning the demands, managers, planners and owners require tools projecting real forest management units, while policy makers need to carry out estimates at national or regional level. Planners and owners require simple guidelines and friendly interfaces. In general stakeholders are interested on timber, cone and biomass production, although managers are highly concerned for the regeneration of the forests, and policy makers on topics as species substitution and fire hazard. Industrials focuses on quantity and quality of raw materials, as cone and timber.

Table 1. Use, demands and requirements of the different groups of potential end-users of models for stone pine

	Forest manager	Forest planner	Policy maker	Forest owner	Nut & timber industry
Knowledge	Wide	Little	Wide	Little	No
Use	Little	No	Little	No	No
Main demands	Project real block units Predict annual cone crop Economic evaluation	Project current state of the forest Simple guidelines Yield tables	Raw estimates at regional level Compatible with NFI Compatible with international demands	Early annual estimates of cone Management to increase cone production	Timber quality and saw classification Pre-crop nut yield & quality
	Identify vulnerable sites Timber and biomass quantification Sensitive to management	Cover all forest tipologies Compatible with management invetories	Species substitution Focus on forest typologies	Simple guidelines	Global forecasting of annual cone production Low cost methods
Output	Cone Timber Biomass Natural regeneration	Cone Timber Biomass	Biomass & CO2 Timber & Cone Vulnerability Fire risk	Cone Timber	Timber Cone Nut yield Wood quality
Spatial scale	Block	Block	Region	Forest	Forest Region
Temporal scale	Annual	Annual Decennial	Annual Decennial	Annual	Annual
Interface	Simulator	Flexible Compatible with large databases	Flexible & homogenous	Friendly apps	Compatible with factory systems

Growth and yield tables represent a simple-orientated guideline, valid as an average value of the observed silvicultural system proposed in a region giving raw estimates on timber an average cone production (not annual cone production). These tools do not consider stand heterogeneity, and are not sensitive to management, thus its validity for managing at block or forest scale is limited. Main current use is in forest planning, thus present simplified guidelines easily implemented, as well as for policy makers, given their large scale utility.

Stand and tree level models, as ORGEST_Pinea and PINEA2 are tools sensitive to silvicultural decisions, thus easily applied at block or forest scale. ORGEST is valid for any type of structure, since the model is calibrated for the whole set of typologies identified in Catalonia. On the contrary, PINEA2 is only valid for pure stands, though calibration for mixed stand is under development. PINEA2 allows annual estimation of cone production, thus useful for owners and forest managers, and it is sensitive to climate, while ORGEST_Pinea uniquely gives average output on cone production. PINEA2 is implemented in a stand level simulator, which give total flexibility for adapting any initial condition of the stand, which makes it compatible with NFI data. While being capable of simulating any different silvicultural schedules, the main limitation is that it requires the simulation of each block/stand within the forest. In the case of ORGEST_Pinea, uniquely a limited number of initial conditions and silvicultural schedules, defined according to forest main objective, are available, but permits to easily define a silvicultural orientation for each block on the stand. PINEA2 permits to estimate end-use of timber production, and can be adapted to include nut yield equations (Morales, 2009), thus can have a moderate interest for industrials.

Process based model PICUS_PINEA allows for annual estimates of cone, timber, biomass and CO_2 fixation at large spatial scales, and it is sensitive to management and climate scenarios. In this sense, it is useful for managing at regional and national scales, as required by policy makers. Its main limitations are linked with complexity of the interfaces, complex inputs and lack of accuracy on cone and timber estimates at small scales, which prevent its use by forest owners, planners and managers working at management unit – forest scale.

Finally, a model focusing on dynamic processes, as the multistage model for natural regeneration, allows identifying the factors governing the whole process and the main bottlenecks that prevent successful regeneration, and permit to estimate the probability of occurrence of established seedlings given a silvicultural schedule. In this regard, models for dynamic processes are useful tools for guiding forest managers and planners in identifying priority areas for regeneration, or proposing specific regeneration techniques. Table 2 show the adequacy of each model to the demands and requirements expressed by the type of potential end-user.

Table 2. Level of adequacy of the different models to each end-user group (ranging from XX minimum adequacy, to √√, maximum adequacy)

Model	Forest managers	Forest Planners	Owners	Nut industry	Policy makers
G&Y table	X	√	X	X X	√
ORGEST	√	√√	√	X	√ √
PINEA2	√ √	√	X	√	X
PICUS	√	X X	X X	X	√ √
PINEA Regeneration	√ √	√	X X	X X	X

IV – Conclusions

Our results confirm the considerable modelling effort carried out with the species *Pinus pinea* in the last 20 years, identifying more than 100 references in the scientific literature. Despite this main progress, several gaps in knowledge have been detected. We observed a geographical gap in the presence of models for the species in countries such as Turkey or Lebanon, where the large production of cone justifies the use of tools supporting management. Also, recent models for the species in European countries as France or Italy are missing. The lack of climate-sensitive growth and yield models, models focusing on processes as mortality or regeneration, as well as process-based and physiological-based models can be referenced as another main gap. In this sense, a main topic of further research should focus on study physiological traits beyond floral induction and cone phenology and development, as well as on the influence of water and nutrients availability in the allocation patterns for roots, leaves, wood and cones for the species.

While the modelling effort has increased in the last two decades, the use of models by final users has not experienced a significant advance. Although a single model cannot meet all the required demands and outputs of every group of users, there are enough models as for covering all these demands. Model users should define their demands, and search among the available models which ones are better suited for their requirements. Forest managers and planners demand growth and yield models, acting at stand or tree level, sensitive to management, climate and flexible to include any initial state, and implemented in friendly interfaces. Forest owners require very simple tables that provide accurate estimates of cone and timber production. Policy makers focus on regional and national estimates, sensitive to climate, which means that simple tables holding large geographical validity, as well as process-based models would meet their requirements. Finally, models focusing on nut yield and quality is a demand from nut industry still uncovered. In any case, apart from this end-user oriented modelling effort, it is important to work on knowledge transfer and continuous feedback between researchers and stakeholders, which should be carried out by means of technical reports in native languages, divulgation sheets, workshops and seminars, web tutorials...

On modellers' side, there is a need to consider the demands from potential users during the modelling design phase. The observed gap on knowledge transfer between modellers and end-users should be taken into account. Modelling design should consider what the expected use of the models is. As a general conclusion of this work, before building our new model, we need to carefully think of why to construct the model, and who is going to use it. Providing that there are still gaps in modelling activity, future models aimed at *Pinus pinea* forests, should be developed with that idea in mind.

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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).

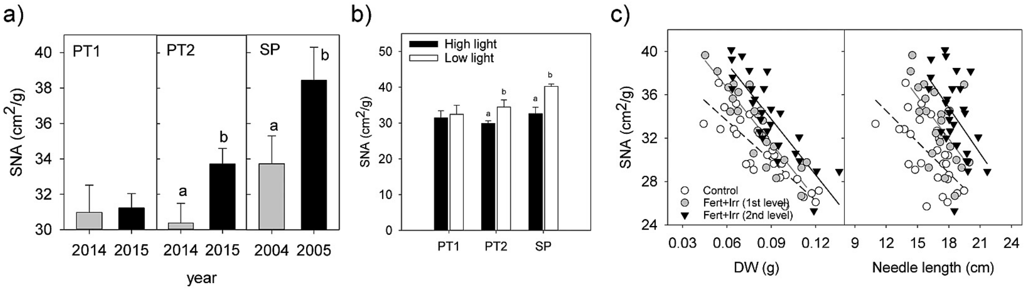

Fig. 1. (a) Average SNA in normal (grey bars) and dry hydrological years (black bars); (b) Average SNA (cm^2/g) for needles growing in low light (white bars) and high light conditions (black bars) in the crown; (c) coorelation between SNA (cm^2/g) and needle dry weight (DW, g) (left) and needle length (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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Pinus pinea above ground biomass estimation with very high spatial resolution satellite images

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Abstract. Above ground biomass is frequently estimated with forest inventory data and an extrapolation method for the per unit area evaluations. This procedure is labour demanding and costly. In this study above ground biomass functions, with crown horizontal projection as the independent variable, were developed. Multi-resolution segmentation method and object-oriented classification based on very high spatial resolution satellite images, were used to obtain the area of tree crown horizontal projection for *Pinus pinea* L. A set of inventory plots were measured and with existing allometric functions for this specie above ground biomass per tree and per plot were calculated. The two data sets were used to fit linear functions to estimate above ground biomass for individual plot and for their cumulative values. The results show a good performance of the models. Errors smaller than 10%, correspond to stand areas greater than 1.4 ha. These functions have the advantages of estimating above ground biomass for all the area under study or surveillance, not requiring forest inventory; allowing monitoring in short time periods and easily implemented in a geographical information system environment.

Keywords. Multi-resolution segmentation – Object-oriented classification – Vegetation mask – Crown horizontal projection – Regression – Biomass estimation.

I – Introduction

Pinus pinea L. is a native of the Mediterranean, occurring from Portugal to Syria (Correia and Oliveira, 1999). In Portugal it is present from North to South, preferring the climates with Atlantic and Mediterranean influence, especially the southern coastal areas. The stands have three main products, namely fruit, timber and resin, and their density varies according to the main production. Presently the first production has more interest due to higher income of the cones when compared with the latter two (*e. g.* Correia *et al.*, 2010; Mutke *et al.*, 2005a). This specie is shade intolerant, with a leader shoot apical dominance weaker than in other conifers which determines their umbrella shape crown (Mutke *et al.*, 2012) and is managed in open growth stands to promote crown growth as flower buds and, consequently, fruit production occurs mainly in the outer crown annual shoots (Mutke *et al.*, 2005b).

The Portuguese National Forest Inventory (IFN5, 2010) estimated an area of pure, dominant and young plantations of *Pinus pinea*, of 130,300 ha, corresponding to about 4% of the forest area, with about 73% of the stands situated in Alcácer do Sal region, southern coastal Portugal. Ground cover of pure stands is larger than 50% in 64% of the area and between 30% and 50% in 20% of the area. About 52% of the stands have areas larger than 10 ha, 34% between 2 ha and 10 ha and 14% between 0.5 and 2 ha. The total biomass (aerial and root) for the Alentejo region is 47.6 t/ha with an error of 20.3% for pure stands and 5.1 t/ha with an error larger than 40% for plantations (IFN5, 2010).

Biomass allometric functions at tree level have been developed for *Pinus pinea* for Portugal (Correia *et al.*, 2010; Correia *et al.*, 2008), Spain (Ruiz-Peinado *et al.*, 2011) and Italy (Cuttini *et al.*, 2013; Tabacchi *et al.*, 2011; Cutini *et al.*, 2009).

The purpose of this study is to provide a simple tool, to assist stakeholders, researches and forest technicians, to quantify above ground biomass, regardless of the existence of inventory data, which can be applied both at small and large scales. The objective is the development of allometric functions to estimate above ground biomass as a function of crown horizontal projection per plot and their cumulative values using very high spatial resolution satellite image data for *Pinus pinea* monospecies stands.

II – Materials and methods

The study area, ca. 35 km^2, is located in coastal southern Portugal between Alcácer do Sal and Setúbal (geographical central coordinate of 8° 40' 28.20''W and 38° 27' 45.71''N). The region is characterized by a Mediterranean climate, and is mainly occupied by *Pinus pinea* and *Quercus suber*, in both pure and mixed stands.

A WorldView2 image (June, 2011) with a spatial resolution of 0.5 m was acquired for this study.

The image is composed by four multispectral bands, three in the visible region (blue, green and red) and one in the infrared region (near-infrared). Ground control points (17 points, evenly distributed by the image) obtained with Global Navigation Satellite System (GNSS) and geodetic vertices, identified on the ground and in the image were used to orthorectified and geometrically correct the image, with ENVI 4.8 (ENVI, 2009). The Root Mean Square Error (RMSE) of the geometric correction was 0.30 m. The radiometric correction was carried out with the dark object subtraction method (Chavez Jr, 1988) with the conversion of the images digital numbers (DN) to Top of Atmosphere (ToA) reflectance and to soil reflectance through the atmospheric correction.

Multi-resolution segmentation method with the Contrast split segmentation algorithm applied in *eCognition* software, version 8.0.1 (Definiens Imaging, 2010), which used as an auxiliary band the Normalized Difference Vegetation Index (NDVI) generated a vegetation mask. The vegetation mask was composed by objects that delimit the tree crowns and separates them from the other land uses. The object-oriented classification with nearest neighbour method was applied to separate the forest species (Sousa *et al*., 2015; Sousa *et al.*, 2010), which in this study were *Pinus pinea*, *Pinus pinaster* and *Quercus suber*.

An area of 2885 ha was selected from the satellite image for the analysis. A square grid of 45 m x 45 m (2025 m^2) was overlaid to the area. The crown horizontal projection of each grid (CHP_{ps}) was calculated using ArcCatalog and ArcMap software version 10, (ESRI, 2010). Grids were classified according to forest species composition and ground cover (GC_s, defined as the percentage of area occupied by the crown horizontal projection of the trees in relation to the grid area). A grid was considered *Pinus pinea* monospecies when all the individuals were of this specie.

The design of the forest inventory was a random stratified sampling by proportional allocation, with strata defined as function of ground cover, namely 10-30%, 30-50% and >50%.The dataset of the forest inventory is composed of 33 monospecies plots of *Pinus pinea*, 5 in the first stratum, 18 in the second and 10 in the third, with a sampled area of 6.7 ha. In each plot, for all the trees with diameter at breast height larger than 5 cm, this measure, total height and crown radii (North, South, East and West directions) were measured (Avery and Burkhart, 1994). The trees geographical location was recorded by a. Tree crown horizontal projection (CHP_t) was calculated as a circle. Its radius is the arithmetic mean of the crown radii measured. The plot crown horizontal projection (CHP_p) has the sum of CHP_t).The tree above ground biomass (W) was calculated using the allometric functions of Correia *et al.* (2008), as the sum of wood (*ww*), branches (*wbr*), leaves (*wl*) and bark (*wb*) biomass ($W = ww + wbr + wl + wb$).

The statistical analysis was based on a correlation analysis with the Spearman test, as normality assumption could not be met (evaluated with Shapiro-Wilk test), and on the linear regression. It was assumed that a null value of above ground biomass corresponds to a null value of crown horizontal projection ($W = \beta x$, where β is the slope). The linear functions were was fitted with ordinary least square linear regression method with the crown horizontal projection calculated from satellite image data as independent variable and above ground biomass estimated from the forest inventory as dependent variable, for their individual plot and cumulative plot values. The allometric functions goodness-of-fit were evaluated with the sum of squares of the residuals (SQR), the coefficient of determination (R^2), the adjusted coefficient of determination (R^2_{aj}). It is recommended that validation is done with an independent set of data. When that is not possible Paulo *et al*. (2015), Myers (1986) and Clutter *et al*. (1983), suggest using predicted residual error. The sum of its square values (PRESS), and the sum of its absolute values (APRESS) as well as their average values (PRESSm and APRESSm), were used as the validation test. The closer to the null value of residuals, the better is the model. Error was evaluated by $error_i$ (%) = $((\hat{y}_i - y_i)/y_i)$ x 100 (where $\hat{y}_i$ and y_i are the estimated and calculated above ground biomass, respectively). Statistical analysis was implemented in R statistical software (R Development Core Team, 2012).

III – Results and discussion

The multi-resolution segmentation and the object oriented classification processes resulted in a vegetation mask with high accuracy (Fig. 1). The agreement between the classification by forest species and the ground truth, evaluated with the Kappa statistics (Congalton *et al*., 1983), was 79% while the global precision was 90%.

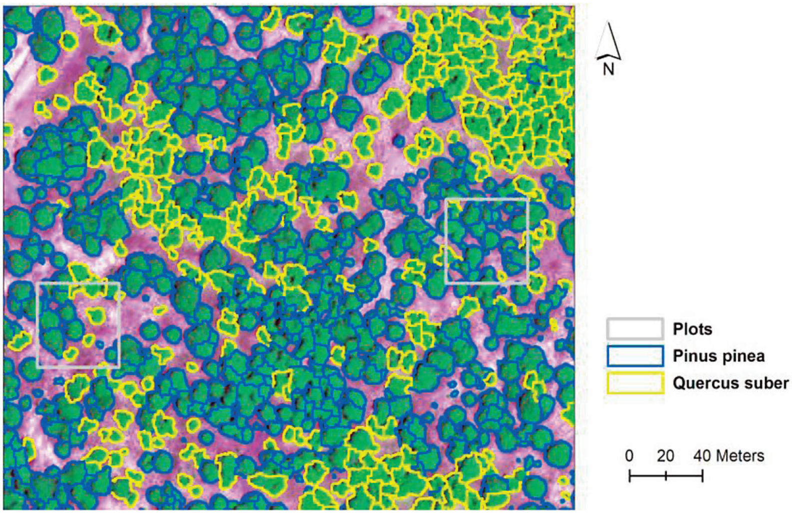

Fig. 1. Illustration of the vegetation mask by forest species over a WorldView-2 image with false colour composite (RGB = red, NIR and blue) and two inventory plots.

The plots have a mean number of trees per hectare of 71 (SD = 23), a mean Chp_{ps} of 885.3 m^2 (SD = 355.1) and a mean W_p of 11792.6 kg (SD = 4200.5). The Spearman's correlation coefficients show that there are strong positive correlations between W and CHP_t (0.851), W_p and CHP_p (0.724), and W_p and CHP_{ps} (0.651). Relation between W_p and CHP_{ps} reflects, at least partially, the inclusion of small patches of soil pixels between tree crowns and also the inclusion of mixed pixels, soil/vegetation and shadow/vegetation in the vegetation mask (Ke and Quackenbush, 2011), which originate a higher area of crown horizontal projection obtained from satellite image when compared with the one of inventory data.

The fitted functions of above ground biomass per plot (W_{ps}) and the cumulative above ground biomass per plot (W_{psc}) have statistical properties that are indicative of their good performance (Table 1), denoted by the large R^2_{aj} and close to zero validation statistics. The variability of the relation of crown horizontal projection and above ground biomass per plot (Fig. 2a) is rather large. This is probably due to *Pinus pinea* growth habit and the competitive relations with their neighbours. Considering for example seven plots with above ground biomass between 40-50 t, the number of trees per hectare varies between 54 and 99 trees/ha, the basal area per hectare between 7.0 m^2 and 8.6 m^2 and ground cover between 17.2% and 48.0%. Spatial distribution pattern in each plot can partially explain the variation in the relation between above ground biomass and crown horizontal projection. It is in the plots where the trees have the crowns touching their neighbours that the relation between CHP and W is smaller while the plots with most of the trees in free growth the inverse occurs. In fact this specie is one of the few pines with weak epinastic control that develops quite wide crowns (Mutke *et al*., 2012). Adult trees can reach 16-20 m of crown diameter. In our study the wider crowns have 15 m of crown diameter. The variation between above ground biomass and crown horizontal projection is related to the available aerial growing space. The trees surrounded by neighbours tend to develop competitive relations, in which high or low shade and branch abrasion, as referred by Oliver and Larson (1996), are the main driving factors. This is especially noticeable in shade intolerant species like *Pinus pinea*, which tend to show crown shyness thus inverting the relation between wood and branch biomass. In opposition, when developing in open growth tend to expand widely their crowns resulting in a higher proportion of branch biomass.

Table 1. Statistical properties of the allometric functions

Allometric function	SQR	R^2	R^2_{aj}	PRESS	APRESS	PRESSm	APRESSm
$W_{pss} = 12.4076 \times CHP_{pss}$	551305965	0.893	0.890	0.000000239	0.0023942	0.000000007	0.000073
$W_{pscs} = 14.1471 \times CHP_{pscs}$	7477064463	0.995	0.995	0.000000006	0.0003334	0.0000000002	0.000010

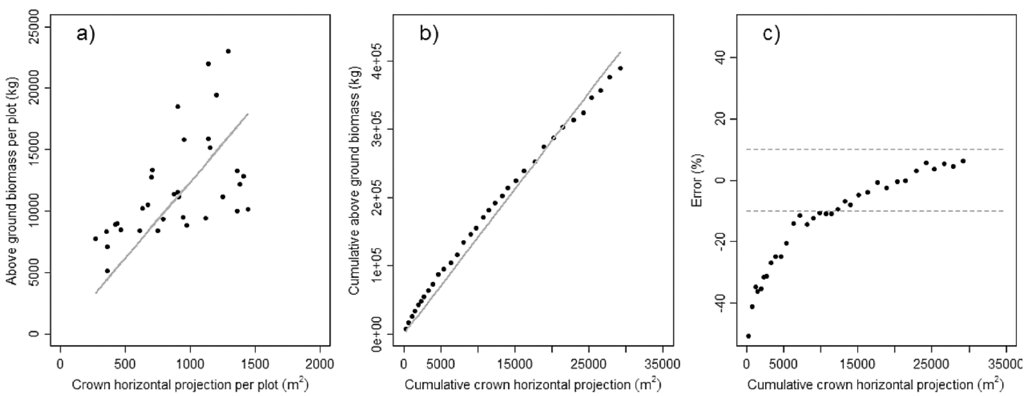

Fig. 2. (a) Crown horizontal projection per tree *vs* above ground biomass (W_{ps}); (b) cumulative crown horizontal projection *vs* cumulative above ground biomass (W_{psc}); and (c) error.

The overall error is 15.4% for W_{ps}, smaller than that of the National Portuguese Forest Inventory (IFN5, 2010). Figure 3 illustrates the estimation of above ground biomass with W_{ps}. The cumulative above ground biomass estimated with Correia *et al*. (2008) functions and the estimated with W_{psc} show some deviation, both negative and positive (Fig. 2b) and errors >20% up to 5000 m^2 of cumulative crown horizontal projection areas (Fig. 2c). The error decreases up to 25000 m^2 stabilising afterwards, and is smaller than 10% for crown projection areas larger than 10000 m^2. Considering the mean crown horizontal projection area obtained from satellite image data (885.3 m^2) and the mean above ground biomass per plot (11792.6 kg), to an error of 10 %, correspond stand areas of 1.4 ha. Noteworthy is that these stand areas cover more than 86% of the stands of this specie.

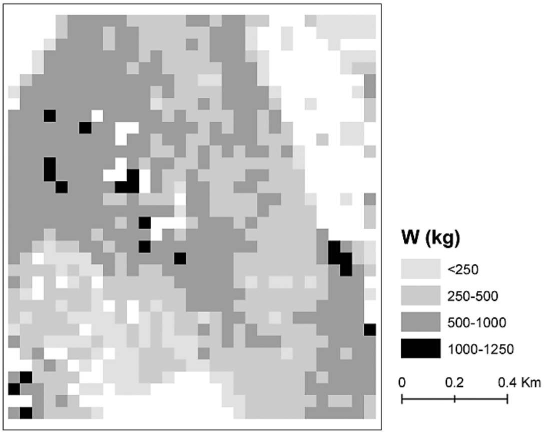

Fig. 3. Illustration of above ground estimation with W_{ps}.

IV – Conclusions

Satellite data with very high spatial resolution images enables above ground biomass estimation, since a vegetation mask per forest species can be obtained with accuracy. Above ground biomass estimation with allometric functions, with crown horizontal projection as the independent variable, can be used for *Pinus pinea* monospecies stand areas equal to or larger than 1.4 ha for an error equal to or smaller than 10%. When compared with the estimation with forest inventory data and an extrapolation method for the per unit area evaluations, three main advantages can be pointed out: all the area can be evaluated without extrapolation; it allows short time periods monitoring; and can be easily implemented in a geographical information system environment.

Nonetheless some limitations can be pointed out. The date of the images acquisition is of the utmost importance. For the Mediterranean region it is during the dry season (June to September) that the higher contrasts between the tree crowns and the understory vegetation are attained, enabling to derive the vegetation mask, by forest species, with high accuracy. Another limitation is that the scenes of WordlView-2 have a swath of 16.4 km at nadir which makes it difficult to evaluate large areas as several scenes have to be worked.

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Estimation of productive areas of stone pine cone in Portugal with geostatistical tools

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Abstract. This work follows previous field studies aiming to define five different stone pine (*Pinus pinea* L.) tree developmental stages, of which three corresponded to cone production stages. Based on collected data of cone production of stone pine stands in 40 georeferenced plots and 330 trees in three production campaigns (2004/05, 2005/06 e 2006/07), geostatistical methods of semivariogram and kriging were used to assess the areas of cone productive classes. The study area is located in the region of Setúbal Peninsula in South-Western Portugal, the denominated Provenance Region V, which is the area with the highest cone production in the country. A simple kriging model with a detrended exponential semivariogram was selected and the results of cone productive classes 1 (299-658 kg.ha^{-1}), 2 (122-298 kg.ha^{-1}), and 3 (lower than 112 kg.ha^{-1}), have a potential extent of 345,056 ha, 189,571 ha, and 148,275 ha, respectively. Considering the actual stone pine area the estimated areas of the three productive classes were 23,196 ha, 29,329 ha and 3559 ha for classes 1, 2 and 3, respectively. Further application of geostatistical methodology can be used to evaluate the potential of expansion of stone pine in this region on areas with other land cover types considering cone and/or kernel productive classes.

Keywords. Kriging – Semivariogram – Stone pine – Cone Productive areas.

Résumé. *Ce travail suit des études visant à définir cinq stades de développement d'arbres de pin parasol (*Pinus pinea *L.), dont trois correspondaient à étapes de la production de cônes. Les données de terrain ont été collectées en 40 placettes géoréférencées avec 330 arbres, pendant trois campagnes de production (2004/05, 2005/06 e 2006/07). L'évaluation des zones de classes productives de cône a été exécuté avec les méthodes géostatistiques de semivariogramme et krigeage. Les placettes ont été installées en peuplements de pin parasol dans la région de la péninsule de Setúbal au sudouest du Portugal, la Région de Provenance V, qui est la productrice plus importante de cônes du pays. Un modèle de krigeage simple, avec une semivariogramme exponentielle et retrait de tendance a été sélectionné, et les résultats des classes productives de cône : 1 (299-658 kg.ha^{-1}), 2 (122-298 kg.ha^{-1}), et 3 (inférieure à 112 kg.ha^{-1}), ont donné des zones d'occupation potentiel de 345 056 ha, 189 571 ha, et 148 275 ha, respectivement. Compte tenu de la surface effective de pin parasol, les zones estimées des trois classes productives sont 23 196 ha, 29 329 ha et 3559 ha pour les classes 1, 2 et 3 respectivement. L'application de la méthodologie géostatistique peut être également utilisée pour évaluer le potentiel d'expansion du pin parasol dans des zones avec d'autres types d'occupation du sol dans cette région, envisageant les classes productives de cône et /ou de pignon.*

Mots-clés. *Krigeage – Semivariogramme – Pin parasol – Zones productives de cône.*

I – Introduction

The area of stone pine (*Pinus pinea* L.) in Portugal has increased 46% since 1995, corresponding currently to about 6% (about 176,000 ha) of the total forest area (ICNF, 2015) and ranking as the fifth species in occupied area. The main source of income for stone pine producers is kernel production for food industry, but environmental benefits in terms of sand dune fixation, soil protection and improvement of degraded ecosystems should also be envisaged. The cone production in Portugal is ca. 65 millions of cones corresponding to about 600 to 700 tons of kernel, mostly aimed at exportation.

This work follows a study that defined five tree developmental stages, of which three corresponded to cone production stages (Carrasquinho *et al.*, 2010), in stone pine stands installed in the Provenance Region V located in South-western Portugal (Cardoso and Lobo, 2001). A complementary work of evaluation of the areas of these productive classes is thereby required. Modeling cone production as a function of stone pine tree biometric variables showed the relationships among these variables (e.g., Calama and Montero, 2005; Freire, 2009; Rodrigues *et al.*, 2014) and the need of evaluation of their spatial variability. Geostatistical tools, such as the ones implemented in Geographical Information Systems (e.g., Geostatistical Analyst tool ArcGis 10.2.2). Geographical Information Systems (GIS) for quantifying spatial variation of clustered data variables, can be used in forest applications. Within this context, this work intended to quantify areas for three cone productive classes, in Provenance Region V, through a geostatistical interpolation of cone production data obtained in field plots. That information should allow for a detailed assessment of the productive potential of the regions abridged by the study.

II – Materials and methods

1. Data collection in the plots

Cone production was obtained from 40 circular georreferenced plots with 330 trees, installed in 2004 and 2005, in eight counties on Provenance Region V, with data production collected in the 2004/05, 2005/06 and 2006/07 campaigns. The data used in this study were the cone production per plot, obtained from the sum of the productions available for each tree in the three campaigns, as it was not possible to obtain production data for the three campaigns from some trees. The productive classes were defined with average productions per ha in the ranges of: lower than 112 $kg.ha^{-1}$ for class 1, 122-298 $kg.ha^{-1}$ for class 2, and 299-658 $kg.ha^{-1}$ for class 3, respectively. The eight counties where plots were located in Chamusca, Coruche, Vendas Novas, Montemor-o-Novo, Setúbal, Alcácer do Sal, Grândola and Santiago do Cacém, respectively (Fig. 1). These counties corresponded to a total area of 682,902 ha. To obtain digital information concerning the area of distribution of stone pine in Portugal, Land Use and Land Cover Map of Portugal mainland 2007 (COS 2007) (DGT, 2007) was used. This digital chart is based on the interpretation of orthorectified aerial photos and also in a multi series satellite imagery enabling a better identification of the vegetation phenology and of the soil occupation. The minimal cartographic unit is 1 ha with a minimum 20 m distance between lines; consequently the grain of analysis, a pixel of 20m width, was used.

2. Data treatment

The productive area estimations were performed using the geostatistic extension implemented in the Geostatistical Tool in the ArcGis package 10.2.2 (Esri Inc.) Geostatistics are based on the concepts of kriging and semivariogam. Kriging is the ultimate spatial linear interpolating model of a spatial random variable $Z(s)$: s D R^2 of the general form (Schabenberger and Pierce, 2002; Gonçalves, 2015):

$$Z(s) = \mu(s) + \delta(s) \qquad \text{(Equation 1)}$$

where $\mu(s)$ is the mean of the random field and is its variance. The variance is evaluated by the semivariogram $\gamma(h)$, h being the Euclidean space between two points, which in its general form is given by:

$$\gamma(h) = ½\, Var\, [\, Z(s) – Z\, (s+h)] \qquad \text{(Equation 2)}$$

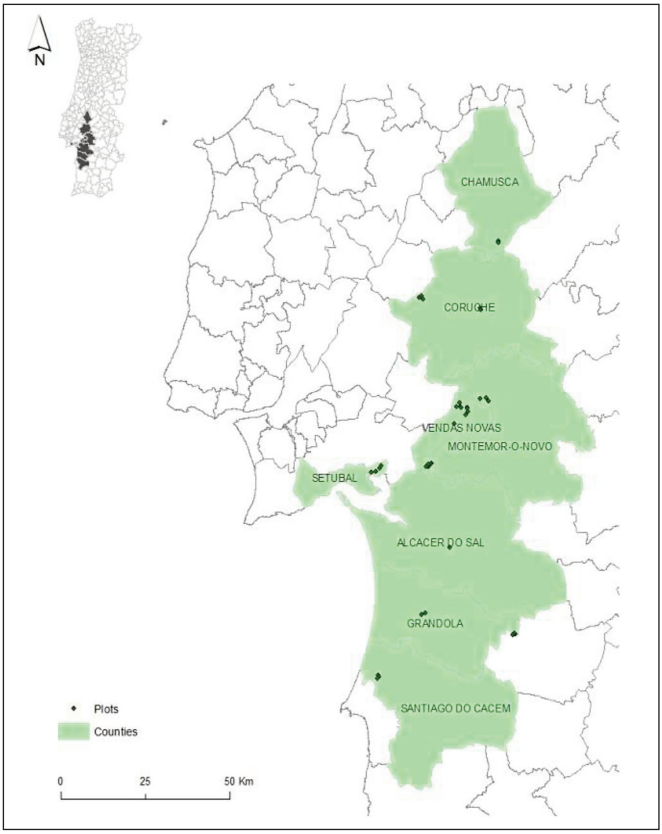

Fig. 1. Counties of stone pine locations.

The semivariogram is thereby representative of the spatial correlation of values of a variable *Z(s)* in the points s and (s+h) and can be modeled by several kinds of equations such as spherical, exponential or Gaussian. The common applications of the semivariogram rest on assumptions of intrinsic or second order stationarity and isotropy. A theoretical configuration of the semivariogram is shown in Fig. 2. It can be noticed that the semivariogram converges monotonically to an asymptote (sill) at a given lag distance (pratical range) representative of the distance from the first point.

In real conditions a discontinuity at the origin, the so-called nugget effect, can exist, due for example to measurement error and the convergence occurs on a partial sill (Fig. 3).

In this study we considered the geostatistical interpolation by simple, ordinary and universal krigings. General equation 1 allows to define simple kriging if the mean of equation $\mu(s)$ is known, ordinary kriging if the mean is unknown, or universal kriging if the mean is given by an expression like:

$$\mu(s) = x'(s)\beta \qquad \text{(Equation 3)}$$

with β unknown. The kriging predictors of $Z(s)$ with a general form $p(Z\ s_o)$ are estimated as a general sum:

$$p(Z;\ s_o) = \lambda' Z(s) \qquad \text{(Equation 4)}$$

where λ is the vector of kriging weights, representative of the contribution of each of the measured points to the estimated spatial variation. The predictors are calculated in order to minimize parameters error parameters such as the mean prediction error:

$$\frac{Z(s_o) - \sum_{i=1}^{n} \lambda(s_t) Z(s_t)}{n} \quad \text{(Equation 5)}$$

where n is the number of plots. Other statistics for kriging error evaluation are the mean prediction error, the average standard error, the root mean square error and the standardized root mean square (Johnson *et al*., 2001). A good spatial model requires that the mean prediction error values should be close to zero, root mean square values as lower as possible, average standard errors close to and root mean square and standardized errors close to 1. Detrending and kernel treatment of semivariograms were also applied in this study. Indeed experimental semivariograms can follow a curve pattern distinct from the Fig. 2 and Fig. 3 above, for example as smooth concave curves that approach the origin with decreasing gradients or that increase sharply after reaching the sill. These patterns are indicative of a global trend or rift which is imposed on the short range variation in the spatial variable. The global trend is an overriding process that affects the measurements on a deterministic way. The superficial trend can be represented by an equation (e.g., a polynomial) removed to pay attention to the stochastic spatial structure submitted to kriging processes and replaced before final predictions are made. Experimental semivariograms can also present some high frequency white noise fluctuations, which can be smoothed through convolution with the so-called kernel functions (e.g., exponential, Gaussian or spherical).

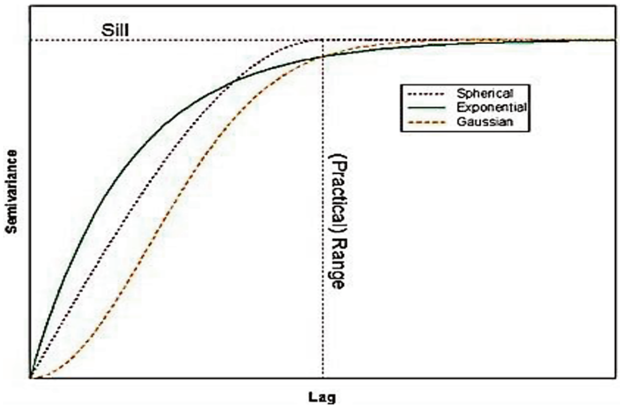

Fig. 2. Theoretical configuration of a semivariogram (adapted from Johnston, 2001).

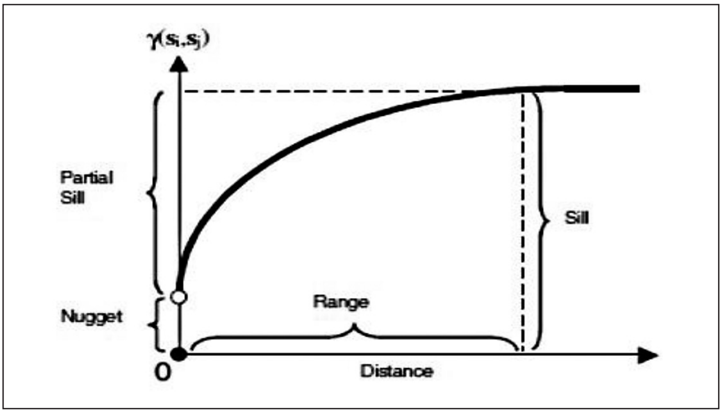

Fig. 3. Semivariogram with a nugget and partial sill (adapted from Bohlig, 2001).

Following the mentioned geostatistical principles, 24 kinds of kriging were applied to evaluated areas for cone production, differing about the type of kriging (simple, ordinary or universal) semivariogram (spherical, exponential or Gaussian), kernel and trend removal.

III – Results and discussion

The spatial model chosen, which optimized the four error criteria aforementioned, was a simple kriging with an exponential semivariogram, a lag size of 11.86m, a second order polynomial trend function and an exponential kernel function (Fig. 4). The exponential function provided a convenient

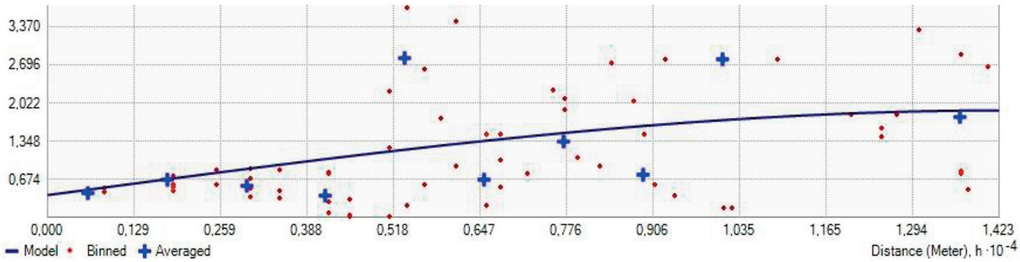

Fig. 4. Semivariogram chosen for the modeling of spatial variability of cone production.

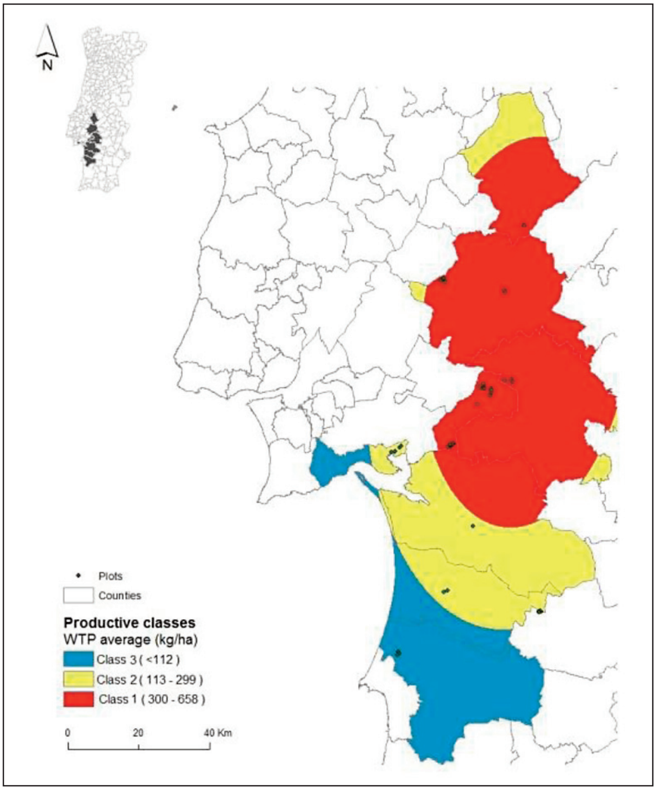

Fig. 5. Kriging results for the three cone productive classes.

smoothing of point autocorrelation. The values of mean prediction error root mean square error, average standard error and standardized root mean square were -0.61 kg, 66.57 kg, 2.3 kg and 0.94 respectively. In the total 682,902 ha counties' area, the results from kriging of cone productive classes 1 (299-658 kg.ha^{-1}), 2 (122-298 kg.ha^{-1}), and 3 (lower than 112 kg.ha^{-1}), gave potential occupation areas of 345,056 ha, 189,571 ha, and 148,275 ha, respectively (Fig. 5).

The magnitude of these areas is indicative of the potential of this region for stone pine cultivation. After intercepting these kriging results with the actual pine stone areas in the 8 counties, using COS 2007, the areas for productive classes 1, 2, and 3 were 23,196, 29,329 and 3558 ha, respectively (Fig. 6). The red (dark) area concerning to higher production (1), corresponded to transect of Chamusca, Coruche, Vendas Novas Novas, Montemor o Novo and Alcácer do Sal. The blue (grey) area concerning the lower productive class (3) was located mainly in Santiago do Cacém County. The yellow (brighyt) area concerning productive class 2 was located in Grândola, Northern Alcácer and Southern Chamusca. The digital chart COS 2007 was therefore a valuable tool to overlap and compare the theoretical kriging results with the actual soil occupation.

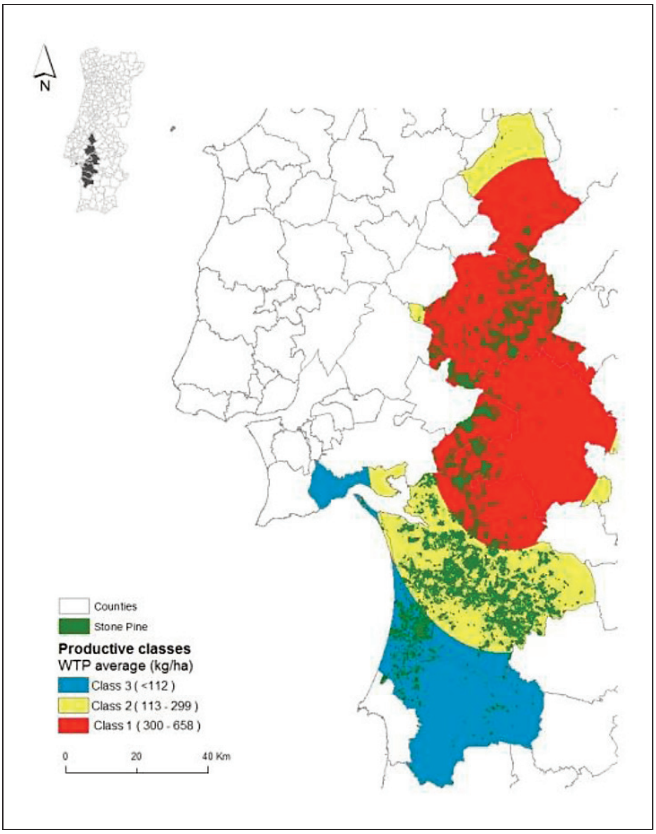

Fig. 6. Actual predicted areas of distribution the three cone productive classes.

IV – Conclusions

This study allowed a prediction of the areas of three productive classes of stone pine in the Provenance Region V, respectively 23,196, 29,329 and 3558 ha, for cone productive classes 1, 2 and 3, located in a continuous longitudinal transect from Chamusca to Alcácer do Sal. The results also showed the potential of geostatistical tools, integrated with GIS, for evaluating of forest biometrical and environmental variables, such as soil and topography. In particular, for stone pine, two interesting field studies that can be envisaged with these tools are the variability of kernel production with biometrical, soil and climate variables and also the potential expansion of stone pine to areas actually occupied with maritime pine or grassland. With these studies an improved validation of the geostatistical predictions should also be achieved.

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Reproductive phenology of *Pinus pinea*

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Abstract. Stone pine (*Pinus pinea*) is one important species for pine nut production worldwide, assuming an extreme economic, cultural and environmental importance in the Mediterranean Basin due to pine nut production. In Portugal, 70% of the national production comes from Alentejo region, where edafoclimatic conditions are propitious to high productivity and quality, rendering high economy revenue. Stone pine has a peculiar reproductive cycle, with approximately 3 years from bud differentiation to maturation. Recent reports point to a decrease in cone production and pine nut productivity, which could be associated with damages in specific phases of stone pine reproductive phenology. During two consecutive years, reproductive phenology was monitored in three plots, one located in Alcácer do Sal and two in Coruche. Observations and images were acquired over two growing periods, from beginning of March 2012 to end of August 2013. The periodicity of the observations ranged between one and three weeks depending on the time of the year. Male phenology was described in 3 main phases and female phenology was described in 7. The knowledge of reproductive phenology is important for relating production and productivity with climatic data or even in a major scale with climatic changes. Through plant phenological stages it is possible to assess damage that may be caused by insects that compromise the pine nut production.

Keywords. Stone pine – Phenological stages.

I – Introduction

Stone pine (*Pinus pinea*) is one important *Pinus* species worldwide, assuming an extreme economic, cultural and environmental importance in the Mediterranean Basin (Costa *et al.*, 2008). Pine nut production represents the most valuable and profitable activity for stone pine forests in Spain, Portugal, Italy and Turkey, where pine nuts have high commercial value (Fady *et al.*, 2004; Martinez *et al.*, 2004). Half of the Portuguese cone production comes from the Setúbal district, where edaphoclimatic conditions are propitious to high pine nut productivity and quality, rendering high economic income (Evaristo *et al.*, 2002; Costa *et al.*, 2008).

Recent reports from the main producers and industries (Pimpão, 2014) point to a decrease in cone production and pine nut productivity in the latest years, reflected by a huge number of aborted flowers and cones, leading to a production decrease, and an abnormal increase of pine cones with empty seeds, responsible for the productivity decrease. Reproductive development is one of the most important stages of a plant life cycle. Considering the kind of damages reported, it is evident that not only one reproductive stage is being affected. The absence of previous studies for stone pine in Portugal, though not in other countries (Italy-Francini, 1958; Spain-Abellanas and Pardos, 1989; Mutke *et al*., 2003), alerts to the importance of phenological and ontogenetic studies on this species, in order to associate the damages to biotic or abiotic factors. It is also very important to understand the type of damage infringed and the causal agent associated. Similar reports from Italy, Spain and Turkey refer that productivity decrease is essentially related following the introduction of an alien pest, the western conifer seed bug *Leptoglossus occidentalis* (Roversi *et al*., 2011; Bracalini *et al*., 2013).

Our objective is to contribute for a better understanding on the anatomy, morphology and timing of male/female reproductive phases which is intended to fill fundamental knowledge gaps about the impacts of biotic and abiotic factors and their interactions with the stone pine reproductive cycle.

II – Materials and methods

1. Phenology

This study was performed in three pure stone pine plots, one located in Alcácer do Sal (Portugal, 38º23'22.6" N, 8º29'22.6"W; 64 m a.s.l.) and two located in Coruche (Plot 1 - 38°55'37.1"N, 8° 31' 25.4"W; 20 m a.s.l.; Plot 2 - 38°55'34.5"N, 8° 31' 25.6"W; 19 m a.s.l.). Phenological observations were conducted during 2 consecutive years, since March 2012 to end of August 2013, where a group of 70 trees were monitored with a frequency between 1 and 3 weeks, depending on the time of the year. The plot Alcácer do Sal had 30 trees and the 2 plots in Coruche had 40 trees (20 trees/plot). Trees were 12 years old in all plots, with a planting distance of 5 m × 5 m, where the selected trees are grafted. The tree diameters and heights were about 25 cm and 7 m, respectively. The phenological monitoring was performed in two layers: on the top of the crown, one branch was selected in 4 positions (N, S, W, E) for female structures observations and data collection; in the central zone other 4 branches for the same positions were selected for male structures. For each tree the most representative phenological state was considered in relation to the 4 positions. In field, images were acquired with a Canon® SX 30 IS digital camera. Some samples were brought to the laboratory and the detailed photos were taken with a Leica® DMS 1000 digital microscope with image acquisition software LAS V4.4.

2. Ontogeny

Samples of reproductive structures from the different phenological stages were fixed, during at least 48 hours, in FAA 1:1:18 - formalin: acetic acid: ethanol (70%) at 4°C (Johansen, 1940; Ruzin, 1999). Dehydration was achieved through progressive ethanol/water series. Samples were included in paraffin, 10µm thin sectioned using a rotary microtome (Leica RM2255) and were stained with Heidenheim Hematoxylin. Digital images of the histological sections were obtained with a ProgRes® CapturePro 2.8 - JENOPTIK Optical Systems coupled to an Olympus BX41 microscope, and were electronically processed using the software ProgRes® Systeme - JENOPTIK Optical Systems.

III – Results and discussion

1. Phenology

Male phenology is described by 3 main phases (Fig. 1): **M1** - Male strobili visible but without apparent pollen production; **M2** - Male strobili are yellow and pollen dehiscence occurs. The branches produce a notable pollen cloud when moved; **M3** - Most pollen has been released. Male strobili are dark brown and the fall is eminent.

Female phenology was described by 7 main phases (Fig. 2): **F1** - Female cones are evident but not fully developed; **F2** - Female cones are fully developed. Cones have a bright yellow/green colour. Receptivity; **F3** - Seed cones showing thickened scales sealing the cone; **F4** - Female strobili lignify and grows slightly, about 1 cm diameter the 1st, 2 cm the 2nd year (quiescent stage); **F5** - Growth restart. The scales junction area becomes greenish as a result of the activity resumption; **F6** - Fully developed, green cone, commercially mature; **F7** - Mature, brown cone opening in spring.

A chronogram for stone pine reproductive phenology was established after 2 years of observations (Fig. 3). The phenological behavior was homogeneous both within and between plots, as well between the two consecutive years. From mid-April to end of May, male conelets are dehiscent and female conelets are synchronously receptive in the first year of development, being the pollination the main event. In the second year, the female reproductive structure remains nearly quiescent, the conelets just increasing in size. Only in the third year the structure resume the development and the fecundation occurs. Cone maturation takes place during October and November (Fig. 3).

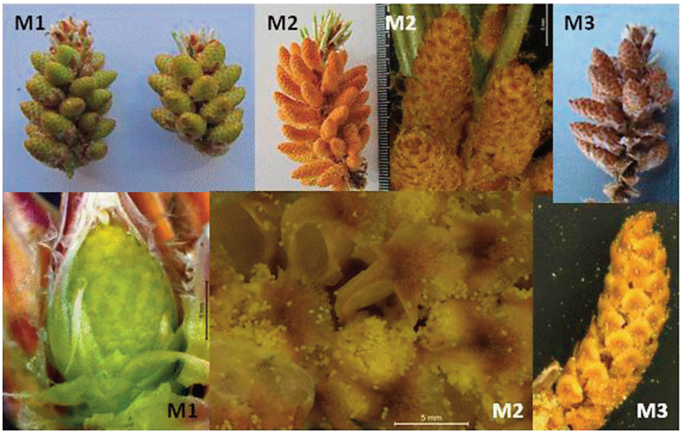

Fig. 1. Male phenological phases.

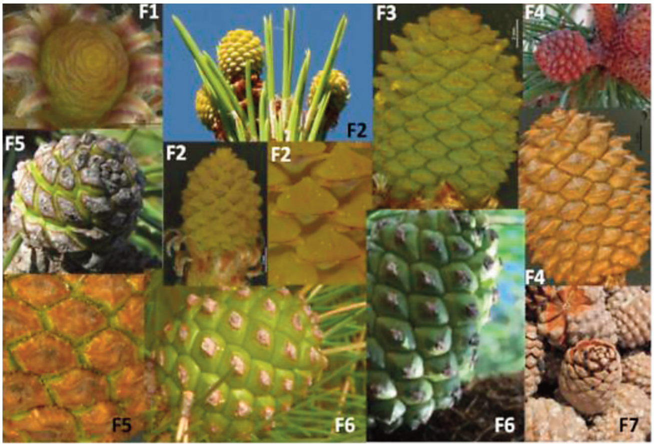

Fig. 2. Female phenological phases.

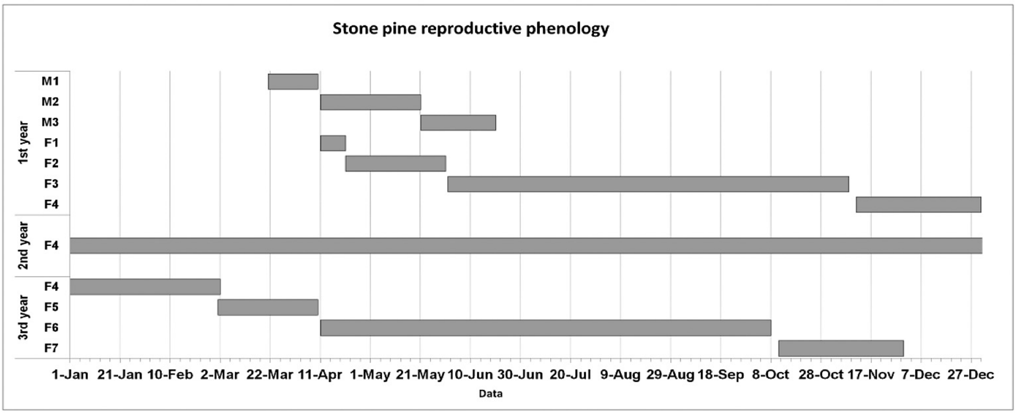

Fig. 3. Chronology of male and female reproductive structures evolution for the 3 years of development. M1 to M3 male structures. F1 to F7 female structures. Adjusted values for 2012-2013.

2. Ontogeny

Histological studies reveal the main internal sequential events of female reproductive structures development (Fig.4). **F1** - Beginning of scales formation; **F2** - Cone scales are separated, primordia of ovule differentiation are visible and structure is ready for pollination; **F3** - Cone scales become imbricated and ovule development continue. **F4** - Quiescence, the longest stage, when few morphological or anatomical changes in the ovules are observed; **F5** - Female gametophyte develops and the entire reproductive structure grows; **F6** - Ovule is ready for fecundation. After this stage it was not possible to process with histological observations due to the hardness of pine nut shell.

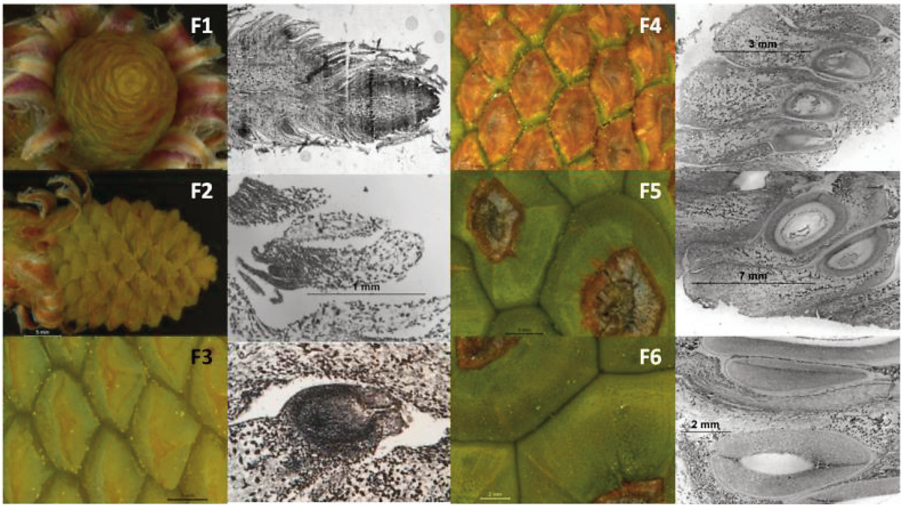

Fig. 4. Female phenological stages and correspondent longitudinal histological sections.

No major differences were observed between the phenology in these two years in the main area of pine nut production in Portugal and the data obtained by Abellanas and Pardos (1989) and Montero *et al.* (2004) in Spain.

Considering phenological and ontogenetic studies in other pine species, such as *Pinus contorta* Dougl. (Owens, 2006) and *Pinus monticola* Dougl. (Owens, 2004), *Pinus pinea* needs one year more than most pine species, implying longer vulnerability of reproductive structures to biotic and abiotic factors.

Adverse weather conditions during pollen release (M2) can compromise pollination, causing a production decrease. However, considering the recent producer reports on the production and productivity decrease, female reproductive structures are the most sensitive. In the initial female flowering phases (F1 to F3), the effect of biotic factors may void the structure development, leading to early death and directly affecting the production. If this damage is caused in a more advanced phenological stage (F4 to F6), where pollination has already occurred and megagametophyte is developing, ovules abortion may occur, causing a productivity decrease (high number of empty nuts).

IV – Conclusions

With these results it was possible to adapt the timing of stone pine reproductive phenology to Portuguese climate conditions (Fig. 5). We have represented five years instead of three, where year 0 corresponds to reproductive structures differentiation; year 1 to reproductive organ formation and pollination; year 2 represents the quiescent stage; in year 3 occurs the fecundation and cone maturation; the year 4 corresponds to the natural dehiscence. Despite this last year being part of the natural cycle, it is eliminated by commercial cones collection at maturity in winter, before dehiscence.

The establishment of a reproductive phenological model is an important tool for: (i) breeding as a base for performing controlled crosses; (ii) climate changes due to phenology being a fingerprint of climate; (iii) pollination efficiency between clones through flowering synchronization; (iv) modeling and prediction of production to improve the producers ability to plan management practices; and (v) relation with pests and diseases assessing damages in reproductive structures and contributing to identify the causes of pine nut production and productivity losses.

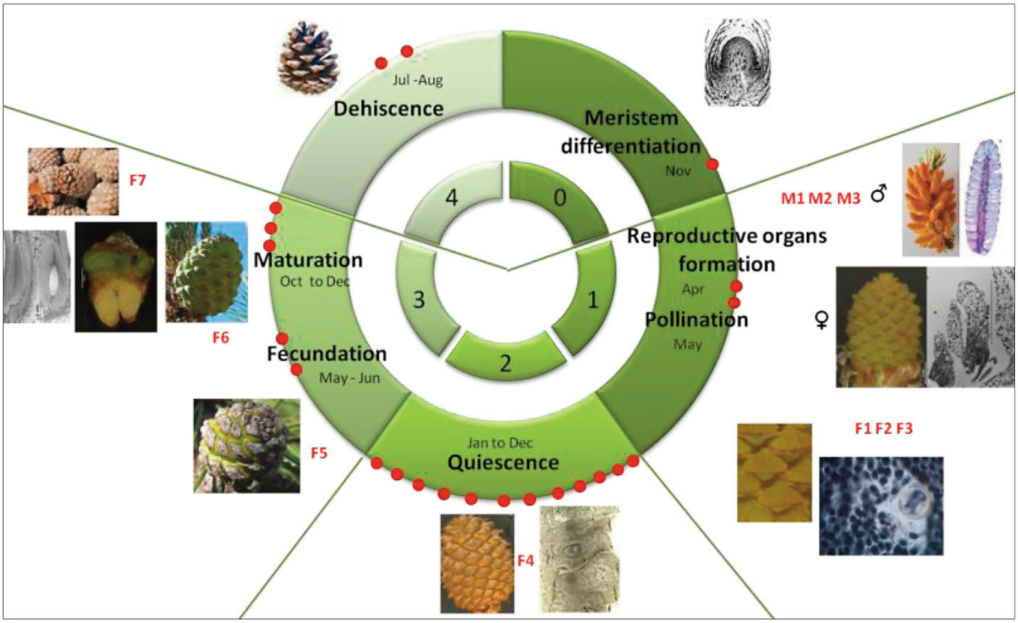

Fig. 5. Stone pine reproductive phenological model.

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Session 3

Genetics

Selection and identification of Spanish elite clones for Mediterranean pine nut as orchard crop

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Abstract. Since 19th century, Mediterranean stone pine area has been expanding to nearly one million hectares. Half of this increase has been on private lands in the last decades, especially in Portugal and western Turkey, where its current extension is 176,000 and 195,000 ha, respectively fourfold the original areas. For establishing profitable stone pine plantations as Mediterranean pine nut crop, grafting improved genetic materials is of major interest. Since 1990, several hundred Spanish clones have been evaluated in grafted common garden trials. They had been selected for superior cone production by plus tree prospection in forests. Analyses of cone yield series in the common trials have allowed ranking these pre-selected clones. However, the legal admission of clones in the National Register of basic materials for the production of reproductive material certified as 'qualified' or 'tested' requires individual identifiability by distinctive characters. In 2015, the Spanish Ministry for Agriculture, Food and Environment has released the first 15 elite clones individually characterised by molecular markers. Field trial data have allowed estimating genetic gain in 10-30% over average cone yield. This catalogue of basic materials has been a first step in building a supply chain, followed by the establishment of mother plant orchards for certified 'tested' or 'qualified' scion supply to commercial nurseries and plantations. As next step, external quality standards must be defined for grafted plants obtained in nursery.

Keywords. *Pinus pinea* – Forest genetic materials – Grafting – Elite clones.

I – Introduction

Plantations of Mediterranean stone pine, *Pinus pinea* L., for soil protection and forest restoration, as well as for producing Mediterranean pine nuts, have expanded threefold the area occupied by the species in the Mediterranean area, exceeding currently 960,000 hectares. Half of this increase has been done by private landowners in the last 40 years, especially in Portugal and western Turkey, where maritime influence with annual rainfalls exceeding 500 mm favours cone productivity (Loewe-Muñoz *et al*., 2016). The current and ongoing expansion of stone pine in both countries is already more than fourfold the original area to 176,000 and 195,000 hectares, respectively (ICNF, 2013; Kilci *et al*., 2014; Santos, 2015; Can, 2016). Due to the excellent world market expectation for pine nut kernel, one of the world's most expensive nuts, for instance Portuguese forest owners have invested in the last fifteen years more than 40 million euros in stone pine plantations and forests (Calado, 2014).

In Spain, expansion of stone pine area has been nearly threefold during 20th century to 490,000 ha, but afforestation with this species aimed mainly at soil protection and forest restoration on wasteland, barren slopes and dunes. Until the European Common Agricultural Policies reform in 1992 favouring farmland set-aside, in Spain stone pine plantation on arable land had been minor, due to less favourable climate that limits revenue expectations from cone yields. Therefore in Spain, virtually all cones are harvested still from pine forests, not in orchards. Anyway, little efforts have been made for its proper domestication as a nut crop. No defined cultivars are known, an absence maybe related to the extremely low genetic diversity found in the species (Prada *et al*., 1997; Vendramin *et al*., 2008; Mutke *et al*., 2012).

Cultivation of stone pine as agroforestry system or orchard crop, including grafted plantations, allows the forest owners getting revenues from annual cone yields even on lands not adequate for most agricultural crops. Moreover, resistance of stone pine to the pine wilt nematode *Bursaphelenchus xylophilus*, one of the most dangerous pest that has been provoking decay of maritime pine *P. pinaster*, facilitates the restauration of affected pinewoods in Portugal by changing the main species to stone pine (Nunes-da-Silva *et al*., 2015; Zas *et al*., 2015).

For establishing profitable plantations as pine nut crop, reproduction of grafted plants is of major interest for allowing massive propagation of genetic improved material, aiming toward domestication of this forest tree (Mutke *et al*., 2013). The standardised use of grafted trees requires the implementation of a legal, technical and commercial framework for supplying legally admitted forest reproductive materials under strict quality standards. The present contribution exposes the state of the art in Spain for establishing grafted *Pinus pinea* orchards.

II – Clone selection in grafted comparative field trials

Since the early nineties, several Spanish regional and national programmes for selecting outstanding stone pines have been developed by forest administrations for enhancing cone production (Abellanas *et al*., 1997; Iglesias 1997; Mutke *et al*., 2000). Within this framework, several grafted comparative clonal trials have been established for Mediterranean stone pine in Spain, evaluating the performance of candidate clones (Table 1). The ortets of these candidate clones had been selected as plus trees in pine forests, by phenotypic traits, namely their outstanding cone yield and good environmental adaption (Gordo, 2004). Comparative trials targeted for characterization of the cone yield of each clone, in quantity and quality, in common garden conditions. The relevance of genetics *vs* environment factors for seed-yield, quantity and quality was evaluated, for instance the clonal degree of genetic determination H^2 and the expected genetic gain for individual clones (Mutke *et al*., 2003b, 2005a, 2007a).

Table 1. Test sites of comparative clonal trials for *Pinus pinea* in Spain

Code	Municipality (province)	Mean annual temperature [°C]	Mean annual rainfall [mm]	Nº of clones tested (since)
B23PH	Madrid (Madrid)	14.2	440	331 (1991)
B23MN1	Quintanilla de O. (Valladolid)	10.1	508	98 (1991)
B23MN2	Tordesillas (Valladolid)	12.3	450	66 (1998)
EC-1	Almonte (Huelva)	16.2	642	120 (1990)
EC-2	Villaviciosa de C. (Córdoba)	15.7	745	120 (1990)
EC-3	Aroche (Huelva)	15.0	792	120 (1993)
EC-4	Arcos de la Frontera (CA)	17.8	740	120 (1993)

Comparison of estimated clonal values among test sites allows also estimating genotype by environment interactions, that is, the relative performance of evaluated genotypes (clones) in different agro-climatic zones.

Recorded data from grafted replicates of each clone for growth and cone yields in the trials, as well as cone and seed characterisation, have allowed ranking productively the pre-selected genotypes. Results of evaluated trials (Table 1) allowed estimating the degree of genetic determination for the annual average cone yield (kg/tree) between 15-38%, resulting in an expected genetic gain of 12-39% if selecting the top 10% of the tested genotypes from each provenance. The genetic correlations between cone yield and cone or seed size were always positive (r = 0.17-0.47), hence no trade-off between crop quantity and quality was observed (Mutke *et al*., 2005a; Mutke *et al*., 2007b; Guadaño and Mutke, 2016).

Comparing the performance of each clone in different comparative trials, the genotype by environment interactions were found to be significant, with some clones performing well at one test site, but only average at others, and *vice versa*. This result implies that the network of trials must be extended into different agro-climatic zones for a neater characterisation of each genetic material (Guadaño and Mutke, 2016).

III – Distinctness and identification of clones by molecular markers

One of the mandatory requirements for the inclusion of clones in National Register of Basic Materials for producing Forest Reproductive Materials is the identifiability of each clone by distinctive characters approved and registered with the official body, following the *Council Directive 1999/105/EC on the marketing of forest reproductive material, Annex IV*. Those distinctive characters can be morphological, phenological or molecular markers, and are required for allowing future post-hoc identification of marketed planting stocks as traceability control of providers and traders.

Nevertheless, in case of stone pine, morphological, phenological and molecular diversity has been found extremely low (Mutke *et al*., 2003a, 2005b, 2010, 2012; Vendramin *et al*., 2008). Only recently, a set of molecular markers, six polymorphic nuclear microsatellites (nSSRs) (Pinzauti *et al*., 2012), has allowed profiling the elite clones selected in the Spanish programmes.

Table 2. Estimated genetic gain for registered stone pine clones over the average cone yield in the respective comparative trial (Guadaño and Mutke, 2016)

Clone	Name of Spanish unit of approval	Genetic gain
	'Qualified' Basic Materials (evaluated at only one test site)	
2004	CL-Q-23/Hoyo de Pinares	19%
2048	CL-Q-23/Almorox	19%
2068	CL-Q-23/San Martín de Valdeiglesias	24%
3029	CL-Q-23/El Provencio	31%
3048	CL-Q-23/Pozoamargo	21%
3057	CL-Q-23/Casas de Haro	23%
3063	CL-Q-23/El Picazo	22%
6010	CL-Q-23/Santa Coloma de Farners	9%
6015	CL-Q-23/Llagostera	11%
6053	CL-Q-23/Dosrius	9%
	'Tested' Basic Materials(evaluated at 2-3 test sites)	
1011	CL-C-23/Portillo-11	+25-27%
1012	CL-C-23/Portillo-12	+12-29%
1073	CL-C-23/La Vega	+12-17%
1123	CL-C-23/Íscar	+11-20%
1201	CL-C-23/Valdegalindo	+15-18%

50 Spanish stone pine clones had been singled out for outstanding cone production in the grafted comparative trials, and passed hence to be the next phase to be genotyped by molecular markers, following the protocol of Pinzauti *et al*. (2012) that combines six nSSRs.

Nevertheless, the six markers used allowed only a distinctive characterisation for some of these clones, while other, even better performing clones didn´t show an own distinct genetic profile in these six markersa and in consequence could not be admitted legally as basic materials.

In 2015, the first fifteen Spanish elite clones have been released and registered officially in the National Catalogue of Basic Materials for *Pinus pinea* by the *Resolución de 21 de abril de 2015 de la Dirección General de Desarrollo Rural y Política Forestal* (B.O.E. 13/05/2015) and *Resolución de 3 de diciembre de 2015 de la Dirección General de Desarrollo Rural y Política Forestal* (B.O.E. 19/12/ 2015) (Table 2).

IV – Conclusions

The approval of the first stone pine clones in the Spanish National Register of Basic Materials will allow marketing their scions and grafted plants as *qualified* and *tested* forest reproductive materials under European regulation (*Council Directive 1999/105/EC on the marketing of forest reproductive material*, whose Annex I includes *Pinus pinea)*.

However, the availability of elite clones as registered basic materials is only the first step in the supply chain for planting stock. The next step is the establishment of officially admitted mother plant hedges to supply scions to commercial nurseries and plantations, followed by the definition of commercial standards for external quality of planting stocks, such as scions, rootstocks and grafted plants. Another pending research line is the phenotypic characterisation of all clones in different agroclimatic zones.

In a recently published monograph, the authors of the present contribution have drawn the balance of their professional experiences gained by their implication in genetic improvement programmes for stone pine in Spain over last 25 years (Guadaño and Mutke, 2016).

Acknowledgments

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Session 4

Pests

Impact of the Dry Cone Syndrome on commercial kernel yield of stone pine cones

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Abstract. The economic relevance of Mediterranean stone pine is based on the harvest of its cones for extracting the edible Mediterranean pine nuts kernels. Recently, a severe loss of kernel-per-cone yield has been reported from cone processing industries: up to half of the extracted seeds are empty or contain only withered remains of the kernel. Additionally, a high percentage of small unripe conelets abort before maturity. The coincident emergence of both phenomena in several countries has coined the common name *Dry Cone Syndrome* (*DCS*). *DCS* has spread out all over the Mediterranean range of stone pine in the last four years, after first reports from Italy ten years ago. If persisting, *DCS* is regarded as a serious threat for commercial pine nut harvesting, an activity essential for the economic sustainability of Mediterranean pine forests and plantations, as well as for the cone processing industry in Europe, with a market of several hundred million euros annually. Cone processors surveys and reports in the framework of the stone pine group within the FAO-CIHEAM Network on Nuts have allowed plotting the spread of the syndrome throughout the Mediterranean, and its comparison with the invasion of Europe by the exotic seed bug *Leptoglossus occidentalis*, a seed-feeding pests known to produce analogous damages in more than 40 conifer species in the Northern America and Europe.

Keywords. *Pinus pinea* – Processor industry survey – Kernel yield loss – *Leptoglossus occidentalis.*

I – Introduction

Mediterranean stone pine, *Pinus pinea* L., is a characteristic tree of most Mediterranean countries. The economic relevance of its forests and plantations is based on the harvest of its cones for extracting the edible Mediterranean pine nuts kernels (Mutke *et al*., 2012, 2013). But in the last years, a severe loss of kernel-per-cone yield has been reported by processing industries for stone pine cones collected in all main producing countries, namely Portugal, Spain, Italy, Morocco, Turkey and Lebanon: when cracking apparently sane cones, up to half of the seeds are empty or contain only withered remains of the kernel. Additionally, cone pickers have also been observing in the pine crowns a high percentage of small unripe conelets aborted before maturity, decreasing the final number of harvested cones.

The recent coincidence of both phenomena has coined the common name *Dry Cone Syndrome* (*DCS*), suggesting a possible common agent. Awareness has grown about the emergence of *DCS* all over the Mediterranean range of stone pine only in the last four years, after first alarms from Italy ten years ago. Since 2011, the subnetwork for stone pine within the FAO-CIHEAM Network on Nuts has been gathering this information from its members, though local incidence might actually have started several years before awareness rose (Fig. 1). *DCS*, if persisting, is regarded as a serious threat for commercial pine nut harvesting, an activity essential for economic sustainability of Mediterranean pine forests and for cone processing industries, with a market exceeding 200 million euros annually (INC, 2016; Santos, 2015; Sattout, 2016).

Generalised awareness up to alarm in the sector has contrasted with the lack of official data to back up the severity of the problem. Though statistics of non-wood forest products are published by ministries of agriculture in several countries, data are often mere estimates for actual amount of annual crops, based on processing industries' declarations or extrapolated from public forests, lacking detailed information from private forests which prevail widely e.g. in Spain or Portugal. Small private forest owners, as well as cone pickers and first processors which are as mostly self-employed workers or small family enterprises, are for fiscal reasons quite reluctant to declare their actual turnovers and revenues to third parties, and traceability systems have been built only few years ago and are not yet fully implemented and fulfilled, persisting hence a significant informal sector. Also in Turkey, small-sized private plantations exceed widely public forests, 132,000 ha versus less than 60,000 ha state forests that give the published annual yield series (Can, 2016).

National import-export data are not sound either, because statistics codes under TARIC or Combined Nomenclature subsume within the same code NC 0802 90 50 any kind of '*pine nuts, fresh or dried, whether or not shelled*'. From average prices it can be deduced that data for shelled kernel (25-45 €/kg), pine nuts in shell (2-5 €/kg) and even cones (less than 1 €/kg) are completely mingled. E.g., part of export from Portugal to Spain has been in form of unprocessed cones, not pine nuts, and hence adds to apparently "Spanish" pine nut kernel production (re)-exported. Moreover, code NC 0802 90 50 doesn't even allow for distinction between Mediterranean pine nuts (*Pinus pinea*) and imports of lower-priced seeds of other, Asiatic pine species, such as *P. koraiensis*, *P. sibirica* or *P. gerardiana*, whose global trade volumes exceed the genuine Mediterranean pine nuts widely (Pastor, 2014; Agri-Ciência, 2014).

In this context, the primary source for raw data would be the cone pickers and processors that trace usually the geographic origin of different incoming cone lots, as well as the final pine nut and kernel yields obtained from each lot. Especially cooperatives as next-level corporations are disposed for more transparency and less secretiveness than individual self-employed or family-based enterprises.

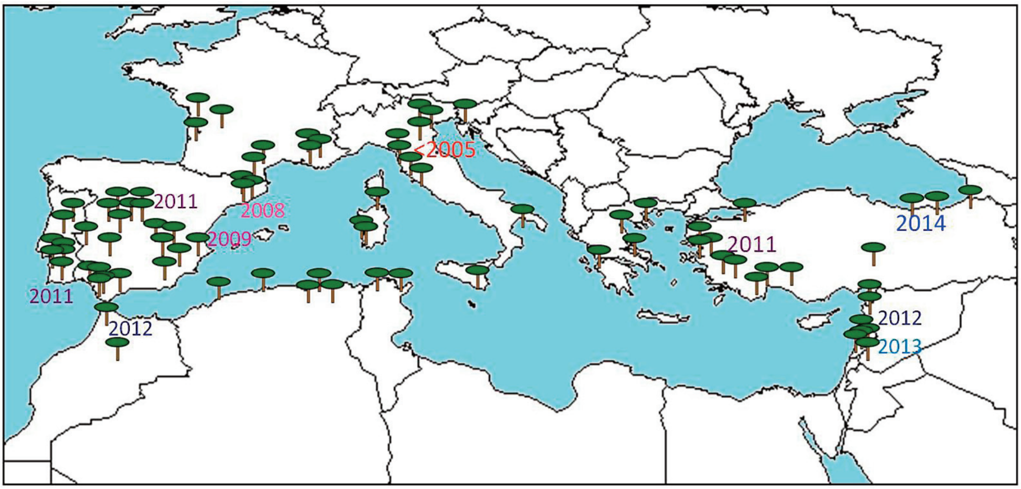

Fig. 1. Distribution of stone pine, and year of first regional report of *Dry Cone Syndrome*.

II – Materials and methods

In 2014, a first survey of cone processing industries about the seed and kernel-per-cone yield had produced a short dataset (Mutke *et al.*, 2014), which has now been updated and enlarged, in order to assess the impact of DCS on the pine nut sector in Mediterranean countries. Nevertheless, the quoted lack of transparency and traceability of the regional, national and international pine nut supply chains has resulted in a short number of responses from cone processors, with regional seed-per cone and kernel-per cone yields data from only four Spanish and two Portuguese processors or processor cooperatives, though their cone supply spans a wide geographic range and includes all relevant stone pine growing areas on the Iberian peninsula, namely Castilla y León, Madrid, Castilla-La Mancha, Catalonia, Andalusia, and Portugal. The Lebanese Pine Farmers Association *Nakabet al Farratin* has contributed with yield series of 23 forest management units (FMU), 15 FMU in Baabda district, and 8 FMU in Jezzine district, reported by 15 forest owners who harvest and process their own cones and have registered the proportion of empty seeds in the last 7 years.

III – Results

Available time series of seed per cone yield data from Iberian cone processors showed that average pine nut per cone weight yield has dropped from a stable yield of 17 (16.9-17.5)% before 2010 to 5-12% since 2012, except the single preliminary value from the currently processed yield, 14.7% (Fig. 2). Average kernel per cone weight yield has decreased from stable 3.8 (3.6-4.1)% before 2008 to 2.2-2.8% since 2011 (Fig. 3). Unusual increases in percentage of empty or internally damaged among normal-sized seeds were specified as main reason: historic values were less than 10%, but currently proportions of 30-50% are observed. The same increase of empty seed proportion has been observed in the Lebanese data series, interestingly with a one-year delay between the two pine forest clusters of the country, Baabda and Jezzine, 40 km south, were incidence of DCS has rocketed only last year (Fig. 4).

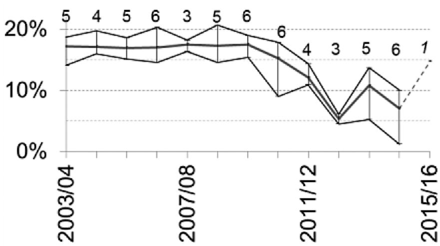

Fig. 2. Annual range and average of pine nut yields in *Pinus pinea* cones from Spain and Portugal [kg seed in shell per kg cones, n 3-6 per year, but only 1 preliminary value in 2015/2016].

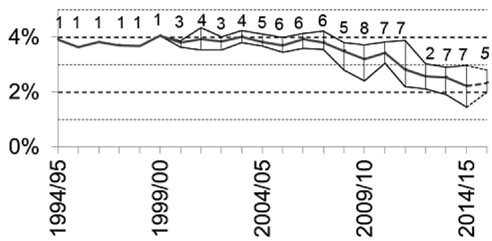

Fig. 3. Annual range and average of pine nut kernel yields in *Pinus pinea* cones from Spain and Portugal [kg kernel per kg cones, n 3-8 per year; 2015/2016 preliminary data].

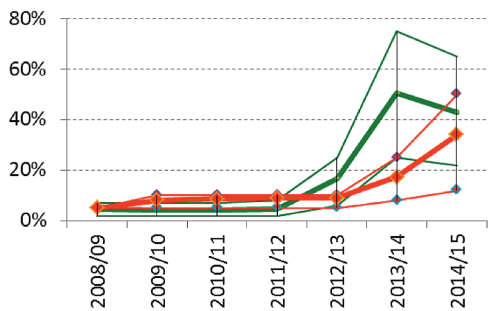

Fig. 4. Annual range and average percentage of empty seeds in *Pinus pinea* cones from Central (green) and South Lebanon (red, diamonds) [% of total seeds; n 16-23 per year].

IV – Discussion

The data reported by cone processors are showing diminishing kernel yields in the last 5 years in cones from Spain and Portugal, the last 2-3 years in Lebanon. These results confirm oral information and published data by colleagues of FAO/CIHEAM Research Network on Nuts and other researchers from all relevant pine nut producing countries (Tiberi, 2007; Bracalini *et al.*, 2013; Calama *et al*., 2016; Can, 2016; Parlak, 2016; Ponce *et al*., 2016) that reflect the spread and prevalence of *DCS* reaching opposite edges of the Mediterranean. Especially interesting is the fine-scale differentiation between the two stone pine areas in Lebanon with a delay in arrival of the syndrome; this suggests the spread of a biotic causal agent, the same as the great picture between countries from Italy outwards (Fig. 1). Putative alternatives like climate factors (for instance, increasing droughts) would not have produced this *"contagious"* pattern.

Not surprisingly, there is an invasive exotic cone pest, the Western Conifer Seed Bug *Leptoglossus occidentalis* of North American origin, pinpointed as putative causal agent of *DCS* as it has been spreading parallelly over Mediterranean and the rest of Europe after its first introduction in Italy in 1999 (Taylor *et al.*, 2001). In 2003, the pest has been first recorded in Spain, probably followed by other introduction events, in 2009 in Turkey and in 2010 in Portugal, although usually it takes a certain time for detecting an alien species because the invasion period takes place before the population densities increase to a critical level causing apparent harm (Fent and Kment, 2011). Types of damages observed in stone pine cones and seeds reported from processing industries do coincide with the whole range of damages caused by *Leptoglossus occ.* feeding on cones of conifers in Europe and Northern America, namely conelet abortion, normal-sized cones with high proportion of fused or aborted seeds, or normal-sized but empty or only partially filled seeds (cf. Strong *et al*., 2001; Bates *et al*. 2002; Strong 2006; Lesieur *et al*., 2014; Boivin and Davi, 2016).

The economic relevance of this problem in Mediterranean stone pine is overwhelming: Formerly, 1 kg of kernel was obtained from 25 kg cones (4%), now about 40 kg cones are necessary for yielding the same amount of kernels (2.5%). Moreover, due to the shortage, cone prices paid to forest owners have increased (Calado, 2012) and profitability of cone processing is jeopardised, pine nut value chain facing a very difficult situation if the syndrome persists.

V – Conclusions

Stone pine cone processors surveys from Portugal, Spain and Lebanon have confirmed a severe decrease of kernel-per-cone weight yield the last years, due to a high proportion of empty seeds or damaged kernels within the cones. These kind of damages observed in factory, together with the general shortage of harvested cones due to massive conelet abortions before ripening, are known as *Dry Cone Syndrome*, reported also from Italy and Turkey.

Observed damages are plainly compatible with the kinds of damage caused by the *L. occidentalis*, confirmed by feeding experiments on caged bugs (Calama *et al*., 2016; Ponce *et al.*, 2016). The prevalent causality of *Leptoglossus* as main biotic agent, however, or possible implications of increasing draughts and phenological shifts due to climatic change (Mutke *et al.*, 2005; Calama *et al.*, 2011) or pathogen fungi like *Diplodia* sp. (Luchi *et al.*, 2011), must be elucidated by ongoing research.

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Leptoglossus occidentalis damages on stone pine female reproductive structures

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Abstract. Stone pine (*Pinus pinea*) is an important forest species in, generating several ecological and economic benefits, particularly with fruit production (pine nuts). The detection of *Leptoglossus occidentalis* in since 2010 seems to be related to a significant decrease in pine cones production and productivity despite the difficulty to detect and quantify its damages. *L. occidentalis* is a sucking insect which feeds by inserting its stylet between the cone scales to reach the developing ovules of several conifer species. In order to understand which stone pine reproductive structures can be affected by the different instars of *L. occidentalis,* measurements were made on the insect stylet, body length and distances between the ovules and cone surfaces from flowering to cone maturation (1st, 2nd and 3rd year cones). The results show that *L. occidentalis* biological cycle overlap with all reproductive structures of stone pine. All development stages of *L. occidentalis* can damage the ovules, except the 1st instar on the 3rd year cones. In the 2nd year cones, cellular damages were also observed in the interior cone scale surface between scales. The stylet length and distance between the ovules and cone surface are determinant factors to allow insect feeding.

Keywords. *Pinus pinea* – Western conifer seed bug – Ovule damages – Stylet length.

I – Introduction

Stone Pine (*Pinus pinea*) is an important forest species in generating relevant economic benefits, particularly through pine nut production. In the latest years an increasing of pests and diseases affecting cones (Sousa *et al*., 2014) was detected demanding the study of its interactions with the stone pine reproductive phenology. Data collected from the main industries (Pimpão, 2014) revealed that in the latest years, cone production and pine nut productivity have dropped to alarming values. The cone production in the 2010-2011 campaign was about 120 million kg, whereas in 2011-2012 was reduced to more or less 25 million kg. The pine nut productivity rounded about 3.5-4% and decreased in the two consecutive campaigns in 2010/2011 (3.3%) and 2011/2012 (2.5%). At the same time, in the field, an increasing number of aborted 1st and 2nd year cones were also detected and were suspectedly related to pests, namely *Leptoglossus occidentalis* (Sousa *et al*., 2012).

L. occidentalis is a sucking insect native to which feeds on seeds of various species of the genus *Pinus* and other conifers (McPherson *et al.*, 1990; Bates *et al.*, 2000a; Strong *et al.*, 2001, Bates and Borden, 2005). The species was first reported in Italy (1999) and in Portugal in 2010 (Bernardinelli and Zandigiacomo, 2001; Sousa and Naves, 2011). Currently it seems to be distributed throughout Portugal and it is usually present in *P. pinea* stands. Several methods have been tested to control the species but an effective method has not been found yet. *L. occidentalis* feeds on developing seeds by inserting its mouthparts between cone scales and affected cones do not show any external damage symptoms (Bates *et al.*, 2000*b*; Strong *et al.*, 2001). The aim of this study is to evaluate *L. occidentalis* feeding capacity in all phenological phases of stone pine female reproductive structures.

II – Material and methods

Measurements were made on the insect stylet and body length (nymphs and adults) and distances between the ovules and cone surfaces from flowering to cone maturation (1st, 2nd and 3rd year cones). The stylet and body length of 5 insects for each 5 nymphal and adult stages were measured using a digital caliber.

For the measurements of the distances between the ovules and cone surface, a field plot with ten trees located in Santa Suzana (Sintra council) originated from natural regeneration was selected (38º 55' 23.2'' N, 9º 22' 46.5'' W; 90-100m a.s.l.). Cone samples of 1st, 2nd and 3rd year were weekly and randomly collected from 19th November 2012 to 21st June 2013 (3 cones per stage). Samples of reproductive structures from the different phenological stages were fixed, during at least 48 hours, in FAA 1:1:18 - formalin: acetic acid: ethanol (70%) at 4 °C (Johansen, 1940; Ruzin, 1999). Dehydration was achieved through progressive ethanol/water series and finally included in paraffin. Histological sections (healthy and damage ovules) of 10µm thickness were obtained using a rotary microtome (Leica RM2255) and stained with Heidenheim Hematoxylin. Image acquisition was performed using a Digital Microscope Leica© DMS1000 with LAS (Leica Application Suite) V4.4 software. Measurements of the distances from the cones surface to ovules (5 ovules/cone) were performed using the software scale bar and ImageJ software in irregular distances.

III – Results and discussion

1. Stylet and body length of *L. occidentalis* development stages

Data from body and stylet length of the five nymphal and adult stages show that stylet length increases by 2.5x from 1st to 2nd instar (2.9 mm to 7.2 mm), then increasing slowly to adult stage (12.3 mm). Body length grows at low rate until 2nd instar, doubling in the 3rd instar and rising slowly until 5th instar, increasing again at adult stage to 16.9 mm (Fig. 1).

The ratio stylet/body length at 2nd instar is 1.74 (the stylet is longer than the body), decreasing until 0.73 at adult stage (stylet shorter than body) (Figs. 1 and 2).

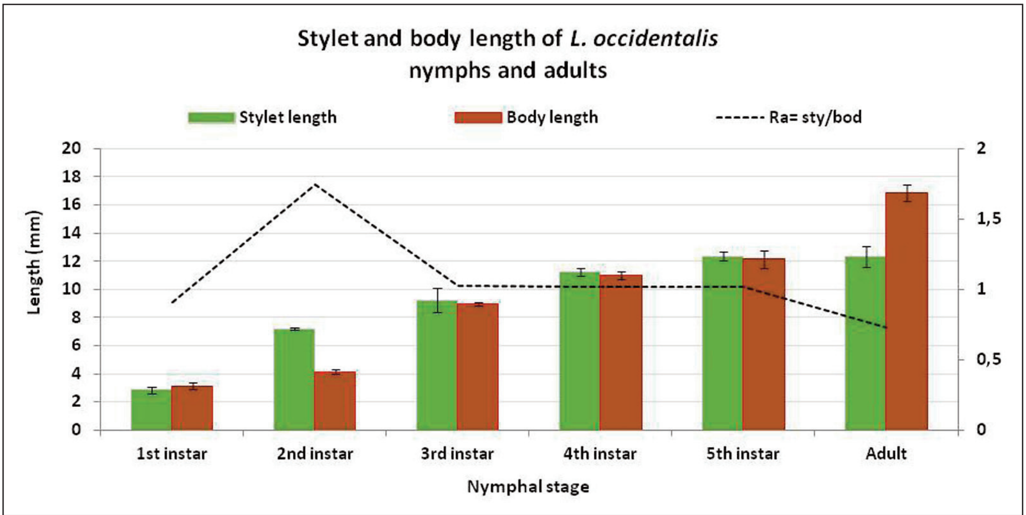

Fig. 1. Stylet and body length (mm), ratio (Ra) (stylet/body) and corresponding Standard Deviation in all *L. occidentalis* development stages.

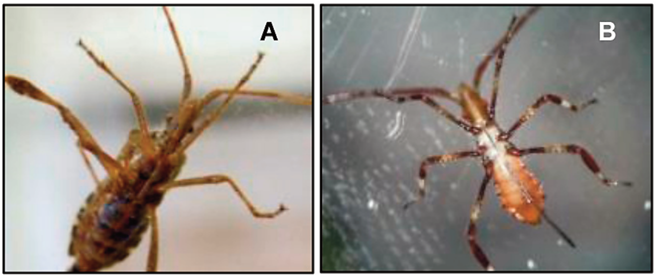

Fig. 2. ***Leptoglossus occidentalis*. A - 2nd instar nymph where stylet length (7.2 mm) (curved in the image) exceeds body length (3.9 mm). B - Adult of *L. occidentalis* where stylet (12.0 mm) is shorter than body (16.1 mm).**

2. Distance from cones surface to ovules

Distance between the cone surface and ovule had usually irregular shape and varied according to cone stage. In the 1st year cones, distance varied from 0.3 to 2 mm, in 2nd year cones from 2 to 2.75 mm. The 3rd year cones remained quiescent (2.75-2.9 mm) until the begin of growth in March, when distance between ovules and cone surface increased from 2,9 to 6,9 mm (Fig. 3).

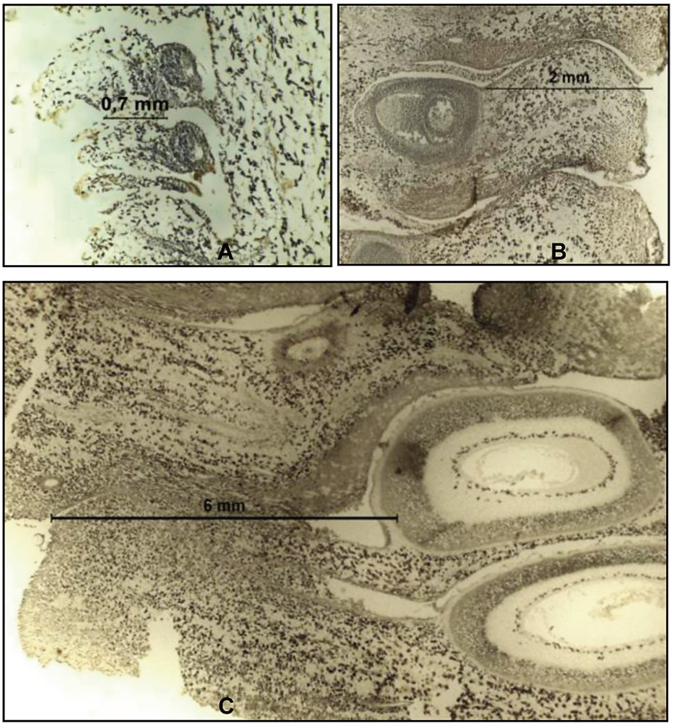

Fig. 3. Distance between the cone surface and the ovules for the 1st, 2nd and 3rd year cones collected in 11th May (2013). Measurements of real distances were performed through ImageJ software. A - 1st year 0.85 mm; B - 2nd year 2.5 mm; C - 3rd year 6 mm.

3. Relation between insect feeding and host damages

The relation between *L. occidentalis* stylet length and the distance from the ovules to the cone surface show that all nymphal stages are able to insert its stylet and suck the content of all cone stages (1st, 2nd and 3th year cones), except the 1st instar nymphs that cannot reach the ovules of the 3rd year cones (Fig. 4).

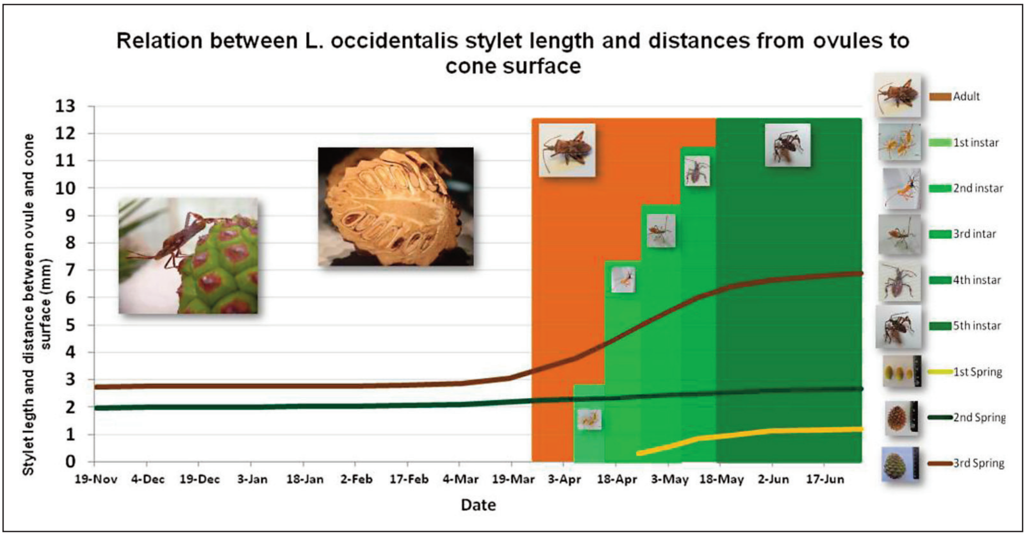

Fig. 4. Stylet length of all *L. occidentalis* stages and distance from ovules to cone surface for the 1st, 2nd and 3rd year cones.

4. Damages in reproductive structures

Internal damages were detected on 2nd year cones (11th May and 1st June 2013) (Fig. 5). Comparison of ovules with and without damage showed ovule cell degradation (Fig. 5B) and cellular damages in the gap between cone scales (Fig. 5C), compatible with *L. occidentalis* that inserted its mouthpart between cone scales to reach the ovules. However, there is uncertainty about the causing agent of ovule and cone scale damages, since cones were collected from the field. These damages are not externally detectable and makes difficult to recognize affected and unaffected cones.

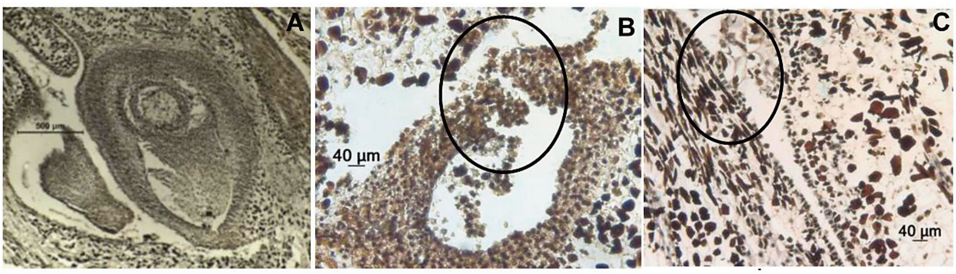

Fig. 5. Cone longitudinal sections where damages were detected in 2nd year cones. A - Developing ovule without damage (11th May 2013); B - Developing ovule with damage (11th May 2013); C - Cone scale surfaces with cellular damage (1st June 2013).

IV – Conclusions

This study reveals that *L. occidentalis* biological cycle overlaps with the development of all stone pine reproductive structures, having the capacity to feed on developing seeds (except the 1st instar on the 3rd year cones). Damages in developing ovules compatible with *L. occidentalis* feeding activity were also detected, suggesting that *L. occidentalis* can cause a significant decrease in cone production and productivity. Further studies are required in order to gather more information about the interaction between the host and this agent.

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Cone pests of stone pine in the Mediterranean Basin

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Abstract. Stone pines in the Mediterranean Basin are one of the most defended pines and only few pest species can cause damage, such as defoliators (*Thaumetopoea pytiocampa* (Schiff.)) or bark beetles (*Tomicus* sp. and *Ips sexdentatus* (Boern.)). In certain situations these insects can weaken or even cause tree mortality, but the main cause for concern are insects like *Pissodes validirostris* Gyll (Coleoptera:Curculionidae), *Dioryctria mendacella* Staudinger (Lepidoptera: Pyralidae) and *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) that feed on cones and seeds, negatively impacting the economy. Two of the insect species are indigenous whereas the third, *L. occidentalis*, is an invasive species originating from America. During seed development substantial resources are used during embryogenesis as seeds provide a higher concentrated source of carbohydrates, fat and proteins and low water content, as compared with young needles. Species like *P. validirostris* and *D. mendacella* burrow through and feed on seed-bearing structures or cones, while *L. occidentalis* suck out the contents of seeds or seed-bearing structures as well as needles, flowers, shoots and twigs. At the same time, these specialized insects have developed diverse ecological strategies to complete their biological cycle. For example, *P. validirostris* completes its immature stages inside the cones, while *D. mendacella* larvae leave the cone to pupate in the soil, and *L. occidentalis* completes its development outside cones. In addition to direct consumption, insect attacks can cause fruit abortion or facilitate introduction of pathogens. For example, the sap-sucking insect *L. occidentalis* causes transmission of the fungus *Diplodia sapinea* (Fr.) Fuckel. Impacts of cone pests are related to reductions in seed production and productivity at the economic level and to reforestation and afforestation programs at the economic and ecological levels. In stone pine forests, phenology and seed production can be highly irregular in both space and time, directly affecting the pest population dynamics. In this paper we summarize the current knowledge on the more important cone pests in the Mediterranean Basin, emphasizing: (i) biological and ecological mechanisms involved in the establishment and spread of these species, (ii) the associated damages and, (iii) strategies available for integrated management with the objective of controlling pest populations.

Keywords. *Pinus pinea* – Seeds – Bioecology – *Pissodes validirostris* – *Dioryctria mendacella* – *Leptoglossus occidentalis*.

I – Introduction

Five regions of the world can be characterized by Mediterranean climate conditions (mild wet winters and warm dry summer conditions) and occur around the Mediterranean Sea (60%) and on portions of the southwest coasts of North America, South America, Australia, and Africa (Di Castri, 1991) (Fig. 1).

According to Quézel and Médail (2003), there are 6 bioclimatic types related to the main vegetation types of the Mediterranean basin (Fig. 2). The Mediterranean climatic zones represent about 2% of the Earth's land mass (World Conservation Monitoring Center, 1992) and are recognized centers of endemism and specialized vegetal communities, accounting for nearly 20% of plant diversity. Stone pines (*Pinus pinea* L.) are endemic to scerophyll forests in sub-humid bioclimate zone of the Mediterranean Basin (600-800 mm of mean annual rainfall and 3-5 months without rainfall), according to Quézel and Médail (2003), together with *Pinus halepensis* Miller, *Pinus brutia* Ten., *Pinus pinaster* Aiton, *Pinus nigra* J.F. Arnold, *Cedrus* sp. and *Quercus* sp.

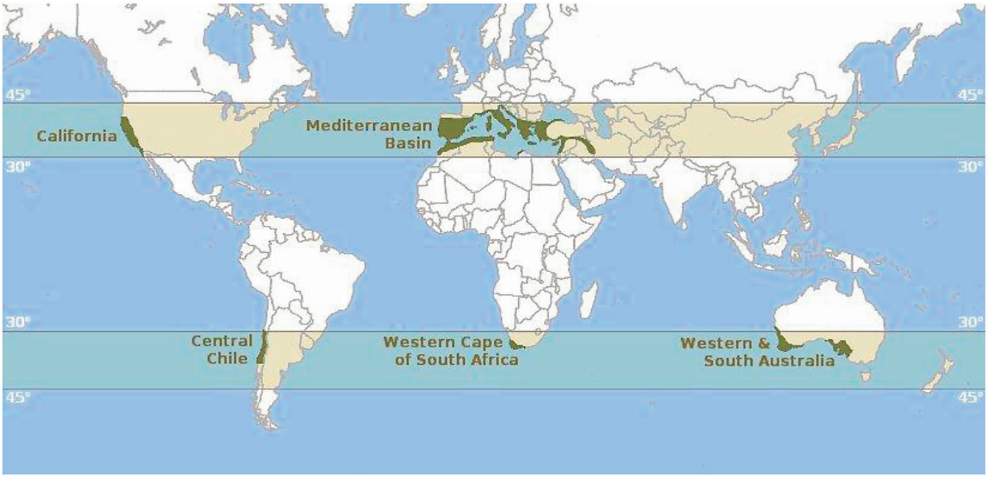


Fig. 1. Mediterranean regions in the world.

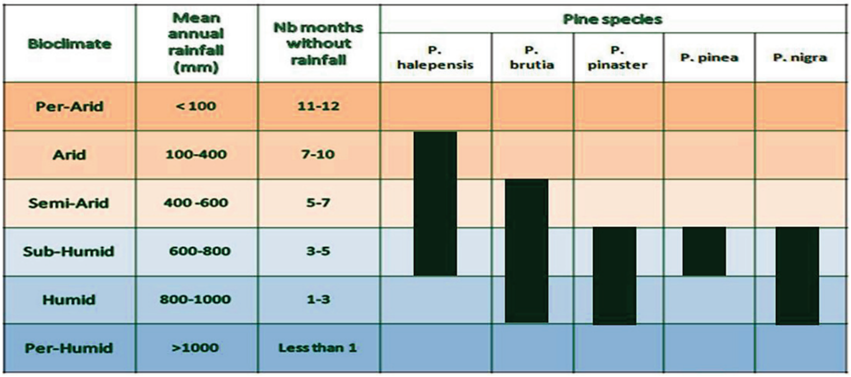


Fig. 2. Bioclimate types and their relationship with main vegetation types of the Mediterranean basin (from Quézel and Médail, 2003).

A characteristic of the dynamics of Mediterranean forests is the intensity and frequency of long term disturbances, defined as temporary changes in the environmental conditions impacting the ecosystem due to complex multi factorial processes of biotic and abiotic origin (anthropogenic effect, pests and diseases, wildfire, drought and frost). These disturbances reshape biodiversity both in terms of composition and structure. However, in the Mediterranean Basin, the stone pine is one of the more defended pines against biotic and abiotic agents. *P. pinea* survival is increased by thick bark and protection of apical buds by terminal needles. Although *P. pinea* is characterised by low genetic variability (Mutke *et al*., 2013), variation for adaptation to fire occurs both between and within tree species. Populations grown in areas of frequent fires appear more adapted than others (Lefèvre and Fady, 2016).

II – Main cone pests of the stone pine

There are only a few species that can cause damage to stone pine, such as defoliators (*Thaumetopoea pityocampa* (Schiff.)) or bark beetles (*Tomicus* sp. and *Ips sexdentatus* (Boern.)). In occasional situations these insects can weaken or even cause tree mortality, but the main concern is for insects that feed on cones and seeds.

Fruiting structures of gymnosperms, i.e. seed cones, generally consist of a complex structure with diverse characteristics (bract, dwarf-shoot, ovules, seed wing, aril, and seed). Contributions of the different insect orders to diversity of insects attacking cones varies greatly among both host groups and genera (Fig. 3). In the Mediterranean Basin, Gymnosperms are colonized by five insect orders and the *Pinus* genus can be colonized by Lepidoptera (11 species), Hemiptera (1 species), Diptera (2 species) and Coleoptera (7 species) (Boivin and Auger-Rozenberg, 2016).

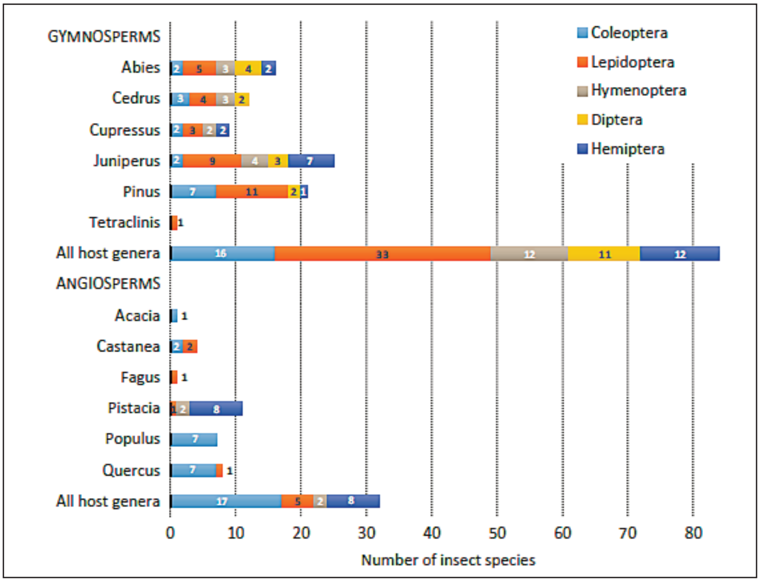


Fig. 3. Order level diversity according to host genera of the entomofauna of tree reproductive structures in the Mediterranean Basin (from Boivin and Auger-Rozenberg, 2016).

For *P. pinea* several conospermatophage species were identified, like *Ernobius impressithorax* Pic, *E. parens* (Mulsant and Rey), (Coleoptera: Anobiidae) and *Dioryctria pineae*. The main species in the Iberian Peninsula are *Pissodes validirostris* Gyll (Coleoptera:Curculionidae), *Dioryctria mendacella* Staudinger (Lepidoptera: Pyralidae) and *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae) which impact the economy. The first two species are indigenous to the Mediterranean basin, while the third is an invasive species originating from America.

Species like *P. validirostris* and *D. mendacella* burrow through and feed on seed-bearing structures or cones, while *L. occidentalis* suck out the contents of seeds or seed-bearing structures as well as needles, flowers or shoots and twigs.

1. Hosts and insect distribution

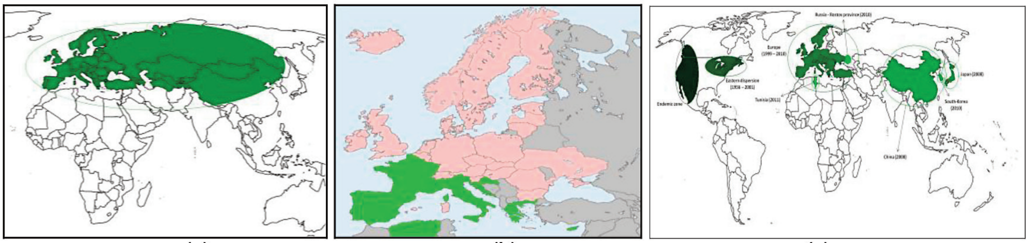
Phytophagous insects are known to preferentially colonize plants that are taxonomically related to their usual host.

P. validirostris (pine cone weevil) is a major cone pest of *Pinus* spp (except the Swiss stone pine *P. cembra* L.) (Dormont and Roques, 2001) having a large distribution from Europe to north eastern China (Annila and Hiltunen, 1977; Roques, 1976, 1983) (Fig. 4a). In Finland, the introduced pine *P. contorta* var. *latifolia* is a prefered host (Annila and Hiltunen, 1977).

D. mendacella (pine cone moth) can attack several pine species (*P. pinea*, *P. sylvestris*, *P. halepensis*, *P. brutia* and *P. pinaster*) (Gomez de Aizpurua, 1991; Knölke, 2007) with a more restricted distribution in Southern Europe and Northern Africa (Karsholt and van Nieukerken, 2013) (Fig. 4b). It is found on *P. halepensis (*Nichane *et al*., 2013) in Algeria.

P. validirostris and *D. mendacella* can attack several pine species but in Portugal they are found mainly on *P. pinaster* and *P. pinea*. In Spain, *P. validirostris* damages several species including *P. pinea*, *P. sylvestris*, *P. halepensis*, *P. nigra* and *P. pinaster* (Cadahia, 1981).

L. occidentalis is native to western North America, from British Columbia to Mexico in latitude, and from the Pacific Coast to Colorado in longitude (Koerber, 1963) (Fig. 4c). It can feed on several species of conifers (it can feed on seeds of over 40 hosts from different genus - *Pinus, Pseudotsuga, Tsuga, Picea, Abies, Cedrus, Juniperus* and *Pistacia)* (Bernardinelli and Zandigiacomo, 2001), *L. occidentallis* and has expanded eastward to the Atlantic Coast (McPherson *et al*., 1990; Gall, 1992). In Europe, the species was first reported in Northern Italy in 1999 (Taylor *et al*., 2001). It expanded its range very quickly and colonized all of Europe within ten years (Lesieur *et al*., 2014), affecting a large host range including native pine species such as *P. sylvestris*, *P. pinaster*, *P. nigra*, *P. halepensis* and *P. pinea*, as well as *Picea abies* (L.) Karst, *Larix decidua* Mill., *Abies* spp., and *Juniperus* spp. in addition to introduced exotic conifers (*Cedrus* spp., *Pseudotsuga menziesii* (Mirb.)) (Taylor *et al*., 2001; Fent and Kment, 2011; Tamburini *et al*., 2012). Moreover, recent detections in Asia (China, Japan and South-Korea) (Zhu, 2010; Ishikawa and Kikuhara, 2009; Ahn *et al*., 2013) and Northern Africa (Ben Jamaa *et al*., 2013) highlight that the pest is a highly successful worldwide invader.



(a) (b) (c)

Fig. 4. World distribution of the main cone pests of the stone pine. (a) *P. validirostris;* (b) *D. mendacella;* (c) *L. occidentalis*.

2. Host-insect interrelationships

Compared to insects that attack other tree structures such as foliage, cone insects require more effective host recognition and more specific selection mechanisms that show high specificity towards cones. Several factors can be associated with the process of host selection (tree/stand characteristics, cones characteristics and cone production). At the same time, these specialized insects have developed diverse ecological strategies to disperse and complete their biological cycle (dispersal capacity, nutrition, biological cycle and natural enemies). The evolution of specialization is favored when pest development is dependent on a single plant organ or tissue for its life cycle (Gaston *et al*., 1992). Overall, these patterns contrast with post-dispersal consumers that feed on a diverse and spatially heterogeneous resource that requires generalist feeding habits (Hulme, 1998).

However, for invasive species, like *L. occidentalis,* successful establishment depends of several factors related with new environments (high densities of favorable hosts, good climatic conditions), their capacity to adapt (good dispersal, good strategies to find susceptible hosts, high capacities of adaptation to new hosts, no endemic predators or parasitoids) and high population levels. All of these factors have undoubtedly contributed towards the success of this pest in attacking conifer seeds in Europe and Asia.

Among the most important parameters which mediate and regulate the attacks of cone pests are tree and stand characteristics, cone characteristics, production and nutritional value, the insect´s biology and dispersal capacity.

A. Tree/stand characteristics

Tree species, silhouette, size and shape play a key role in initiating long-range insect orientation. The contrast between the fruit or seed cone structure and foliage color may also act as a stimulus at the stand scale. Both visual and chemical cues operate sequentially or simultaneously in host location, insect response may also vary among sexes and may depend on the reproductive and nutritional status of individuals.

The variation between trees of the same population may be also due to the size and shape of the crown, motivated by age and social stratum to which the tree belongs; also they influence the quality of the station, the stand density and management techniques.

In many cases, plant chemistry may be responsible for the absence of insect attack on a given species (Bernays and Chapman, 1994). Such relationships were described for *P. validirostris*, a major cone pest of *Pinus* spp that does not attack Swiss stone pine (*P. cembra*) (Dormont and Roques, 2001). Further studies confirmed that this behavior is associated with cone volatile emissions (Dormont *et al*., 1998; Dormont and Roques, 2001). However, second instar weevil larvae that were artificially introduced into Swiss stone pine cones were capable of developing to the adult stage, suggesting that it is only a question of host selection and that this pine species does not contain strong feeding deterrents that could prevent larval development. The greater susceptibility of *P. contorta* in Finland is also associated with differences in emission of volatile monoterpenes by foliage and especially to a lower content of α-pinene than for *P. sylvestris* (Brockerhoff *et al*., 2004). However, it is unclear whether the effect of particular plant chemical defenses prevents insect colonization indefinitely, or simply delays it (Jones and Lawton, 1991).

Morphometric, behavioral and genetic (mtDNA) analyses showed that *P. validirostris* probably does not represent a single generalist species but rather consists of a complex of sibling species specialized to different host pines (Roques *et al*., 2004). Two main groups of *P. validirostris* were identified corresponding to populations developing on northern and alpine pines of the sylvestris section (*P. sylvestris, P. uncinata, P. nigra*) and on Mediterranean pines (*P. pinaster, P. pinea*), respectively (Roques *et al*., 2004).

B. Cone characteristics

One of the main strategies for insects that depend on a single plant organ or tissue for development, is the synchronization between their biological cycle and cone development. The relationships between seed cone phenology and time of colonization have been reported for several Mediterranean conifers of the genus *Abies* sp. *Cupressus* sp. and *Juniperus* sp., and for only for two pine species, *P. brutia* and *P. nigra* (Roques *et al*., 2005). These influence insect species in which both oviposition and larval development occur on immature fruiting structures (*P. validirostris* and *D. mendacella*), as there may be strong selection for synchronizing insect life history with the target stage of the host plant (McClure *et al*., 1998; Harman, 1999).

Cone phenology in stone pine forests can be influenced by several environmental factors like weather conditions during flowering and cone development, giving rise to irregular behavior over both space and time. A similar effect is due to physical damage caused by hail, as the scales affected no longer grow or develop their pinions, leaving a sunken navel on the mature cone.

In the Mediterranean area most of the insect colonization occurs during the second year of cone development, which corresponds to cone growth, although some species may also attack during the first year of development. For short-distance selection, the cone or seed size, structure and chemistry determine the short-range detection and host suitability for mating, feeding and ovipositing by the different pests (Turgeon *et al*., 1994).

It is possible that for some insects, ovarian maturation may be stimulated by the presence of seed cones in the suitable development stage, thereby assuring synchrony between egg production and adequate oviposition sites as has been shown for *P. validirostris* (Roques *et al*., 2005). In fact, the *P. validirostris* oviposition period is relatively limited in time (about a month) and coincides with a specific cone phenological phase (Roques, 1976).

On the other hand, *L. occidentalis* develops entirely outside the cones with a strong capacity of mobility even for earlier stages. This insect is very polyphagous for several conifers and can feed on different cone development phases. Therefore, this insects appears to have less specialized relations with its hosts, although it possesses infra-red (IR) radiation receptive organs to use as host-finding cues towards pine cones, which are warmer and emit more near-, mid- and long-range IR than needles (Takács *et al*., 2009).

C. Production of cones

Insect species that cannot shift to alternative hosts when resource supply is low and when intra- or interspecific competition increases, life histories are under strong selection to offset such heterogeneity in resource availability.

Long term studies on *Juniperus thurifera* growing in Morocco have revealed that fluctuations in cone abundance generally reflect the substantial annual variation in larval populations of cone pests (Roques *et al.*, 2005) (Fig 5).

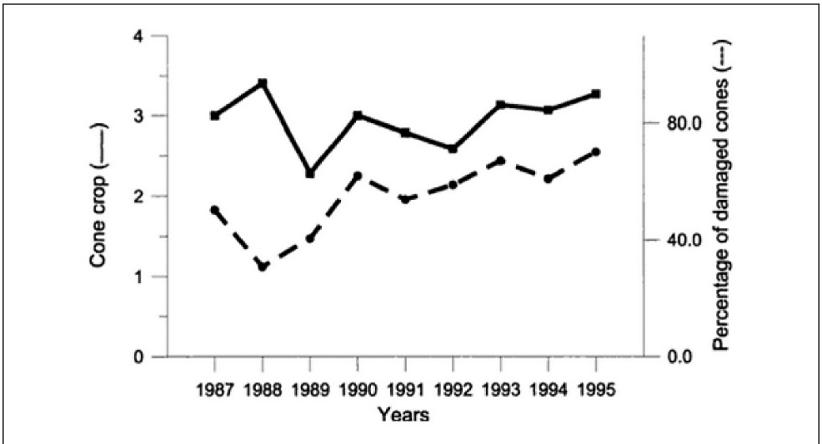


Fig. 5. Comparative variations in annual cone crop and annual pest damage on *Juniperus thurifera* at Tizrag (High Atlas, Morocco) from 1987 to 1995. Cone crop is estimated using a qualitative scale: 1 - slight, 2 - medium, 3 - heavy, 4 - bumper. (Roques *et al*., 2005 modified from El Alaoui and Roques, 2005).

D. Nutritional value of the seeds / cones

During seed development, trees invest substantial resources for embryogenesis. The high nutritional value of fruiting structures is generally associated with a greater investment of plants in physical or chemical defense mechanisms than on vegetative tissues (Janzen, 1971). For example, *P. sylvestris* seeds during cone lignification (phase V) provide a concentrated and rich source of carbohydrates (61%), fat (4%) and proteins (10%) with a low water content (12%), compared to young leaves (4%, 1%, 5% and 76%, respectively) (Jordano, 2000) (Fig. 6).

Internal biochemical changes in cones occur at different stages of development. Water content shows a high increase since the resumption of activity in the second year of cones growth of *P. sylvestris*, with the higher levels in early June before the lignification process (phase IV). Concentrations of cellulose and hemicelluloses are inversely proportional to the concentration of water. Early June corresponds to the period when food reserves are at their maximum, and also to the peak of biological activity of most cone pests.

The cone therefore provides a particular insect habitat in permanent evolution (change of physical and chemical characteristics) while simultaneously being a direct or indirect source of food. However, insects can target very early developmental stages of tree reproductive structures such as male and female flowers (Turgeon *et al.*, 1994). Insects also attacking fruiting structure display two different feeding habits to acquire nutrients from their host plants.

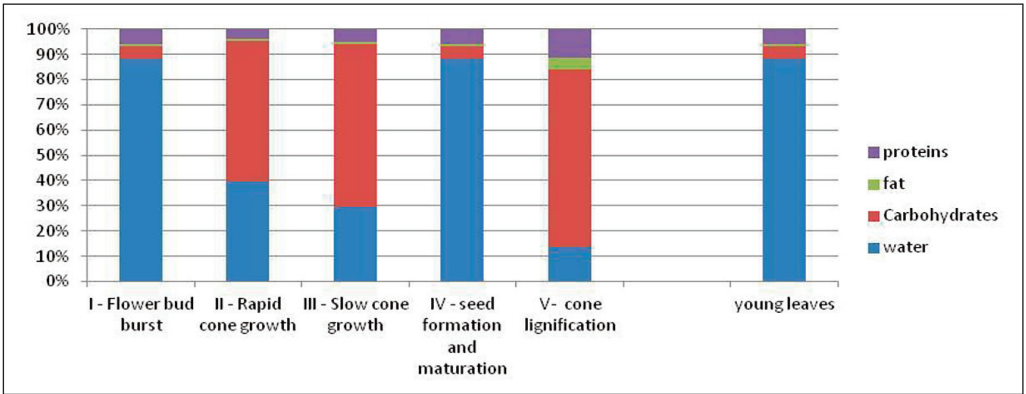


Fig. 6. Differences on *Pinus sylvestris* cone composition during its development (adapted from Jordano 2000).

Larval stages of species belonging to the Coleoptera (*P. validirostris*) and Lepidoptera (*D. mendacella*) feed only on cones (Merlo *et al.*, 2005), while adults and immature nymphs belonging to Hemiptera (*L. occidentalis*) consume individual seeds during the growing season as well as needles, flowers or shoots and twigs (Strong *et al.*, 2001). Insects insert their mouth stylets through the cone tissues directly to the seeds sucking the lipid and protein content (Bates *et al.*, 2001).

E. Biological cycle

In Europe the tree main cone pests (*P. validirostris, D. mendacella* and *L. occidentalis*) show different particularities in their biological cycles, which differ by geographic areas. *P. validirostris* completes all the immature stages inside the cones and is univoltine in most of its distribution range, although in Finland the life-cycle takes 2 years (Annila, 1975). In the case of *D. mendacella*, the larvae leave the cone to pupate in the soil, and this insect has 1.5 or two generations per year in the Mediterranean basin.

L occidentalis is univoltine in Northern California (Koerber, 1963), whereas two to three generations per year are observed in Mexico (Cibrián Tovar *et al.*, 1995). In Europe, recent studies conducted in Italy (Bernardinelli *et al.*, 2006) and Spain (Más *et al.*, 2013) have shown the existence of up to three generations per year (threshold temperature of development: 15°C. Integral thermal 513.72 degree days).

P. validirostris mating and oviposition takes place on cones during April-May when the cone moisture content peaks (Dormont and Roques, 2001). The female lays a variable number of eggs per cone. In *P. pinea* there may be up to 40 eggs in one cone. The eggs hatch in 10-15 days (Romanyk and Bachiller, 1965). Larvae occur in spring and summer, with 4 instars recognised (Roques, 1976). Several larvae can coexist in the same cone implying a higher degree of intraspecific larval competition (Annila, 1975). Pupation occurs within the cones in August and adults emerge in late summer, through a circular exit orifice. Adults can survive for at least 2 years (Annila, 1975) overwintering in the litter and bark crevices, and emerging in the following spring (April-May) (threshold of 12°C and a mass occurrence for more than 15 °C) (Bachiller, 1966, Cuevas and Bachiller, 1970). The adults need a short period of maturation feeding on the pine leader shoots before they are able to lay eggs on cones (Roques, 1976).

Adults of *D. mendacella* emerge in spring/early summer for the 1^{st} generation and in the late summer/very early autumn for the 2^{nd}. Oviposition inside cones and the development of the 1st generation takes around four months, while nine months are required for the 2^{nd}.

One to six larvae can occur in the same cone (Pajares, 2015). Larvae can move from one cone to another. Larvae (5 stages) construct irregular galleries during June to September in the 1st generation and from November to April in the 2nd. Insects overwinter in the larval stage inside the cones. Pupation occurs in the soil, protected by a weak silken cocoon (1 month in summer or 2 to 3 months in winter). *D. mendacella* can be found associated with *P. validirostris* in the same cone (Cuevas and Bachiller, 1969).

L. occidentalis adults mate repeatedly from spring to fall (Koerber, 1963). The female lays eggs (73-80) on the underside of needles (series of 3 to 20). Eggs hatch 10-15 days after laying (Bates and Borden, 2005). Five nymphal stages occur, with a high young nymphal mortality (> 80%) (Koerber, 1963). There is high mobility from one cone to another. The presence of the other pests does not seem to affect *L. occidentalis* (Lesieur, 2014). This insect overwinters in refuge sites, including human-made structures such as wood piles, containers, sheds or houses with an aggregation behavior, grouping up to thousands of individuals (Tamburini *et al.*, 2012), regulated by a pheromone produced by the males (Blatt and Borden, 1996). During the winter they survive using the lipid reserves accumulated in the fall.

F. Dispersal capacity

The dispersal capacities of an invasive population may be a decisive factor to access new and more adequate environments (Travis and Dytham, 2002; Wilson *et al.*, 2009). The dispersal can take different forms, it can be active, involving specific capabilities of each organism (walking or flying, for example). It can also be passive, with insects being transported with their host material.

The history of the European invasion by *L. occidentalis* is complex because it occurred over the past ten years. Recent studies using polymorphic microsatellite markers characterized the invasion scenario by this insect (Lesieur, 2014). It was concluded that the current geographic distribution of *L. occidentalis* in Europe is possibly the result of several independent introductions (Lesieur, 2014), highlighting at least two independent introductions, although additional introductions in Spain, France and Austria are also suggested.

This dispersal can be explained by different mechanisms related to human activity and the specific characteristics of the species. Different means of propagation could underlie its very fast invasion. In this respect, the introduction pathway could be related to timber shipments (e.g. timber logs or wood panels) from the USA (Dusoulier *et al*., 2007; Malumphy *et al*., 2008) and the habits of the bug to aggregate inside man-made structures in the fall to seek shelter for overwintering. Moreover, individuals (eggs, nymphs or adults) may also have been brought from established populations via their host plants with for instance the commercial Christmas tree trade or translocations of ornamental trees (Gall, 1992; Gapon, 2012).

During insect outbreaks, the flight behavior and flight capabilities of invasive species are important for understanding the dynamics of the invasion and progression of the front. Important flight capabilities of *L. occidentalis* could facilitate the spread in European territory. For example, the maximum flight distance of breeding insects in laboratory conditions using flight mills is about 15.5 km for males and 12.5 km for females, with values of around 21.5 km and 22.5 km for insects collected under natural conditions (Lesieur, 2014) (Fig. 7). In the autumn, young insects showed good flight capabilities but the flight abilities in early April (with insects exiting hibernation), suggest that the insects can also actively disperse during spring. Such events can lead to a homogenization of genetic diversity in Europe (Koerber, 1963; Malumphy *et al*., 2008).

For the endemic species, although pine cone weevils are capable of flying, a study on the dispersal of marked adults within a pine stand showed that a high proportion remained on or near the tree where they hibernate, and only a few adults travelled more than 10 m (Annila, 1975). However, all the pine trees in this report contained cones and nothing is known about the weevil's dispersal behaviour when cones are absent in a stand.

For *Dioryctria* spp, recognition and selection of suitable feeding and breeding sites involve both the adult female and its progeny when larvae are capable of moving and finding resources.

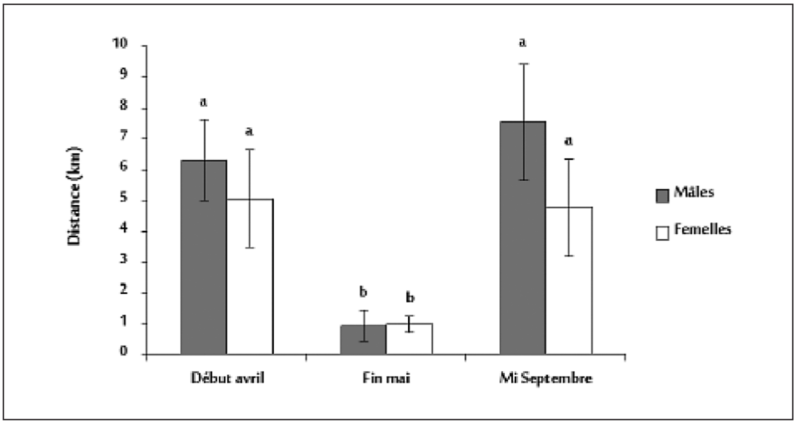


Fig. 7. Evolution of the total flight distance (DP) by individuals of *Leptoglossus occidentalis* depending on the season (from Lesieur, 2014).

3. Associated damage

The demographic and evolutionary consequences of the feeding activities of insects on fruiting structures are likely to differ from most forms of herbivory which only partially remove tissues from individual plants (Hulme, 1998). However until the 1980s this had received little attention because their predominantly cryptic way of life makes them difficult to detect with simple external fruit examination, and because their economic and ecological impact on seed crops was barely quantifiable. However, improvement in tree multiplication and planting programs relying on both certified seed collections and seed orchards progressively created the need for research on the ecology and impact of insects on seeds (Roques, 1983).

Additionally, insect attacks can facilitate introduction of pathogens, such as the transmission of the fungus *Diplodia sapin*ea by the sap-sucking insect *L. occidentalis* (Luchi *et al*., 2012).

P. validirostris was also considered a vector of the highly virulent fungus, *Fusarium circinatum*, (pitch canker) an important pathogen of *Pinus* seedlings recently introduced in Europe (Romon *et al*., 2007). However, more recent work done in South Africa showed that the weevils did not transmit the fungus, although damage caused during feeding facilitated the ingress of the fungus into the host plant (Lennox *et al*., 2009).

The insect's cone and seed consumption have to be looked at in relation to ecological and economic consequences, although damage to cones is not only associated with biotic agents. Schowalter and Sexton (1989) indicated that the major factor in seed losses of Douglas-fir in western Oregon was unexplained developmental failure. In the French Alps during the first year of *P. cembra* conelet development, 66% disappeared mainly because of abiotic factors (85% of the losses) (Dormont *et al*., 1996).

A. Ecological impact

Insects feeding on plant reproductive structures potentially affect abundance, distribution and dynamics of tree populations (Boivin and Auger-Rozenberg, 2016). When they differentially decrease seed production among individuals that vary in some heritable traits, genetic variability can be reduced (Kolb *et al*., 2007). In this way, by directly affecting tree reproductive success, impacts on seed quality and supply for regeneration, reforestation, and conservation purposes can be very important.

These aspects can be very critical for attacks of adult and nymph *L. occidentalis* that feed on cones from a wide range of conifer species (Lesieur *et al*., 2014). In Europe, ecological consequences of its introduction could be significant and its damage is as a serious threat for natural regeneration (Tamburini *et al*., 2012). Roversi and colleagues (2011) estimated that in Italy, the production of edible seeds of *P. pinea* has sharply decreased over the years, and cone crop production collapsed in 2009.

B. Economic impact

In addition to their impact on host ecology and evolution, the economic consequences of insect feeding activity on fruiting structures are diverse, and essentially depend on the developmental stage or tissues that insects target (Roques, 1983). Insect damages on early developmental stages, e.g. flowers or young cones generally inhibit their growth and accelerate their lignification and dehydration, which leads to a premature drop of the fruiting structure. Insect damages on later developmental stages have a weaker influence on cone growth, but they generally lead to intense resin flow that sticks together cone scales, which prevents the release of the unaffected and viable seeds (e.g. the pine cone weevil *P. validirostris*).

P. validirostris and *D. mendacella* have long been known as important agents that damage cones and seeds in several European countries across the Mediterranean basin. After the detection of *L. occidentalis* in Mediterranean countries, a significant decrease in production and productivity of pine cones was reported although attacked mature cones do not show any symptoms of external damage unlike those attacked by many other cone pests. In Portugal this pattern was also seen, and in the last few years a decrease in the yield of pine cone nuts was observed. Data from two major manufacturing industries (Preparadora de Pinhões Ldª and António Pais Ldª) showed stability in pine nut yield during the last 18 years, varying between 3 and 4%. However, in the 2011-2012 season (and subsequent years) the yield dropped to below 2.5%, which had not previously been observed.

However, characterizing the status of empty seeds remained a problem because radiographic interpretations do not allow a clear differentiation between seeds sustaining severe bug damage and empty seeds which have naturally aborted because of a lack of pollination, fertilization problems or any other reasons not linked to insects (Schowalter and Sexton, 1990).

Indeed, overall economic damages due to seed production losses associated with cone pests have not been estimated. Some of the data available from the literature is summarised in Table 1.

Table 1. Examples of cone pests economic damages (*P. validirostris, D. mendacella* and *L. occidentalis*)

P. validirostris	***D. mendacella***	***L. occidentalis***
Spain (Valladolid) - Damages on *P. pinea* between 20% and 56% (Gordo Alonso *et al*., 1997)		**EUA** - damages of 70% on *Pseudotsuga menziesii*. Estimation of a loss of 310 seeds /insect (Bates *et al.,* 2000)
France - Over 80% of the annual production of cones (Roques *et al*., 2004); due to L3 and L4 stages (Roques 1976) **Finland** – About 20 % of cones of *P. sylvestris* and 75% in *P. contorta* (Annila 1975)	**Italy** - About 80% of cones of *P. pinea* (Innocenti and Tiberi 2002)	Abortion 75% of conelets, with a reduction of of 47% of the content in seeds of *P. monticola* (Bates *et al*., 2002) **Mexico** - 30% of cones on *P. cembroides* (Cibrian Tovar *et al*., 1995). **France** - 70% of the seed yield in both *P. nigra* and *P. sylvestris* (Lesieur *et al*., 2014) **Italy** - Production on *P. pinea* sharply on *P. pinea* sharply decreased since its introduction (Roversi *et al.,* 2011) DNA-based diagnostic protocols can be used to quantify damages even when insect excrements or saliva are the only biological traces available (Bracalini *et al*., 2015)

4. Strategies for the integrated management of cone pests

Protection of tree reproductive structures from pest insects is generally a complex process, partly due to the cryptic internal feeding habits of many pest species that makes them difficult to detect and control, and to the spatial heterogeneity of fruiting structures at both tree and stand levels. This is particularly true for insects that spend most of their lives hidden and protected inside pine cones (*P. validirostris* and *D. mendacella*).

If the biology and behavior of pest insects are sufficiently well known, damage can often be controlled by silvicultural, mechanical, biological and genetic measures, without using homologated insecticides.

Repeated spraying or dusting of the trees with chemicals can gradually lead to accumulation of pesticide residues in the seed orchards, upsetting the balance between the pest insects and their enemies. There is also a risk that insects may become resistant to insecticides if chemical control is repeatedly used in seed orchards. Some insecticides may also have phytotoxic effects on seeds, reducing the quality of the seed crop (Annila, 1973).

In Spain, chemical control methods with contact insecticides (deltamethrin) for *P. validirostris* may only be used when adults are in crowns during spring when they leave wintering and fall before wintering. There are no registered insecticides against these pests in Portugal or France. Alternative or complementary methods are being developed and consist mainly of changing the tree species composition in the stand in order to increase ecosystem resistance, and to encourage natural enemies by providing them with both alternative hosts and shelters. Other methods such as seeding or inundating releases of natural enemies (several species are known – *Coeloides melanostigma, Eubadizon atriconis, Scambus brevicornis, Eubazus robustus and Eurytoma annilai, Exeristes ruficolli, Scambus sagax, Scambus sudeticus, Coeloides sordidator, Spathius rubidus*) and mating disruption have been attempted but with limited success (Kenis *et al*., 2004).

As *D. mendacella* larvae are inside the cone at time of collection (autumn/winter), observation of attacked cones and their destruction can be an effective way to decrease populations. Destruction of unharvested cones or fruit left on the soil surface is a complementary measure that serves to reduce overwintering populations of many pest species.

Adult trapping techniques with sex pheromones is expected to be used more frequently in the short term in order to assess emergence and flight periods. *D. mendacella* has been the subject of recent research, having given rise to the knowledge of its pheromonal complex, which will be published in the near future. It is likely that attractive lures will become available within a year or two. These studies show that cone moth females produce (Z,E)-9,11-tetradecadienyl acetate (ZE9,11-14:Ac) and (Z,Z,Z,Z,Z)- 3,6,9,12,15-pentacosapentaene (ZZZZZ3,6,9,12,15-25:H). The former elicits a strong EAG response from males while no response could be recorded for the latter. In field trapping tests, both compounds were individually unattractive to males, but blends of the two compounds were highly attractive (Pajares, 2016).

Control methods against *L. occidentalis* seemed easier at the onset because the populations are always outside the host. Population control in the area of origin mainly comes from the use of broad-spectrum insecticides (Strong *et al*., 2001). The results are variable but chemical control is the only means of effectively controlling the population (Strong, 2006). Luring using the attractiveness of infrared radiation for adults has recently been achieved experimentally (Takács *et al*., 2009). However, no effective trapping technique exists currently.

Only a few studies have dealt with the parasitic spectrum against *L. occidentalis* (*Gryon pennsylvanicum*, *Anastatus pearsalli* and *Ooencyrtus johnsoni* are the most important natural enemies), which appears limited to its area of origin (Bates and Borden, 2004; Maltese *et al*., 2012). It was shown that the rate of egg parasitism can reach 30% with *G. pennsylvaticum* as the dominant species. This species is the subject of recent studies to determine the effectiveness and relevance of a possible European introduction for biological control (Peverieri *et al*., 2012; Roversi *et al*., 2014).

Although parasitoids native to Europe, such as *Anastatus bifasciatus* and *Ooencyrtus pityocampae,* were found parasitizing some egg masses in Italy, it seems that native parasites and parasitoids have yet not adapted to this invasive species (Binazzi *et al*., 2013).

The use of insect pathogenic fungi, *Beauveria bassiana* and *Isaria fumosorosea*, was recently tested in Italy and the Czech Republic, providing encouraging results (Rumine and Barzanti, 2008; Barta, 2010).

5. Regarding the future

Recent decades represent a major turning point for the movement of species and especially the introduction of organisms beyond their areas of origin. For example (Fig. 8), the number of terrestrial arthropod species introduced by year has increased exponentially since the sixteenth century (Roques, 2010) but particularly in recent decades. It can be anticipated that new damaging outcomes of cone pests will occur in the Mediterranean region.

At the same time, in the next decades, Mediterranean forests will be under pressure from climate change and increased demand on ecosystem services related to human expansion in the area (Regato, 2008, Solomon *et al*., 2007, Petit *et al*., 2005). Changes in the biology (insect's developmental cycle, survival and reproduction) and distribution of some forest insects in response to on-going climatic changes is expected and has already been observed during the last 30-50 years (Bale and Hayward, 2010; Menéndez, 2007). Furthermore, the establishment of alien insect species originating from subtropical and tropical climates may increase due to more favorable climatic conditions. In parallel, tree species can also suffer changes in their phenology and vigor, becoming in some cases, more susceptible to native and introduced pests. Additionally, higher temperatures may favor fungi, viruses and nematodes frequently associated with insects. These organisms, which are vectored by the insects, may subsequently weaken host trees, making them more vulnerable to the insect pest attack (Paine *et al*., 1997).

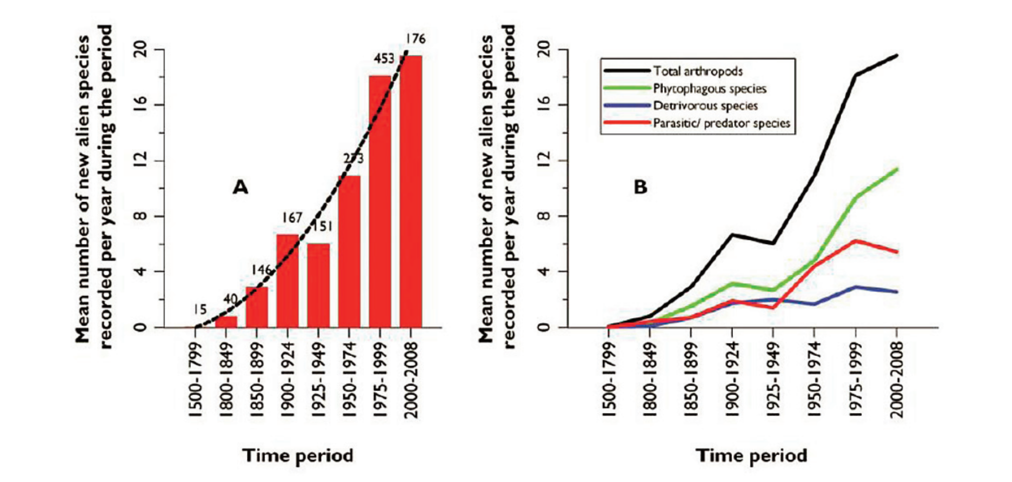


Fig. 8. Temporal changes in the mean number of new records per year of arthropod species alien to Europe from 1500 to 2008. A total arthropods (Best fit: $y = -0.411 - 0.407x + 0.304 x^2$; $r^2 = 0.965$); B Detail per feeding regime (from Roques, 2010).

III – Conclusions

Although considerable knowledge has been gathered over the recent years there is strong need for further knowledge, particularly in some countries of the Mediterranean region. The most important identified knowledge gaps relate to the bio-ecology of pests and their interrelationship with host/stand characteristics, impact of forest management techniques, damage assessment and quantification, and development of eco-friendly and sustainable control methods.

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Session 5

Industry

State of stone pine (*Pinus pinea*) forests in Turkey and their economic importance for rural development

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Abstract. Stone pine is one of the most characteristic tree species in Mediterranean landscape, due to the ancient use of its edible seeds. In Turkey, specifically, stone pine (*Pinus pinea*) is the most valued pine species because of its multiple use. Multi-objective management of stone pine forest focuses on timber and pine nut, as the main products, as well as other beneficial services such as soil protection, recreational use and biodiversity. Among these ecosystem services and products, nut production is currently the most profitable one for the owners of stone pine forests and villagers. Pine nut production in stone pine areas promotes rural development by providing employment and supplementary income for forest villagers. It can play an economic role by increasing income level of forest villagers based on its primary and secondary products. In Turkey, the incomes from pine nut production are around three times greater than the revenues from timber production. In this study, changes in size and structure of stone pine forests in Turkey during 10 years were analyzed in terms of age class, development stage, crown closure, tree mixture, functions and productivity. In addition, the amount of cones in number and weight, in-shell pine nut and kernel production were estimated based on age classes within some regional directorates of forestry (OBM) by using traditional estimation equations of constant per tree cone yields by age classes. Turkey would have a potential to produce about 600,000 tons cones and 15.600 tons pine nut kernels, which with current market prices would be worth US$ 320 million and 550 million, respectively. These figures show that stone pine forests in Turkey can make an important contribution to the rural development and global pine nut market. However, this gross estimation of pine cone or nut was based only on age class distributions and has not taken into account several factors such as climate-driven yield variation or losses due to insect damages. For a better integration of cone or kernel production into forest management plans and the development of a sustainable management oriented to these products will require more realistic empirical yield models. Such models must integrate climate factors and stand variables for allowing more accurate predictions of the actual annual cone and nut production.

Keywords. NWFP-cone production – Rural development – Pine nut export.

I – Introduction

1. Stone pine (Pinus pinea) as a NWFP

Stone pine, *Pinus pinea*, is one of the most characteristic tree species in the Mediterranean ecosystems, specially due to the ancient use of its edible seeds, the pine nut kernel. In Turkey, stone pine is considered as the most valued pine species because of the emergence of multiple use forest management. Multi-objective management of stone pine forest focuses on timber and pine nut as the main products, as well as other beneficial services such as soil protection, recreational use and biodiversity.

Edible kernels extracted from the cones of stone pine are among the most important non-wood forest products (NWFPs). The woody rest of the cone is a renewable resource used as wood based panel (Ayrilmiş e*t al*., 2009). Edible seeds are highly valued for health and gourmet product, with rich flavour and excellent dietetic values. Stone pine nut has about 50% lipids, mainly unsaturated fat acids, and 35% proteins, doubling the protein content of the two other main commercial pine nuts species, Chinese and Pakistani pine (Mutke *et al*., 2012).

Turkey is one of the largest producers and exporters of pine nuts in the world. Based on official production data for 2000-2010, total annual production in Turkey increased from 1,500 to 6,000 metric tons of cones only in state forest, hence not taking into account private forests and plantations (OGM, 2015). Stone pine forests play an essential economic role for forest villagers with nut and timber products in Turkey. Forest villagers can have an employment opportunity and supplementary income. Many rural inhabitants worldwide depend on harvest of pine nuts as they collect and sell them in market, a major economic source for these people (Mutke *et al*., 2012). Thus this product nicknamed "ingenious pine" by rural inhabitants is considered one of the most profitable NWFPs. Furthermore, due to important additional income by picking and selling of this product many landowners do not think of earning their income from timber. Even, in some region of Turkey, like Kozak near Bergama, incomes from pine nut production (about 7 million US$) are about three times greater than incomes from timber (Geray *et al*., 1993). The local forest administration allows local people to collect pine cones for household consumption. But, for commercial purposes, they have to pay a stumpage prices. In fact, the price in effect is not a real stumpage price and is deliberately determined by General Directorate of Forestry (OGM) as quite low to support rural development. However, increasing revenue from pine nut trade causes intense collection of this product. Thus, sustainable management of the product within the framework of ecosystem based multiple use planning is paramount.

Since the initiation of forest management planning in Turkey (1960s), timber has been considered the major forest product, while NWFPs, such as pine nuts, have been considered as "minor" products. Because of complexity of inventory, planning and harvesting of these products, they have not been well integrated into forest management plans up to now. In cases where these products have wide distribution and economic value, "harvesting plans" were mandated to be prepared by the forest management department. In the plans average yield was estimated by information taken just from a few sample points. With the adoption of new ecosystem-based multiple use planning approach, the interest to those products increased over the last decade. New forest management planning guidelines include and consider stone pine forests for cone production, too. However, management plans to integrate NWFP such as cone production have not been well prepared because the lack of field data and the other necessary information, such as optimal rotation ages for cone production, relationships between yield variation and environmental variables, summarized as yield models.

Even though some empirical models analyzing the relationship between cone or nut yield and environmental parameters were developed in other countries (Calama *et al*., 2008; 2011), in Turkey we don't have any yield models for pine cone yet. Some regional studies have discovered empirical relationships between pine cone production and stand age (Sülüşoğlu, 2004; Fırat, 1943; Çukur, 1994; OGM,1995, 2013).

In this study, changes in size and structure of stone pine forests in Turkey during 10 years were analyzed in terms of age class, development stage, crown closure, tree mix, function and productivity. In addition, the amount of cones in number and weight, the resulting hypothetic production of pine nuts in shell and finally of shelled pine nut kernels were estimated based on age classes within regional directorates of forestry (OBM) by using traditional per- tree or per-hectare estimations. Applying current market prices, figures for value of estimated cone and nut production were calculated separately, and they were also referred per-inhabitant for each OBM. In this way, the potential of pine nut on rural development was analyzed.

II – Materials and methods

The data from digitized database of Turkish forests (including state and private areas), demographic information and statistical data of international trade for pine nut were used. Digital database built with Arc/Info GIS for 2015 year was provided by OGM. The names of villages in or adjacent to the stone pine forests were gathered from each OBM. Demographic information about each village for

2015 was supplied from address based registration system by Turkish Statistical Institute (TUIK, 2015b). To estimate of total amount of cone (in number and weight), and corresponding amount of nut and shelled kernel, the following assumptions were used:

- Stone pine stands in the first age class (0-10 years) don't produce cone (Genç, 2004).
- In the second age class (11-20 years): 50 cones/tree (Sülüşoğlu, 2004).
- 30-80 years: 300 cones/tree, corresponding 100-120 kg and 6-8 kg kernel (OGM, 1995, 2013).
- Above 80 years: 200 cones/tree (Çukur, 1994).
- For degraded areas, 1 ha includes 30 trees, 1 tree produces 50 cones and 1 cone produces 10 g kernel.
- For the rest of classes, 70 productive trees per hectare were assumed (OGM, 2006).
- 1 cone produces about 15 g kernel, (Çukur, 1994).
- 4 kg nut in-shell produces about 1 kg shelled kernel (OGM, 2013a).

For the economic value estimation, current market prices for pine cone (0.53 US$/kg) and kernel (35.5 US$/kg) were used. In addition, the international market price for pine nut kernel was provided by TUIK (2015a) as 51 US$/kg.

III – Results and discussions

1. Stone pine forest areas and structure in Turkey

The total area of stone pine forests in Turkey was about 70,000 ha ten years ago (OGM, 2004), but today it approaches 200,000 ha (Table 1). Some OBM hadn't had any stone pine forests in 2004, but they have included the species in 2015 forest plans due to new pine nut plantations. The huge increase in area allocated for stone pine plantations is thanks to various utilization opportunities of stone pine from bark, wood, cones and resin, and also use for aesthetic and soil conservation purposes (Anşin, 1994), though the present interest for pine nuts a business opportunity is overwhelming. According to spatial forest database in 2015, 140,863 ha of stone pine forests are pure stands (71%), more than two times the area occupied by mixed stands with stone pine (56,686 ha, 29%).

Although an ecosystem-based multiple-use planning approach has been adapted in Turkish forestry for almost a decade, analysis of forest plan data indicates that most stone pine forests (58%) are still oriented toward maximum timber production. In only 9% (18,315 ha) of stone pine forests, planning is oriented to maximal pine nut production. Another 12% are devoted in the first place to ecological purpose such as nature conservation, erosion control and climate protection, while in the resting 12%, priority is given to social and cultural ecosystem services, including drinking water provision, community health by preventing air pollution and noise, landscape and recreation, as well as scientific research. Some areas have nature conservation status such as national park, nature protection areas, genetic reserves or for wildlife conservation, and others are mapped for the elevated social pressure they are suffering for not authorized or illegal uses.

Given the threefold expansion of stone pine forests from 70,000 to nearly 200,000 ha only in the last 10 years, it can be easily seen that in terms of development stages, approximately 63% (123,934.8 ha) are still considered regenerated, including those new plantations, whereas 9%, 8% and 6% are quite balanced between young, mature and old-growth, respectively (Table 2). Due to this recent expansion, also in terms of crown closure, most area (54%) still corresponds to low coverage, less than 50%. If crown cover were maintained so low in a future, it would be a positive development for pine nut production, due to positive effect of low coverage on crown diameter and cone formation (Mutke *et al.*, 2012).

Table 1. Total stone pine areas in 2004 and 2015 years according to Regional Directorates of Forestry (OBM)

Regional Directorates of Forestry	Stone pine areas (ha)		Regional Directorates of Forestry	Stone pine areas (ha)	
	2004	2015		2004	2015
Izmir	18,639	59,329	Zonguldak	41	1,041
Muğla	25,517	50,312	Amasya	100	891
Balikesir	6,821	25,523	Ankara	–	721
Istanbul	790	12,121	Kütahya	–	617
Çanakkale	8,560	9,427	Kastamonu	–	366
Bursa	3,145	8,968	Isparta	65	341
Kahramanmaraş	–	8,903	Eskişehir	10	216
Adana	422	7,500	Artvin	100	125
Antalya	–	4,790	Trabzon	–	123
Sakarya	12	3,168	şanliurfa	–	15
Denizli	100	1,665	Bolu	–	11
Mersin	913	1,355	**TOTAL**	**69,294**	**197,550**

Table 2. Turkish stone pine forests according to development stages

Development stages (dbh)	Total areas (ha)
Degraded areas	12,686
a (regenerated, < 8 cm)	123,926
b (young, 8 - 19.9 cm)	17,450
c (mature, 20 - 35.9 cm)	15,820
d (old growth, > 36 cm)	12,404
Unknown	15,265

Some studies about pine nut production indicated that stone pine trees begin to bear cones at the age of 10 years, the ability to seed will continue until the age of 100 and cone production is maximum between 40-50 ages (Genç, 2004). In this study, all stone pine stands in Turkey were evaluated according to the age class distribution obtained from the digital database. In the mixed stone pine stands where long rotation forestry dominates, age classes are ranged according to 20 years. 46% of stone pine forests in Turkey are still in the first, immature age class (under 10 years) without cone production, and only 4.5% exceed 50 years (Table 3).

Table 3. Dominant age class distribution of Turkish stone pine forests

	Total Areas (ha)		
Age classes	Short rotation	Long rotation	Total
I (0-10 year)	90,440	1,294	91,734
II (10-20 year)	35,594	61	35,654
III (20-30 year)	13,875	41	13,916
IV (30-40 year)	6,687	–	6,687
V (40-50 year)	12,605	–	12,605
VI (50-60 year)	7,665	40	7,705
VII (60-70 year)	405	–	405
VIII (70-80 year)	152	–	152
IX (80-90 year)	11	–	11
X+ (90-100 year)	692	–	692
Unknown	26,157	1,794	27,951

Regarding site quality, estimated from dominant height-age relation, stone pine forests in Turkey were classified predominantly (83%) in the medium II. and III. quality y classes, whereas both higher and poorer qualities are quite rare (1.5% and 0.5%). This homogeneity will facilitate future studies on growth and come yield modeling, both known to depend strongly on site quality (Calama *et al*. 2008, 2011).

2. Estimation of potential cone and nut production in Turkish stone pine forest

The potential production of stone pine in Turkey, estimated in the present study roughly by assuming aforementioned theoretical fixed per-tree average productivities for different age classes, would hypothetically mean as much as 600,000 t cones and 15,600 tons pine nut kernels from stone pine areas in Turkey (Table 4).

Table 4. Predicted cone yield in number and weight, corresponding amount of pine nuts in shell and shelled kernel (t) for each regional directorates of forestry (OBM)

OBM	Stone pine Area (ha)	Potential cone production (Units)	Potential cone production (t)	Pine nuts in shell (t)	Pine nut kernels (t)
Adana	7,500	51,612,745	36,431	3,095	774
Amasya	891	3,120,100	2,872	179	45
Ankara	721	1,429,750	2,860	86	21
Antalya	4,790	46,133,550	25,002	2,744	686
Artvin	125	436,800	146	26	7
Balikesir	25,523	54,423,700	53,736	3,238	809
Bolu	11	16,350	20	0.7	0.16
Bursa	8,968	11,331,750	12,701	675	169
Çanakkale	9,427	20,542,215	18,194	1,232	308
Denizli	1,665	4,018,485	6,552	241	60
Eskişehir	216	771,050	380	46	12
Isparta	341	2,088,100	1,933	125	31
Istanbul	12,121	44,807,300	23,216	2,685	671
Izmir	59,329	234,324,500	178,320	13,584	3,396
Kahramanmaraş	8,903	12,735,100	7,608	762	191
Kastamonu	366	625,450	246	38	9
Kütahya	617	836,150	1,025	50	13
Mersin	1,355	3,883,800	2,693	233	58
Muğla	50,312	555,440,400	225,772	33,040	8,260
Sakarya	3,168	1,612,000	2,356	96	24
Şanliurfa	15	52,850	106	3	0.8
Trabzon	123	619,800	411	35	9
Zonguldak	1,041	2,548,700	1,757	153	38
Turkey	**197,550**	**1,053,410,645**	**604,335**	**62,366**	**15,592**

These estimations for potential production are extremely high when compared with official Turkish forestry statistics, which report only about 2,000-6,000 t of cones each year between 2006 and 2015 (OGM, 2015). However, these official statistics refer only to the legal production from state forests, that is, 89,000 ha (OGM, 2013b), only 45% of total stone pine area in Turkey, giving a mean productivity of less than 60 kg cones per hectare, though computing herein also non-harvested regeneration stands, protection forests etc. As a matter of fact, Turkish export statistics report about

1,500 t pine nut kernel exports annually (TUIK, 2015a), and only 90% of Turkish kernel output are exported (Bilgin, 2012). Applying a kernel-cone weight ratio of 4%, plausible annual cone production exceeds 40,000 t, that is about 200 kg/ha of total stone pine area, but more than 600 kg/ha when referred to areas in 2004, excluding hence immature newer plantations (Table 1). These averages values are in the same order of magnitude as references in other countries (Calama *et al*., 2008, 2011; Mutke *et al*., 2012). Nevertheless, the hypothetic productive potential of Turkish pine forests given here exceeds more than tenfold this value, and more than twofold the actual production of Mediterranean pine nut kernel in the world (Mutke *et al*., 2012). Possible causes are their calculation depending just on age classes, assuming per-hectare average cone yields up to 7,000 kg/ha, while other important factors that limit yields, such as climate or insect damages, could not be reflected in the gross prediction at regional OBM scale.

Anyway, Muğla is, and was predicted correctly as, the most productive OBM among all because of the extension of its mature stone pine stands. Though Izmir has today a wider stone pine area, for the moment it would produce less cones and pine nuts than Muğla, due mainly to its age classes centred in the first and second one (less than 20 years). Indeed, OBM Izmir has more than tripled its stone pine area only since 2004 (Table 1). On the other hand, in OBM Muğla many stone pine forests are between 30-80 years old, the most productive age classes for cones.

According to the information from the address-based registration system in 2015, the total rural population in the villages or neighborhood located in or adjacent to stone pine forests is about 881,000. This is about 1% of total population of Turkey. Regionalized yield estimations (Table 4) imply a total market value of those potential cone and kernel yields from Turkish forests exceeding US$ 320 million and 550 million, respectively, even US$ 790 million if all kernel were exported – assuming no world market price decay. Referring the market values of hypothetic annual average cones and kernels yield estimations to regional rural population in each OBM, per-inhabitant figures are about US$ 360 and 630, respectively. But even assigning only the reverse-estimated mean annual cone production considered plausible, 40,000 t, market value of cones exceeds US$ 20 million, kernels US$ 55 million, this is US$ 24 and 62 per rural inhabitant, respectively. In fact, official exportation statistics have computed annual pine nut export for US$ 25-68 million between 2007 and 2013 (TUIK, 2015a). More interesting, if in cone harvesting an average man-day yield of 400 kg cones is estimated, cone harvest season will require about 5,000 man-month of local, rural employment each year.

IV – Conclusions

This study has evaluated the potential of cone or pine nut kernel production in Turkey, supposing that postulated per-tree and per-hectare can be matched. The exportation incomes from pine nuts are higher than the other important NWFPs such as bay laurel or lime tree when they are compared. Therefore, stone pine forests might be allocated primarily for production though always based on compatible multiple use forest management planning approach. Stone pine forest areas are drastically increasing over the last decade. Though it is a good progress, the quality of the expanded stone pine areas to produce high rate of cones and nuts need to be improved too.

In addition, traditional prediction methods for cone or nut production from fixed per-tree yields per age-class are not effective estimations, because this coarse calculation does not take into account yield variation due to soil or climate factors, silviculture (stand density) or insect damages and could not reflect real production. Integration of cone or nut production into forest management plans and the development of a sustainable management of these products will require therefore sound empirical yield models which are still to be developed. Such models built by climatic and stand variable would allow us to accurately predict the cone or nut production. Thus, the first step in integration of the products into forest management is to focus on modelling the production of pine cone or nut. The integration of these models to decision support systems will help forest managers and planner to specify management activities for optimal co-productions of various forest values. Empirical models and decision support systems will have a great potential to support the nut industry. Such approach would provide good opportunities for developing countries' economy and rural development. Besides, management treatments such as reforestation and rehabilitation of degraded areas should keep going. Because more added value can be obtained from kernel sale than cone sale based on this study results, the last suggestion would insist for the establishment of processing plants working on pine nut in the rural areas.

Acknowledgments

The authors gratefully thank for the material support from OGM, OBMs and TUIK.

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Insights on the value chain and management practices of stone pine forests in Lebanon

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Abstract. Drawing the value chain of stone pine (*Pinus pinea* L.) forests (SPFs) in Lebanon requires defining habitat characteristics and vegetation composition, mapping stakeholders and forests governance structure, understanding local and international markets as well as collecting time series data on forests production and revenue. Remote sensing techniques and vegetation surveys were used to draw the ecological profile of SPFs. Information gathering on the extracted goods and annual cost and revenues of pine nuts resulted in a 20-years time series data (1996-2016). Data mining revealed limitations for the designed research study and prevailing narratives on the total annual production of pine nuts. Rapid appraisals with government representatives, land tenants and managers defined the existing administrative and management framework of these forests. The community of labors is regulated by the Order of Labors and Agriculture of SPFs. The workforce is mainly composed of foreigners with 400 forestland's tenants. The average annual production generated from 6-years life cycle for pruning reaches up 1,100 t (metric ton) of pine nut kernel. The average local market price of pine nuts remaining higher than the imported one, increased from US$ 20 to 90 between 2000-2016. Turkey and Italy are the major importers of Lebanese pine nuts. The net revenue was estimated at US$ 37.8 million in 2012 and US$ 5.38 million in 1996. Since 2014, drop in the annual production was estimated at 500 t/year. The decrease in production is attributed to the Western Conifer Seed Bug (WCSB, *Leptoglossus occidentalis*). The insect has been affecting not only the annual production of nuts but also the land tenancy of SPFs.

Keywords. Nut – Value Chain – Net revenue – Lebanon.

I – Introduction

In Lebanon, pine nut is recognized as the "white gold" among managers and tenants of stone pine forests (SPFs). Stone pine, representing the second emblematic tree for Lebanese population next to Lebanese cedar, was initially introduced for soil stabilization in the 16th century (Saghieh, 2001). Those forests occupy 9.5% of the total forest cover in Lebanon (12,755 ha) (FRA, 2010) with 5,400 ha of productive area (Sattout *et al.*, 2005). They grow ideally between 800 to 1,500 meter altitude on sandstone and limestone soils, in the thermo-, eu- and supra-Mediterranean life zones (Abi Saleh *et al*., 1996). While these forests are threatened by anthropogenic activities, they feature important ecological functions and socio-economic values. SPFs provide the highest gross revenue generated from direct forest goods in the country, followed by hunting (Sattout *et al.*, 2005, Sattout, 2014). The high demand of pine kernels results from its use in Mediterranean cuisine and delicacy as well as Arabic sweets and pastry in Jordan, Lebanon, Palestine, Syria and Turkey. The total annual Lebanese pine nuts production is estimated at 75 to 280 kg/ha with total annual revenues ranging from US$ 16.5 to 52.5 million (Sattout *et al.*, 2005; Awad *et al.*, 2014; Sattout, 2014). The national market is being affected by the import of pine nuts from Turkey, which price is less than half the local production as it was the case in early nineties (Darwish *et al.*, 1996). The main goal of this study is to set the scene for a long-term research program on socio-ecological functioning and economic values of SPFs. The objectives are first to define the abiotic characteristics of SPFs and plant species composition, second to identify their management structure and practices, and third to estimate the net revenue generated from pine nuts over the past twenty years period.

II – Materials and methods

1. Habitat characterisation and floristic composition

Remote sensing data acquired by satellite sensors including daily MODIS LST products MOD11 (EARTHDATA NASA), CHIRPS released by US Geological Survey (USGS) and Climate Hazards Group (CHG) scientists (Funk *et al.*, 2015), CHIRPS 2.0 and combined daily MODIS snow products were processed. The aim was to obtain the most recent accurate information on temperature, rainfall and snowfall in each of the districts of Metn, Shouf and Jezzine using the software ARCGIS 10.3.

Data collection on floristic composition of SPFs was performed in March and April 2016. Surveys were performed in 20x20m quadrats located in six different locations in Metn and Shouf. One sampled quadrat was randomly selected in the defined site. Sites selection aimed at capturing the different vegetation communities growing in SPFs.

2. Data collection and estimation of net revenue

Data collection and information gathering on pine nuts production, governance and management, as well as market dynamic including cost and revenue were done during winter and spring 2016. The methodology relied on Rapid Appraisals (RAs) with representatives from the Ministry of Agriculture (MoA) and the Order of Labours and Agriculture (OLA) of SPFs as well as forestlands tenants and managers in the Metn and Shouf districts.

The calculation of the Net Revenue of pine nuts production and associated byproducts (i.e. scales, shell, shell dust) took into account the cost of management and processing, in addition to their revenue. It was calculated over a period of 20 years based on time series data provided by the OLA of SPFs.

Net Revenue = (Rpn + Rcp) - (Cch + Cpc + Cmgt)

Rpn	Revenue pine nuts
Rcp	Revenue cones byproduct
Cch	Cost of cones harvesting and collection
Cpc	Cost of cone processing
Cmgt	Cost of maintenance including pruning and understorey clearing

Data on the importers and exporters were provided by the Chamber of Commerce, Industry and Agriculture of Beirut (CCIAB). The available database was restricted to the years 2012-2015.

III – Results and discussion

1. Habitat and vegetation composition

SPFs are found at high density in Mount Lebanon in districts of Kesrouan, Metn, Baabda, Aley; in Shouf, Jezzine districts in South Lebanon; and in Marjeyoun and Hasbaya in Nabatieh. Stands with low density are found in Saida, Jbeil, Akkar and Batroun (Fig. 1). These forests are distributed mainly on leptosols and arenosols. They grow in area with an average temperature ranging from 12˚C to 23˚C and an average rainfall of 800-1,400 mm/year (Fig. 1). The six sites featured a variety of vegetation communities including *Quercus coccifera* L. and *Q. infectoria* G. Oliv., *Arbutus unedo* L., *Cercis siliquastrum* L., *Calycotome villosa* (Vahl.) Link, *Juniperus oxycedrus* L., *Cistus salviifolius* L. *and C. creticus* L., *Calluna vulgaris* (L.) Hull., *Myrtus communis* L., *Lavendula stoechas* L., *Origanum ehrenbergeri* Boiss. and *O. syriacum* L., *Salvia officinalis* L., and *Corydothymus capitatus* (L.) Reich.

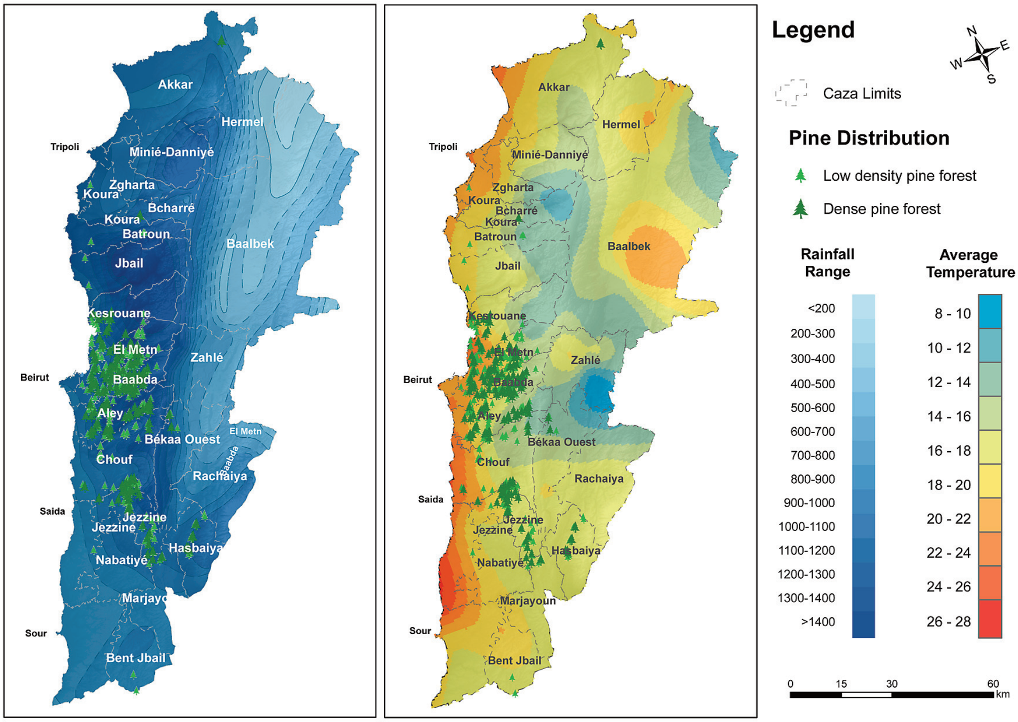

Fig. 1. Distribution and habitat characteristics of SPFs in Lebanon.

2. Governance

A. Land ownership and workforce

Stone pine forests are distributed on communal, religious and private lands. Religious lands constitute 35 to 40% of the total area of SPFs. Those are subject to long-term land lease contracts. Communal lands occupy 20% of SPFs. The municipalities manage those lands in close coordination with the MoA. Following auctions procedures, they are leased on short-term basis with strict term of conditions. Private lands extend over 40% of SPFs, whereby the land's owners are direct users and managers in most of the cases, otherwise they sign short-term leasing contracts. The forestland tenants are around 400 in number. The workforce is mainly composed of foreigners because of the low labor's cost. Labors community falls under the stewardship of OLA of SPFs and workers are granted a yearly health insurance.

B. Management practices

In Lebanon, SPFs are rainfed and traditionally managed. They exclude any agricultural practices such as fertilization, pulverization and mechanization. Thinning and understorey clearing are executed in the managed forests. Over the years, the management practices are still relying on manpower and exclude the use of new technologies and/or adoption of sophisticated protocols. The forests are pruned at 5-years interval, with restrictions on the removal of dead trees. The different pruning techniques revealed to impact yearly cone production, the know-how is based on skilled SPFs managers. Lately, the banning imposed on clearing dead wood contributed to the rapid spread of the pests (*Tomicus destruens*) to nearby SPFs. Consequently, the outdated forestry laws and existing regulatory measures are the main constraints to foresee the implementation of sustainable forest management in general. And in particular, they will affect the leasing of stone pine forestlands, in addition to the quality and quantity of their production.

3. Industry chains and revenues

A. SPFs goods and services

The good and services provided by SPFs could be classified under four business models (Fig. 2). The agroforestry and gardening industries constitute the outlet for forest provisioning services. The cone's scales and nut shells are used in gardening aesthetic, water and soil conservation and weed control. Dust resulting from cone processing is used as soil amendment. The 'ecological functioning' industry targets regulating services while the tourism industry combines under its umbrella the SPFs' landscape character and its aesthetic value as well as amenity services. Even though, some uses were not identified through the RAs done in the context of this study, it is worth mentioning that the resin is applied to goats as insecticide (Masri in Saghieh, 2001).

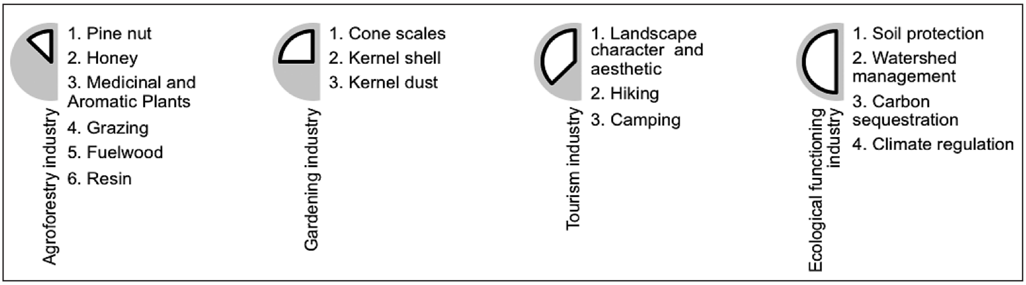

Fig. 2. Industry outlets of SPFs good and services.

B. Production and net revenue

Over the past two decades, the annual average pine nuts production at 6-years interval for pruning was approximately estimated as 1,100 t. The highest annual production reached up to 1,300 t and the lowest 500 t. An important drop in the quantity of pine nuts produced was observed during the last cycle starting 2014 whereby it ranged between 500 and 900 t (Fig. 3). All over the years, the fluctuation is attributed to the prevailing practices in SPFs management coupled with the production cycle of trees affected by biotic and abiotic conditions of sites. The RAs with managers revealed that total annual pine nuts production could reach up more than 2,500 t (Mr. Neaimeh E., personal communication – April 2016).

The average local market price increased from US$ 20 to 40 between 2000 and 2010, to reach US$ 90 in 2016 due to shortage. This year, the international market price reached US$ 70. The main importers of pine nut are Italy, Turkey, Jordan, Qatar, Kingdom of Saudi Arabia, Brazil and United States. Revenues from transactions range from US$ 9,000 to 12,250,000 between 2012 and 2015 (Eng. Massoud E., personal communication - CCIAB, 2016).

Time series data calculation of the net revenue showed the highest value in 2012 (US$ 37.8 million) and the lowest in 1996 (US$ 5.38 million) (Fig. 4). In the last few years, SPFs have been subject to the Western Conifer Seed Bug (WCSB) observed across the entire Mediterranean basin. In Lebanon, the pest affected not only the annual pine nuts production but also the forestlands lease. Tenants are abandoning the lands because of the depreciating ratio of cost/benefit.

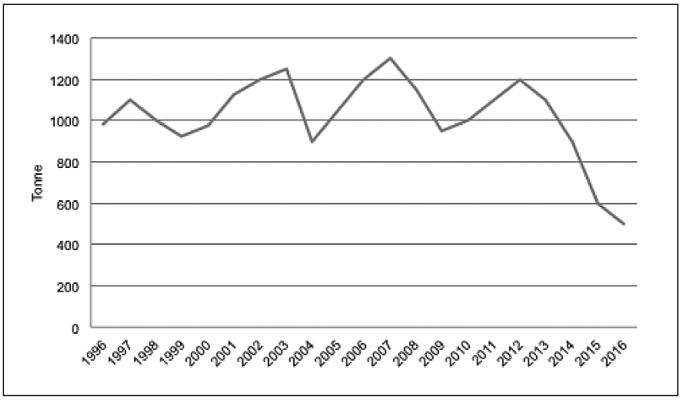

Fig. 3. Average annual production of pine nut kernel 1996-2016 (t).

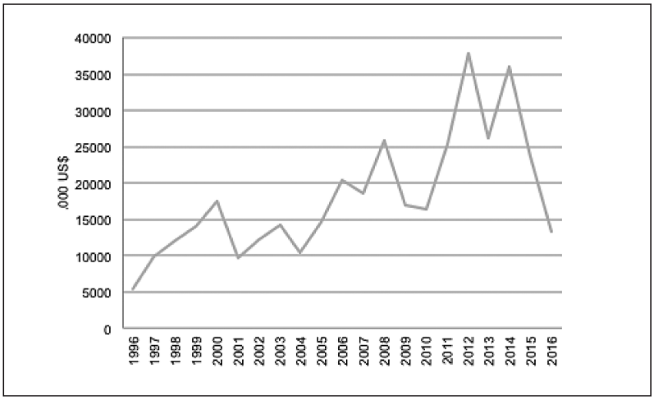

Fig. 4. Variation of net revenue of pine nuts production 1996-2016 (.000US$).

IV – Conclusion

The average Lebanese pine nuts production was approximately estimated at 1,100 t. Land tenants and skilled managers believe that annual pine nuts production could reach up more than 2,500 t. Those constructed narratives might be true or they may be overshooting the real value of total annual pine nuts production in the country. Insights on what could be lurking behind those spoken accounts, bring to the forefront various issues among which protection of stone pine forests from development projects, reforestation policy and conservation of SPFs landscape character. Those latters have an impact on real estate value.

With the time series data mining emerged the prevailing limitations for a complete study at this stage. Further research needs to foresee the validation of the time series data and consolidation of the existing national databases. The ambitious goal as expressed by local stakeholders highlights the importance of first acquiring a better understanding of the ecological conditions and management practices of SPFs and their impact on all services provided by these forests; second revisiting existing forestry laws (Law Nb. 85-12/9/1991, Law 558-24/7/1996) and national forest policy to ensure better an inclusive planning approach for the management of SPFs; and setting the path for their sustainable management.

Acknowledgments

We thank Mr. Abboud Freiha from the Lebanese Ministry of Agriculture and Mr. Elias Neaimeh from the Order of Labors and Agriculture of Stone Pine Forests for their support on the time series data. We acknowledge the support provided by Dr. Micheline Wehbe from the Lebanese University on data collection.

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FAO-CIHEAM Network on Nuts
IAMZ-CIHEAM
INIAV
ISA
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INIA
IUFRO RG 1.08.00 - Silviculture for production of edible fruits
FAO Silva Mediterranea

PROGRAMME

WEDNESDAY, 18TH MAY 2016

09:00 REGISTRATION

09:45 OPENING SESSION

10:15 The FAO-CIHEAM Interregional Cooperative Research Network on Nuts

10:30 **Session 1. MANAGEMENT OF STONE PINE FOR CONE PRODUCTION IN AGROFORESTRY**

Keynote Speaker: **Margarida Tomé, ISA (Portugal)**

11:00 COFFEE BREAK

11:30 Spatial and temporal changes in the pine nut forest of Turkey: a case Study in Ayvalik Forest Planning Unit. **D. Mumcu Küçüker, KTU (Turkey)**

11:45 Effect of fertilization on the mineral composition of pine stone needle. **F. Calouro, INIAV (Portugal)**

12:00 The needles as drivers of tree productivity: relationship with growth, vitality and cone production in *Pinus pinea.* **A. C. Correia, ISA (Portugal)**

12:15 Determination of effect of fertilizing on cone yield rate of stone pine (*Pinus pinea* L.) in Kozak province/Turkey. **M. Özçankaya, EFRI (Turkey)**

12:30 Trials on *Pinus pinea* and *Pinus halepensis* rootstocks from Tunisia and Spain. **M. Piqué, CTFC (Spain)**

12:45 DISCUSSION

13:00 LUNCH

14:30 **Session 2. GROWTH AND YIELD MODELLING**

Keynote Speaker: **Rafael Calama, INIA (Spain)**

15:00 Development of a spatial tree growth model for stone pine – PINEAFITS. **J. Ribeiro, ICNF (Portugal)**

15:15 Tree growth and gas exchange of stone pine (*Pinus pinea*) in northeastern Tunisia. **S. Fkiri, INRGREF (Tunisia)**

15:30 Stone pine responses to the combined effects of drought stress and warming. **R. Lobo-do-Vale, ISA (Portugal)**

15:45 Does management improve the photosynthetic capacity of stone pine (*Pinus pinea* L.) in northwest Tunisia? **T. Rzigui, Silvopastoral Institute /INRGREF (Tunisia)**

16:00 Evaluation of interannual growth prediction in *Pinus pinea* stands from multi-temporal UAV imagery datasets. **J. Guerra Hernández, ISA (Portugal)**

16:15 DISCUSSION

16:30 COFFEE BREAK

17:00 POSTER SESSION

THURSDAY, 19th MAY 2016

09:30	**Session 3.** **GENETIC IMPROVEMENT, SELECTION AND BREEDING OF STONE PINE**
	Keynote Speaker: **Bruno Fady, INRA (France)**
10:00	Reproductive phenology of *Pinus pinea.* **T. Valdiviesso, INIAV (Portugal)**
10:15	Establishment of clonal stone pine orchards as nut crop. **S. Mutke, INIA (Spain)**
10:30	*Pinus pinea* clonal orchards: contributions for genetic improvement. **C. Silva, AFPC (Portugal)**
10:45	COFFEE BREAK
11:15	Variability of kernel yield in Portuguese *Pinus pinea* L. stands from Provenance Region V **I. Carrasquinho, INIAV (Portugal)**
11:30	DISCUSSION
11:50	**Session 4.** **BIOTIC RISKS AND THEIR IMPACT ON STONE PINE PRODUCTS**
	Keynote Speaker: **Edmundo Sousa, INIAV (Portugal)**
12:20	Distribution of *Leptoglossus occidentalis* Heidemann (1910) in Turkey and its impact on stone pine forests. **S. Parlak, Bursa TU (Turkey)**
12:35	Genetic structure and invasion pathways of the Western Conifer Seed Bug, *Leptoglossus occidentalis*, in the Iberian Peninsula. **A. Farinha, ISA (Portugal)**
12:50	Pine nut damage on immature cones of *Pinus pinea* L: evidences for Leptoglossus causality. **R. Calama, INIA (Spain)**
13:10	LUNCH
14:30	Seasonal damage on stone pine cones and seeds caused by feeding of *Leptoglossus occidentalis* (Hemiptera: Coreidae). **J. Pajares, U. Valladolid (Spain)**
14:45	Does forest management have an effect on cone pest damage? **P. Naves, INIAV (Portugal)**
15:00	DISCUSSION
15:20	**Session 5.** **PINE NUT INDUSTRY AND MARKETS**
	Keynote Speaker: **Pedro Amorim, PineFlavour, Lda (Portugal)**
15:50	Local population's role in the collection and processing of stone cones in Rimel Forest (Bizerte, Tunisia). **S. Sebei, INRGREF (Tunisia)**
16:05	Value chain of stone pine forests in Lebanon: insights on good management practices. **E. Sattout, U. Balamand (Lebanon)**
16:20	State of stone pine (*Pinus pinea*) forests in Turkey and their economic importance for rural development. **D. Mumcu Küçüker, Karadeniz T.U. (Turkey)**
16:35	COFFEE BREAK
17:05	Stone pine in Portugal: main policies and recent developments. **C. Ferreira, ICNF (Portugal)**
17:20	DISCUSSION
17:40	FAO *Silva Mediterranea* Working Group 2 on Non-Wood Forest Products Agenda
17:50	FAO/CIHEAM Stone pine subnetwork follow-up session
18:10	CLOSING SESSION

FRIDAY, 20th MAY 2016

08:30	FIELD TRIP Alcácer do Sal, Alentejo. *Departure from Praia Mar Hotel, return* 17:30 Lisbon Airport

THE FAO-CIHEAM INTERREGIONAL COOPERATIVE RESEARCH NETWORK ON NUTS

M. Rovira, S. Mutke and A. López-Francos

FAO-CIHEAM Research Nut Network on Nuts

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The Research Network on Nuts was established in 1990, after an expert consultation organized by FAO (REU, RNE and AGPS). Exchange of scientific information, joint applied research, exchange of germplasm, and establishment of links between researchers were identified as the main objectives. In 1996, FAO and CIHEAM agreed to cosponsor the Network. CIHEAM was already involved in fostering nut tree research activities.

The Network structure is based on a Coordination Centre (Coordinator and Secretary), supported by different Subnetworks (Working Groups) with the mission of fostering and coordinating specific activities. IRTA Mas de Bover has been the Coordination Centre from the start of the Network activities, in 1990. Today, the Network has 7 Subnetworks (6 tree crop species and 1 miscellaneous): Almond, Chestnut, Hazelnut, Pistachio, Stone Pine, Walnut, all of them having a Liaison Officer as Coordinator, and one miscellaneous, including Pecan, Genetic Resources and Economics, which is included in the general coordination. Two representatives, one from each supporting institution (FAO and CIHEAM) are also integrated in managing the Network.

The general activities of the FAO-CIHEAM Research Nut Network are proposed, discussed, agreed and planned in the Technical Consultations (participation of representatives of the member countries) and at the Coordination Board meetings (FAO and CIHEAM Officers, Network Coordinator and Subnetwork Liaison Officers).

The main activities carried out during the last 20 years have been: promotion of R&D activities; edition of proceedings, reports, and the NUCIS Newsletter; edition of inventories of germplasm and research lines; organization of meetings, workshops, and two international courses on “Nut Production and Economy”, and providing training grants for young researchers.

Within the network, stone pine (*Pinus pinea*) is particular. Being a conifer, its *pine nuts* are seeds, not true nuts; its cones, not seeds, are gathered, and until recently, they had been wild-collected, not orchard-cropped, without any defined cultivars. Only in the last 20 years have new specific plantations for cone production come into production, and first elite clones have been registered recently as basic materials for graft scion supply. In 2011, AgroPine2011 was held in Spain as First International Meeting on Mediterranean Stone Pine for Agroforestry, co-organised by our stone pine Subnetwork together with several institutions. The meeting brought together about forty experts, researchers, public and private forest managers, land owners, and representatives of pine nut processing enterprises, from Spain, Portugal, Tunisia, Turkey and Lebanon, with contributions also from France and Chile, reviewing the current state of the art in Mediterranean pine nut production in forests and orchards.

Welcome to *AgroPine2016*, the 2nd Meeting on Mediterranean Stone Pine for Agroforestry! The main objectives of this meeting are to connect the different researchers from the Mediterranean region, to update, share and transfer current knowledge and to foster further links between researchers, industrials and potential users of this species. We invite all of you to participate, discuss the challenges of the future, and propose ideas and activities to keep the Stone Pine Subnetwork alive.

Mercè Rovira, Nut Network Coordinator

Sven Mutke, Liaison Officer for Stone Pine

Antonio López-Francos, CIHEAM Representative

CIHEAM

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Mediterranean pine nuts from forests and plantations

Edited by:
I. Carrasquinho, A.C. Correia, S. Mutke

OPTIONS méditerranéennes

Mediterranean pine nuts kernels, the seeds of *Pinus pinea*, are among the world's most expensive nuts, with a value chain of several hundred million euros annually. Cones are still mainly wild collected from 0.7 million hectares stone pine forests in the Mediterranean area. In the last twenty years, its cultivation as nut crop has been increasing, approximating 0.3 million hectares of new plantations in its home range, and incipiently in New Zealand, Australia and Chile. Domestication is advancing and first registered clones with outstanding cone production have been released for graft scions.

In this panorama, the 2nd International Meeting on Mediterranean Stone Pine for Agroforestry - AgroPine 2016 took place on 18th-20th May 2016 in Oeiras, Portugal. The meeting brought together more than 80 experts, researchers, public and private forest managers and land owners, as well as pine cone processing enterprises from Portugal, Spain, Tunisia and Turkey, with some participants from France, Italy, Lebanon, and Australia.

The five topics discussed during the meeting were: Management for cone production in forests and agroforestry; Growth and yield; Genetic improvement; Biotic risks and their impact on stone pine products; and Pine nut value chain. The present issue of *Options Méditerranéennes Series A* comprehends the proceedings of the meeting, with 14 full articles from the contributions presented at the Meeting.

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