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SHORT COMMUNICATION

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Tomography as a method to study umbrella pine (*Pinus pinea*) cones and nuts

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Abstract

Aim of study: Umbrella or stone pine (*Pinus pinea*) nuts are one of the most valuable and expensive non-wood forest products in Portugal. The increasing market and landowner's interest resulted on a high expansion of plantation areas. This study tests the feasibility of using tomography to characterize pine cones and nuts.

Area of study: The research was carried out in pine stand, with nine years, grafted in 2011, on Herdade of Machoqueira do Grou, near Coruche, in Portugal's central area.

Material and methods: Starting in June 2015, ten pine cones in their final stage of development, were randomly monthly collected, and evaluated with tomography equipment commonly used in clinical medicine, according to Protocol Abdomen Mean. A sequence of images corresponding to 1mm-spaced cross-sections were obtained and reconstructed to produce a 3D model. The segmented images were worked using free image processing software, like RadiAnt Dicom Viewer, Data Viewer and Ctvox.

Main results: The cone's structures were clearly visible on the images, and it was possible to easily identify empty pine nuts. Although expensive, tomography is an easy and quick application technique that allows to assess the internal structures, through the contrast of materials densities, allowing to estimate pine nut's size and empty nut's proportion. By analysis of ninety images, it was obtained, an estimated mean value of 25.5 % empty nuts.

Research highlights: Results showed the potential of tomography as a screening tool to be used in industry and research areas, for analysis and diagnostic of stone pine cone's structures.

Keywords: pine cone; pine nuts; stone pine; tomography; CAT.

Abbreviations: CAT: computerized axial tomography; 3D: three dimensional; HU: Hounsfield Units.

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Introduction

Pinus pinea L., known as umbrella or stone pine, is a Mediterranean tree species with great importance in Portuguese forestry where the areas for fruit production have steadily increased in the last decades (Calado, 2012).

The seeds of stone pine, or pine nuts, are eatable and have a recognized nutritional and organoleptic value, reaching high prices in the market. However, they suffer strong competition from pine nuts of other species, with lower quality and price (Costa & Evaristo, 2014), namely from *Pinea koraensis*. The production of cones and pine nuts has decreased in the recent years according to the Portuguese industry. This may be consequence of the presence of *Leptoglossus occidentalis*, a sucking insect that feeds on seeds from various species of conifers (Tamburini *et al.*, 2012).

The harvest season for pine cones is regulated (Decree-Law 77/2015, from May 12th) and only mature cones (third year cones) are harvested, in winter. There are however some knowledge gaps regarding phenology and maturation of cones and nuts. The process of opening cones in the laboratory to evaluate nut maturation and yield determination is very time consuming. Here we evaluate the possibility of using tomography, a quick, easy and non-destructive (without opening cone) technique, as an indirect method to estimate pine nuts yield, by determining the total number of nuts in the cone and the number of empty or unhealthy nuts. To our knowledge this is the first time that computerized axial tomography is used for this type of evaluation in pine cones, although this technique has been applied at other related research areas e.g. in conifer seeds and cones (Cresswell *et al.*, 2007; Gee, 2013), wood and fibers (Bensadoun *et al.*, 2014) and cork (Oliveira *et al*., 2015).

Material and methods

Third year stone pine cones were used. A sample of 10 cones was randomly collected every month between June and September 2015 in Herdade of Machoqueira do Grou, where a stone pine research project is underway (PINEA project). A total of four samples, each one with 10 repetitions (cones), were examined.

The samples were taken to the laboratory where each pine cone was measured regarding weight, volume, crossed diameters and length (Figure S1 [online supplement]).

Tomography and image processing

Each group of cones was submitted to tomography monthly just after harvesting and measurement in order to avoid preservation in cold and prevent accumulating water in the structures, damaging quality of images obtained.

Computerized axial tomography (CAT), or computed tomography (CT) is an X-ray emission technique that gives information on the amount of radiation absorbed by the study object, which is related to its density. A sequence of cross-sectional views is obtained with a determined gap. Images in gray scale reflect the density variations in the object, allowing to distinguish them. Each pixel has a gray color given by an average absorption value that may be expressed in Hounsfield Units (HU). In this scale, 0 matches the water density, -1000 the air density and +1000 corresponds to high density materials (Knipe *et al.,* in http://radiopaedia.org/articles/computed-tomography). Cross-sectional scans can then be reconstructed in a 3D model by an image software.

The CAT scans were carried out at Faculdade de Medicina Veterinária, Universidade de Lisboa, using a medical use device (Toshiba equipment). Given the size and structure of the samples, CAT was performed according to Protocol Abdomen Mean. The cones were placed on the scan table, spaced over 88 mm in length, supported on the base and with the top up (Figure S2 [online supplement]). Images were collected at 1 mm spacing and computer processed (Figure S2 [online supplement]).

The segmented images were worked using free image processing software, as RadiAnt Dicom Viewer, Data Viewer and Ctvox, depending on the type of analysis. The RadiAnt Dicom Viewer software (RadiAnt DICOM Viewer 1.9.16.7446 build on May 23, 2014; available in http://www.radiantviewer.com/) (Figure 1) was used for measurements, e,g, pine nut dimensions (Figure 2 a) and evaluation of different cone structures through their densities (Figure 3), expressed by HU mean value determinations. The Ctvox software allows a 3D image reconstruction that provides a global view of the pine cone. In the present study, the analysis of different tissues focused in each batch on cone number 4 and in its central area. In this way, in total four images were analyzed, one for each month. The HU mean value was estimated for the different cone's structures (i.e. cone's scales and axis, pine nut's shell and kernel, and empty pine nuts) (Figure 3). The pine nut's dimensions were also calculated (Figure 2). The number of nuts was counted on all the 40 pine cones, and in each cone three axial sections corresponding to 25, 50 and 75% of the cone where all the normal pine nuts were identified i.e., whose interior was clearly visible. The empty or unhealthy pine nuts were also counted. The images were analyzed using the mode option "Negative", because the contrast was clearer. Finally, the proportion of empty seeds per cone and per month was estimated.

Results and discussion

Tomography produced a sequence of images with good quality (Figure 1) that clearly allowed the identification of the different structures within the cones. Since several projections (axial, coronal and sagittal) can be made it was possible to view the cone in three dimensions and easily distinguish the different structures in the cones in these planes. The difference between empty and not empty pine nuts was obvious (Figure 1). It was also possible to perform measurements and estimate the values of HU of these structures.

The pine nuts (over shell) were 17.87 mm in length and 8.54 mm in width (mean values), respectively ranging between 16.89 mm and 18.81 mm, and between 8.06 mm and 9.17 mm. The lowest values were observed in September and the highest value in July. The number of pine nuts in the analyzed section by cone ranged between 7 (July) and 12 (August) (Figure 2b).

The determination of the HU values was made by selecting a study polygon with a certain area (cm^2) and number of existing pixels (px), and calculating the mean, standard deviation, maximum and minimum

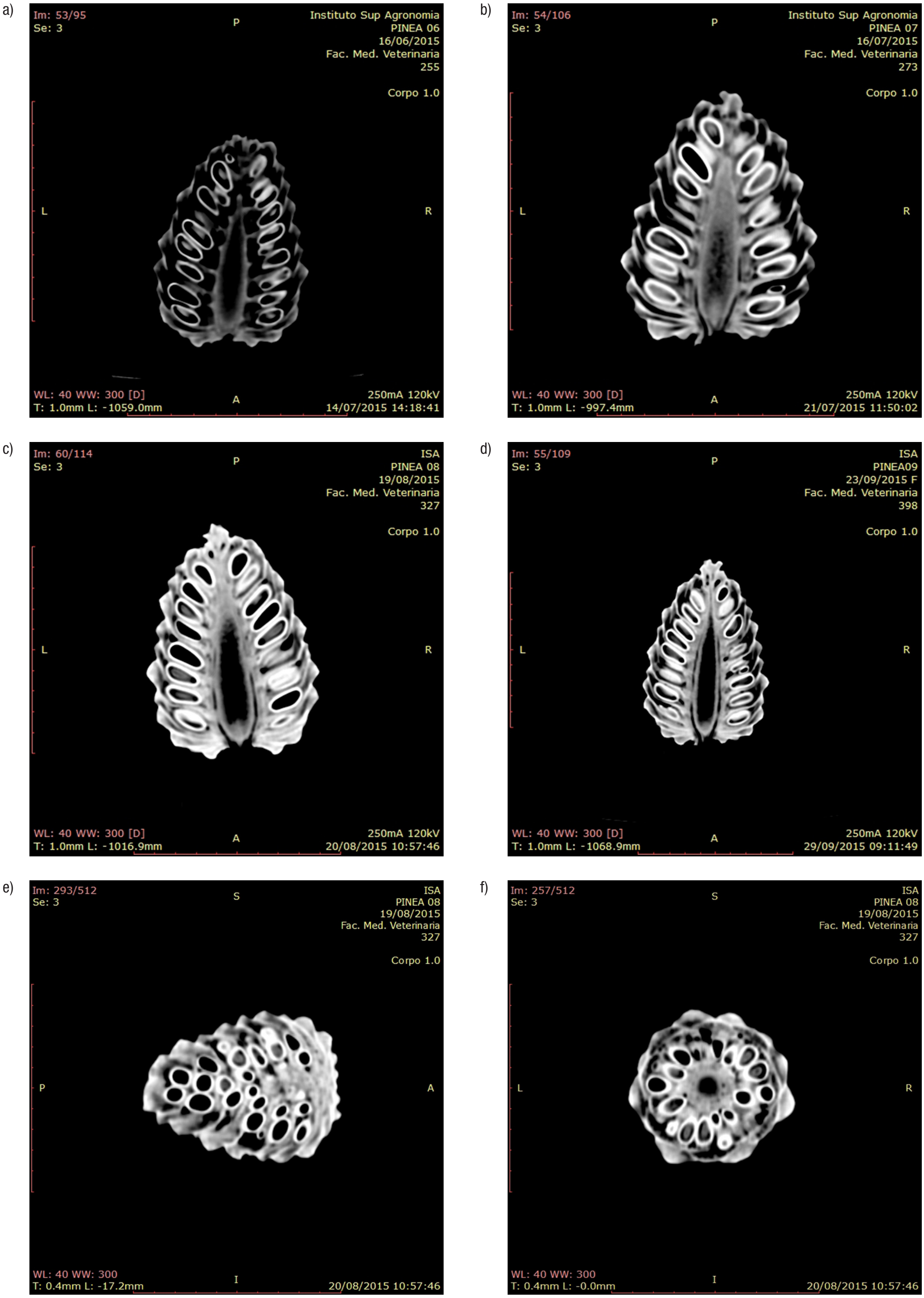

Figure 1. Monthly CAT greyscale views with RadiAnt Dicom Viewer: in June (a), July (b), August (c, e, f), and September (d). Axial view (a, b, c, d), sagittal view (e) and coronal view (f).

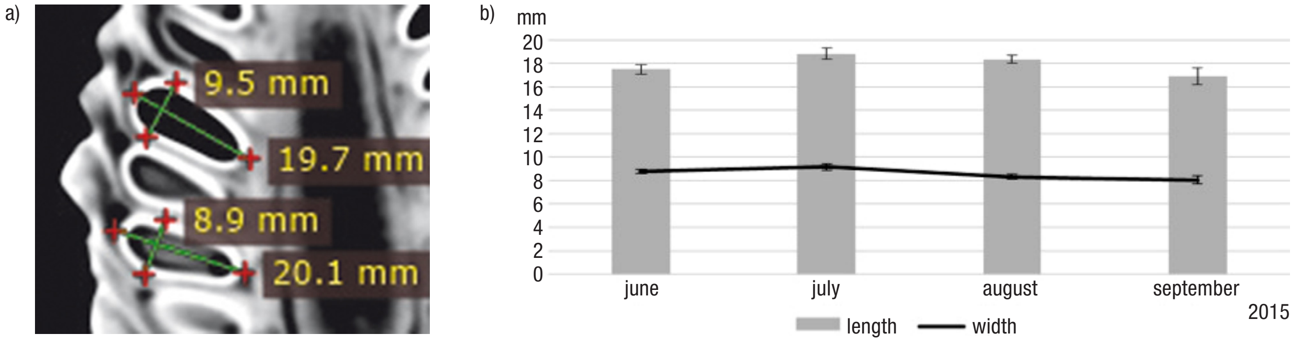

Figure 2. Pine nut's length and width, measured with RadiAnt Dicom Viewer: Illustrative CAT greyscale view of pine nut's measures (a) and mean of measurements per month (b).

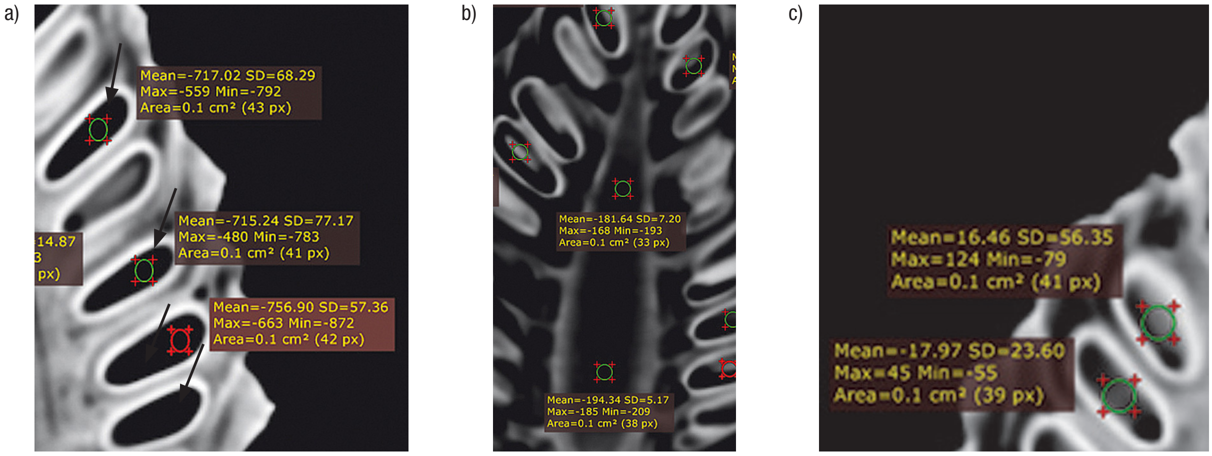

Figure 3. CAT greyscale views: determination of HU values with RadiAnt Dicom Viewer in empty pine nuts (a) (label: the empty pine nuts were signaled with an arrow), pine cone's central axis (b) and pine nut's kernel (c).

values of HU in the polygon (Figure 3). The data show that there exists a clear and monthly variation in the HU mean values of the pine nut's kernel. The mean HU value per month decreased from June (67.40 HU), to September (27.90 HU); in July and August the mean value was 64.30 HU and 33.00 HU, respectively. Within the cone no trend in HU values was observed.

The HU values observed at the cone axis and the empty pine nuts were negative, revealing a low density that indicates a high presence of air: the empty pine nuts had values around -700 HU and the cone axis around -200 HU (Figure 3 a and b).

The number of normal nuts in each section and the number of empty pine nuts (without kernel or unhealthy) as well as the proportion of empty pine nuts (Table 1) were determined. The number of pine nuts varied between cones, without any apparent relationship with maturation time and the same occurred with the proportion of empty nuts (Table 1). The estimated mean values of empty pine nuts (%) was 21.0 % in July, 38.5% in August and 16.9% in September. In June, it was not possible to identify the number of empty pine nuts because there was interference in the contrast, apparently due to the presence of water in tissues.

Table 1. Number of normal and empty nuts, and empty pine nut's proportion, estimated per month, with RadiAnt Dicom Viewer in CAT greyscale images.

Month	Number of pine nuts (identified in images)					Number of empty pine nuts (identified in images)					Empty pine nuts (%)			
	Total	Average	Stdev	Maximum	Minimum	Total	Average	Stdev	Maximum	Minimum	Average	Stdev	Maximum	Minimum
June	382	38	15,9	63	20	–	–	–	–	–	–	–	–	–
July	380	38	16,0	70	13	67	7	5,0	15	1	21,0	21,6	75,0	2,9
August	425	43	9,8	59	29	168	17	14,2	41	3	38,5	28,8	82,1	5,9
September	561	56	7,6	69	47	91	9	5,9	23	2	16,9	12,1	45,1	3,8

The resulting images showed that CAT provides an excellent visualization of the pine cone and its internal structure in two or three dimensions. It allows to count and measure pine nuts, and to estimate the proportion of healthy pine nuts. This seems to be the major advantage of the use of CAT compared to the traditional method of opening cones. This may be good news for the industry, since they can get a quick first assessment of the amount of healthy pine nuts per batch of cones.

The images were processed using only free software and a better image processing software would certainly improve the outputs of this study.

The values estimated for the pine cone and pine nuts need to be validated after opening the pine cones by the traditional method. This will provide more information such as color and damage and may be supplemented by chemical and physical analysis, with determination of density and moisture in pine nuts, and shell resistance along the ripening time. In fact, the monthly variation in pine nut's kernel HU values, i.e. the variation in density maybe due to a change in the chemical composition of the pine nut along the maturation process. Further research should compare HU values observed in the CAT scans with chemical analysis and NIR spectroscopic data.

The empty pine nut's proportion, estimated by pine cone, should also be validated after manual opening of pine cones and nuts.

In this exploratory trial we verified the potential of computerized axial tomography as a screening tool for pine cones and pine nuts in industry and research.

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