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TREE-RINGS IN MEDITERRANEAN PINES – CAN WE ASCRIBE THEM TO CALENDAR YEARS?

PRIRASTNE PLASTI V MEDITERANSKIH BORIH – ALI LAHKO UGOTOVIMO, V KATEREM KOLEDARSKEM LETU SO NASTALE?

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Abstract / Izvleček

Abstract: *A tree ring is defined as a band of xylem-cell layers produced in one year. In Mediterranean trees cambium often produces several bands of alternating early- and latewood during one calendar year, resulting in Intra-Annual Density Fluctuations (IADFs). We present some characteristics of growth layers in* Pinus pinea *(stone pine) growing on Vesuvius Mountain (near Naples, Italy). The trees showed uninterrupted wood production from January 2015 till the end of January 2016. The ring formed in this period contained five different layers of wood types. Therefore, the wood formed between January and March 2015 would be falsely ascribed to latewood of the tree ring 2014, and the wood formed between March 2015 and the end of January 2016 would be classified as tree ring 2015. We also showed differences in cambium activity in late autumn between years: in November 2015 and 2018 the cambium was still active and not active anymore, respectively. Furthermore, we present the structure of the wood formed in the year 2017 affected by a wildfire in July. Such cases where the growth layer cannot be exactly ascribed to a calendar year should be considered in dendrochronological studies of* Pinus pinea *and other Mediterranean species.*

Keywords: *tree ring, tree ring boundary, stone pine=*Pinus pinea*, earlywood, latewood, Intra-Annual Density Fluctuation, xylogenesis, Italy*

Izvleček: *Branika v lesu je plast ksilemskih celic, nastalih v enem letu. V drevesih, rastočih v Sredozemlju, kambij pogosto proizvede več plasti ranega in kasnega lesa v enem koledarskem letu in prirastna plast posledično vsebuje gostotne variacije (IADF). Predstavljamo nekatere značilnosti prirastnih plasti lesa pinij (*Pinus pinea*), s pobočij Vezuva pri Neaplju v Italiji. Drevesa so od januarja 2015 do konca januarja 2016 izkazovala neprekinjeno nastajanje lesa. Les, ki je nastal v tem obdobju, je vseboval pet različnih slojev. Les, nastal med marcem 2015 in koncem januarja 2016 bi opredelili kot braniko 2015, les, ki je nastal med januarjem in marcem 2015, pa bi zmotno prišteli kasnemu lesu branike 2014. Prikazali smo tudi razlike v delovanju kambija v pozni jeseni; novembra 2015 je bil kambij še aktiven in je proizvajal les, novembra 2018 pa se je aktivnost že zaključila. Predstavljamo tudi zgradbo lesa, nastalega v letu 2017, ko je drevesa v juliju prizadel gozdni požar. Primere, ko rastne plasti ni mogoče natančno pripisati koledarskemu letu, je treba upoštevati v dendrokronoloških raziskavah pinije in drugih sredozemskih drevesnih vrst.*

Ključne besede: *branika, letnica, pinija=*Pinus pinea*, rani les, kasni les, gostotna variacija v prirastni plasti, ksilogeneza, Italija*

1 INTRODUCTION
1 UVOD

A tree ring, also called annual ring or annual growth ring, is defined as a layer of cells produced in one year in the xylem or phloem (Kaennel & Schweingruber, 1995). In temperate wood species, it usually consists of early- and latewood and is demarcated by clear growth ring boundaries. The formation of temperate tree rings usually takes place between spring and autumn (Gričar & Čufar, 2008; Prislan et al., 2013; Useros et al., 2017; Martinez del Castillo et al., 2018). In Mediterranean-types ecosystems, warm winters, hot dry summers and frequent wildfires affect cambial activity, and thus lead to structural "anomalies" within tree rings. Therefore, it is often not clear what a tree ring is and what its boundaries are. This challenges the application

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of dendrochronology, which is the science of dating tree rings and needs to clarify in which calendar year a particular tree ring was formed.

In Mediterranean trees, the process of tree ring formation is not necessarily synchronised with the calendar year. Vascular cambium can show similar seasonality as in temperate trees, can stop more than once per year, reactivate or remain active throughout one or more years. Such patterns of cambial rhythm affect the wood structure, leading to irregular alternations of earlywood and latewood bands in the xylem. Successive layers of earlywood and latewood forming tree rings which are not synchronised with the calendar year are referred as false rings, or Intra-Annual Density Fluctuations (IADFs) (Bräuning 1999; Rigling et al., 2001; Cherubini et al., 2003; De Micco et al., 2016a). Recently, numerous studies have been published on the occurrence of IADFs in Mediterranean woods, especially in conifers (Campelo et al., 2007; De Luis et al., 2007, 2011; De Micco et al., 2016b; Novak et al., 2016) and a complete description and classification of IADFs is reviewed in De Micco et al. (2016a).

IADFs occurring in Mediterranean conifers are mainly classified into four types (De Micco et al., 2016a) according to their relative position within the early- and latewood: 1) *E-IADF* as a band of latewood-like cells in the earlywood occurring in the first half of the ring; 2) *E+-IADF*, as cells with features intermediate between true earlywood and true latewood cells, called transition cells, occurring at the end of the earlywood; 3) *L-IADF* as earlywood-like cells in the latewood occurring in the second half of the ring; 4) *L+-IADF* as a band of earlywood-like cells with narrower lumen and thicker walls than true earlywood, occurring between the latewood of one ring and the earlywood of the next ring.

IADFs, if not recognised, can cause problems in tree ring dating and consequent errors in dendroecological investigations. In some species, the occurrence of specific anatomical features, such as the increased incidence of thin-walled parenchyma cells, narrow marginal parenchyma bands, and resin canals, can help distinguishing IADFs from true tree ring boundaries (Campelo et al., 2007; Schweingruber 2007); however, only monitoring of wood formation (xylogenesis) can assure the identification of IADFs in relation to the time of their formation (De Micco et al., 2019).

The latest studies report that the application of microcoring to follow xylogenesis helps to define the timing of IADF formation (De Micco et al 2016b; Balzano et al., 2018 a, b), and to detect the environmental conditions triggering their formation.

Here we present three examples where different rhythms of cambial activity and different wood anatomical traits within growth layers, either due to climatic constraints or extreme events such as fire, affect the structure of tree rings in *Pinus pinea* and their dating.

2 MATERIALS AND METHODS
2 MATERIAL IN METODE

In 2014 we selected six dominant trees of *Pinus pinea* (stone pine, Pinaceae) on Vesuvius Mountain, Naples (Southern Italy) (Figure 1), at a site characterised by a Mediterranean climate. They grew in a stone pine forest with a stand density of 2,500 trees per hectare. The climate at the site is Mediterranean, with hot and dry summers followed by mild and wet winters (for details refer to Balzano et al., 2018a). The trees were used for a wood formation study based on microcores taken at regular intervals from January 2015 until January 2016 (Balzano et al., 2018a).

In addition, we collected cores and microcores from the same trees in November 2018. The cores were collected at the breast height of the trees with an increment borer, polished and observed under a stereo microscope (OLYMPUS SZ-STUZ) to identify the tree rings and observe their features. Additionally, we scanned them at 1200 dpi using an HP Scanjet 4890 in accordance with the procedures used in dendrochronology (e.g., Čufar et al., 2017).

The microcores containing phloem, cambium and new formed xylem increment, were taken at the breast height. After sampling, the microcores were stored in FAA (mixture of formalin, acetic acid and ethanol) and then dehydrated in a graded ethanol series (70, 90, 95 and 100 %), cleaned with D-limonene and embedded in paraffin blocks (tissue processor Leica TP1020-1, Nussloch, Germany). We cut cross-sections (9 µm thick), using a semi-automatic rotary microtome RM 2245 (Leica, Nussloch), subsequently stained with a safranin and astra blue water solution (Prislan et al., 2013) and mounted in Euparal (Bioquip Rancho Dominguez, CA, USA).

Under a Nikon Eclipse 800 light microscope we observed the slides and microphotographs were taken by a digital camera (DS-Fi1) connected with the NIS-Elements BR 3 image Analysis System (Melville, NY, USA). We analysed wood cells in different phases of xylogenesis: cambium cells (CC), post-cambial cells (PC), and cells with a developing cell wall (SW) (Gričar, 2007). IADFs were characterised in accordance with De Micco et al. (2016a).

Intra-annual variability of anatomical traits was examined with specific attention to the tree rings corresponding to 2014/2015, 2017 and 2018.

3 RESULTS AND DISCUSSION
3 REZULTATI IN RAZPRAVA

3.1 DURATION OF WOOD FORMATION IS NOT ALWAYS SYNCHRONISED WITH THE CALENDAR YEAR
3.1 NASTAJANJE LESA NI VEDNO USKLAJENO S KOLEDARSKIM LETOM

The monitoring of xylogenesis showed that cambium was active from January 2015 until January 2016 in all investigated trees of *P. pinea*. The highest peaks of cambial activity occurred in spring and autumn, while the production was lower during

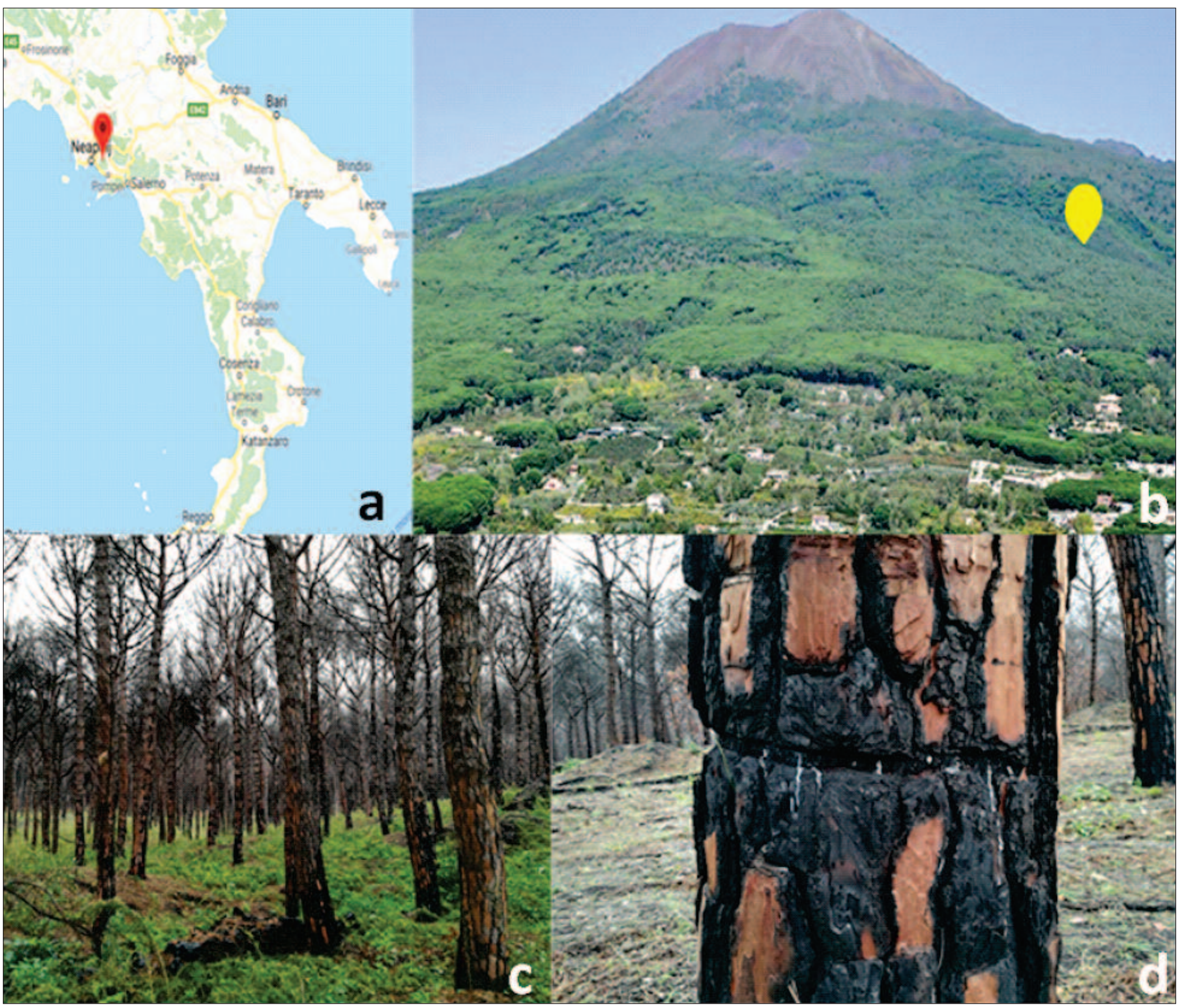

Figure 1. Sampling site located near Naples, Italy: (a) sampling site on the map, (b) sampling location in Vesuvius National Park, (c) Pinus pinea *stand, and (d) one of sampling trees injured by the fire that occurred in July 2017.*

Slika 1. Rastišče pri Neaplju v Italiji: (a) lega na zemljevidu, (b) mesto vzorčenja v nacionalnem parku na pobočju Vezuva, (c) sestoj pinij in (d) eno od raziskanih dreves, ki je bilo poškodovano v požaru julija 2017.

summer and winter (Balzano et. al, 2018a). Wood anatomical inspection showed that between January and March 2015, earlywood-like cells were produced; they had narrower lumina and thicker cell walls than regular earlywood, but wider lumina than latewood (Figures 2, 3). Formation of earlywood-like wood between January and March 2015 was presumably triggered by abundant rain in December 2014. The above described tissue corresponds to so-called transition wood or L+-IADFs (De Micco et al., 2016a; Balzano et al., 2018a). True earlywood production started at the end of March and continued till the onset of latewood formation in June (Figure 2), probably triggered by the onset of summer drought events. Earlywood-like cell production, forming L-IADF, restarted in October (Figure 2), concurrently with a rainy period, and lasted till the new production of latewood at the end of December (Figure 2). The sampling in January 2016 showed that the cambium production did not stop at the end of the calendar year 2015, but continued with the production of latewood cells in January 2016. In this case, wood formation lasted longer than one calendar year (more than 13 months). The ring formed in this period contained five different layers which could be described as: earlywood-like, earlywood, latewood, earlywood-like and latewood (Figure 2).

All five layers could not be clearly detected by observing the wood of the polished cores under magnification, which is usual in dendrochronological investigations (Figure 3), therefore the wood increment formed between March 2015 and the end of January 2016 would be defined as one annual ring, i.e. tree ring 2015 (Figure 2). Without knowing the duration of xylogenesis, the earlywood-like wood

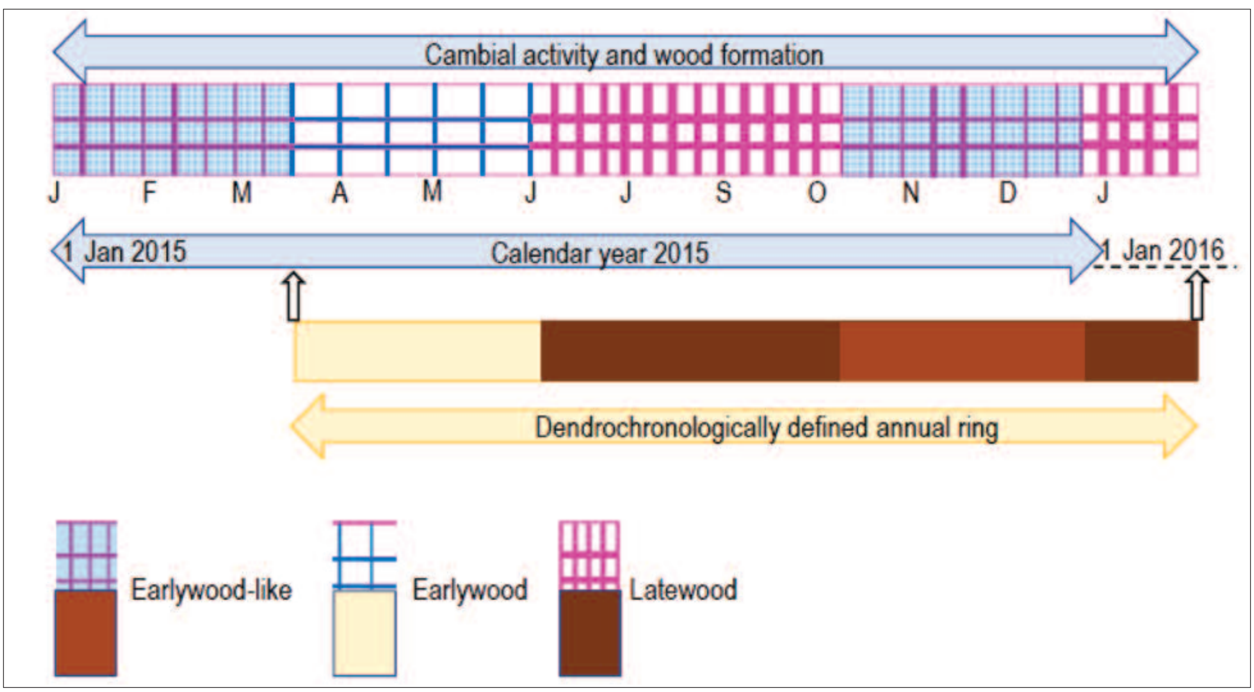

Figure 2. Time perspective of wood production between January 2015 and the end of January 2016 in Pinus pinea*: (a) cambial production was uninterrupted during the entire period; (b) cambium produced five bands with different wood types (earlywood-like, earlywood, latewood, earlywood-like, latewood); (c) wood formed between March 2015 and end of January 2016 would be defined as tree ring 2015 if observed with the methods and magnifications normally used in dendrochronology.*

Slika 2. Časovni prikaz produkcije lesa med januarjem 2015 in koncem januarja 2016 v piniji: (a) kambijeva produkcija je bila v celotnem obdobju neprekinjena; (b) kambij je proizvedel pet pasov oz. plasti lesa z različno anatomijo (podoben ranemu lesu, rani les, kasni les, podoben ranemu lesu, kasni les; (c) les, ki je nastal med marcem 2015 in koncem januarja 2016, bi bil opredeljen kot branika 2015, če bi ga opazovali pri povečavah, ki jih običajno uporabljajo v dendrokronologiji.

formed from January until March 2015, would be falsely ascribed to tree ring 2014. This observation contradicts the definition of a tree ring – which is considered to be the amount of wood produced in one year in temperate environments.

The alternation of different types of wood can be observed in thin cross-sections obtained from the microcores or on smoothly polished wood observed under the stereo microscope at high magnifications (Figure 3).

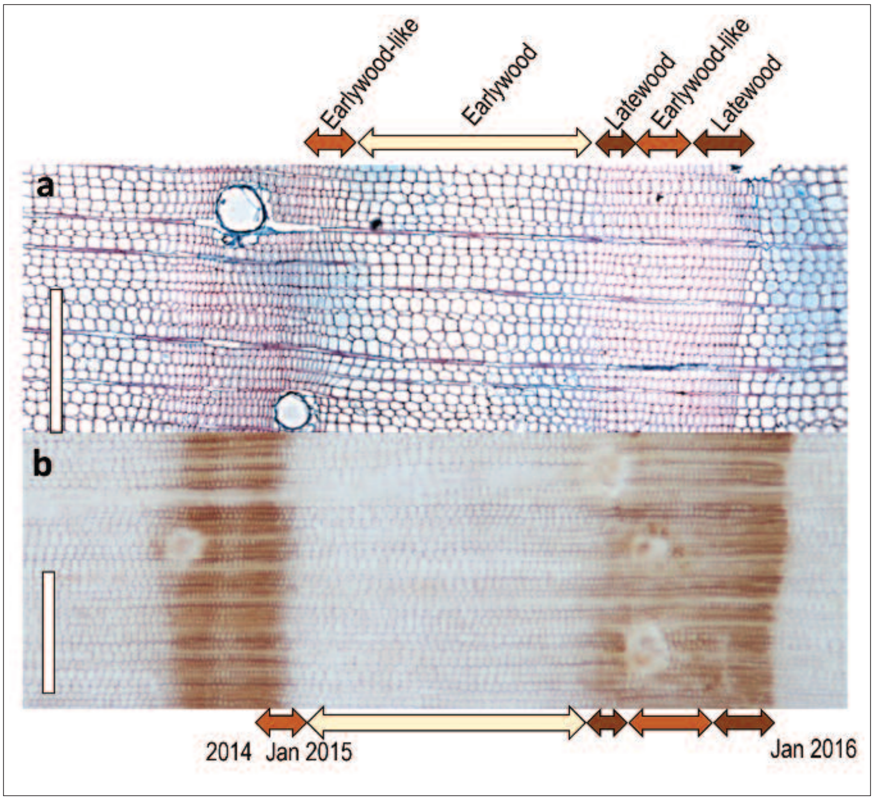

Figure 3. Anatomy of wood produced between January 2015 and the end of January 2016 in Pinus pinea*: (a) cross-section under a light microscope and (b) polished surface under a stereo microscope. There is no sharp boundary between the increments of 2014 and 2015. Scale bars = (a) 500 μm, (b) 800 μm.*
Slika 3. Anatomija lesa, nastalega med januarjem 2015 in koncem januarja 2016 v piniji: (a) prečni prerez pod svetlobnim mikroskopom in (b) polirana površina lesa pod stereo mikroskopom. Med prirastki 2014 in 2015 ni ostre meje (letnice). Merilne daljice = (a) 500 μm, (b) 800 μm.

Figure 4. Increment cores of Pinus pinea *polished and scanned at the resolution of 1200 dpi. Black dots indicate the tree rings dated 2010 (left) and 2015 (right). Intra-annual density fluctuations (IADFs) in tree ring 2015 cannot be clearly seen, the wood formed between March 2015 and the end of January 2016 would be considered as the annual ring for 2015. Wood produced between January and March 2015 would be ascribed to the 2014 increment (c.f. Figures 2 and 3).*
Slika 4. Izvrtki pinije zglajeni in skenirani pri ločljivosti 1200 dpi. Črne pike označujejo prirastne plasti 2010 (levo) in 2015 (desno). Gostotne variacije (IADF) v prirastni plasti 2015 ni mogoče jasno videti, les, ki je nastal med marcem 2015 in koncem januarja 2016, bi zmotno opredelili kot braniko 2015. Les nastal med januarjem in marcem 2015, bi pripisali prirastni plasti 2014 (prim. sliki 2 in 3).

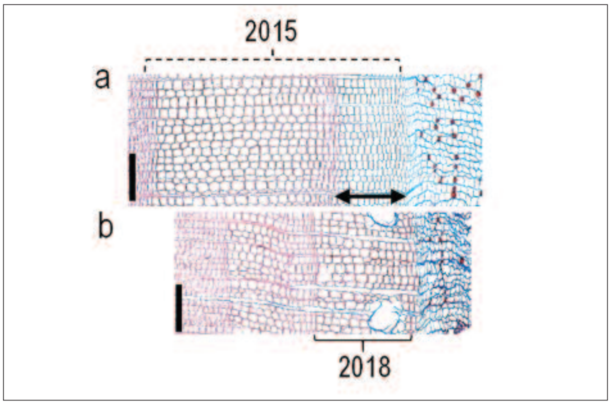

Figure 5. Cross-sections of cores of the same Pinus pinea *tree: (a) in November 2015 the cambium was productive and part of the xylem ring was not yet differentiated (arrow), and (b) in November 2018 the cambium was not productive and the last formed wood was fully differentiated. Scale bars = 250 μm.*

Slika 5. Prečni prerezi tkiv istega drevesa Pinus pinea*: (a) novembra 2015 je bil kambij produktiven in del ksilemske prirastne plasti še ni bil diferenciran (puščica) in (b) novembra 2018 kambij ni bil produktiven, zadnji nastali les pa je bil popolnoma diferenciran. Merilni daljici = 250 μm.*

3.2 CAMBIAL ACTIVITY IN THE SAME TREE AND MONTH IN DIFFERENT YEARS

3.2 AKTIVNOST KAMBIJA V ISTEM DREVESU IN MESECU V RAZLIČNIH LETIH

In 2015, the cambial activity did not end at the end of the calendar year. Indeed, the cross-sections from the microcores taken in November 2015 showed active cambium producing earlywood-like cells forming an L-IADF (Figures 4, 5, 6).

However, the same tree showed no cambial activity in November 2018. At that time, a narrow 2018 tree ring, consisting of earlywood and very narrow latewood, was already complete and contained no IADFs (Figures 5, 6).

Consequently, a comparison of the wood formed in 2015 and 2018 indicates a completely different anatomical structure (Figures 5, 6) and highlights high variability among years in tree rings of *P. pinea* growing on a typical Mediterranean site.

3.3 EFFECT OF FIRE ON TREE RING STRUCTURE

3.3 UČINEK POŽARA NA ZGRADBO PRIRASTNE PLASTI

During the sampling in November 2018 we noticed that the trees were severely damaged by fire

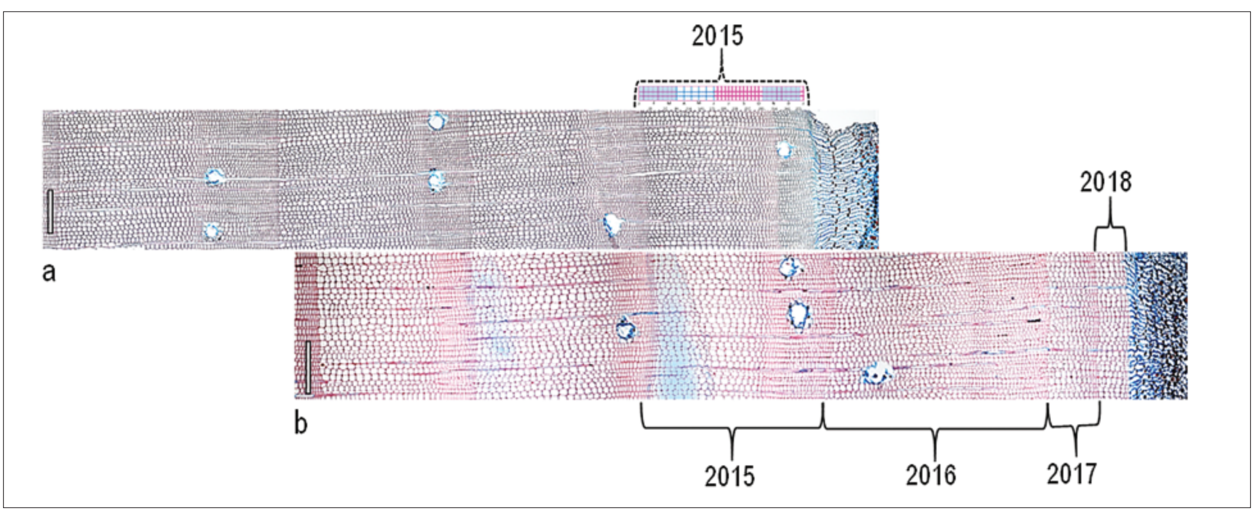

Figure 6. Cross-sections of Pinus pinea *from microcores collected from the same tree in November 2015 and 2018: (a) the incomplete ring for 2015 and previous rings, and (b) the ring for 2018 and previous rings including the 2017 one (when the tree was injured by fire) and the fully differentiated 2015 ring. Scale bars = 500µm.*

Slika 6. Prečni prerezi Pinus pinea *iz mikroizvrtkov, odvzetih iz istega drevesa novembra 2015 in 2018: (a) nediferencirana prirastna plast 2015 in predhodne prirastne plasti, in (b) diferencirana prirastna plast 2018, plast 2017, ko je julija drevo poškodoval požar in predhodne prirastne plasti, vključno z diferencirano plastjo 2015. Merilne daljice = 500µm.*

(Figure 1), which occurred in July 2017. This offered us the opportunity to inspect the anatomy of 2017 and the following 2018 ring at the location where the cambium was not injured, but was probably affected by high temperature.

By the analysis of cores (Figures 4 and 7) and microcores (Figure 6) we found that the ring for 2017 was narrower than the previous ones, with very narrow latewood and no IADFs. Such a structure is a consequence of an abrupt cessation of wood formation in July, due to fire. The cambium seemed to stop its production due to heat; although it did not seem to be injured as it formed no wound wood. Latewood consisting of only a few cells indicates that the cambium probably stopped wood production and did not reactivate after the abrupt stop due to fire. Our finding is in accordance with the observations of De Micco et al. (2013), who reported that the tree rings of *P. halepensis* formed after summer fire mainly contained earlywood cells.

4 CONCLUSIONS
4 ZAKLJUČKI

In this study we present details of the high variability in tree ring structure of *Pinus pinea* (stone pine) growing at a typical Mediterranean site in Southern Italy.

Cross-sections of tissues taken by microcoring and observed under a light microscope showed that cambium can remain productive over the entire calendar year.

When cambium was active for more than 13 months, it produced an increment consisting of five different layers with different wood structures (including an IADF).

If we observed the same increment on polished surfaces of wood under a stereo microscope, IADFs could not be clearly distinguished. Therefore, the wood formed between March 2015 and the end of January 2016 would be defined as the tree ring for 2015, and the wood formed between January and March 2015, would be falsely ascribed to the latewood of the 2014 tree ring.

Furthermore, sampling in November 2015 and 2018 showed that cambium can be still productive, with the latest formed wood in the differentiation phase, or the cambial activity can be ended, with the latest formed xylem fully differentiated.

Finally, we found that cambium in trees affected (but not injured) by wildfire in July 2017 may have abruptly stopped producing xylem cells, which resulted in narrower increment consisting mainly of earlywood cells with no reactivation of cambial activity to produce an IADF within latewood.

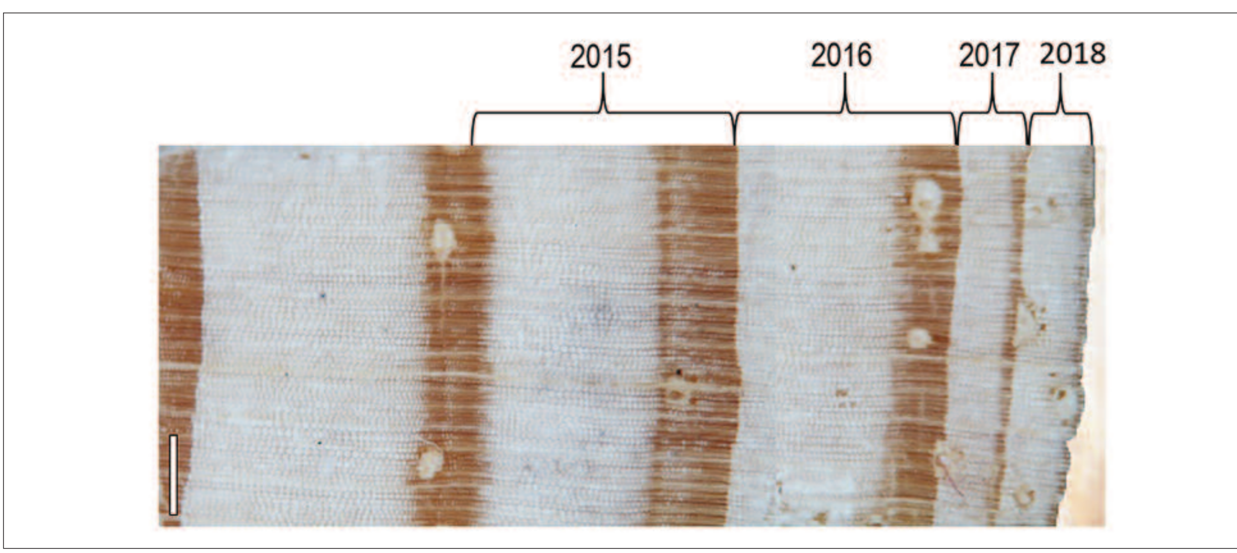

Figure 7. P. pinea *wood of a core sampled in November 2018. Note the rings for 2018, 2017 (formed in the year when the fire occurred, in July 2017) and the 2015 tree ring.*

Slika 7. Les izvrtka pinije, odvzetega v novembru 2018 z zadnjo prirastno plastjo, plastjo 2017 (nastalo v letu, ko je julija požar prizadel sestoj) in predhodnimi plastmi.

We showed that monitoring of wood formation can help us to exactly define when a particular growth layer has formed. The importance of monitoring wood formation should thus be taken into account in dendrochronological studies of Mediterranean wood species.

5 SUMMARY

5 POVZETEK

Branika v lesu je prirastna plast lesa, nastalega v enem letu. V drevesih, rastočih v Sredozemlju, kambij pogosto proizvede več plasti bolj ali manj tipičnega ranega in kasnega lesa v enem koledarskem letu in zato prirastna plast vsebuje gostotne variacije, imenovane tudi IADF (Intra-Annual Density Fluctation). IADF, ki se pojavljajo v sredozemskih iglavcih, večinoma razvrščamo v štiri tipe (De Micco et al., 2016a) glede na njihov relativni položaj v ranem in kasnem lesu: 1) E -IADF, kot pas kasnemu lesu podobnih celic, ki se pojavlja v prvi polovici prirastne pasti t.j. v ranem lesu; 2) E+ -IADF, kot celice z značilnostmi med ranim in kasnim lesom (prehodni les) ob koncu ranega lesa; 3) L -IADF kot ranemu lesu podobne celice v kasnem lesu, ki se pojavljajo v drugi polovici prirastne plasti; 4) L+ -IADF kot pas ranemu lesu podobnih celic z ožjimi lumni in debelejšimi stenami kot jih ima pravi rani les, ki se pojavlja med kasnim lesom in ranim lesom naslednje prirastne plasti. V slednjem primeru meja med prirastnimi plastmi (letnica) ni ostra.

IADF, če jih ne prepoznamo, lahko povzročijo težave pri datiranju prirastnih plasti in napake v dendroekoloških raziskavah. Najnovejše študije poročajo, da uporaba metode odvzema mikro izrvtkov za spremljanje ksilogeneze (nastajanja lesa) omogoča natančno opredeliti čas nastanka IADF (De Micco et al., 2016b; Balzano et al., 2018 a, b) in s tem pojasniti okoljske razmere, ki sprožijo njihovo nastajanje.

Cilji te raziskave so predstaviti tri delne študije, ki prikazujejo, kako različni ritmi kambijeve produkcije vplivajo na anatomsko zgradbo lesa, ta pa na prepoznavnost prirastnih plasti in njihovo datiranje. Raziskave so bile opravljene na odraslih pinijah (*Pinus pinea*), s pobočij Vezuva pri Neaplju v Italiji, s tipično sredozemsko klimo.

Na izbranem rastišču (slika 1) smo od januarja 2015 do januarja 2016 v rednih časovnih razmakih odvzemali mikro izvrtke za spremljanje nastajanja lesa (Balzano et al., 2018a). Vzorčenje smo ponovili v novembru 2018. Izvrtke smo vklopili v parafin in izdelali anatomske preparate, obarvane s safraninom in astra modrim po uveljavljenem postopku (Prislan et al., 2013). Prečne prereze tkiv smo opazovali s svetlobnim mikroskopom Nikon Eclipse 800 in jih posneli z digitalno kamero (DS-Fi1), povezano s sistemom za analizo slike NIS Elements BR 3 (Melville, NY, ZDA). Analizirali smo nastajajoče celice lesa (ksilema) v različnih fazah ksilogeneze: kambijeve celice (CC), postkambijske celice (PC) in celice z razvijajočo se celično steno (SW) (Gričar, 2007). IADF-e smo opredelili v skladu z De Micco et al. (2016a).

Na istih drevesih smo v novembru 2018 s prirastoslovnim svedrom odvzeli izvrtke. Te smo gladko zbrusili in nato pregledali s stereo mikroskopom (OLYMPUS SZ-STUZ). Izvrtke smo tudi skenirali pri ločljivosti 1200 dpi s pomočjo skenerja HP Scanjet 4890 v skladu s postopki, ki so v uporabi v dendrokronologiji (npr., Čufar et al., 2017).

V prvem primeru smo prikazali nastanek prirastne plasti, ko je na toplem in suhem sredozemskem rastišču kambij neprekinjeno deloval vse leto oz. več kot 13 mesecev. V tem primeru je kambij proizvedel pet plasti lesa z različno anatomijo (podoben ranemu lesu, rani les, kasni les, podoben ranemu lesu, kasni les (slika 2). Če bi les opazovali na zglajeni površini izvrtka, pri povečavah, ki jih običajno uporabljajo v dendrokronologiji, bi les, ki je nastal med marcem 2015 in koncem januarja 2016, napačno opredelili kot braniko 2015.

Med prirastki 2014 in 2015 ni bilo ostre meje (letnice), zato bi les, nastal med januarjem in marcem 2015 zmotno pripisali prirastni plasti 2014 (slike 2, 3, 4).

V istem drevesu smo prikazali aktivnost kambija v različnih letih, 2015 in 2018. Novembra 2015 je bil kambij še aktiven, les zadnje prirastne plasti pa je bil še v fazi diferenciacije, novembra 2018 pa se je aktivnost kambija že zaključila, zadnji nastali les je bil popolnoma diferenciran, prirastna plast pa je vsebovala rani in kasni les brez IADF.

Med vzorčenjem v letu 2018 smo ugotovili, da so bila drevesa prizadeta zaradi požara v juliju 2017. To nam je omogočilo študij zgradbe lesa, nastalega v letu 2017, ko je drevesa v juliju prizadel požar, in v letu 2018 po požaru. Pri tem naj poudarimo, da kambij ni bil vidno poškodovan, zelo verjetno pa je bil izpostavljen visokim temperaturam.

Z analizo izvrtkov (sliki 4 in 7) in prečnih prerezov iz mikro izvrtkov (slika 6) smo ugotovili, da je bila prirastna plast 2017 ožja od predhodnih, imela je zelo ozek kasni les in je bila brez IADF. Takšno zgradbo pripisujemo nenadni zaustavitvi kambijeve aktivnosti in nastajanja lesa v juliju 2017 zaradi požara, pri čemer se kambij ni ponovno aktiviral. Naše ugotovitve so v skladu z ugotovitvami De Micco et al. (2013), ki so poročali, da so v alepskem boru (*Pinus halepensis*), ki je doživel poletni ogenj, zasledili prirastne plasti, ki so večinoma vsebovale rani les.

Raziskave so pokazale, da v primerih, ko branike ne nastajajo strogo sezonsko, tako kot v zmerni klimi, le spremljanje nastajanja lesa in opazovanje zgradbe lesa pod mikroskopom omogoča natančno določitev, kdaj je nastala posamezna prirastna plast.

Primere, ko prirastne plasti ni mogoče natančno pripisati koledarskemu letu, je treba upoštevati v dendrokronoloških raziskavah pinije in tudi ostalih sredozemskih vrst.

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