Morphology, anatomy and essential oil characterization of Pinus radiata needles in the conditions of the Southern Coast of the Crimea

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Abstract. The paper presents data on the dendrometric parameters, a needle anatomy and an essential oil characterization of a radiate pine (Pinus radiata D. Don) in the conditions of the Southern Coast of the Crimea. The research was carried out on the model trees (age 70-80 years) growing in the territory of the Montedor Park. For research collected pine needles radiant cuts were made on the freezing microtome (MZ-2, Ukraine), were stained with Sudan III and examined with a light microscope Mikmed-5 (LOMO, Russia) equipped with a digital camera MS-3 (LOMO, Russia). The essential oil from the needles was extracted by hydrodistillation on Ginsberg devices and examined on a gas chromatograph 6890N (Agilent Technology, USA). The studies showed that radiata pine trees achieve their genetically determined parameters, compared to those in the nature habitats in Monterey (California, USA). A needle anatomical investigation clearly demonstrated typical structure. In the essential oil composition, predominance of monoterpenes, in particular α- and β-pinenes with a large content of the latter was demonstrated. The peculiarity of P. radiata essential oil under the conditions of the Southern Coast of the Crimea, compared to some other regions, was the presence of limonene, which determined the stronger coniferous smell.

1. Introduction

Radiata pine (Pinus radiata D. Don) is a representative of the Trifoliae Duhamel section and Attenuatae Van Der Burgh subsection, an endemic species of California flora, naturally grows in the hazy belt at an altitude of 400 m above the sea level. The area of natural populations of P. radiata is no more than 8,000 ha [1, 2], however, this species is the most widespread throughout the world [3]. Due to its adaptation potential to arid conditions and rapid growth, P. radiata is widely cultivated outside its natural area in Australia, New Zealand, Spain, Argentina, Chile, Uruguay, Kenya and South Africa [4]. For the Southern Coast of the Crimea (SCC), as a moisture-deficient region, it is of particular importance to search for promising tree introduced species that can be successfully cultivated in the climatic conditions with the increased thermal regime and a low water ability [5]. The early polycyclic P. radiata growth observed before [6] was characteristic of ability to respond to various fluctuations of climatic phenomena, which is of considerable interest for studying the growth characteristics of this species under introduction [7].

Needles are the most important assimilating organs that determine the productivity and vital state of trees. Essential oil is the product of secondary metabolism, formed mainly in the process of
photosynthesis, accumulates inside needle tissues. All the representatives of the genus Pinus are characterized by accumulation of α-, and β-pinene [8], which have broad pharmacological properties (fungicidal, antimicrobial, antiviral anti-inflammatory, analgesic, etc.) [9]. Both the adaptation ability and presence of essential oil with useful characteristics, are determined the prospect of P. radiata usage in landscaping. Therefore, the objective of our investigation was to study biological aspects of P. radiata plantings, the accumulation of essential oil in needles and its component composition in the conditions of the SCC.

2. Materials and methods
The studies were conducted on the model trees (age 70-80 years), growing in the territory of Montedor Park (Nikitsky Botanical Garden). For the investigations, the needles from pine radiata trees were collected and processed on freezing microtome (MZ-2, Ukraine). Obtained sections were stained with Sudan III and investigated under Mikmed-5 light microscope (Lomo, Russian Federation) equipped with MC-3 camera (Lomo, Russian Federation). On sections quantitative parameters as well as inclusion square with essential oil were determined in UTHSCSA ImageTool software version 3.0. The essential oil from the needles was isolated by hydrodistillation on Ginsberg devices and investigated on 6890N gas chromatograph (Agilent Technology, USA) with 5973N mass-spectrometry detector (Agilent Technology, USA). Data were analyzed statistically with PAST software [10].

3. Results and discussion
At the SCC, Pinus radiata trees grow as separated individuals or in groups. The dendrometric investigation showed that the highest tree was 19 m in height with the diameter 0.59 m, the mean values was 9.9 ± 0.87 m and 0.27 ± 0.03 m respectively. Mean crown size was 4.7 ± 0.5 × 6.1 ± 0.6 m (n = 10). All radiata pine trees were characterized by a good living condition. Shoots were formed annually, conifers with seeds also were observed. The anatomical investigation revealed semi-oval (from binate fascicle) or triangle needle form (from ternate fascicle) and typical pine structure that shown on Figure 1. Epidermis with thick cell walls had well developed cuticle on periphery. Under epidermis, hypodermis cells joined into 2 or 2-4 layers in the corners were revealed. In mesophyll (2-3 layers) with plicate cells, two, rarely three, resin ducts were observed. In the central part of a needle section, endodermis and transfusion tissue with two bundles differentiated into xylem and phloem was.

![Figure 1. Needle cross-sections of Pinus radiata: a – semi-oval form; b – sectorial form, Ep – epidermis, Hyp – hypodermis, Mc – mesophyll cells, Rd – resin duct, End – endodermis, Tt – transfusion tissue, Xy – xylem, Ph – phloem (light microscopy, × 100).](image-url)
In P. radiata, analysis of the needle quantitative parameters revealed its variations. The most changed parameters were resin duct area (29.7 ± 0.7%) and bundle area (25.1 ± 2.2%), other were characterized by lower coefficient of variation in range 11-17% (Table 1). In needles, essential oil was detected with Sudan III as oval, brown-colored inclusions in mesophyll cells, lining cells of resin ducts and bundles (Figure 2).

**Table 1.** Anatomical parameters of the pine needle cross-sections (mm).

| Parameter                        | 2015               | 2016               | 2017               | 2018               | Statistical score |
|----------------------------------|--------------------|--------------------|--------------------|--------------------|-------------------|
| Number of resin ducts            | 2.03 ± 0.003       | 2.03 ± 0.003       | 2.00 ± 0.003       | 2.03 ± 0.003       | M ± m CV%         |
| Cross-section area               | 8.57               | 8.57               | 20.26              | 8.57               | CV%               |
| Central cylinder area            | 0.90 ± 0.002       | 0.88 ± 0.004       | 0.97 ± 0.005       | 1.06 ± 0.003       | M ± m CV%         |
| Bundle area                      | 11.95              | 11.91              | 15.52              | 10.21              | CV%               |
| Resin duct thickness (vertical)  | 0.18 ± 0.005       | 0.19 ± 0.006       | 0.22 ± 0.005       | 0.28 ± 0.004       | M ± m CV%         |
| Mesophyll thickness (horizontal) | 0.18 ± 0.010       | 0.18 ± 0.006       | 0.16 ± 0.008       | 0.19 ± 0.004       | M ± m CV%         |
| Mesophyll thickness (horizontal) | 17.46              | 19.07              | 19.51              | 12.45              | CV%               |
| Resin duct area                  | 0.34 ± 0.001       | 0.33 ± 0.003       | 0.35 ± 0.003       | 0.34 ± 0.003       | M ± m CV%         |

**Figure 2.** Needle transversal section fragments of Pinus radiata: Mc – mesophyll cells, Lc – lining cells, Tt – transfusion tissue cells, Vb – vascular bundles. Arrows indicate inclusions with essential oil (light microscopy, Sudan-III staining). Bars – 50 µm.

Square inclusion measurement (n = 50) showed that its size was 3.8 ± 0.39 µm² that reached 5.02 ± 0.44% from cell with mean value 2.529.41 ± 191.58 µm². In the essential oil of P. radiata 64 components were identified (Table 2). The major components were β-pinene with a specific content of 29.57%, α-pinene – 21.2%, and limonene – 12.41%. Also, in composition α-terpeniol, β-myrcene, pinocarveol, mirtenol, δ-kadinen, germacren D and γ-3-karen were revealed.

The stand dendrometric parameters are determined by species genetics and also influenced by other endo/exogenous factors. The growth rate of radiata pine in nature is slow, compared with its growth in plantations outside their natural habitats. In Monterey (California, USA) the heights of mature 20 years old trees were in the range 9-37 m, with the tallest trees occurring on better soils in gullies. For
comparison, the tallest trees on Guadalupe (French) and Cedros Islands (United Mexican States) were 33 and 32 m, respectively. 35-years-old unmanaged first-rotation stands in central North Island (Seychelles), New Zealand were characterized by top height of 42 m [4]. At the SCC, age of pine trees was 70-80 years and according to our data, they achieved their genetically determined parameters, compared to natural habitats in Monterey.

Table 2. The composition of the Pinus radiata essential oil in the conditions of the Southern Coast of Crimea.

| No. | Component                                      | RI   | Pinus radiata D. Don |
|-----|-----------------------------------------------|------|---------------------|
| 1   | ethanol                                       | 819  | 3.01                |
| 2   | tricycylene                                   | 931  | 0.17                |
| 3   | α-tuyen                                       | 934  | 0.05                |
| 4   | α-pinene                                      | 940  | **21.20**           |
| 5   | camphene                                      | 947  | 0.80                |
| 6   | 1-isopropyl-4-methylenebicyclo[3.1.0]hex-2-ene| 950  | 0.12                |
| 7   | sabinen                                       | 966  | 0.12                |
| 8   | β-pinene                                      | 970  | **29.57**           |
| 9   | β-myrcene                                     | 981  | 1.84                |
| 10  | γ3-caren                                      | 996  | 2.05                |
| 11  | α-terpineolate                                | 1001 | 0.14                |
| 12  | p-cymene                                      | 1004 | 0.18                |
| 13  | β-phellandrene                                | 1010 | 0.79                |
| 14  | limonene                                      | 1013 | **12.41**           |
| 15  | trans-otsimen                                 | 1030 | 0.85                |
| 16  | γ-terpinene                                   | 1038 | 0.13                |
| 17  | limonene oxide                                | 1046 | 0.11                |
| 18  | terpinolen                                    | 1065 | 0.75                |
| 19  | α-thujone                                     | 1072 | 0.33                |
| 20  | epoksiterpinolen                              | 1076 | 0.71                |
| 21  | β-thujone                                     | 1083 | 0.07                |
| 22  | fenhol                                        | 1086 | 0.36                |
| 23  | α-kamfolenal                                   | 1090 | 0.29                |
| 24  | pinon                                         | 1092 | 0.27                |
| 25  | camphor                                       | 1103 | 0.14                |
| 26  | pinocarveol                                   | 1108 | 1.86                |
| 27  | verbenol                                       | 1116 | 0.43                |
| 28  | pinocamphone                                   | 1133 | 0.25                |
| 29  | isoborneol                                    | 1136 | 0.64                |
| 30  | terpinen-4-ol                                 | 1148 | 0.46                |
| 31  | myrtenal                                      | 1152 | 0.83                |
| 32  | α-terpineol                                   | 1161 | 4.51                |
| 33  | myrtenol                                      | 1167 | 1.50                |
| 34  | fenhyl acetate                                | 1191 | 0.12                |
| 35  | methylthymol                                  | 1204 | 0.18                |
| 36  | bornyl acetate                                | 1254 | 0.14                |
| 37  | mirtenyl acetate                              | 1292 | 0.10                |
| 38  | α-ilangen                                     | 1330 | 0.10                |
| 39  | α-copaen                                      | 1354 | 0.21                |
Our needle anatomical data of radiate pine was compared to those from Monterey (USA) [11] and Kaingaro (New Zealand) [12]. Its common anatomical structures were similar, that is normal and evidence of a genetic stability manifestation. The secondary needles were two or more per fascicle and semi-oval or sectorial in cross-sections. The uniform, thick-walled epidermis was separated from plicate mesophyll cells by one or more hypodermal layers. There are usually two medial resin canals. However, in needle cross-section from the SCC accession of radiate pine three canals were noted occasionally. A uniform layer of endodermal cells bounds the stellar portion with two vascular bundles embedded in transfusion tissue. The quantitative parameters were not compared due to absent of the similar data.

P. radiata as a representative of Attenuatae sub-section of Trifoliae section of the genus Pinus is characterized by predominance of monoterprenes in essential oil [8], in particular α- and β-pinenes with a large specific weight of the latter. Thus, when cultivated in Greece and Ecuador, the amount of pinenes was 56.6% [8], and 57.1% [13], respectively. This trend can also be seen in the results of our research (under the conditions of the SCC, the amount of pinenes is 50.77%) and is consistent with the data obtained by other authors [14]. The peculiarity of P. radiata essential oil under the conditions of the SCC is the presence of limonene (12.41%), which determines the stronger coniferous smell of the essential oil. Diterpenes and sesquiterpenes in P. radiata essential oil are present in small amounts under different environmental conditions. Comparing the available data, we can conclude that the component composition of this type of essential oil is quite stable.

| No. | Component                  | RI  | Pinus radiata D. Don
|-----|----------------------------|-----|-------------------------|
|     |                            |     | The quantitative content (% rel.) of the identified components in the essential oil |
| 41  | β-bourbonen                | 1361| 0.05                    |
| 42  | β-cubeben                  | 1366| 0.06                    |
| 43  | β-elemens                  | 1368| 0.11                    |
| 44  | trans-karyofillen          | 1392| 1.04                    |
| 45  | bergamotene                | 1412| 0.23                    |
| 46  | cubeben                    | 1420| 0.10                    |
| 47  | humulene                   | 1423| 0.22                    |
| 48  | β-cadinenne                | 1443| 0.14                    |
| 49  | germacren D                | 1448| 2.48                    |
| 50  | bicyclo sesqui phelanderen | 1456| 0.24                    |
| 51  | germakren B                | 1462| 0.56                    |
| 52  | α-muurolen                 | 1470| 0.42                    |
| 53  | α-amorphene                | 1478| 0.30                    |
| 54  | γ-cadinene                 | 1480| 0.24                    |
| 55  | kalamene                   | 1482| 0.20                    |
| 56  | δ-cadinene                 | 1488| 1.14                    |
| 57  | cadina-1,4-diene           | 1495| 0.12                    |
| 58  | nerolidol                  | 1526| 0.14                    |
| 59  | spatuenol                  | 1529| 0.55                    |
| 60  | caryophyllene oxide        | 1531| 0.27                    |
| 61  | cubenol                    | 1575| 0.24                    |
| 62  | tau muurolol               | 1586| 1.23                    |
| 63  | α-cadinol                  | 1595| 1.10                    |
| 64  | manoyl oxide               | 1778| 0.58                    |

Note: RI – component retention index.
4. Conclusion

Our data showed that P. radiata trees grown in groups or as simple specimen at the Southern Coast of the Crimea achieved their genetically determined parameters compared to those in the nature habitats in Monterey. The needles’ anatomical structures were similar, that was normal and evidence of the genetic stability manifestation. However, on the cross-sections of one, two, three-years old needles, variations of the anatomical structure sizes were revealed with the higher coefficients for resin ducts and vascular bundles.

On the needle cross-sections, essential oil was detected as oval, brown-colored inclusions in mesophyll cells, lining cells of resin ducts, bundles and biochemically was characterized by a predominance of monoterpens, in particular α- and β-pinenes with a large specific weight of the latter. Diterpenes and sesquiterpenes in P. radiata essential oil were present in small amounts. The peculiarity of P. radiata essential oil under the conditions of the SCC is the presence of limonene, which determines the stronger coniferous smell of the essential oil.

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