



Long-term pathological consequences of resin tapping wounds on stems of Scots pine (*Pinus sylvestris* L.)

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Abstract

Key message After 5–6 decades since inflicting resin tapping wounds, overmature (> 120 years old) *Pinus sylvestris* stems remain undecayed and vigorous.

Abstract Overmature trees of *Pinus sylvestris* bearing large wounds made by resin tapping decades ago are still present in woodlands of south-eastern Baltic Sea region. The aim of the present study was to investigate health condition of those trees focusing on fungal infections and to estimate impact of the injury on radial stem growth. The study was conducted in Latvia in three overmature stands of *P. sylvestris*, resin-tapped in 1950–1970 s. On the studied ninety 120–167-year-old trees, exposed sapwood constituted from 1140 to 7755 cm² per individual stem. Of the 127 wounds sampled, 52 (41%) showed wood discoloration. The discoloration in its extent was limited, expanding beyond wound margins approx. 1 (max 3) cm in radial, and 6–7 cm in longitudinal directions. Of the 127 wood samples/wounds subjected to fungal isolations, 96% resulted in fungal growth, yielding 236 fungal isolates that represented 47 fungal taxa. The most common among macrofungi was basidiomycete *Porodaedalea pini*, which was isolated from 9% of stems. The fungus is currently classed not as a tree pathogen, but instead as an indicator species for woodland sites to be considered for nature conservation. Data from tree ring widths have revealed that tree reacted to the resin tapping injury by increasing radial increment of the un-affected part of the circumference of the stem. Current study demonstrated that even on the long term, resin tapping has little influence on health condition and vitality of *P. sylvestris*, even at the very old age. This should be considered as a supporting message in case resin tapping practices in the region are to be revived.

Keywords *Pinus sylvestris* · Resin tapping · Stem wounds · Fungal infection · *Porodaedalea pini*

Introduction

In Latvia, experimental resin tapping from stems of Scots pine (*Pinus sylvestris* L.) was initiated in year 1903, and already in year 1913, a total of 2290 kg of resin has been obtained from 1280 trees. Since then (based on experience from Germany) resin tapping started to be implemented on an industrial scale. Yet systematic research in this respect was launched in 1923 but only since 1950s, the resin tapping has become a large-scale activity acquiring an important role

in forest management (Rasins and Vilsons 1960). Optimal pine age for resin productivity was estimated to be 70–80 years; however, in practice, resin tapping was usually accomplished in 90-year-old and older stands. Stands from all quality classes were selected (site index I–V; Bušs 1997), estimated yield of resin from low productive individual pine being 400–600 g, and from highly productive, 3200–4800 g per season. In all, from 1950 to 1980 total yield of resin in Latvia comprised 2800–3000 tons annually, and in all over 20,000 ha of pine stands were used for resin tapping (Rasins and Vilsons 1960; Baumanis et al. 2014). Since 1980, due to large production of cheaper synthetic resins, resin tapping in pine stands of Latvia has been abandoned.

Resin tapping was done during the vegetation season, normally from May to September. Upon initiating the procedure, surface layer of outer bark had to be scratched off, simultaneously delimiting size of the wound to be inflicted. Then, two opposite approx. 5 mm wide × 5 mm deep

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diagonal (30° – 45° angle) sapwood scars were incised in a pairwise manner, encompassing a total of approx. 40 cm of stem circumference. Subsequently, each tree was visited about 3 times per week, during each visit making a pair of new scars, normally at 1–1.5 cm distance either above or below from the two respective previous ones (Rasins and Vilsons 1960; Racinskas 1995). Thus, depending on duration of a tapping season and number of incised scars, size of a wound inflicted on and individual stem per season/year could potentially be very large. For example, in case of 3 visits per week during 5 weeks of harvesting, it would result in a total of 15 pairwise incisions, comprising

approx. 40 cm-wide \times 25–30 cm-long “fishbone-like” sapwood-exposing injury, thus comprising an open sapwood wound exceeding 1000 cm^2 in size. In case if resin harvesting from a given tree would be accomplished during 5 seasons (years; “usual protocol” being 8 to 10 years), this would result in a wound comprising accumulative length of over 2 m, exposing thousands of cm^2 of sapwood for decades to come (Fig. 1a, b). Moreover, a common practice was to inflict two tapping wounds per individual stem (Fig. 1c). In certain cases, to enhance resin yield, chemical substances as chloride-lime (5 years before felling) and



Fig. 1 Sixty-three-year-old resin tapping wounds on 135-year-old stem of *Pinus sylvestris* (Site 1—Kalsnava); **a** wound on a standing tree; **b** close photo of wound surface; note width of scars of approx. 0.5 cm, and “step” between scars of approx. 1 cm; **c** cross section of

two resin tapping wounds showing approx. 3–4 cm lateral closure from each margin in about 4 decades; **d** longitudinal section of a wound showing up to 1 cm-deep reddish sapwood discoloration

sulfuric acid (2 years) were applied on a cut wood surface (Rasins and Vilsons 1960; Racinkas 1995).

Despite the forestry regulations requiring to cut resin-tapped trees soon after the completion of resin harvesting (1–2 years), considerable numbers of those had remained uncut, thus currently overmature pines bearing large old wounds are still present, and can be found throughout the whole country (Fig. 1a). Moreover, similar situation is being observed also in neighboring countries with the long resin tapping history, as Lithuania, Poland and eastern part of Germany Racinkas 1995; van der Maaten et al. 2017, and references therein). Consequently, such trees currently constitute characteristic (albeit fragmented) feature of overmature pine forests of south-eastern region of the Baltic Sea, nowadays as a rule subjected to nature protection regimes. Surprisingly, to date no information is available regarding impact on tree health of such injuries (e.g., eventual occurrence of associated stem rots, insect attacks, etc.), despite considerable interest by foresters, nature conservationists, but also concerns by a wider society (e.g., while encountering such trees during recreational activities). Moreover, the existing data on the impact of resin tapping on pine growth are fragmented and to a certain extent contradictory Auzins 1995; Genova et al. 2014; van der Maaten et al. 2017, and references therein). The aim of the present study was to investigate fungal infections to old resin tapping wounds on stems of *P. sylvestris*, and to estimate impact of the injury on radial stem growth.

Materials and methods

Field work

The study was conducted in Latvia in three overmature stands of *P. sylvestris*, resin-tapped in 1950–1970 s, all of them growing on mineral soil: Site 1—Kalsnava, approx. 140 years old (56° 7246 N, 25° 9167 E), Site 2—Ventspils, approx. 167 years old (57° 4495 N, 21° 8070 E), and Site 3—Zvirgzde, approx. 140 years old (56° 6863 N, 24° 4462 E). In each stand, 30 living resin-tapped *P. sylvestris* were randomly selected. For each tree, the diameter at 1.3 m height (DBH) was measured (twice in both directions, estimating mean), and the number of individual injuries per stem recorded (Table 1). For each of the 127 recorded injuries, maximal length and width and position in relation to ground level (height of its lowest margin) were measured. The area of exposed sapwood (wound) was estimated based on its length and width. From each of the 127 wounds, a single wood sample (bore core length, 10–26 cm) was taken using an increment borer, targeting 2 cm below the injury. Bark was removed from the core immediately after extracting the sample. After each sampling, the borer was sterilized in 70% ethanol. Immediately after sampling, each wood core was assessed for the presence or absence of discoloration. After assessment, all samples were individually placed in sterile plastic tubes and transported to the laboratory for fungal isolation.

Table 1 Mean parameters (mean \pm SD) of analyzed *Pinus sylvestris* trees and resin tapping wounds

Parameters	Site 1 (Kalsnava)	Site 2 (Ventspils)	Site 3 (Zvirgzde)	All
Stems				
No. examined	30	30	30	90
Stem diameter at 1.3 height (cm)	49 \pm 9^a	38 \pm 6	38 \pm 6	42 \pm 9
Distance of damage from ground (cm)				
Lowest point	63 \pm 8	129 \pm 11	46 \pm 9	74 \pm 44
Highest point	180 \pm 22	225 \pm 10	261 \pm 11	219 \pm 39
No. of stems with one/two/three wounds	9 / 20 / 1	29 / 1 / 0	15 / 15 / 0	17.7 / 12 / 0.3
Exposed sapwood per stem (cm ²)	3753 \pm 1907	2985 \pm 777	9419 \pm 2981	5361 \pm 3538
Wood discoloration (% of stems)	60	43	60	54
Wounds^b				
No. examined	52	31	42	42
Wound width (cm)	18 \pm 4	30 \pm 4	30 \pm 2	25 \pm 7
Wound length (cm)	117 \pm 23	95 \pm 11	214 \pm 11	146 \pm 58
Exposed sapwood per wound (cm ²)	2168 \pm 994	2889 \pm 590	6422 \pm 583	3818 \pm 2065
Wood discoloration (% of wounds)	42	42	41	41

^a Values printed bold indicate that the differences between the means are statistically significant (Wilcoxon signed-rank test, $p < 0.05$)

^b On Site 1 (Kalsnava), resin tapping was accomplished in years from 1955 till approx. 1965. We do not know exact timing for Sites 2 and 3 but, judging accordingly wound width, the tapping should have been taking place during 1970s. This investigation was accomplished in the year 2018

Isolation of fungi

Isolation and identification of fungi followed our routine procedures (Arhipova et al. 2015; Burnevica et al. 2016). In the laboratory, each tree core was split into approx. 8 cm-long pieces and, after sterilization by flame, each piece was individually placed in a 9 cm plastic Petri dish on malt agar media (15 g malt extract, 12 g agar, and 1000 mL H₂O). Petri dishes with samples were incubated at room temperature and inspected in every 3 days for fungal growth; all emerging mycelia were subcultured on individual Petri dishes and grown as pure cultures. After 4–5 weeks of growth, all pure cultures were examined under a microscope and grouped into mycelial morphotypes. Isolates, obtained during the study, are deposited in fungal culture collection of Latvian State Forest Research Institute Silava.

DNA extraction, amplification and sequencing

One to three representatives of each distinct mycelial morphotype were subjected to molecular identification following procedures as in our previous studies (Arhipova et al. 2011, 2015). One to three representatives of each distinct mycelial morphotype were subjected to molecular identification following procedures as in our previous studies (Arhipova et al. 2011, 2015). DNA extraction and PCR amplification were made according to established protocols (Kåren et al. 1997; Padutov et al. 2007).

DNA was extracted using a modified CTAB method (Padutov et al. 2007). For DNA extraction, fungal mycelia were collected from Petri dishes using flame sterilized scalpel and placed into 2 ml microcentrifuge tubes with 150 µl 2% CTAB extraction buffer and three flame sterilized metal bead were added in each of the tubes, and the samples were ground twice in the buffer using a Bead-Beater homogenizer (Mixer Mill MM 440, Germany) for 45 s at 29 r/s. Then the samples were centrifuged at 12,000 rpm for 8 s. An additional volume of 650 µl of 2% CTAB extraction buffer was added to the suspension and the samples vortexed (Bio Vortex V1, Latvia) for 5 s. Then samples were incubated in water bath at 65 °C for 60 min with intermittent shaking every 20 min (MultiSUB Maxi, UK). After heating, the samples were centrifuged at 12 rpm for 20 min and 700 µl of the supernatant was transferred to a new 2 ml microcentrifuge tube with 700 µl of chloroform. The mixture was shaken for 30 s and samples were centrifuged at maximum speed for 20 min. A total of 550 µl of the aqueous phase was moved into a new 2 ml microcentrifuge tube, but the chloroform phase was discarded. One-fifth volume of a 5% CTAB buffer, preheated to 65 °C in the water bath, was added to the aqueous phase and thoroughly mixed. Then samples were incubated in water bath at 65 °C for 15 min with intermittent shaking after 7 min during incubation. The equal volume

of chloroform (1:1) was added and the mixture was shaken for 30 s after incubation. Then samples were centrifuged at maximum speed for 20 min. The upper aqueous phase were transferred to a new 2 ml microcentrifuge tube and the DNA was precipitated with 2 volumes of isopropanol. Samples were gently mixed by inversion and incubated for 30 min at +4 °C and then centrifuged 30 min at 13,000 rpm. Isopropanol was removed and the DNA pellet washed with 900 ml of 70% cold ethanol to eliminate salt residues adhered to the DNA. The tubes were centrifuged at 13,000 rpm for 5 min and ethanol was removed from the top of each tube. The samples were put in the fume hood for 30 min with the lid open to dry the ethanol out. 50 µl of TE buffer (pH 8.0) was added and samples were put at +40 °C for 24 h. The DNA concentration was determined using spectrophotometer NanoDrop 8000 (Thermo Fischer Scientific, US).

PCRs were performed in a volume of 10 µl containing approximately 50 ng DNA, 2 µl HOT FIREPol® Blend Master Mix (Solis BioDyne, Estonia) (containing 10 mM MgCl₂), 0.3 µM ITS 1 F (CTTG GTCATTTAGAGGAAG TAA) and ITS4 (TCCTCCGCTTATTGATATGC) primers (Kåren et al. 1997). PCR was carried out in a thermocycler (Eppendorf Mastercycler EP gradient) using the following protocol: initial predenaturation step at 95 °C for 15 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 35 s, and 72 °C for 1 min and a final extension step of 72 °C for 10 min.

PCR fragments were visualized UV Transilluminator (FireReader V10, U) after electrophoresis on 2% agarose gel stained with 0.5 µg/ml ethidium bromide.

The PCR products were purified using FastAP Thermo-sensitive Alkaline Phosphatase and Exonuclease I (EF0651 and EN0581, ThermoFisher Scientific, US) and sent to Macrogen Europe (Amsterdam, the Netherlands) for further Sanger sequencing. Sequencing was conducted in one direction using the universal primer ITS4 for every specimen. BLAST searches were performed using the GenBank sequence database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed 10 April 2019). Sequences were manually edited using the BioEdit software (version 7.0.9.0). To determine fungal taxons (presumed species), the internal transcribed spacer (ITS) sequence homology was set at 98–100%. For delimiting at the genus level, the ITS sequence homology was set at 94–97%. All ITS sequences obtained in this study were deposited in GenBank (MK801309–MK801355).

Sample tree

To accomplish detailed examination, on Site 1 (Kalsnava), one pine was felled, 135 years of age, 42.4 cm DBH, showing two resin tapping wounds. The first resin tapping injury on the stem was inflicted in year 1955, thus 63 years previously to the investigation. The larger was 184 cm in length, 15 cm in

width; area of exposed sapwood was approx. 2760 cm². Its lower point was located at 110 cm from root collar, and the higher at 294 cm. The second wound was 115 cm in length and 14 cm in width; area of exposed sapwood was approx. 1610 cm². Its lower point was located at 100 cm from root collar, and the higher at 215 cm. The vertical and radial extent of wood discoloration, and extent of mechanical damage inflicted to stem by resin tapping was estimated by cutting the stem into two sections in the middle of the larger wound, after that each section was dissected longitudinally, and radial spread, and the total length of discoloration column was recorded. Extent of wound closure in lateral and longitudinal directions was measured.

Dendrochronological measurements

From 15 resin-tapped pines and from 10 control pines wood cores were taken. The trees were spatially distributed within the same stand (Site 1, Kalsnava) and were of a similar age, being approx. 140 years old (age varied from 120 to 150). A core was extracted from each stem at 1.3 m height using an increment borer (each bore core reached the center of a stem, or more). From resin-tapped stems, the cores were taken from the stem side directly opposite to the wound (in case of a single wound per stem), or, in cases of two wounds per stem, from the middle part in-between of those. Tree-ring width was measured with the precision of 0.01 mm using measurement system LINTAB 5 (RinnTECH, Germany). Measurements were made using WinCELL 2007 software (Regent Instruments, Canada). Sample images, with 24-bit color depth and 1200 dpi resolution, were acquired using EPSON GT 15,000 scanner.

Statistics

Data were inspected for normality using the Shapiro–Wilk test and by manually evaluating Q–Q plots. Using these criteria, obtained data (diameter of trees, size of wounds, exposed sapwood per tree and radial increment of resin-tapped trees) were considered to be not normally distributed. Wilcoxon signed-rank test was performed to compare differences of diameter of trees, size of wounds, exposed sapwood per tree in three stands and to compare tree ring width of resin-tapped trees to non-tapped trees. The statistical analysis was performed in the “R” environment, R studio Version 1.3.1093 (RR Core Team 2020).

Results and discussion

Wounds

The present study examined wounds on overmature *P. sylvestris* stems inflicted during resin tapping 60–50 years prior

to the investigation. Characteristics of examined trees and wounds are presented in Table 1. The results demonstrated that, despite the five decades since the injury to the stems has occurred, areas of exposed sapwood had remained extremely large, on average constituting 5361 cm² per individual stem. Sizes of individual wounds also varied to great extent, minimal and maximal size of a wound corresponding to 1140 and 7755 cm², respectively, indicating that occlusion of resin tapping wounds on *P. sylvestris* stems is slow. This was further confirmed by the analysis of the sample tree, in an examined wound of which since about six decades after the injury, only 3–4 cm lateral and 5–20 cm longitudinal closure from its respective margins was observed, corresponding to a total of approx. 8 and 25 cm closure in each direction, respectively (Fig. 1c, d). This can be compared with the occlusion rates of mechanical wounds inflicted to stems of Norway spruce (*Picea abies* (L.) Karst.), which are also notoriously slow. Here, it has been demonstrated that in numerous cases even 3 cm wide artificial mechanical stem wounds failed to occlude over the two decades, and none of initially 15 cm wide has occluded (Vasaitis et al. 2012; Vasaitis 2013). Yet, in case of resin tapping wounds of *P. sylvestris* examined during the present work, we do not know whether the investigated injuries were subjected to chemical treatment for the tapping, although it might have had negative impact on the closure rates.

Fungi, insects and windbreaks

Another restricting factor for the occlusion could be potentially imposed by infections of wood-inhabiting fungi. However, of the 127 wounds sampled in the current study, only 52 (41%) showed (reddish) wood discoloration. Analysis of the sample tree has demonstrated that, in that particular stem, the discoloration in its extent was limited, expanding beyond wound margins about 1 (max 3) cm in radial, and 6–7 cm in longitudinal directions (Fig. 1c, d). Of the 127 wood samples/wounds subjected to fungal isolations, 96% resulted in fungal growth, yielding 236 fungal isolates that represented 47 fungal taxa (Table 2). The most common among macro-fungi, was basidiomycete *Porodaedalea pini* (Brot.) Murrill, which was isolated from 9% of stems. Characteristic for this fungus are the latent infections to trees through natural pathways (e.g., dead twigs), symptomless persisting in wood for many years, and subsequent development of heart-rot in aging overmature stems (Vasaitis 2013, and references therein). Consequently, *P. pini* is currently classed not as a tree pathogen, but instead as an indicator species for woodland sites to be considered for nature conservation (Nitare 2000). Notably, in USA, the fungus has even been used for artificial inoculations of conifers to promote stem decay in trees to be subsequently used as wildlife habitats (Filip

Table 2 Fungi detected in stem wounds of *Pinus sylvestris*

Fungal taxa	GenBank accession no.	Frequency of occurrence (%)	
		In wounds (n = 127)	In stems (n = 90)
Basidiomycota			
<i>Bjerkandera adusta</i>	MK801310	1.6	2.2
<i>Coprinellus disseminatus</i>	MK801349	0.8	1.1
<i>Fomitopsis pinicola</i>	MK801342	0.8	1.1
<i>Phlebia acerina</i>	MK801347	0.8	1.1
<i>Porodaedalea pini</i>	MK801329	7.9	8.9
<i>Trichaptum abietinum</i>	MK801344	0.8	1.1
Ascomycota			
<i>Aequabiliella effusa</i>	MK801313	2.3	3.3
<i>Alternaria alternata</i>	MK801345	0.8	1.1
<i>Alternaria infectoria</i>	MK801346	0.8	1.1
<i>Ascocoryne sarcoides</i>	MK801322	14.2	14.4
<i>Botrytis cinerea</i>	MK801334	0.8	1.1
<i>Chaetothyriales</i> sp. P38	MK801333	1.6	2.2
<i>Cladosporium</i> sp. P12	MK801318	0.8	1.1
<i>Cladosporium xylophilum</i>	MK801316	0.8	1.1
<i>Coniochaeta ligniaria</i>	MK801323	0.8	1.1
<i>Coniochaeta velutina</i>	MK801324	0.8	1.1
<i>Crumenulopsis pinicola</i>	MK801332	1.6	2.2
<i>Dothideomycetes</i> sp. P11	MK801317	3.1	4.4
<i>Dothideomycetes</i> sp. P47	MK801337	1.6	2.2
<i>Dothideomycetes</i> sp. P49	MK801338	0.8	1.1
<i>Eurotiales</i> sp.	MK801350	6.3	7.8
<i>Mariannaea elegans</i>	MK801319	0.8	1.1
<i>Paraconiothyrium fuckelii</i>	MK801348	0.8	1.1
<i>Penicillium chrysogenum</i>	MK801311	1.6	2.2
<i>Penicillium maclellaniana</i>	MK801309	18.9	25.6
<i>Penicillium</i> sp. P76	MK801352	1.6	2.2
<i>Penicillium spinulosum</i>	MK801351	1.6	2.2
<i>Penicillium thomii</i>	MK801325	8.6	12.2
<i>Pestalotiopsis</i> sp. P14	MK801320	2.4	3.3
<i>Pyxidiophorales</i> sp. P77	MK801353	1.6	2.2
<i>Sarea difformis</i>	MK801315	37.8	50.0
<i>Sarea resiniae</i>	MK801326	8.7	11.1
<i>Sydowia polyspora</i>	MK801331	0.8	1.1
<i>Symbiotaphrina microtheca</i>	MK801314	2.4	3.3
<i>Thyronectria pinicola</i>	MK801321	0.8	1.1
<i>Tolypocladium inflatum</i>	MK801343	0.8	1.1
<i>Tolypocladium pustulatum</i>	MK801328	2.4	2.2
<i>Trichoderma harzianum</i>	MK801327	11.8	13.3
<i>Trichoderma viride</i>	MK801354	0.8	1.1
<i>Tymphanis</i> sp. P35	MK801330	2.4	2.2
<i>Tymphanis</i> sp. P46	MK801336	0.8	1.1
Unident. Ascomycota P43	MK801335	0.8	1.1
Unident. Ascomycota P50	MK801339	0.8	1.1
Mucormycota			
<i>Absidia caerulea</i>	MK801340	1.6	2.2

Table 2 (continued)

Fungal taxa	GenBank accession no.	Frequency of occurrence (%)	
		In wounds (n = 127)	In stems (n = 90)
<i>Absidia glauca</i>	MK801341	1.6	2.2
<i>Umbellopsis</i> sp. P5	MK801312	1.6	2.2
<i>Umbellopsis isabellina</i>	MK801355	15.0	21.1

et al. 2011). Moreover, during the present study, fruit bodies (sporocarps) of *P. pini* were observed on stems of five trees, growing either close to the edge of a wound or higher on a stem. Interestingly, in our previous work in Latvia, the fungus was occasionally isolated from much younger (30-year-old) stems of *Pinus contorta* Douglas ex Loudon, exhibiting bark stripping damage caused by game (Arhipova et al. 2015). Yet neither in that nor in the current study, the isolations of *P. pini* were associated with decay.

Among other fungi, characteristic were Ascomycetes from the genera *Ascocoryne*, *Alternaria*, *Cladosporium*, *Penicillium*, *Sarea* known as endophytes and saprotrophs, some of which were reportedly associated with wood discoloration in bark stripping wounds on stems of *P. contorta* (Arhipova et al. 2015). Moreover, several fungi were detected that can be pathogenic to trees, namely *Botrytis cinerea*, *Pestalotiopsis* and some unidentified Dothideomycetes (Table 2). However, representatives of those groups of fungi are known mainly as foliar pathogens and/or decomposers of wood in the last stages of degradation (Capieau et al. 2004; Ferrari et al. 2021; Wang et al. 2019). Yet, a limitation is that the ITS rDNA sequence data are insufficient to delineate species in some ascomycete tree pathogens, and in particular of those from *Ophiostoma* species clusters (Linnakoski et al. 2016). Thus, the possibility cannot be excluded that also during the present work certain ascomycetes have been identified at the species *sensu lato* level. On the other hand, none of ophiostomatoid fungi, and/or other potential tree pathogens apart of discussed above, have been detected by us in wounded stems of *P. sylvestris*. We conclude that the sequences of full ITS region were sufficient for taxon identification for fungi within the focus of the current work.

Exit holes of insects on wood and bark of a tree indicate not only the incidence of the attack as such, but also that insect life cycle in a given substrate has been completed. On living tree stems, this symptom remains exposed for decades. During the present study, no insect exit holes on examined wounds or on stems at wound height have been detected. Windbreaks and symptoms of bacterial tree diseases, e.g., wetwood (Griffiths 2013) were not observed.

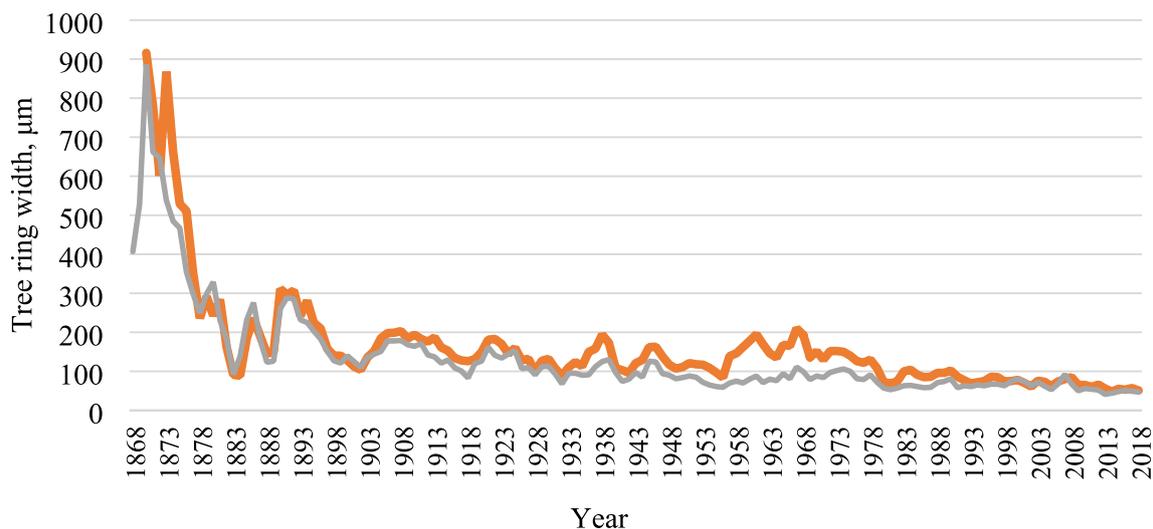


Fig. 2 Mean tree ring width (μm) for resin tapped and control pines. Orange bold line—tree ring width of resin tapped trees, gray line—tree ring width of control trees

Radial increment

Impact of resin tapping on radial increment was investigated on Site 1 (Kalsnava), where the tapping was initiated in year 1955 and lasted for approx. 10 years afterwards. Data from tree ring widths have revealed that radial increment of resin-tapped trees, as compared to non-tapped ones, in years thereafter has notably increased ($p < 0.001$) (Fig. 2). Similarly, increased radial growth at wounded and deformed parts of a stem was observed in resin-tapped *P. sylvestris* stems in German study, and explained by fact that in such instance wood formation is concentrated on the living portion of the bole, as after tapping there is no growth taking place on the exposed sapwood due to removal of cambium (van der Maaten et al. 2017). Yet in this respect, a negative impact of resin tapping on wood quality is asymmetrical stem growth, which notably devalues its otherwise the most commercially valuable part (Auzins 1995). However, resin-tapped stems should be subjected to the final felling soon after completion of the tapping (Rasins and Vilsons 1960). Under current circumstances, resin-tapped *P. sylvestris* trees at least in woodlands of the south-eastern Baltics are the components of sites of overmature forests under nature protection regimes, thus very unlikely to be harvested for wood.

Conclusions

Current study demonstrated that, even on the long term, resin tapping has little influence on health condition and vitality of *P. sylvestris*, even at the very old age. In fact, the present work provided new insights and confirmed that those trees could indeed be of importance for nature

conservation. Moreover, in recent years, interest for Non-Wood Forest Products, relevant in supporting sustainable forest management and rural development is being noted in Europe and that, as reported from Spain and Portugal, also includes the option for resin tapping of, e.g., maritime pine (*Pinus pinaster* Ait.) (Genova et al. 2014; Silva et al. 2020). The possibility cannot be excluded that the interest in such renewable natural resource as pine resin will come into agenda also to other areas of Europe, e.g., to more northern parts, where *P. sylvestris* is grown. Should this be the case, current study herewith provides valuable (and supportive) insights regarding the options, outcomes, and consequences of the related eventual activities.

Author contribution statement AZ, RV designed the experiment and made conceptualization; AZ, ZS, RRR collected and analysed the data; AZ, TG, RV developed the first draft; AZ led the writing for the subsequent revisions. All authors contributed critically to the drafts and gave final approval for publication.

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Data availability statement Datasets obtained in this study are openly available in GenBank (MK801309–MK801355).

Declarations

Conflict of interest Authors disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication.

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