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Terpene and resin acid contents in Scots pine stem lesions colonized by the rust fungus *Cronartium pini*

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Abstract

Cronartium pini causes economic losses especially on Scots pine in northern Europe. Scots pine reacts to rust infection by resin flow. The chemicals enriched in wood after *Cronartium* infection have not been investigated before. We investigated resin acids and mono- and sesquiterpenes produced in *Cronartium*-infected wood. *Cronartium*-infected wood was extracted with acetone, and the extractives were analysed by GC-mass spectrometry (GC-MS) and compared to those from control wood. Among resin acids, abietic acid, levopimaric acid, palustric acid, dehydroabietic acid and neoabietic acid were the richest (32–68 mg/g) in *Cronartium*-infected wood. Among monoterpenes, concentration of α-pinene was the highest (49 mg/g) in *Cronartium*-infected wood. Concentrations of all monoterpenes and resin acids and most sesquiterpenes were significantly higher (1.3- to 108-fold) in *Cronartium*-infected wood compared to control wood. In the control wood, the extractive content was greater (1.1- to 14-fold) than in the literature suggesting that the chemical processes were strongly affected by the rust. The results suggest that terpenes and resin acids are produced by the host to protect it from *Cronartium* rust.

KEYWORDS
gas chromatography–mass spectrometry, *Pinus sylvestris*, rust disease, secondary chemicals, wood chemistry

1 | INTRODUCTION

Cronartium rusts are serious pathogens of *Pinus* causing significant losses to pine forests in the northern hemisphere. *Cronartium pini* (Willd.) Jørst, the most important rust pathogen of *Pinus sylvestris* L. in northern Europe (Gäumann, 1959; Kaitera, 2000), consists of a heteroecious life-cycle form that spreads via alternate host plants, and an autoecious life-cycle form that spreads from pine to pine. A severe rust epidemic was reported in northern Sweden and Finland recently (Kaitera, 2000; Samils et al., 2010). The rust reduces pine growth (Martinsson & Nilsson, 1987), volume and value of timber trees (Kaitera et al., 1994), and in cases with over 20% of infected trees, the stand is recommended to be regenerated before full maturation. If uncontrolled, the rust may threaten northern forest ecosystems.

Cronartium rusts form spermogonial and aecial stages on pine (Figure 1a), from which they spread to alternate host plants in early to mid-summer. The rust forms uredinia on the plant leaves and spreads by urediniospores from plant to plant in mid-summer. In late summer, telia develop on the plant leaves and form after germination basidia with basidiospores that infect pine needles (Cummins & Hiratsuka, 1991; Gäumann, 1959). Disease symptoms appear

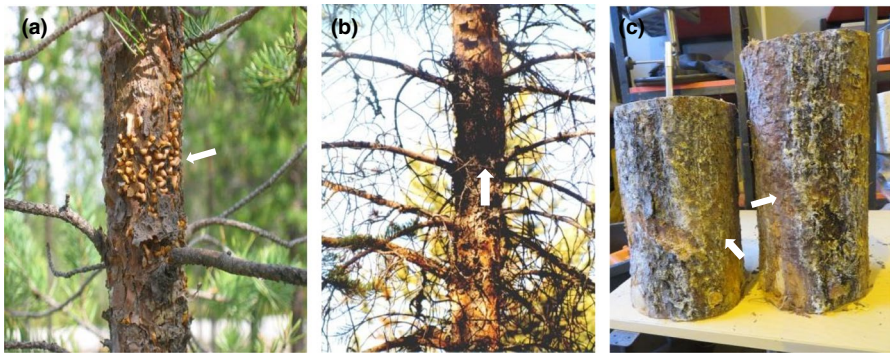

FIGURE 1 *Cronartium pini* infection on Scots pine stem. (a) yellowish aecial blisters (white arrow). (b) a resinous *Cronartium* stem lesion (white arrow). (c) cut sample bolts from a resinous *Cronartium*-infected stem lesion (white arrows)

as lesions that may expand for decades in pine stem or branches (Figure 1b). Eventually, the rust may girdle the leader above the lesion. The anatomical description of a *C. pini* lesion on Scots pine has been presented in van der Kamp (1969). The rust includes also another life-cycle form which has spermogonial and aecial stages on pine and spreads directly from pine to pine.

Cronartium pini can infect shoots and needles of Scots pine by wounds and needles also directly through stomata (van der Kamp, 1970; Olembo, 1971). In a *C. pini* lesion, rust hyphae grow first along the outer edge of the functional phloem and across the active cambium. Active hyphal growth continues in the resin ducts of the xylem. Changes in xylem tissues become evident 2–4 years after invasion of the cambium. The hyphae subsequently grow outwards into the older phloem and bark (van der Kamp, 1969). Lesions can be parasitized by *Tuberculina maxima* Rostr., which can reduce the aecial sporulation of *C. pini* by two-thirds (van der Kamp, 1970).

Cronartium pini can infect and sporulate on over 50 different plant species belonging to 14 plant families (Kaitera et al., 2012, 2015). Among these families, members of Orobanchaceae are particularly susceptible: *Melampyrum* is the most important genus in northern Fennoscandia (Kaitera, 1999; Kaitera & Hantula, 1998; Kaitera et al., 1999), of which the most important susceptible species is *M. sylvaticum* L. (Kaitera et al., 2005).

The physiological basis for rust resistance in alternate host plants is currently poorly studied. Variation in susceptibility among species may correlate with quality and quantity of secondary chemical compounds in leaves. These chemicals are released by stress factors, but they are also known as defensive agents. Resin acids are important chemicals in decay resistance of Scots pine heartwood (Belt et al., 2017; Tomppo et al., 2011). Defence chemicals can be formed rapidly after infection: infection by the grey mould fungus *Botrytis cinerea* Pers. led to 38-fold increase in stilbene synthase activity within one day in Scots pine seedlings (Gehlert et al., 1990). Wounding also induces a similar defensive chemical pattern that occurs in natural Scots pine heartwood (Nilsson et al., 2002). In the heartwood of Scots pine, the total phenolic concentration correlates with the resistance against *Coniophora puteana* (Shum.: Fr.) P. Karst (Harju & Venäläinen, 2006; Harju et al., 2009). Variation in total concentration of phenolics can also be used to select Scots pine provenances resistant to *C. puteana* (Harju & Venäläinen, 2006). Scots pine wood contains mainly α-pinene and 3-carene monoterpenes that have especially high concentrations in northern pine provenances (Manninen et al., 2002). Concentrations of monoterpenes, resin acids and total phenolics vary within the season. In the spring, 3-carene, α-pinene, α-pinene, (+)-sabinene and total amount of monoterpenes occur in Scots pine shoots of seedlings at high concentrations. In autumn, the resin acids levopimaric and dehydroabietic acid can occur at high concentrations (Nerg et al., 1994). In the north, palustric and neoabietic acids occur more commonly in Scots pine shoots than in the south (Nerg et al., 1994). Origin of seed material is, however, not as important as environmental factors for concentrations of secondary compounds (Nerg et al., 1994).

Phenolic synthesis is promoted in leaves of *Vaccinium myrtillus* L. in sites with high light intensity compared to forests. Leaves from high altitudes and latitudes contain more soluble phenolics and flavonols, higher antioxidant capacity and lower content of chlorogenic acid derivatives than leaves from lower altitudes and latitudes (Märtz et al., 2010). Also, the concentrations of terpenoids and soluble phenolics, monoterpenoids, proanthocyanidins and flavonols in needles of *Juniperus communis* L. increase along altitude and latitude and show good activity against *Staphylococcus aureus* Rosenbach bacteria (Märtz et al., 2009). High limonene and myrcene contents in the shoots of *Pinus nigra* J.F. Arnold protect pines from *Gremmeniella abietina* (Lagerb.) M. Morelet infection (Stephan et al., 1984). *Pinus elliottii* Engelm. provenances with a low β-phellandrene content are more susceptible to *Cronartium quercuum* f.sp. *fusiforme* Burds. & G.A. Snow than those with a high content (Michelozzi et al., 1991).

The aim of this study was to investigate the variation in quality and quantity of resin acids, mono- and sesquiterpenes in *Cronartium*-infected wood with rust symptoms compared to control wood. The hypothesis was that specific compounds were enriched in Scots pine wood as defence chemicals after rust infection. Some of these substances are also present in the wood already before the fungal infection, as has been shown for phenolics in some other plants (Witzell & Martín, 2008).

2 | MATERIALS AND METHODS

2.1 | Wood and bark material

The infected wood material with bark, phloem, cambium and outer sapwood (stem lesions) was collected from a severely rust-infected stand in Jaurakkajärvi [Pudasjärvi, Northern

Ostrobothnia (65°08'N, 27°35'E)]. Sample trees (age >60 years) were felled prior to the final-cutting stage. The stand consisted of a mixture of Scots pine and Norway spruce trees growing on a moist site (VMT; *Vaccinium-Myrtillus* type). The ground vegetation in the stand included *Melampyrum sylvaticum*, *M. pratense* L. and *Euphrasia stricta* Wolff ex Lehm that are known alternate hosts of *C. pini* (*C. flaccidum*). In a plant sample, 85% of *M. sylvaticum* (total of 66 sampled plants) and 6% of the leaves (3442) carried telia of *C. pini* in mid-August (19th) 2017. Similarly, 40% of *Euphrasia stricta* (116 sampled plants) and 2.5% of the leaves (2928) carried telia of the rust. Therefore, as *C. pini* was common in the stand, we concluded that *C. pini* had caused the stem lesions on Scots pine. The high frequency of *C. pini* on Scots pine in connection with high frequency of alternate hosts has been shown in northern Finland earlier (Kaitera et al., 2015). After the sanitary cutting, 50 pine trees carrying rust disease symptoms (lesions) were removed and piled by the roadside next to the stand in late September (28th) 2017. The rust symptoms consisted of a long, blackish resinous lesion of various length on the stem, where the fungus had grown for years and spread along the stem (Figure 1a-c). At the time of cutting, no aecial sporulation occurred anymore in the lesions. Within less than a week after the sanitary cutting, the infected trunks were cut into 30–60 cm long bolts using a chain saw (Husqvarna 550 XP) and transported in plastic bags to the laboratory of Luke (Oulu). In an inner hall, mixed-tissue samples characterized by resin flow and involving the bark, phloem, cambium and darkened xylem (outer sapwood) were cut longitudinally from the bolts with a frow (Fiskars). In addition, such mixed-tissue samples but without any visible disease symptoms were cut from each infected bolt at the opposite side of the trunk as control. In the wood laboratory, the resinous wood from 10 samples was cut into small 0.5–2 cm slices using a sterilized (100% ethanol) bandsaw (Einhell TC-SB 200/1) and stored at –20°C. A 1- to 2-mm slice was removed at the distal parts of the sample to remove wood possibly contaminated by oil from the chain saw. Therefore, the infected samples contained various amounts of resin flow in bark, phloem, cambium and outer sapwood depending on the time the rust had been present and how deep the lesion had reached in the inner wood, estimated as a visible darkened reaction area.

In addition to stem lesion samples, a dead top of Scots pine killed by *C. pini* was cut from a dry site in the Oulu City area in August 2017 and prepared in early October as described above. In this bolt, the dead distal part and the decayed wood next to alive wood were removed and only the proximal alive resinous basal part of the stem was processed further. In this bolt, no healthy control wood was available for the analyses.

2.2 | Sample preparation and acetone extraction

Sample preparation for the acetone extraction, and subsequent GC-MS analysis was done in Luke Espoo laboratory by modifying their routine methods (Jyske et al., 2014). For the mono- and sesquiterpene analyses, the fresh samples were ground using an analytical mill (Kinematica, Littau/Luzern) equipped with a cooling unit (–20°C). The samples for the resin acid analyses were freeze-dried for 72 h and ground with a ball mill prior to the chemical analyses.

Acetone was used to extract both resin acids and terpenes to avoid two sample preparation steps. According to the laboratory's practical experience, acetone has been found to be efficient enough for extracting both resin acids and terpenes. Fifty milligrams of finely powdered samples were sonicated (USC300TH) with 5 ml of acetone (p.a.) for 30 min. After sonication, the sample tubes were centrifuged to get the extract solution. For the resin acid analysis, 1 ml of extract was evaporated into dryness under nitrogen flow and silylated with 0.5 ml of 20% N-trimethylsilyl imidazole (TMSI in pyridine, Aldrich). Heptadecanoic acid (C:17) and 1-chlorodecane were used as internal standards for resin acids and terpenes, respectively.

2.3 | Gas chromatography–mass spectrometry analysis

Acetone extracts were analysed using gas chromatography (GC, Agilent Hewlett 13 Packard 6890)–mass spectrometry (MS, Hewlett Packard 5973 MSD, EL-MS 70 eV) equipped with an HP-5 capillary column (Hewlett Packard, 30 m × 0.25 mm i.d., 0.25 μm film thickness). The chromatographic conditions for resin acids were as follows: initial temperature 180°C, temperature rate 5°C/min, final temperature 300°C for 5 min, injector temperature 280°C and split ratio 1:20, MS interface temperature 300°C and ion source temperature 230°C. For the analysis of mono- and sesquiterpenes, 1 ml of the original extract solution (without evaporation and silylation) was used with the following GC-MS conditions: initial temperature 30°C, rate 10°C/min, temperature 230°C, hold time 5 min, rate 40°C/min, hold time 2 min and final temperature 300°C. For the terpene analysis, humulene was used as an external standard. The compounds were identified on the basis of their mass spectra by using Agilent ChemStation software (MSD ChemStation E.02.00.493) with the mass spectral libraries NIST14.L and WILEY275.L. Quantitative analysis of identified compounds was done by using an internal standard method. The concentrations of compounds were compared between *Cronartium*-infected sample and control sample without visible rust symptoms.

2.4 | Statistical analysis

The mean concentrations of monoterpenes from stem lesions were compared between rust-infected samples and control samples using t test. Statistical analyses were conducted using R program (3.4) and SPSS (IBM SPSS Statistics 22) software. The means of mono- and sesquiterpenes from *Cronartium*-infected stem wood samples were also compared to the respective amounts from the dead-top sample. Correlation matrix was calculated for single mono- and sesquiterpenes in *Cronartium*-infected samples. The concentrations of resin

acids were below the detection limit in control samples, and thus, no statistical comparisons could be done between *Cronartium*-infected samples and control samples.

3 | RESULTS

3.1 | Resin acids in wood samples

The total concentration of resin acids varied greatly between 185 and 364 mg/g in the *Cronartium*-infected samples (Table 1). The infected samples were especially rich (>20 mg/g) in abietic acid, levopimaric acid, palustric acid, pimaric acid, dehydroabietic acid and neoabietic acid (Table 1), of which abietic acid was the most common resin acid. The samples contained also small concentrations of isopimaric acid, hydroxydehydroabietic acid, sandaracopimaric acid, 7-hydroxy-dehydro-abietic acid and hydroxy-resin acid. Concentrations of all single resin acids and the total concentration of resin acids were higher in the sample that was severely infected by the rust (dead top) compared to the mean amount of resin acids in the *Cronartium*-infected stem samples. Concentrations were higher especially for hydroxy-resin acid (29% higher), x-hydroxyabietic acid (26%), neoabietic acid (20%), abietic acid (17%), 7-hydroxy-dehydroabietic acid (16%), hydroxydehydroabietic acid (14%), pimaric acid (14%) and the total concentration of resin acids (11%), while for the rest of the resin acids, the concentrations were nearly equal (1%–4%). However, different samples varied greatly in concentration of resin acids: in five samples, concentrations of single resin acid compounds were higher in *Cronartium*-infected stem wood samples compared to the sample from the dead top.

In the control sample, concentrations of most of these resin acids were very low and below the detection level (0.1 mg/g), which did not allow any statistical comparisons with *Cronartium*-infected samples. However, concentrations of resin acids were significantly higher in *Cronartium*-infected sample compared to control sample, which could be seen visually without any statistical comparisons.

3.2 | Mono- and sesquiterpenes in the samples

α-pinene was the most frequent monoterpene, forming on average 57% of the total concentration of mono- and sesquiterpenes. Its concentration varied between 22–91 mg/g in *Cronartium*-infected samples (Table 2). High concentrations (>5 mg/g) of 3-carene, limonene and terpineol were detected from the respective samples. The rest of the monoterpenes and all the sesquiterpenes were extracted in small concentrations, with less than 3 mg/g per sample. Concentrations of many monoterpenes like tricyclene, γ-terpinene, bornyl acetate and α-terpinyl-acetate and of the sesquiterpenes α-murolene, copaen, cubene, σ-cadinene, cubenol and T-murolol were less than 1 mg/g per sample. Concentrations of most monoterpenes were higher from the dead-top sample compared to the average concentrations from infected *Cronartium*-stem wood samples: especially concentrations of β-pinene (110% higher), myrcene (65%), 3-carene (278%), limonene (93%), γ-terpinene (181%), terpinolene (78%), α-terpinyl-acetate (147%) and γ-terpineol (248%) were higher. Among sesquiterpenes, concentration of longipinene was 811% higher in the dead-top sample compared to *Cronartium*-infected stem wood samples, while concentrations of other substances were lower in the dead-top sample. Six to sixty-nine per cent lower

TABLE 1 Mean concentrations and standard deviations (Std) of resin acids in *Cronartium*-infected lesions and control samples of Scots pine after acetone extraction and quantitative analysis using GC-MS

	Cronartium-infected lesion			Control sample	
	Std[a]	Stem Mean[a]	Dead top[b]	Std[a]	Stem Mean[a]
Resin acid	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**
Pimaric acid	5.59	21.53	24.64	4.75	2.06
Sandaracopimaric acid	1.09	4.03	4.20	0.49	0.31
Isopimaric acid	6.47	15.61	15.76	3.42	1.43
Palustric acid	9.04	39.86	40.34	10.33	4.67
Levopimaric acid	10.02	36.23	37.96	15.11	6.43
Dehydroabietic acid	8.43	32.07	33.46	4.93	2.27
Abietic acid	15.32	67.96	79.63	16.88	7.61
Neoabietic acid	10.12	34.45	41.17	6.79	3.18
x-hydroxyabietic acid	3.60	10.82	13.62	<0.1	<0.1
7-hydroxy-dehydroabietic acid	1.62	4.63	5.37	<0.1	<0.1
hydroxydehydroabietic acid	1.22	2.93	3.41	<0.1	<0.1
Hydroxy-resin acid	2.61	7.86	10.17	<0.1	<0.1
Total resin acids	55.11	277.99	309.33	63.20	27.64

[a]Includes 10 trees.

[b]Includes one tree.

TABLE 2 Mean monoterpene[a] and sesquiterpene[b] concentrations and their standard deviations (Std) in *Cronartium*-infected lesions and control samples of Scots pine after acetone extraction and quantitative analysis using GC-MS

	Cronartium-infected lesions			Control sample	
	Std[c]	Stem mean[c]	Dead top[d]	Std[c]	Stem mean[c]
Terpene	**mg/g**	**mg/g**	**mg/g**	**mg/g**	**mg/g**
Tricyclene[a]	0.09	0.17	0.16	0.003	0.01
α-pinene[a]	20.62	48.76	53.80	5.027	2.58
Camphene[a]	0.10	1.66	1.30	0.083	0.04
β-pinene[a]	0.92	2.33	4.89	0.199	0.10
Myrcene[a]	0.72	1.27	2.10	0.04	0.03
3-carene[a]	12.48	9.46	35.76	5.416	2.73
Limonene[a]	3.55	5.16	9.97	0.227	0.13
γ-terpinene[a]	0.21	0.26	0.73	0.089	0.09
Terpinolene[a]	1.43	2.48	4.41	0.759	0.38
4-terpineol[a]	1.88	2.41	1.81	0	0
Terpineol[a]	4.83	5.51	3.20	0	0
Bornyl acetate[a]	0.10	0.12	0.60	0	0
α-terpinyl-acetate[a]	0.14	0.17	0.42	0	0
γ-terpineol[a]	0.33	0.50	1.74	0	0
Longipinene[b]	0.23	0.19	1.73	0	0
α-murolene[b]	0.06	0.12	0.08	0	0
Copaen[b]	0.15	0.18	0.08	0	0
Longifolene[b]	0.30	0.36	0.11	0.008	0.11
Cubene[b]	0.14	0.17	0.09	0.036	0.12
γ-murolene[b]	1.07	1.14	0.74	0.013	0.35
Germacrene[b]	1.35	1.72	0.61	0	0
α-cadinene[b]	1.06	1.35	0.77	0.332	0.16
σ-cadinene[b]	0.16	0.16	0.13	0	0
Cubenol[b]	0.18	0.22	0.10	0	0
T-murolol[b]	0.16	0.18	0.10	0.003	0.05
Total	26.42	86.03	125.33	-	-

[c]Includes 10 trees.

[d]Includes one tree.

concentrations were detected in the dead-top sample compared to average concentrations in stem wood samples for tricyclene, camphene, 4-terpineol, terpineol, α-murolene, longifolene, cubene, γ-murolene, germacrene, α-cadinene, σ-cadinene, cubenol and T-murolol (Table 2).

When comparing the average concentrations of mono- and sesquiterpenes between *Cronartium*-infected samples and control samples, tricyclene, α-pinene, camphene, β-pinene, myrcene, 3-carene, limonene, γ-terpinene, terpinolene, longifolene, γ-murolene, α-cadinene and T-murolol had statistically significantly higher concentrations in *Cronartium*-infected samples (t test; Table 3). Cubene was the only sesquiterpene that did not differ in concentration between the rust-infected and control samples. The concentrations of the previously mentioned substances were 1.3- to 45-fold higher in *Cronartium*-infected samples compared to control samples. Especially concentrations of myrcene (45-fold), camphene and limonene (27-fold), β-pinene (20-fold), tricyclene (17-fold) and α-pinene (13-fold) were extremely high. For the rest of the monoterpenes (11 in number; Table 2), the concentrations were also higher in *Cronartium*-infected samples compared to control samples, but due to low concentrations, below the detection level or complete absence of a compound, statistical comparisons could not be done. Many monoterpenes had high concentrations in *Cronartium*-infected samples (e.g. in samples nos. 4, 46 and 49).

TABLE 3 Comparison of mean concentrations of monoterpenes[a] and sesquiterpenes[b] in *Cronartium*-infected lesions and control samples of Scots pine using t test

Compound	t	df	*p*-value
Tricyclene[a]	5.76	10	<0.001*** [0.0001817]
α-pinene[a]	6.94	10	<0.001*** [0.0000398]
Camphene[a]	5.25	10	<0.001*** [0.0003745]
β-pinene[a]	7.55	10	<0.001*** [0.0000195]
Myrcene[a]	6.21	10	<0.001*** [0.0001004]
3-carene[a]	2.13	10	<0.01** [0.05929]
Limonene[a]	4.89	10	<0.001*** [0.0006319]
γ-terpinene[a]	3.93	10	<0.005** [0.002804]
Terpinolene[a]	6.69	10	<0.001*** [0.0000541]
Longifolene[b]	2.59	10	<0.05* [0.02691]
Cubene[b]	0.77	10	NS [0.461]
γ-murolene[b]	2.47	10	<0.05* [0.03328]
α-cadinene[b]	3.04	10	<0.05* [0.01255]
T-murolol[b]	2.76	10	<0.05* [0.02012]

Note: Significance levels: *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.
Abbreviation: NS, non-significant.

Correlation matrix revealed that composition of tricyclene, α-pinene, camphene, α-murolene, copaen, longifolene, cubenene, germacrene, σ-cadinene, T-murolol and α-cadinene correlated in most cases statistically significantly with one another in *Cronartium*-infected samples (Table 4). Concentrations of most of these mono- and sesquiterpenes were lower in the dead-top sample compared to *Cronartium*-infected stem wood samples. The monoterpenes β-pinene, myrcene, bornyl acetate and the sesquiterpene longipinene correlated also significantly with one another (Table 4).

4 | DISCUSSION

This is the first study exploring the chemical content of wood infected by *Cronartium* rust. The role of the now explored mono- and sesquiterpenes and resin acids in rust resistance is poorly known. Bioactive phenolic compounds have been linked to defence responses of boreal tree species in prior studies (Witzell & Martín, 2008). A detailed chemical analysis showed that the relative concentrations of several

TABLE 4 Correlation matrix of monoterpenes[1] and sesquiterpenes[2] in *Cronartium*-infected wood

Terpene	Tricyclene[1]	α-pinene[1]	Camphene[1]	β-pinene[1]	Myrcene[1]	3-carene[1]	Limonene[1]	γ-terpinene[1]	Terpinolene[1]	4-terpineol[1]	Terpineol[1]	Bornyl acetate[1]
Tricyclene[1]	1.00											
α-pinene[1]	0.76**	1.00										
Camphene[1]	0.80**	0.95***	1.00									
β-pinene[1]				1.00								
Myrcene[1]				0.72*	1.00							
3-carene[1]						1.00						
Limonene[1]							1.00					
γ-terpinene[1]						0.73*		1.00				
Terpinolene[1]						0.63*		0.96***	1.00			
4-terpineol[1]	0.81**		0.66*							1.00		
Terpineol[1]											1.00	
Bornyl acetate[1]				0.79**	0.70*							1.00
α-terpinyl-acetate[1]												
γ-terpineol[1]						0.62*		0.84**	0.72*			0.65*
Longipinene[2]				0.62*				0.73*				0.81**
α-murolene[2]	0.70*	0.77**	0.79*									
Copaen[2]	0.64*	0.74**	0.73*									
Longifolene[2]		0.73*	0.78**									
Cubene[2]	0.63*	0.75**	0.73*									
γ-murolene[2]												
Germacrene[2]	0.61*	0.72*	0.71*									
α-cadinene[2]		0.73*	0.69*									
σ-cadinene[2]	0.73*	0.86***	0.86***							0.63*		
Cubenol[2]												
T-murolol[2]	0.65*	0.76**	0.73*									

Note: Only significant correlations at $p < .001$***, $p < .05$** and $p < .01$* are shown.

mono- and sesquiterpenes were significantly higher in *Cronartium*-infected samples than in control samples. Concentrations of many terpenes recorded in this study from diseased wood samples were 60- to 300-fold higher than in stem wood samples of healthy young Scots pines close to the study site (Nerg et al., 2004). Although extraction methods slightly vary between these studies, the results clearly indicate the great impact of the rust on upregulation of terpene production in the infected wood. The function of terpenes in rust resistance may be either direct toxicity or general antioxidant activity, which can protect the cells from, for example, free radicals and lipid peroxidation. On the other hand, slow-canker growth has been considered as a resistant response to *Cronartium ribicola* J. C. Fisch on *Pinus monticola* D. Don (Hunt, 1997).

Terpene quality and quantity varies according to stress type, intensity, time within and among years and even between trees on *Pinus* (Kopaczyk et al., 2020). Most previous studies, however, deal with emissions of volatile chemicals or focus on young seedlings or needles and therefore are not strictly comparable to our results of chemicals from wood of mature pines. Among monoterpenes, α-pinene was the most common substance in *Cronartium*-infected samples. It was also the major monoterpene in needles and wood of young Scots pines (Manninen et al., 2002). It is a bicyclic monoterpene that exhibits antiulerogenic activity by reducing gastric lesion induced by ethanol. α-pinene and β-pinene are common monoterpenes in sapwood of many pines, for example *Pinus radiata* D. Don (McDonald et al., 1999). α-pinene concentration correlates well with the gastroprotective effect of essential oils from *Hyptis* species (Pinheiro et al., 2015) and is known to act as a plant defence chemical against insect herbivory. High transcription level of genes encoding α-pinene synthase has also been linked to increased level of necrotic lesion formation in Scots pine in response to *Heterobasidion annosum* (Fr.) Bref. s.l. (Mukrimin et al., 2019). Among the other monoterpenes found at high concentrations in *Cronartium*-infected wood, camphene reduces plasma cholesterol and triglycerides in hyperlipidemic rats (Vallianou et al., 2011). β-pinene and limonene also exhibit antiherpetic activity by reducing viral activity and could, therefore, be used as potential antiviral agents (Astani & Schnitzler, 2014). β-pinene was also the major component of the oil extracts from *Salvia officinalis* L. that showed good antifungal activity against pathogenic *Candida* spp. yeasts (Badiee et al., 2012). The number of eggs of *Hylotrupes bajulus* (L.) was also shown to correlate positively with

α-terpinyl-acetate[1]	γ-terpineol[1]	Longipinene[2]	α-murolene[2]	Copaen[2]	Longifolene[2]	Cubene[2]	γ-murolene[2]	Germacrene[2]	α-cadinene[2]	σ-cadinene[2]	Cubenol[2]	T-murolol[2]
1.00												
0.72*	1.00											
	0.92***	1.00										
			1.00									
			0.96***	1.00								
			0.79**	0.78**	1.00							
			0.95***	0.99***	0.75***	1.00						
							1.00					
			0.94***	0.99***	0.78**	0.99***		1.00				
			0.95***	0.99***	0.72*	1.00***		0.99***	1.00			
			0.93***	0.95***	0.83**	0.95***		0.94***	0.93***	1.00		
			0.87***	0.91***		0.93***		0.91***	0.99***	0.78**	1.00	
			0.96***	1.00***	0.76**	0.99***		0.99***	0.99***	0.95**	0.92***	1.00

low β-pinene concentration, total concentration of monoterpenes and β-pinene:α-pinene ratio in Scots pine wood (Nerg et al., 2004). In phloem of Scots pine, the amounts of β-pinene and limonene were reported to increase after infection by the blue-stain fungi *Leptographium wingfieldii* Morelet and *Ophiostoma canum* (Münch) H. and Sydow (Fäldt et al., 2006), while the amounts of α-pinene and 3-carene were decreased or remained unaffected. As we did not analyse separately chemicals from different layers of the resinous lesions, we cannot directly compare our results to previous ones. Among other monoterpenes, α-terpinyl-acetate and γ-terpineol, and among sesquiterpenes, α-murolene, longifolene, α-cadinene and cubenol, have also shown antimicrobial characteristics. In addition, myrcene is a precursor to formation of other secondary terpenes, 3-carene is a flavouring ingredient, and γ-terpinene has aromatic effects as a strong antioxidant. Terpinolene from oil of *Pinus mugo* Turra prevents effectively low-density lipoprotein (LDL)-oxidation (Grassmann et al., 2005). 4-terpineol is common especially in pines. T-murolol is an active sesquiterpenoid with great activity against *Candida* sp. Limone and 4-terpineol were shown to have anti-insect and antimicrobial activity against *Ceratitis capitata* (Widemann) and *Triatoma infestans* Klug. The oil from *Baccharis darwinii* Hook. & Arn. exhibited antifungal activity against yeasts and dermatophytes of *Candida* spp. (Kurdelas et al., 2012). Therefore, the high concentrations of most of the monoterpenes recorded now in *Cronartium*-infected samples in comparison to control samples imply a strong defence reaction by the *Pinus* host to rust infection.

In this study, the most common resin acid detected was abietic acid, which occurred at a concentration that was 10-fold higher than in healthy young Scots pine trees from the study area (Nerg et al., 2004). Also, the rest of the most commonly detected resin acids, palustric acid, levopimaric acid, dehydroabietic acid and neoabietic acid, had ca. 15- to 100-fold higher concentrations in the *Cronartium*-infected samples compared to concentrations in the wood of healthy young Scots pines (Nerg et al., 2004). Generally, the resin acids detected at high concentrations in *Cronartium*-infected lesions (this study) were the same as detected commonly in wood of young Scots pines (Nerg et al., 2004). Therefore, the rust induces great increase in resin acid concentrations in Scots pine wood after infection.

Recently, the relative concentrations of apigenin flavonoids were reported to be higher in the *Cronartium*-resistant alternate host

M. pratense than in the *Cronartium*-susceptible *M. sylvaticum* (Kaitera & Witzell, 2016). One of these flavonoids, acacetin, has been reported to inhibit formation of penetration structures and the colonization of arbuscular mycorrhizal fungi on roots of *Lycopersicon esculentum* L. (Scervino et al., 2005), indications of antimicrobial effect. Flavonoids are also known for their strong antioxidant and radical scavenging activity (Burda, & Oleszek, 2001; Pietta, 2000). In order to understand how the substances in wood may contribute to resistance of Scots pine to *Cronartium* rusts, further studies are needed to describe the structure of the chemical compounds and their absolute concentrations. Moreover, the bioactive effects of chemical substances from infected wood on germination of spores of *Cronartium*, or growth of their axenic cultures (Moricca & Ragazzi, 1996), should be investigated.

In conclusion, our results indicate that especially monoterpenes and resin acids occur at high concentrations in *Cronartium*-infected lesions as a response of pine to *Cronartium* rust. In particular, further research should be conducted to clarify which of the chemicals are connected to rust defence, how *Cronartium* rust reacts to these chemicals, and whether the colonization process could be affected. As the chemical processes were abnormal also in the visually healthy wood in rust-infected trees, these processes should be further studied in the future. *Cronartium*-infected wood is a promising source of terpenes, resin acids and some other chemicals for further utilization in medical or cosmetic industry.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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