



Inheritance of specific secondary volatile metabolites in buds of white birch *Betula pendula* and *Betula pubescens* hybrids

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Abstract

Despite the differences in ploidy and in the timing of flowering, two species of white birch, *Betula pendula* and *Betula pubescens* often form viable hybrids in places of their joint growth. Recognition of such hybrids by their morphological features is very difficult or impossible. On the other hand, it could be assumed that substantial differences can be found in the secondary metabolites whose composition is determined mainly by genetic factors. However, the inheritability of biosynthesis of secondary birch metabolites is unknown. We investigated the bud volatile organic compounds (VOCs) of the artificial hybrids of these birches and compared them with specific metabolites of pure parent species. The bud VOCs were determined by a combination of head-space microextraction with gas chromatography-mass spectrometry. As the result, we found that: (a) hybrids clearly show dominant inheritance along the maternal line and (b) the buds of hybrid plants have characteristic features of VOCs composition, which allows for definitive conclusions about hybrid origins in some cases.

Keywords *Betula pendula* · *Betula pubescens* · Hybrids · Buds · Chemical composition

Introduction

Birch trees (*Betula* L.) are among the most common deciduous trees in the boreal and temperate zones of the Northern Hemisphere. According to different authors, in these zones there are more than 65 species of the genus *Betula*, however, European forests are dominated by two species: silver birch (*Betula pendula* Roth) and downy birch (*Betula pubescens* Ehrh.) (Hynynen et al. 2010). High morphological variability greatly complicates the distinction between these two species (Keinänen et al. 1999; Lahtinen et al. 2006; Migalina et al. 2010), and it is further complicated by their hybridization (Brown et al. 1982; Kennedy and Brown 1983).

Both species belong to the section *Albae*, subgenus *Betula* (Keinänen et al. 1999); however, they are distinguished by ploidy level: *B. pendula* is a diploid ($2n = 28$), whereas

B. pubescens is a tetraploid ($2n = 56$). According to some authors, downy birch is an ancient allotetraploid, formed by a cross between a *B. pendula*-like species and another diploid species (Johnsson 1945; Howland et al. 1995). In Poland, silver birches bloom in April, approximately 10 days earlier than downy birch; in Finland and Karelia, birches bloom in early May, and the difference between species in flowering periods between the species is 7–10 days. In spite the difference in ploidy as well as flowering times, these two species are capable of forming hybrid forms.

The occurrence of plants that are morphologically intermediate between different birch species has been reported in many publications (Scoggan 1978; Atkinson 1992; Anamthawat-Jónsson and Thórsson 2003). For this reason, natural hybridization of different species of plants in the family *Betulaceae* has been and still remains the objective of research by specialists from different countries (Johnsson 1945; Natho 1959; Stern 1963; Brown et al. 1982; Anamthawat-Jónsson and Thórsson 2003; Banaev and Bažant 2007; Vetchinikova and Titov 2017). The study of this phenomenon allows us to better understand the evolutionary role of natural hybridization, and it allows us to solve a number of theoretical and practical problems, such as the level of compatibility for natural crosses between the two birch species or the genetic improvement of birches.

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A strong specificity of the secondary metabolite composition has been previously established for extracts from buds of *B. pendula* and *B. pubescens* (Isidorov et al. 2014, 2016), which makes it possible to use this distinction in chemotaxonomy. The extent to which the chemical compositions of different species of parent plants are transmitted to the progenies from crosses would be interesting to discover. To clarify this issue, we conducted a comparative study of the chemical composition of volatile organic compounds (VOCs) emitted into the gas phase by artificial hybrids and parental birch species using modern high-performance analytical techniques, which consisted of a combination of solid-phase microextraction with chromatography-mass spectrometry (HS-SPME/GC–MS) (Agelopoulos and Pickett 1998). The study of the VOC composition of birch buds and their volatilomes is of particular interest because these volatiles show anti-microbial and anti-herbivore activity and can serve to protect valuable reproductive parts of plants, such as buds (Holopainen 2004; Dudareva et al. 2004, 2006; Peñuelas and Munne-Bosch 2005; Rennenberg et al. 2006; Schwab et al. 2008; Karl et al. 2008).

Materials and methods

Plant material

The buds were collected from artificial hybrids of *B. pendula* Roth and *B. pubescens* Ehrh. growing at the field experimental station (FES) of the Forest Research Institute of the Karelian Research Centre of the Russian Academy of Sciences (KarRC RAS) near Petrozavodsk (61°45'N, 34°20'E). Hybrids (F_1) were produced in 1969 in a series of crosses involving *B. pendula* seed parents and *B. pubescens* pollen parents and vice versa (Ermakov 1975, 1986). Crossing was carried out on trees in natural populations located in the southern part of the Republic of Karelia (North-West Russia) (61°19'N, 35°29'E). Besides, buds of downy birch and silver birch were gathered from 3–5 trees belonging to the same populations in April–May and August–September 2018 at the FES. In addition, buds were gathered from December 2017 to January 2018, from trees growing in the Leningrad region (59°45'N, 30°04'E), Latvia (57°17'N, 26°34'E), and north-eastern Poland (53°32'N, 22°43'E) (Fig. 1). A previously described method based on the nuclear DNA isolation and sequencing was used to identify the birch species (Isidorov et al. 2014). Voucher specimens were deposited with the herbarium of the Forest Research Institute KarRC RAS (PTZ) and the Department of Pharmacognosy, of the



Fig. 1 Map giving the position of birch bud sampling

Medical University of Białystok, Poland (nos. BP-17034 and BO-17035).

HS-SPME sampling and GC–MS determination of VOCs

The previously described analytical procedure, HS-SPME/GC–MS, was used for VOC investigation (Isidorov et al. 2012, 2014). In these works, the comparison of PDMS 100, carboxen/PDMS (CAR/PDMS) and divinylbenzene/carboxen/PDMS (DVB/CAR/PDMS) sorption fibres was performed, and the best effectiveness of the extraction–desorption cycle was obtained by the later. The chromatograms registered after the exposition of PDMS 100 fibres demonstrated hardly any peaks of compounds with low boiling temperatures (light carbonyls and alcohols). In turn, fibres CAR/PDMS do not completely return high-boiling-point components, such as sesquiterpenes, during the desorption stage, (Isidorov et al. 2012).

Harvested buds (0.5 g) were placed into a of 16 mL headspace vial and immersed into a thermostat at 50 °C. The membrane of the screw-cap was pierced by the needle with DVB/CAR/PDMS fibre and exposed to a headspace gas phase. After 50 min of exposition, the fibre was introduced for 10 min into the injection port of the GC–MS apparatus. The latter was operated at 250 °C in the splitless mode. The helium flow rate through the column was 1 mL min⁻¹ in constant flow mode. The initial column temperature was 40 °C and rose to 220 °C at a rate of 3 °C min⁻¹. The MSD detector acquisition parameters

were as follows: the transfer line temperature was 280 °C, the MS source temperature was 230 °C and the MS quad temperature was 150 °C. The electron impact mass spectra were obtained at 70 eV of ionization energy. Detection was performed in the full scan mode. After integration, the fraction of separated components in the total ion current (TIC) was calculated.

To identify the components, both mass spectral data and the calculated retention indices were used. Mass spectrometric identification was carried out with an automatic system of GC–MS data processing supplied by NIST mass spectra library, as well as by computer search libraries containing the mass spectra and retention indices from Adams' (2007) and Tkachev's (2008) collections.

To determine the retention times of reference compounds, a SPME fiber was inserted for 2–3 s into the headspace vial with a mixture of C₅–C₁₈ *n*-alkanes, which were separated under the conditions described previously. The linear temperature-programmed retention indices (RI) were calculated from the equation:

$$RI = 100 \left[n + \frac{(t_x - t_n)}{(t_{n+1} - t_n)} \right],$$

where t_x is the retention time of the analyte, t_n is the retention time of the *n*-alkane eluting directly before the analyte, and t_{n+1} is the retention time of the *n*-alkane eluting directly after the analyte. Calculated retention indices of the registered components were compared with the above-mentioned collections, as well as with the NIST (2013) collection. The identification was considered reliable if the results of the computer-based search of the mass spectra library were confirmed by the experimental RI values, i.e., if their deviation from the averaged published values did not exceed ± 10 u.i. Mass spectrometric identification not confirmed by the retention index was considered as putative.

Results and discussion

The chemical composition of bud VOCs

The chemical compositions of VOCs emitted into the gas phase by buds of artificial hybrids of silver and downy birch species (12 samples) growing in Karelia were determined with the aid of HS-SPME/GC–MS. VOC compositions were also studied with the same technique for eleven samples of silver birch and eleven samples of downy birch buds. The geography of these samples encompasses a latitudinal interval of northeastern Europe from 61°N to 53°N (meridian distance ca. 890 km).

On the obtained chromatograms were registered 224 peaks which belonged to C₁–C₁₈ organic compounds of different classes. The number of compounds detected in the volatiles of both birch species was nearly the same: 157 in

silver birch emissions and 156 in downy birch emissions. Hence, the composition of their volatiles overlapped only partially; some components were found in the emission of only one species. It is remarkably that VOCs of both crossed variants were substantially less diverse: only 88 compounds in the *B. pendula* ♀ × *B. pubescens* ♂ emissions and 87 compounds in the *B. pubescens* ♀ × *B. pendula* ♂ emissions (107 compounds in total). The difference in the VOC compositions of downy and silver birch buds and their hybrids is demonstrated in Fig. 2.

Table 1 presents the averaged semi-quantitative composition (TIC fraction) of bud volatiles detected in emissions of all 34 samples that were investigated. It also contains some analytical parameters that were used to confirm the identification results: experimental (RI^{Exp}) and literature (RI^{Lit}) values of retention indices, *m/z* values of the most intensive ions in the mass spectra (in order of decreasing intensity), and mass numbers of molecular ions, M⁺, if registered in the mass spectra. The content of the compounds in Table 1 vary widely from traces (<0.01% of TIC) to tens of percent. These variations can be explained by the circumstances under which the samples were collected: different seasons and populations growing in different climatic zones.

The VOCs in Table 1 are divided into 11 groups according to their chemical structures; each group lists compounds in order of their retention indices. Although genes and enzymes specifically involved in the synthesis of these birch bud volatiles have not yet been investigated, some considerations according to their synthesis pathways can be presented based on data available in the literature.

The most comprehensive group of the birch bud VOCs is formed by terpenoids: 22 mono- and 93 sesquiterpenoids. Moreover, terpenoids are characterised by the higher content (80–90% of TIC). It is known that all terpenoids derive from isopentenyl diphosphate and dimethylallyl diphosphate (Croteau et al. 2000). Terpene synthases are responsible for catalyzing the formation of terpenoids from these substrates. Many other enzymes participate in the transformation of initial products by their oxidation, hydroxylation, dehydrogenation and other processes (Croteau et al. 2000; Dudareva et al. 2004; Qualley and Dudareva 2009).

The majority of monoterpene compounds belong to minor components; only limonene, β-ocimene isomers and linalool oxide isomers were at quantities higher than 1% of TIC. Camphene, β-pinene, and myrcene were only registered in the VOCs of downy birch. In turn, 1,8-cineole was characteristic of the emissions of silver birch.

The assignment of some compounds to C₁₅H₂₄O, C₁₅H₂₄O₂ and C₁₅H₂₆O₂ sesquiterpenoids was based only on the MS data and should be considered putative. The majority of these substances belong to minor components, which share in the total ion current of the chromatogram not exceed 1% of TIC, as a rule. Qualitative composition

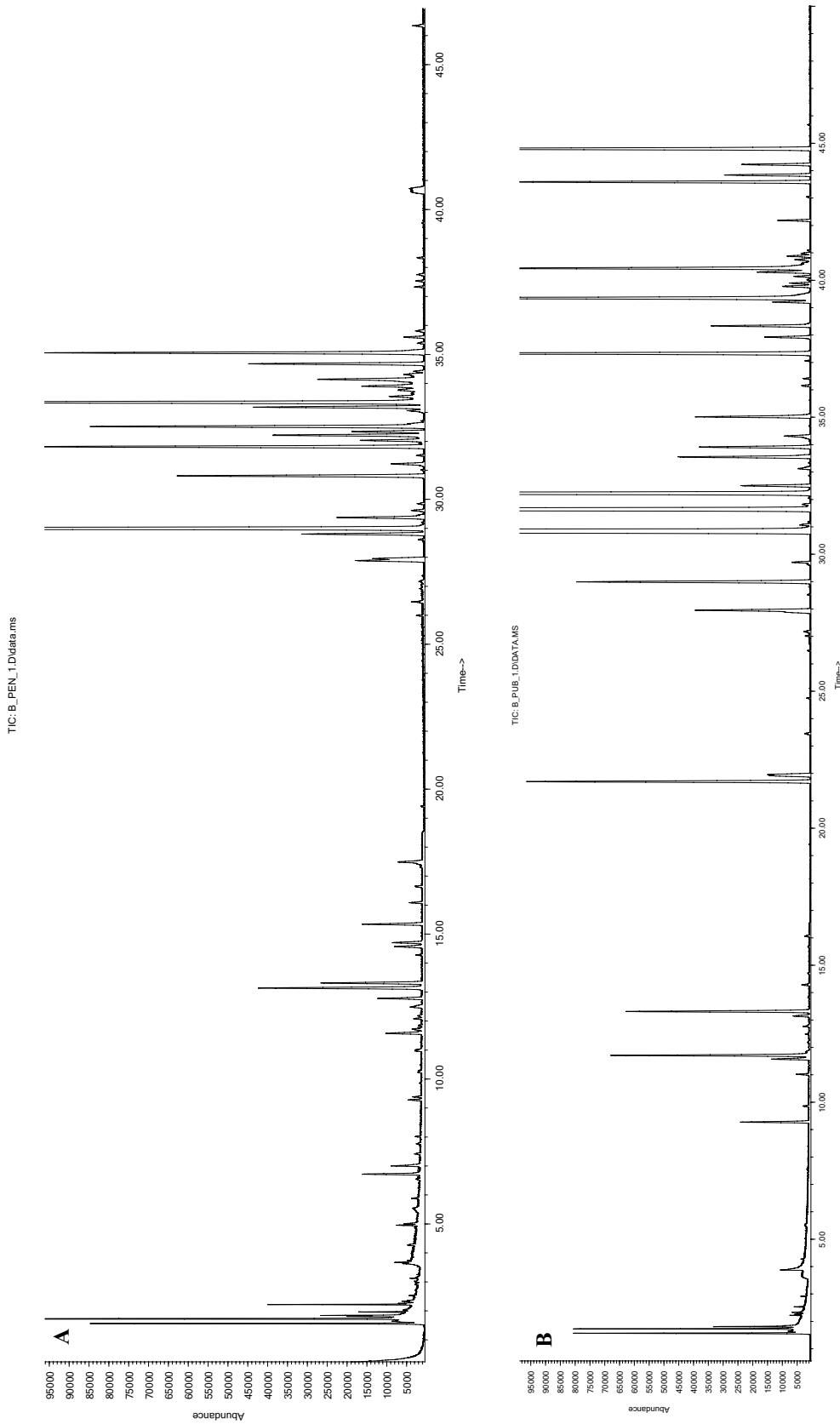


Fig. 2 Typical chromatograms of VOCs emitted from buds of *B. pendula* (a), *B. pubescens* (b), *B. pendula* ♀ × *B. pubescens* ♂ (c), and *B. pubescens* ♀ × *B. pendula* ♂ (d) hybrids

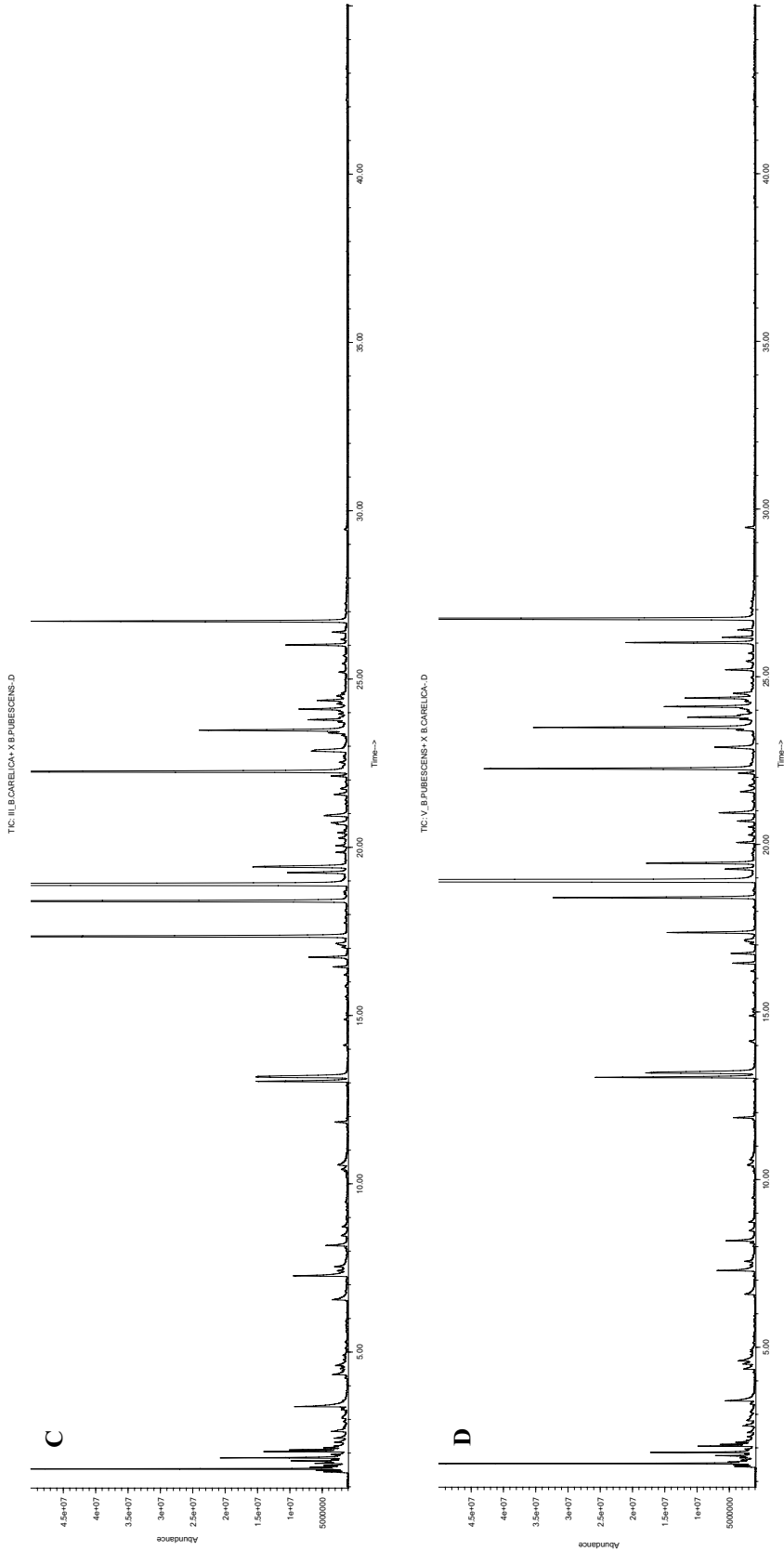


Fig. 2 (continued)

Table 1 Qualitative and semi-quantitative (% of TIC) composition of VOCs emitted by buds of two birch species and their hybrids

Compounds	CAS	<i>m/z</i>	M ⁺	RI ^{Exp}	RI ^{Lit}	<i>B. pubescens</i> (<i>n</i> = 11)	<i>B. pendula</i> (<i>n</i> = 11)	<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (<i>n</i> = 5)	<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (<i>n</i> = 7)
Monoterpenoids									
α-Pinene	80-56-8	93,91,77,92,41	136	932	936	<0.01–0.06	<0.01–0.14	–	–
Camphene	79-92-5	93,121,79,91,41	136	946	946	<0.01–0.02	–	–	–
β-Pinene	127-91-3	93,41,69,91,77	136	973	973	<0.01–0.05	–	–	–
Myrcene	123-35-3	41,93,69,39,136	136	991	991	<0.01–1.19	–	0.10–0.21	0.55–1.32
3-Carene	13466-78-9	93,91,77,79,121	136	1012	1011	<0.01	<0.01	–	–
Limonene	138-86-3	68,93,67,107,136	136	1026	1028	0.07–0.90	0.39–1.77	0.21–0.37	0.51–1.06
1,8-Cineole	470-82-6	43,81,108,111,71	154	1030	1031	–	0.01–1.34	–	–
<i>cis</i> -β-Ocimene	3338-55-4	93,41,79,91,77	136	1038	1038	0.02–0.07	0.07–1.72	<0.01–0.12	<0.01
<i>trans</i> -β-Ocimene	3779-61-1	93,41,79,91,77	136	1047	1048	0.02–0.26	<0.01–0.40	<0.01–0.03	<0.01–0.03
γ-Terpinene	99-85-4	93,91,136,121,77	136	1058	1058	<0.01–0.04	<0.01–0.38	–	–
<i>cis</i> -Linalool oxide	34995-77-2	59,43,94,68,111	–	1072	1073	0.01–0.31	0.02–1.26	–	≤0.01
<i>trans</i> -Linalool oxide	5989-33-3	59,43,42,69,94	–	1088	1088	<0.01–0.05	<0.01–0.17	–	–
Terpinolene	586-62-9	93,121,136,91,79	136	1088	1089	<0.01–0.06	–	–	–
Linalool	78-70-6	71,93,55,43,41	154	1101	1101	<0.01–0.09	<0.01–0.17	–	≤0.01
4-Terpineol	562-74-3	71,93,111,43,86	154	1179	1177	<0.01–0.51	<0.01–0.31	–	–
α-Terpineol	98-55-5	59,93,121,136,67	–	1189	1189	<0.01–0.03	–	–	–
Nerol	106-25-2	69,41,68,93,67	154	1229	1228	–	<0.01–0.03	–	–
Citronellol	106-22-9	69,41,67,81,82	156	1231	1229	<0.01–0.18	<0.01–0.02	–	–
Linalyl formate	115-99-1	69,41,136,93,121	–	1245	1241	<0.01–0.42	<0.01–0.28	–	–
Geraniol	106-24-1	69,41,68,123,93	–	1257	1255	<0.01–0.11	<0.01–0.19	–	–
Bornyl acetate	76-49-3	95,43,121,93,136	196	1286	1285	<0.01–0.03	–	–	–
Geranial	5392-40-5	69,41,84,94,83	152	1273	1274	–	<0.01–0.03	–	–
Sesquiterpenoids									
Sesquiterpene C ₁₅ H ₂₄	–	81,91,204,93	204	1320	–	<0.01	<0.01–0.13	0.66–0.70	0.43–0.52
δ-Elementene	20307-84-0	121,136,93,41,91	204	1338	1338	–	0.02–1.04	–	–
α-Cubebene	17699-14-8	105,161,119,41,91	204	1349	1349	0.02–0.29	0.94–1.76	–	–
Sesquiterpene C ₁₅ H ₂₄	–	95,147,96,79,189	204	1351	–	0.12–0.73	0.26–1.27	–	0.01–0.56
α-Ylangene	14912-44-8	105,119,93,91,41	204	1373	1372	<0.01–0.04	0.57–2.30	<0.01–0.06	–
α-Copaene	3856-25-5	161,119,105,91,204	204	1376	1376	0.26–2.13	11.85–22.42	6.67–7.31	0.87–1.63
β-Bourbonene	5208-59-3	81,80,123,161,79	204	1387	1387	<0.01–0.05	0.02–1.46	<0.01	<0.01
β-Cubebene	13744-15-5	161,105,91,120,41	204	1391	1392	<0.01–0.07	0.01–0.59	0.25–0.43	<0.01
β-Elementene	515-13-9	81,93,68,107,41	204	1393	1393	<0.01–0.44	0.01–1.54	0.24–0.46	<0.01
Sesquiterpene C ₁₅ H ₂₄	–	161,105,119,91,81	204	1396	–	–	<0.01–0.13	–	–
α-Gurjunene	489-40-7	105,161,204,189,91	204	1410	1412	<0.01–0.04	<0.01–0.03	–	–
<i>cis</i> -α-Bergamotene	64727-43-1	41,93,119,91,105	204	1416	1415	–	<0.01–0.74	–	–
β-(<i>E</i>)-Caryophyllene	87-44-5	93,133,91,41,69	204	1420	1418	6.40–35.09	0.77–4.60	27.17–42.02	3.18–37.27
γ-Elementene	29873-99-2	121,177,107,69,93	–	1425	–	<0.01–0.05	–	–	–
β-Copaene	18252-44-3	161,105,91,41,119	204	1429	1432	<0.01	0.10–0.66	0.13–0.30	–
α-Bergamotene	17699-05-7	93,119,41,91,69	204	1436	1435	–	0.10–5.38	–	–
Caryophylla-4(12),8(13)-diene	136296-38-3	120,69,93,91,41	204	1434	1430	<0.01–0.11	–	–	–
Birkenal	N/A	107,93,121,41,69	206	1440	1443	10.21–53.51	–	9.15–17.83	23.00–38.71
Guaia-6,9-diene	36577-33-0	93,107,81,91,204	204	1444	1445	<0.01	1.73–6.42	–	–
Sesquiterpene C ₁₅ H ₂₄	–	161,91,105,204,133	204	1449	–	<0.01	0.80–2.44	–	–
Geranyl acetone	689-67-8	43,69,41,151,136	–	1453	1454	<0.01–0.01	–	–	–
α-Humulene	6753-98-6	93,80,91,121,147	204	1454	1454	0.79–9.47	0.68–3.97	6.55–9.89	3.16–7.92
Selina-4(15),6-diene	N/A	161,105,204,91,133	204	1456	1454	<0.01	<0.01–1.05	–	–
Alloaromadendrene, dehydro-	85048-01-7	41,105,91,93,107	204	1456	1459	–	<0.01–3.37	–	–
Alloaromadendrene	25246-27-9	161,41,91,93,105	204	1462	1464	–	1.76–6.35	1.15–3.74	<0.01–0.02
Sesquiterpenol C ₁₅ H ₂₆ O	–	127,43,136,107,95	222	1467	–	–	<0.01–0.26	–	–

Table 1 (continued)

Compounds	CAS	<i>m/z</i>	M ⁺	RI ^{Exp}	RI ^{Lit}	<i>B. pubescens</i> (<i>n</i> = 11)	<i>B. pendula</i> (<i>n</i> = 11)	<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (<i>n</i> = 5)	<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (<i>n</i> = 7)
Sesquiterpenoid C ₁₅ H ₂₂	–	159,145,202,160,131	202	1473	–	–	<0.01–0.32	–	–
Drima-7,9(11)-diene	N/A	105 > 91,107,93,121	204	1475	1471	<0.01	–	–	–
<i>trans</i> -Cadina-1(6),4-diene	20085-11-4	161,105,204,81,134	204	1477	1476	–	0.23–1.18	–	0.1–0.56
γ-Muurolene	30021-74-0	161,105,119,93,204	204	1478	1480	–	0.90–2.56	0.13–0.37	–
Germacrene D	23986-74-5	161,105,91,41,119	204	1482	1480	<0.01–0.11	1.83–26.81	0.63–2.93	–
β-Selinene	17066-67-0	41,204,105,93,107	204	1487	1488	<0.01–0.89	<0.01–0.76	<0.01–0.20	<0.01–0.24
α-Guaiene	3691-12-1	105,107,93,147,79	204	1489	1490	–	<0.01–1.15	–	–
<i>trans</i> Muurola-4(15),5-diene	–	161,204,189,105,91	204	1493	1494	–	≤0.01	<0.01–0.10	–
γ-Amorphene?	6980-46-7	161,105,119,93,91	204	1495	1496	–	0.01–1.10	<0.01–0.17	–
α-Selinene	473-13-2	189,93,105,81,133	204	1496	1496	0.01–0.77	≤0.01	<0.01–0.07	≤0.01
4- <i>epi</i> -Cubebol	38230-60-3	207,161,105,93,119	222	1497	1495	–	<0.01–1.30	–	–
α-Muurolene	10208-80-7	161,119,105,93,91	204	1498	1499	–	0.02–2.33	0.12–0.27	<0.01–0.05
β-Cadinene	523-47-7	161,204,119,189,105	204	1507	1515	–	<0.01–0.49	–	–
β-Bisabolene	495-61-4	69,93,41,204,161	204	1509	1507	<0.01	–	–	–
δ-Amorphene	189165-79-5	161,204,119,134,105	204	1510	1509	–	<0.01–0.30	–	–
γ-Cadinene	39029-41-9	161,204,105,119,91	204	1517	1517	–	1.01–3.22	0.26–0.59	–
Birkenol	N/A	121,93,177,91,105	208	1523	1523	0.52–1.61	–	–	–
δ-Cadinene	483-76-1	161,134,119,105,41	204	1527	1527	–	1.55–6.87	0.65–1.02	–
Cadina-1,4-diene	38758-02-0	119,105,161,204,91	204	1535	1536	–	0.01–0.42	<0.01–0.05	–
des-4-Methylcaryophyll-8(14)-en-5-one	N/A	79,82,55,41,124	206	1540	1538	<0.01–0.13	–	–	≤0.01
α-Cadinene	24406-05-1	105,161,204,91,119	204	1541	–	–	<0.01–0.50	<0.01–0.08	–
<i>cis</i> -α-Bisabolene?	495-61-4	93,119,121,80,204	204	1544	–	<0.01–1.32	–	–	–
α-Calocorene	21391-99-1	157,142,200,115,156	200	1546	1546	–	<0.01–0.75	<0.01–0.08	–
Cyclocaryophyllene aldehyde	N/A	135,164,93,79,105	–	1553	1555	<0.01–0.41	–	<0.01	<0.01–0.41
Salviadienol	N/A	123,131,91,109,107	220	1557	1555	–	0.03–1.15	–	–
Sesquiterpenoid C ₁₅ H ₂₂ O	–	79,96,41,109,123,138	218	1559	–	<0.01–0.19	–	<0.01–0.15	–
Dihydrocaryophyllene-5-one	68330-80-3	79,96,41,138,69	220	1565	1562	<0.01–0.13	–	<0.01–0.15	≤0.01
β-Calocorene	50277-34-4	157,142,156,105,200	200	1566	1565	–	<0.01–0.34	–	–
Mint oxide	N/A	96,123,95,81,159	220	1570	1568	–	0.01–0.37	–	–
Germacren D4-ol	74841-87-5	81,43,41,161,123	204	1582	1580	–	<0.01–0.40	–	–
Caryophylla-4(12),8(13)-dien-5-one	N/A	134,69,91,107,41	218	1579	1576	0.01–0.68	–	0.10–0.21	<0.01–0.48
Caryophyllene oxide	1139-30-6	43,79,41,93,91	220	1584	1582	2.63–6.56	0.03–2.29	0.17–6.94	2.37–4.63
β-Copaen-4α-ol	126060-41-1	41,159,91,131,117	–	1586	1584	–	<0.01–1.40	–	–
Sesquiterpenoid C ₁₅ H ₂₂ O ₂	–	91,159,93,105,107	234	1591	–	–	<0.01–5.26	–	–
Sesquiterpenoid C ₁₅ H ₂₄ O	–	93,159,41,79,107	220	1593	–	–	≤0.01	–	–
Salvia-4(14)-en-1-one (mint ketone)	N/A	123,81,177,93,107	220	1596	1598	–	0.03–2.39	0.13–0.32	–
Sesquiterpenoid C ₁₅ H ₂₂ O	–	107,91,135,41,218	218	1597	–	<0.01–0.25	–	–	–
Humulene epoxide I	N/A	93,80,121,107,138	220	1604	1598	<0.01–0.80	0.01–0.41	<0.01–0.11	–
Sesquiterpenoid C ₁₅ H ₂₄ O	–	105,91,93,131,159	220	1608	–	–	<0.01–0.57	–	–
Humulene epoxide II	19888-34-7	109,138,96,67,43	220	1611	1608	0.33–2.86	0.05–1.66	0.55–1.41	≤0.01–0.33
Sesquiterpenoid C ₁₅ H ₂₄ O	–	105,91,93,131,159	220	1615	–	–	<0.01–1.61	<0.01	0.17–0.29

Table 1 (continued)

Compounds	CAS	<i>m/z</i>	M ⁺	RI ^{Exp}	RI ^{Lit}	<i>B. pubescens</i> (<i>n</i> = 11)	<i>B. pendula</i> (<i>n</i> = 11)	<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (<i>n</i> = 5)	<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (<i>n</i> = 7)
Guaia-6,10(14)-diene-4β-ol	N/A	119,159,162,43,91	220	1632	1632	–	<0.01–2.54	–	–
Birkenyl acetate	N/A	105,119,43,147,93	250	1632	1635	<0.01–0.19	–	–	–
Caryophylla-4(12),8(13)-diene-5β-ol	N/A	136,69,41,91,109	220	1639	1641	<0.01–0.56	–	0.23–0.49	0.12–0.30
6-Hydroxy-β-caryophyllene	N/A	69,41,109,81,136	220	1640	1636	1.39–14.47	<0.01–0.03	2.96–5.36	1.33–6.14
Sesquiterpenoid C ₁₅ H ₂₂ O	–	150,107,135,108,218	218	1648	–	0.01–1.67	<0.01–0.67	<0.01–0.25	0.17–2.02
Sesquiterpenoid C ₁₅ H ₂₄ O	–	123,177,81,82,43	220	1650	–	–	<0.01–0.99	–	–
Betulenal	N/A	69,79,91,105,133,121	218	1652	1653	0.01–1.95	–	0.25–0.53	0.29–2.03
Sesquiterpenoid C ₁₅ H ₂₄ O	–	159,91,95,121,81	220	1658	–	–	<0.01–2.59	–	–
Caryophylla-3,8(13)-diene-5α-ol	N/A	91,93,107,41,43	220	1662	1662	0.01–1.11	–	<0.01–0.02	≤0.01
<i>cis</i> -10-Hydroxycalamenene	N/A	157,133,203,92,176	218	1662	1662	–	<0.01–0.46	–	–
Sesquiterpenoid C ₁₅ H ₂₄ O	–	109,69,94,95,81,41	220	1663	–	<0.01–2.17	–	<0.01–0.47	0.21–0.62
14-Hydroxy-β-caryophyllene	N/A	91,69,79,105,93	220	1667	1665	1.08–7.51	<0.01–0.04	0.86–1.61	0.66–2.91
Cadalene	483-78-3	183,198,168,153,184	198	1679	1677	–	<0.01–0.31	–	–
14-Hydroxy-β-isocaryophyllene	N/A	91,41,69,79,105	220	1680	1678	0.12–2.47	–	0.18–0.53	0.19–1.32
Sesquiterpenoid C ₁₅ H ₂₄ O ₂	–	121,177,93,107,205	236	1683	–	<0.01–2.02	–	<0.01–0.24	–
Sesquiterpenoid C ₁₅ H ₂₄ O?	–	91,105,41,79...205	–	1686	–	<0.01–0.35	–	–	–
Cadina-3,10(15)-dien-5β-ol	N/A	159,91,109,105,220	220	1690	1685	–	<0.01–0.11	<0.1–0.33	–
3β-Hydroxymurola-4,9-diene	N/A	159,177,160,131,220	220	1705	1701	–	≤0.01	–	<0.01–0.26
Caryophylla-3,8(13)-dien-5α-ol acetate	32214-91-8	131,105,43,187,91	–	1719	1720	<0.01–1.05	–	–	–
Sesquiterpenol C ₁₅ H ₂₄ O acetate	–	69,109,41,82,79	262	1739	–	0.02–1.61	–	–	0.01–0.47
Caryophylla-4(12),8(13)-dien-5β-ol acetate	N/A	131, 43,187,91,105	–	1744	1739	0.02–0.67	–	<0.01–0.12	<0.01–0.18-
6-Hydroxy-β-caryophyllene acetate	N/A	133,43,131,69,91	262	1758	1758	0.37–5.01	–	0.33–0.56	0.48–1.43
3-Hydroxy-β-caryophyllene acetate	N/A	159,131,43,91,105	262	1764	1762	0.07–2.01	–	<0.01–0.10	0.01–0.65
14-Hydroxy-α-humulene acetate	N/A	43,80,134,119,133	–	1773	1771	0.01–3.08	–	0.11–0.21	<0.01–0.48
14-Hydroxy-β-caryophyllene acetate	N/A	91,43,131,105,159	262	1787	1787	2.38–24.24	–	1.84–3.04	4.14–15.81
14-Hydroxy-4,5-epoxycaryophyllene acetate	N/A	43,93,91,79,41	–	1925	1925	<0.01–0.24	–	–	0.16–0.43
C ₁₃ H ₂₂ and C ₁₃ H ₂₀ norisoprenoids									
Bicyclo[5.2.0]nonane, 4,8,8-trimethyl-2-methylene, C ₁₃ H ₂₂ ?	N/A	93,107,122,79,163	178	1216	–	0.02–4.22	–	<0.01–0.34	0.29–0.75

Table 1 (continued)

Compounds	CAS	<i>m/z</i>	M ⁺	RI ^{Exp}	RI ^{Lit}	<i>B. pubescens</i> (<i>n</i> = 11)	<i>B. pendula</i> (<i>n</i> = 11)	<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (<i>n</i> = 5)	<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (<i>n</i> = 7)
Megastigma-7(E),9,13-triene, C ₁₃ H ₂₀ ?	81983-67-7	105,91,120,176,161	176	1211	–	0.51–3.02	–	0.15–0.92	0.92–2.58
Megastigma-4,6(Z),8(Z)-triene, C ₁₃ H ₂₀	71186-25-9	105,120,91,41,79	176	1248	1251	< 0.01–0.13	–	< 0.01–0.09	≤ 0.01
Megastigma-4,6(Z),8(E)-triene?	51468-85-0	105,91,133, 176,161	176	1276	–	0.01–0.12	–	–	–
Megastigma-4,6(E),8(E)-triene, C ₁₃ H ₂₀	51468-86-1	105,161, 41,176,79	176	1362	1360	< 0.01–0.41	–	0.03–0.19	0.01–2.41
C ₁₃ H ₂₀	–	105,91,120,133,161	176	1461	–	0.24–1.82	–	–	–
Aliphatic carbonyl compounds									
Acetaldehyde	75-07-0	29,44,43,42	44	< 500	–	< 0.01–0.03	< 0.01–0.10	–	–
Acetone	67-64-1	43,58,42	58	500	500	0.02–0.52	0.01–2.40	0.10–0.86	0.30–0.97
2-Methylpropanal (isobutanal)	87-64-2	43,41,39,42,57	–	550	552	< 0.01–0.32	< 0.01–2.06	< 0.01	0.10–0.32
Methyl acroliene	78-85-3	41,70,39,42,38	70	564	566	< 0.01–0.27	< 0.01–0.44	< 0.01–0.56	0.10–0.27
2-Butanone	78-93-3	43,72,57	72	606	605	< 0.01–0.03	< 0.01–0.20	–	–
2-Butenal	4170-30-3	41,70,39,69,42	70	632	632	< 0.01–0.10	< 0.01–0.32	< 0.01–0.46	≤ 0.01–
3-Methylbutanal	590-86-3	44,43,41,58,71	86	648	649	< 0.01–1.34	< 0.01–3.18	–	0.53–1.34
2-Methylbutanal	96-17-3	57,41,29,58,39	86	658	658	< 0.01–1.15	< 0.01–4.20	0.71–1.11	0.31–1.06
3-Pentanone	96-22-0	57,29,86,27	86	688	688	< 0.01–0.02	< 0.01	–	–
Pentanal	110-62-3	44,58,57,41,71	86	695	696	< 0.01–0.03	< 0.01–1.14	< 0.01–0.06	< 0.01–0.03
2-Pentanone	107-87-9	57,29,86,27	86	698	700	< 0.01–0.23	< 0.01	–	–
3-Methyl-2-butenal (prenal)	107-86-8	84,83,55,41,39	84	778	783	< 0.01–0.11	< 0.01–0.04	< 0.01–0.09	< 0.01–
Hexanal	66-25-1	44,56,41,43,57	–	802	801	0.02–1.14	0.02–5.19	0.59–1.00	0.27–1.38
(Z)-3-Hexenal	6789-80-6	41,39,55,69,83	98	805	807	0.05–0.50	< 0.01–0.83	0.03–0.61	0.08–0.39
(Z)-2-Hexenal	–	41,55,69,39,83	98	844	841	0.01–0.45	< 0.01–0.40	–	–
(E)-2-Hexenal	6728-26-2	41,55,39,69,83	98	854	854	< 0.01–0.35	< 0.01–0.54	–	–
2-Heptanone	110-43-0	43,58,71,27,114	114	984	892	–	< 0.01–0.15	–	–
6-Methyl-5-henten-2-one	110-93-0	43,41,108,69,111	126	987	986	< 0.01–0.47	< 0.01–2.41	≤ 0.01	≤ 0.01
Heptanal	111-71-7	44,70,43,41,55	–	902	902	< 0.01–0.07	< 0.01–0.22	< 0.01–0.03	< 0.01–0.04
(Z)-2-Heptenal	57266-86-1	41,55,83,57,56	112	956	953	< 0.01	< 0.01–0.22	–	< 0.01
Octanal	124-13-0	43,44,41,56,84	–	1004	1002	< 0.01	< 0.01–0.56	–	–
(E,E)-2-Heptadienal	4313-03-5	81,110,41,53,67	110	1014	1012	< 0.01	< 0.01	–	–
(E)-2-Octenal	2548-87-0	41,55,70,83,57	–	1060	1060	< 0.01	0.01–1.20	–	–
Nonanal	124-19-6	57,43,41,56,44	–	1102	1104	< 0.01–0.21	0.02–2.02	–	–
6-Methyl-3,5-heptadiene-2-one	1604-28-0	109,81,57,79,39	124	1105	1107	0.03–0.35	< 0.01	–	< 0.01
(E)-2-Nonenal	18829-56-5	43,55,70,41,83	–	1161	1161	< 0.01–0.02	< 0.01–0.22	–	–
Decanal	112-31-2	43,41,55,57,29	156	1209	1205	< 0.01–0.06	< 0.01–0.39	–	–
Aliphatic alcohols									
Methanol	67-56-1	31,32,29	32	< 500	–	0.01–0.33	0.02–1.20	0.14–0.31	0.20–0.34
Ethanol	64-17-5	31,45,46,29,27	46	< 500	–	0.13–4.97	0.05–11.04	0.61–1.54	0.51–4.34
Isobutanol	78-83-1	43,41,42,33,31	74	622	617	< 0.01–0.17	< 0.01–0.02	–	–
1-Penten-3-ol	616-25-1	57,29,43	86	683	688	< 0.01–0.02	< 0.01–0.48	< 0.01–0.14	0.01–0.19
3-Methylbutanol (isopentanol)	123-51-3	55,43,42,41,70	–	729	726	< 0.01–0.39	< 0.01–1.35	< 0.01–0.26	< 0.01
2-Methylbutanol	137-32-6	57,56,41,29,70	–	728	728	–	< 0.01	< 0.01	< 0.01
1-Pentanol	71-41-0	42,55,70,41,31	–	767	767	–	≤ 0.01	–	< 0.01–0.02
(Z)-3-Hexen-1-ol	928-96-1	41,67,82,55,39	–	855	857	0.05–0.69	< 0.01–1.83	0.53–0.88	0.38–0.89
(E)-2-Hexen-1-ol	6728-26-3	57,41,82,67,44	–	864	864	< 0.01–0.25	–	< 0.01–0.70	< 0.01–0.12
1-Hexanol	111-27-3	56,43,41,55,42	–	867	865	< 0.01–1.05	0.01–1.35	0.24–0.67	0.14–1.29

Table 1 (continued)

Compounds	CAS	<i>m/z</i>	M ⁺	RI ^{Exp}	RI ^{Lit}	<i>B. pubescens</i> (<i>n</i> = 11)	<i>B. pendula</i> (<i>n</i> = 11)	<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (<i>n</i> = 5)	<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (<i>n</i> = 7)
1-Penten-3-ol, 4-methyl-	763-89-3	41,69,67,399,70	100	869	868	≤ 0.01	–	–	–
6-Methyl-5-hepten-2-ol	1569-60-4	95,41,45,69,128	128	993	995	< 0.01–0.04	< 0.01–1.11	< 0.01–0.17	–
Aliphatic esters									
Methyl acetate	790-20-9	43,74,42,59,44	74	508	510	< 0.01–0.44	< 0.01–7.80	< 0.01–0.23	0.01–4.46
Ethyl acetate	141-78-6	43,45,61,70,88	88	609	606	0.02–2.21	0.01–2.54	< 0.01–0.36	0.90–2.21
Ethyl 2-(<i>Z</i>)-butenoate	6776-19-8	69,41,99,39,114	114	835	830	–	< 0.01–0.07	–	–
Isopentyl acetate	123-92-2	43,70,55,61,87	–	875	874	< 0.01–0.06	–	–	–
2-Methyl-1-butyl acetate	624-41-9	43,70,55,61,87	–	878	877	< 0.01–0.03	–	–	–
Methyl hexanoate	106-70-7	74,87,43,99,41	–	924	921	< 0.01–0.07	–	–	–
Ethyl hexanoate	123-66-0	88,43,99,60,73	–	1002	1005	< 0.01–0.22	< 0.01–1.10	–	–
(<i>Z</i>)-3-Hexenyl acetate	3681-71-8	43,67,82,41,39	–	1007	1009	< 0.01–0.02	–	–	–
Hexyl acetate	142-92-7	43,56,55,84,61	–	1015	1014	< 0.01–0.04	–	–	–
(<i>Z</i>)-3-Hexenyl butanoate	16491-36-4	82,67,43,71,41	–	1187	1186	< 0.01	< 0.01–0.03	–	–
(<i>E</i>)-2-Hexenyl butanoate	53398-83-7	71,43,55,82,67	170	1195	1193	–	< 0.01–0.37	–	–
(<i>Z</i>)-3-Hexenyl pentanoate	35852-46-1	67,82,43,41,71	–	1237	1238	< 0.01–0.22	< 0.01–0.04	–	–
Hexyl 2-methylbutanoate	10032-15-2	57,103,43,41,85	–	1238	1238	< 0.01–0.04	–	–	–
Hexyl 3-methylbutanoate	10032-13-0	85,103,84,57,43	–	1241	1243	< 0.01–0.80	0.01–0.20	–	–
Hexyl pentanoate	1117-59-5	85,103,56,43,57	–	1245	1247	< 0.01–0.09	< 0.01–0.15	–	–
Ethyl tetradecanoate	124-06-1	88,101,43,41,55	256	1741	1741	–	0.01–0.65	–	–
Ethyl hexadecanoate	628-97-7	88,101,43,41,55	284	1994	1996	–	< 0.01–0.16	–	–
Aliphatic acids									
Acetic acid	64-19-7	43,45,60	60	606	602	0.02–1.98	< 0.01–1.56	0.41–0.57	0.17–0.66
Isovaleric acid	503-74-2	60,41,43,87,45	–	840	838	< 0.01–0.12	–	–	–
Hexanoic acid	142-62-1	60,73,87,41,43	–	990	989	< 0.01–0.09	< 0.01–0.81	–	–
Aromatic compounds									
Toluene	108-88-3	91,92,65,51,63	92	760	760	< 0.01–3.22	≤ 0.01	< 0.01	< 0.01
Ethyl benzene	100-41-4	91,106,65,77,51	106	854	857	–	< 0.01–0.62	–	≤ 0.01
Styrene	100-42-5	104,103,78,51,77	104	878	882	< 0.01–0.09	≤ 0.01	–	≤ 0.01
Benzaldehyde	100-52-7	106,105,77,51,50	106	953	958	0.01–0.84	0.02–2.14	0.39–0.52	0.07–0.64
<i>p</i> -Cymene	99-87-6	119,134,91,120	134	1022	1022	0.02–0.19	< 0.01–2.24	≤ 0.01	< 0.01–0.19
Benzyl alcohol	100-51-6	108,79,107,77,91	108	1033	1033	< 0.01–0.13	0.01–1.53	< 0.01–0.19	≤ 0.01
2-Phenylacetaldehyde	122-78-1	91,92,120,65,39	120	1043	1043	< 0.01–0.30	< 0.01–0.60	0.01–0.04	≤ 0.01
<i>n</i> -Butyl benzene	104-51-8	91,92,134,65,105	134	1055	1053	–	< 0.01–0.40	–	–
2-Phenylethanol	60-12-8	91,120,92,65	120	1112	1112	0.01–0.16	0.02–7.74	–	≤ 0.01
Benzyl acetate	140-11-4	108,91,90,43,79	150	1165	1161	< 0.01–0.04	–	–	–
Methyl salicylate	119-36-8	120,92,152,121,65	152	1198	1199	< 0.01–0.04	< 0.01–1.39	–	–
Phenylethyl acetate	103-45-7	104,43,91,105,65	–	1258	1258	< 0.01–0.02	≤ 0.01	–	–
Ethyl salicylate	118-61-6	120,166,92,65,121	166	1274	1270	< 0.01–0.01	–	–	–
Methyl anthranilate	134-20-3	119,151,92,120,65	151	1345	1344	< 0.01–0.04	–	–	–
Eugenol	97-53-0	164,103,77,149,131	164	1358	1358	0.01–0.89	0.01–4.08	0.18–0.54	≤ 0.01
Hexyl benzoate	6789-88-4	105,123,77,84	206	1575	1576	–	< 0.01–0.19	–	–
Benzyl benzoate	120-51-4	105,91,77,51,65	212	1766	1760	< 0.01–0.09	–	–	–
Aliphatic hydrocarbons									
<i>n</i> -Hexane	110-54-3	57,43,41,29,56	–	600	600	< 0.01–0.16	< 0.01–0.20	< 0.01	< 0.01
(<i>E</i>)-1,3-Pentadiene, 2-methyl-	926-54-5	67,82,41,39,65	82	624	627	< 0.01–0.48	< 0.01	–	–
2,3-Dimethylpentane	565-59-3	56,43,57,41,71	–	666	665	–	< 0.01–0.08	–	–

Table 1 (continued)

Compounds	CAS	<i>m/z</i>	M ⁺	RI ^{Exp}	RI ^{Lit}	<i>B. pubescens</i> (<i>n</i> = 11)	<i>B. pendula</i> (<i>n</i> = 11)	<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (<i>n</i> = 5)	<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (<i>n</i> = 7)
<i>n</i> -Octane	111-65-9	57,71,43,85,41	114	800	800		<0.01–1.05	–	<0.01
2,3-Dimethylbicyclo[2.2.1]heptane?	20558-16-1	81,43,124,109	124	883	–	<0.01–0.03	–	–	–
<i>n</i> -Decane	124-18-5	71,43,85,41,99	142	1000	1000	–	<0.01–0.09	–	–
<i>n</i> -Undecane	1120-21-4	57,71,43,85,41,99	–	1100	1100	<0.01	<0.01–0.1	–	–
(<i>E</i>)-4,8-Dimethyl-1,3,7-nonatriene	N/A	69,41,81,79,150	150	1117	1117	<0.01–0.15	<0.01–1.42	–	≤0.01
<i>n</i> -Tridecane	638-67-5	57,71,43,85,99	324	1300	1300	<0.01–0.01	–	<0.01–0.06	–
<i>n</i> -Pentadecane	629-62-9	57,43,71,85,41	–	1500	1500	<0.01–0.29	≤0.01	0.12–0.26	<0.01–0.05
<i>n</i> -Hexadecane	544-76-3	57,71,43,85,99	226	1600	1600	<0.01–0.16	–	–	–
<i>n</i> -Heptadecane	629-78-7	57,71,43,85,41	–	1700	1700	<0.01–0.08	–	–	–
Other miscellaneous compounds									
Dimethyl sulfide	75-18-3	47,62,61,35	62	520	518	–	<0.01–0.02	–	–
Carbon disulfide, CS ₂	110-54-3	76,78,44,38	76	538	536	–	<0.01–0.46	–	–
2-Methylfuran	534-22-5	82,81,53,39	82	608	604	<0.01–0.22	<0.01–0.19	<0.01	<0.01–0.22
Acetoin	513-86-0	45,43,88,55,42	88	707	709	<0.01–0.44	<0.01–1.84	–	0.01–0.25
2- <i>n</i> -Pentylfuran	3777-69-3	81,82,138,53,41	138	995	996	<0.01–0.72	<0.01–4.80	<0.01–0.09	0.23–1.26
Non-identified compounds									
NN	–	122,107,121,77,79	–	934	–	–	<0.01–0.10	–	–
NN	–	119,91,105,93,175	190	1333	–	<0.01–0.17	–	–	–
NN	–	173,131,188,145,115	–	1428	–	–	<0.01–0.45	–	–
NN	–	152,177,109,43,119	–	1574	–	–	<0.01–0.66	–	–
NN	–	123,163,81,43,191	–	1591	–	–	<0.01–0.88	–	–
NN	–	71,43,111,159,243	–	1599	–	<0.01–0.25	–	–	–
NN	–	123,81,109,43	–	1623	–	–	<0.01–0.85	–	–
NN	–	173,55,41,119,202	–	1636	–	–	<0.01–0.31	–	–
NN	–	91,109,93,41,131	–	1658	–	<0.01–0.32	–	<0.01–0.15	–
NN	–	69,109,41,79,93,82	–	1690	–	<0.01–0.34	–	–	–

of the positively identified sesquiterpenoids in birch bud VOCs is highly contrasted. Figure 3 presents the chemical structures of the main components of volatile emissions from buds of both birch species. It was determined that the sesquiterpenoids of *B. pendula* are structurally diverse; the main components of VOCs are monocyclic germacrene D, bicyclic compounds with cadaline (cadinenes, amorphenes, calcorenes and muurolenes) and guaiazulane (guaia-6,9-diene, α -guaiene, salviadienol) skeletons, as well as tricyclic (α -, β -copaenes, aromadendrenes, β -bourbonene and α -ylangene) compounds. In contrast, principal VOCs in *B. pubescens* emissions belong to bicyclic compounds with nor-caryophyllane (birkenal, birkenol, and des-4-methylcaryophyll-8(14)-en-5-one) and caryophyllane skeletons. Monocyclic germacrene D, as well as tricyclic α - and β -copaenes, β -bourbonene and α -ylangene were also present in VOCs of *B. pubescens*, but their share of the TIC is much smaller. In turn, β -caryophyllene and its 6- and 14-hydroxy derivatives belong to the minor components of *B. pendula* and to the main components of *B. pubescens* emissions.

Among the volatiles of *B. pubescens*, we registered for the first time a C₁₃H₂₂ hydrocarbon tentatively identified (based on the MS data) as 4,8,8-trimethyl-2-methylene bicyclo[5.2.0]nonane (Fig. 3). If we accept the idea that the above-mentioned nor-caryophyllanes, birkenal, birkenol and des-4-methylcaryophyll-8(14)-en-5-one in the buds of *B. pubescens* are the ring-contracted products of β -caryophyllene (Klika et al. 2004), we can speculate that the C₁₃H₂₂ bicyclic hydrocarbon is the product of further ring-contracting.

Another previously undescribed characteristic feature of *B. pubescens* is the emission of C₁₃H₂₀ hydrocarbons, which were identified as megastigmatriene isomers based on mass spectral and chromatographic characteristics. In the volatile emissions of the *B. pubescens* buds, these hydrocarbons are represented by five isomers, the total content of which in VOC was 0.9–3.2%. These hydrocarbons were found for the first time among the volatile components of purple passion fruit (*Passiflora edulis*) juice. The biosynthetic pathway of their formation was postulated to occur successively through β -ionone and β -ionol, which quickly undergoes dehydration

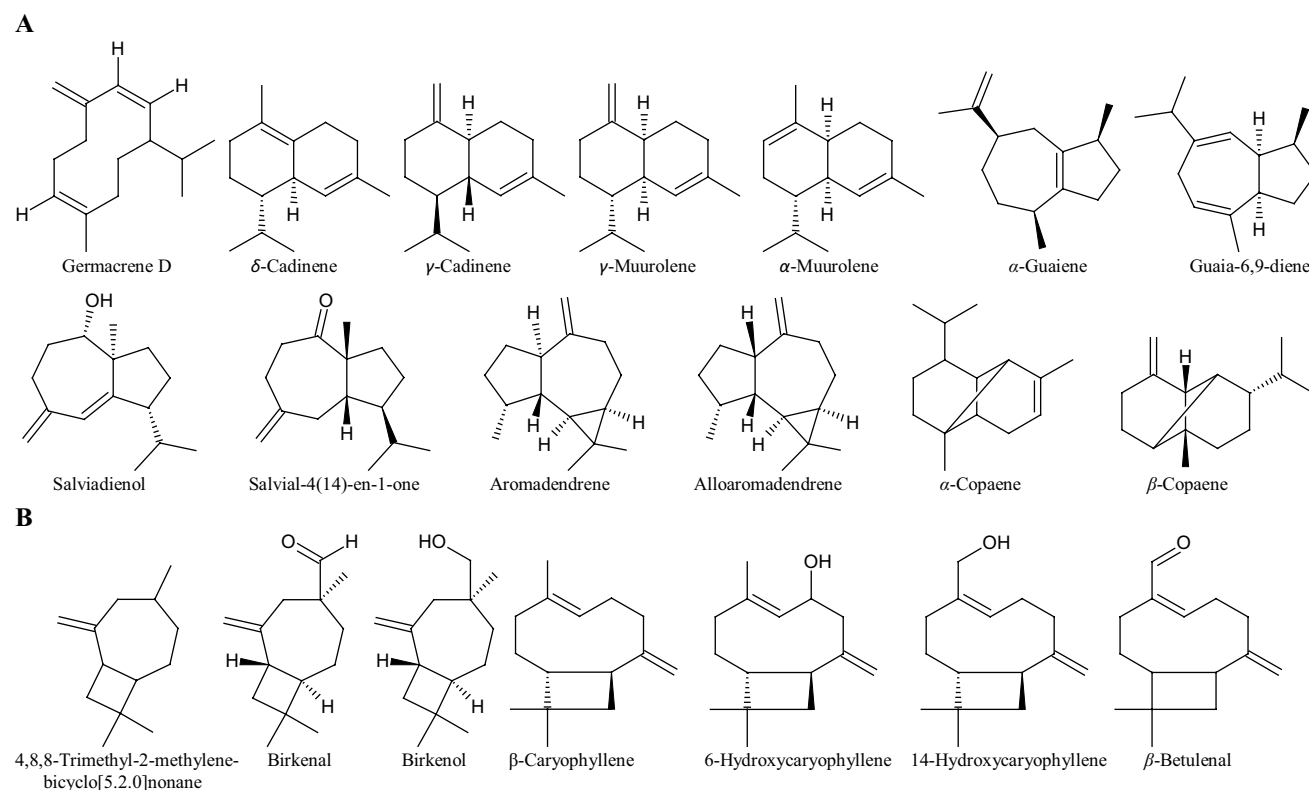


Fig. 3 Sesquiterpenoids and nor-sesquiterpenoids typical for VOCs of *B. pendula* (a) and *B. pubescens* (b) buds

(Camisir et al. 1981; Simkin et al. 2004). However, β -ionol itself was not detected among the volatile components of the downy birch buds (as well as among the VOCs of passion fruits). Therefore, the assumption of their origin, as well as the role of $C_{13}H_{20}$ hydrocarbons, requires further study.

The next comprehensive group of VOCs was formed by carbonyl compounds, which included 27 aliphatic saturated and unsaturated C_2 – C_{10} aldehydes and ketones, as well as two aromatic aldehydes: benzaldehyde and benzene acetaldehyde. The short-chain aldehydes play important roles in the plant defence strategies. Volatile unsaturated aldehydes, as well as some unsaturated alcohols and their esters [e.g. (*Z*)-3-hexenol and (*Z*)-3-hexenyl acetate] originated from unsaturated fatty acids, which have been found in birch buds in significant amounts (Vetchinnikova 2004; 2005; Vedernikov and Roshchin 2009; Isidorov et al. 2018). In their biosynthesis through the oxylipin pathway (Matsui 2006; Stumpe and Feussner 2006), several different enzymes are involved: lipoxygenases (LOX), hydroperoxyde lyase (HPL), isomerase, and alcohol dehydrogenase.

LOX catalyse the regio- and enantio-selective dioxygenation of linoleic and α -linolenic acids. We assumed that only 13-LOX enzymes participate in hydroperoxidation in birch buds and lead to the 13-hydroperoxy derivatives, (*Z*)-3-hexenal and products of its former transformations

as listed in Table 1: (*E*)-2-hexenal, hexanal, and (*E*)-2-hexen-1-ol, (*Z*)-3-hexen-1-ol and (*Z*)-3-hexen-1-ol acetate (Matsui 2006). However, we did not find typical products of 9-LOX hydroperoxidation such as isomeric nonadienals and nonadienols among birch bud VOCs.

It can be assumed that products created by HPL in birch buds are subjected to *Z,E*-isomerisation and reduction to unsaturated alcohols by alcohol dehydrogenases (ADH). Unsaturated and saturated C_6 alcohols are capable of producing a wide range of esters by alcohol acyl transferases (AAT). We observed (*Z*)-3-hexenyl acetate, (*Z*)- and (*E*)-hexenyl butanoates, and (*Z*)-3-hexenyl pentanoate among bud volatiles (Table 1).

Interestingly, the lists of carbonyl compounds and esters identified in the emissions of hybrids were much shorter than those of the parent birch species. For example, out of 27 aldehydes and ketones detected from parent species, only 14 were presented in detectable amounts among the VOCs of hybrids, and out of 17 esters, only two (methyl and ethyl acetate) were registered in emissions of hybrids.

Saturated short chain linear carbonyls, alcohols acids and their esters were detected among VOCs of birch buds. They could be derived from saturated fatty acids through repeated β -oxidative cycles (Schwab et al. 2008). Branch-chain volatile carbonyls and alcohols can also be formed

in the degradation process of branch-chain aliphatic amino acids. In addition, aromatic compounds such as 2-phenylethanol and 2-phenylacetaldehyde can be derived from phenylalanine (Kaminaga et al. 2006).

In this investigation, we established that the chemical composition of VOCs emitted from the buds of silver and downy birches is species-specific regardless of both the geographical origin of the trees (at least within the boundaries of the boreal and mid-latitude zone), as well as the time of bud collection. This difference is evident in the dendrogram in Fig. 4. The left part of the picture (dendrogram A) is clearly divided into two groups; one of which includes VOCs from buds of silver birch (BB-11–BB-36), whereas the other shows the VOCs of downy birch buds (BO-11–BO-35). To construct the dendrograms, we used the data of Table 2, which reflected a generalized composition of the main bud VOCs, i.e., sesquiterpenoids belonging to different groups: compounds with a bicyclic caryophyllane- and norcaryophyllane-type skeleton, with cadalene and muurolane structures (cadinenes and muurolenes) and tricyclic copaenes, as well as $C_{13}H_{20}$ hydrocarbons.

Taxonomic implications of sesquiterpenoid variation in the VOCs of hybrid birch buds

The differences we observed indicate the existence of some connection between the composition of VOCs and the genetic characteristics of the birch trees studied that had different ploidies. Notably, despite the difference in these birches' number of chromosomes, many authors have demonstrated the possibility of interspecies crossings to produce fertile seeds (Natho 1959; Clausen 1963; Gardner 1984; Ermakov 1986; Vetchinnikova 2004, 2005; Vetchinnikova et al. 2013). For example, when the silver birch (♀) was crossed with the downy birch (♂) in Karelia (Russia), germination of seeds was about 27%. Conversely, when the downy birch (♀) was crossed with the silver birch (♂), up to 32% germination was achieved (Ermakov 1975, 1986). Allotetraploidy of downy birch, or the presence of the silver birch genome in the genotype, in all likelihood “facilitates” the hybridization between these species even with different ploidies. At the same time, morphological features of shoots and leaves characteristic of these species (hairy leaves and stems, resin glands on vigorous shoots, toothiness of leaves) are often erased (Ermakov 1986; Vetchinnikova 2004, 2005). Consequently, these features

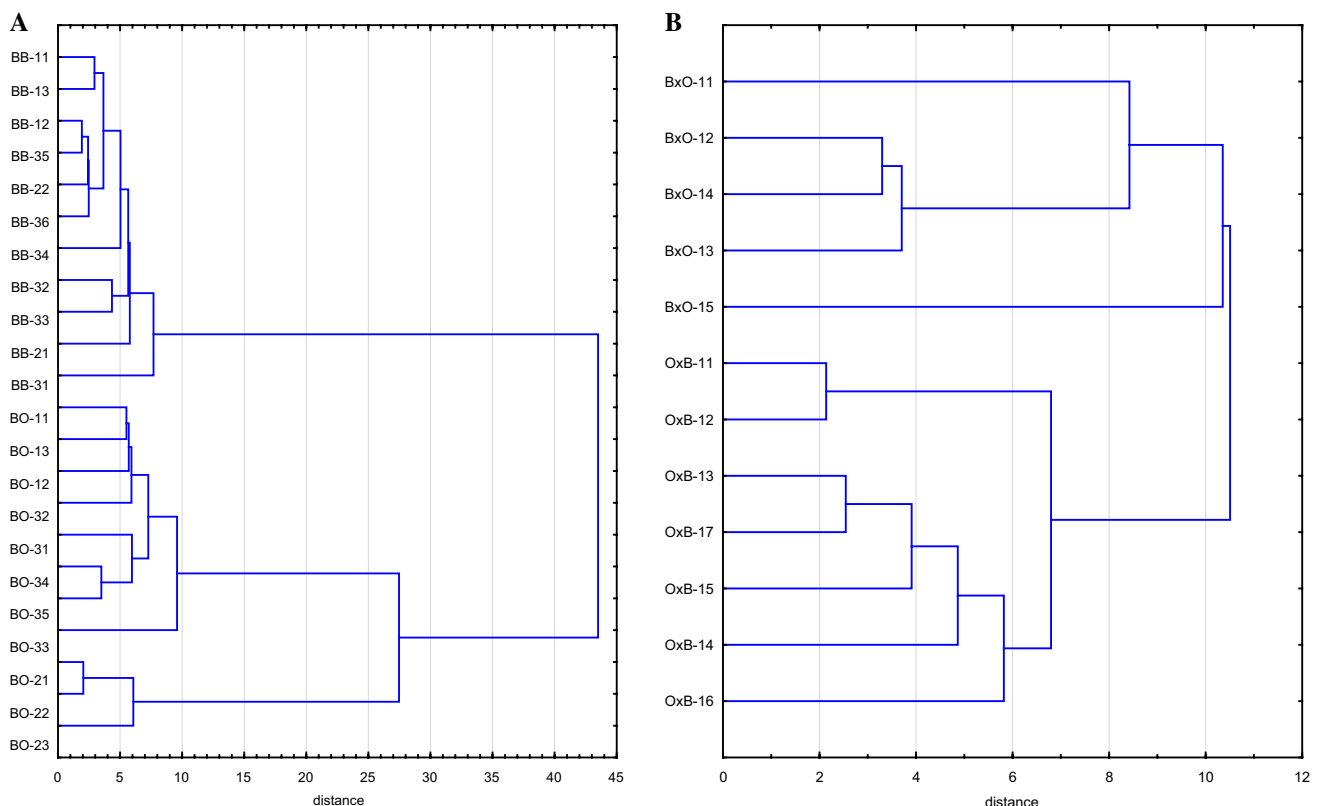


Fig. 4 Dendrograms of the chemical similarity of silver birch (BB) and downy birch (BO) volatiles (a), and hybrids *B. pendula* ♀ × *B. pubescens* ♂ (B × O) and *B. pubescens* ♀ × *B. pendula* ♂ (O × B) (b) (Hammer et al. 2001)

Table 2 Relative group composition (% of TIC) of volatile compounds from buds of two birch species and their hybrids

Species and hybrids	Code (sample designation)	Mono-terpe-nes	Sesquiterpenes					Hydro-carb-ons C ₁₃ H ₂₀	
			Total	Caryophy-llanes	Nor-caryo-phyllane	Cadina-nes	Murol-anes		Copae-nes
<i>Betula pendula</i> (Russia, Petrozavodsk region)	BB-11	1.75	56.80	2.15	–	3.93	3.79	27.2	–
	BB-12	1.05	54.80	2.61	–	6.75	4.72	22.40	–
	BB-13	2.56	65.72	3.46	–	5.56	1.75	27.52	–
<i>Betula pendula</i> (Latvia, Madona region)	BB-21	4.66	74.48	5.65	–	3.61	1.39	20.59	–
	BB-22	3.58	75.75	3.95	–	9.44	4.89	22.14	–
<i>Betula pendula</i> (Poland, Bialystok region)	BB-31	0.88	89.30	7.72	–	18.72	3.40	14.78	–
	BB-32	0.93	91.50	4.47	–	10.23	4.01	16.63	–
	BB-33	0.54	75.81	4.38	–	12.12	3.86	12.72	–
	BB-34	0.81	78.82	3.91	–	10.27	6.10	27.70	–
	BB-35	4.50	56.37	3.48	–	7.76	4.34	23.73	–
	BB-36	6.80	56.70	2.38	–	4.82	5.07	23.90	–
<i>Betula pubescens</i> (Russia, Petrozavodsk region)	BO-11	3.32	87.20	51.69	28.37	–	–	0.71	1.68
	BO-12	3.18	83.56	45.33	31.81	–	–	0.93	1.96
	BO-13	5.32	83.14	46.61	26.26	–	–	1.05	1.81
<i>Betula pubescens</i> (Russia, St.-Petersburg region)	BO-21	0.63	70.85	20.89	48.02	–	–	1.04	3.20
	BO-22	0.47	74.10	20.10	49.00	–	–	1.23	1.51
	BO-23	0.59	81.70	25.83	51.15	–	–	1.15	1.49
<i>Betula pubescens</i> (Latvia, Madona region)	BO-31	2.33	92.89	59.94	18.62	–	–	1.39	1.67
	BO-32	1.79	65.56	54.32	23.16	–	–	1.22	0.85
	BO-33	2.65	72.23	38.68	21.60	–	–	2.13	0.86
<i>Betula pubescens</i> (Poland, Bialystok region)	BO-34	0.51	94.75	68.72	16.02	–	–	0.45	1.89
	BO-35	0.48	90.05	65.79	17.92	–	–	0.55	1.85
<i>B. pendula</i> ♀ × <i>B. pubescens</i> ♂ (Russia, Petrozavodsk region)	BxO-11	0.60	86.5	47.40	17.80	1.08	0.25	7.44	0.34
	BxO-12	0.21	91.8	54.50	11.00	1.11	0.39	7.36	0.20
	BxO-13	0.60	88.13	49.29	9.61	2.80	1.03	6.97	0.23
	BxO-14	0.40	78.90	51.90	11.01	1.30	2.30	8.01	0.29
	BxO-15	1.63	90.60	63.60	15.48	2.73	1.67	7.12	0.25
<i>B. pubescens</i> ♀ × <i>B. pendula</i> ♂ (Russia, Petrozavodsk region)	OxB-11	2.11	86.32	52.69	24.08	–	–	0.89	1.73
	OxB-12	1.87	90.17	54.30	23.00	–	–	1.63	1.21
	OxB-13	2.38	86.90	47.50	31.80	–	–	0.90	2.58
	OxB-14	1.93	88.49	44.89	38.71	–	–	0.87	3.54
	OxB-15	1.40	86.22	48.68	35.76	–	–	0.87	2.76
	OxB-16	0.88	77.03	46.28	26.28	–	–	0.80	1.20
	OxB-17	0.50	82.60	45.78	33.4	–	–	1.50	1.81

cannot reliably be used to detect cases of natural hybridization between different birch species.

A natural question arises: if the composition of the bud VOCs of the parent plants are so different that they can serve a chemotaxonomy purpose, is it possible to distinguish their hybrids on this basis? This question becomes more important in connection with the fact that due to the warming and instability of the climate observed in past decades, the flowering times of both birches increasingly overlap, thus eliminating the phenological isolation typical of these species and thereby fostering hybridization.

The results of the study of hybrid offspring (F1) presented in Table 2 and on the right part of Fig. 4 (dendrogram B), indicate that the buds of hybrid plants have a characteristic composition of volatile substances, which allows a definitive conclusion about the hybrid origin in some cases. As can be seen on dendrogram B, all hybrids investigated are clearly divided into two groups depending upon the type of crossing. From the data of Table 2, it can also be seen that the ability of species to accumulate specific components in the buds to a greater or lesser extent was consistently preserved in the offspring. At the same time, hybrids clearly show dominant

inheritance along the maternal line in both crossbreeding variants: the *B. pendula* ♀ × *B. pubescens* ♂ hybrid that resembled silver birch and the *B. pubescens* ♀ × *B. pendula* ♂ that resembled downy birch.

However, the pollen of tetraploid downy birches substantially affects the composition of the VOCs of the progenies; in volatile secretions from buds of plants, when the pollen donor was downy birch (hybridization variant of B × O), there were significant amounts of nor-caryophyllenes and C₁₃H₂₀ hydrocarbons, which were uncharacteristic for the maternal species, silver birch (Table 2). The mechanism of the phenomenon of gene transfer through pollen described for oaks is called “pollen swamping” (Petit et al. 2003). Another distinctive feature of this hybridization variant is a noticeable decrease in the relative content of components typical for silver birch, e.g., cadinanes, muurolanes, and copanenes. On the other hand, in the second crossing variant (O × B), the influence of the pollen donor (diploid silver birch) on the VOCs composition was nearly undetectable.

Conclusion

The study of the composition of volatile secretions of birch buds reliably reveals only those hybridization variants for which the pollen donor was the plant with the higher ploidy, i.e., downy birch. Identification of the second hybridization variant requires a more detailed study of the composition of secondary metabolites, including non-volatile compounds and those contained in other plant organs and tissues. For these purposes, the molecular cytological mapping of ribosomal genes and species-specific DNA may also be useful (Anamthawat-Jónsson and Thórsson 2003).

Author contribution statement Conceptualization: VI, Sample collection and preparation for analysis: LV, MS, Acquisition of data: VI, MS, Analysis and interpretation of data: VI, LV, Writing—original draft: VI.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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