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Analysis of Leaf Surface Sesquiterpenes in Potato Varieties

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A comparative study of potato leaf sesquiterpenes was carried out. GC, GC-MS, and NMR analyses were used to identify and quantify the sesquiterpenes in the leaf surfaces of 10 potato (*Solanum tuberosum*) varieties. Two sesquiterpene alcohols and 17 sesquiterpene hydrocarbons were identified and quantitatively determined. The distribution of the sesquiterpenes was found to be variety-specific. The sesquiterpene contents of the different potato varieties were subjected to cluster and principal component analyses. The eight potato varieties of the main chemotype cluster were dominated by β-caryophyllene (9−148 ng/cm^2), germacrene D (2−46 ng/cm^2), germacrene D-4-ol (0.4−31 ng/cm^2), β-sesquiphellandrene (1−34 ng/cm^2), and an unknown sesquiterpene alcohol III (0.2−37 ng/cm^2). Chemometric classification distinguished two varieties, Mila and Vistula, from a major cluster. The Vistula variety was distinguished from the others by its high contents of β-caryophyllene, α-humulene, germacrene D, and germacrene D-4-ol and the Mila variety by β-elemene, *trans*-α-bergamotene, (*Z*)-β-farnesene, (*E*)-β-farnesene, *trans*-β-bergamotene, β-sesquiphellandrene, and unknown sesquiterpene alcohols I, II, III.

KEYWORDS: ***Solanum tuberosum*; potato; leaf sesquiterpenes, GC; GC-MS; NMR; cluster and principal component analysis**

INTRODUCTION

The potato (*Solanum tuberosum*) is one of the most commonly cultivated commercial plants in the temperate zone. It is an important crop in many countries, including Poland. However, viruses, mycoplasma, bacteria, fungi, insects, and slugs can significantly deplete the crop. The ability of the potato plant to resist pathogens and herbivores depends on the physical and chemical barriers it can present. Potato plants accumulate a wide variety of secondary plant metabolites, including glycoalkaloids, terpenes, sesquiterpenes, phenolic compounds, phytoalexins, protease inhibitors, and stress proteins as protection against the adverse effects of bruising and injury by phytopathogens. Both primary and secondary plant metabolites play a role in host finding and acceptance by herbivores. The structure and chemical characteristics of the leaf surface are also important.

Defense responses in plants against insects are generally triggered by volatiles (*1*−*3*). Sesquiterpenes act as semiochemicals, providing built-in protection against invading organisms. It therefore seems reasonable to search among this group of substances for components capable of controlling insect infestation. In both physiological and pathological states, potato plants emit a spectrum of volatile compounds, including sesquiterpenes. Some of the emitted sesquiterpenes have been identified (*4*−*6*). Sophisticated tools have recently been applied to plant−insect interaction studies at the molecular level. Gas chromatography−electroantennogram experiments (GC-EAG) show that the Colorado potato beetle is capable of detecting not only green leaf volatiles but also the sesquiterpene fraction (*7*).

The composition of potato leaf sesquiterpenes has already been the subject of several studies (*8*, *9*), but not all of the sesquiterpenes have yet been identified. Furthermore, these studies focused only on the qualitative compositions of the leaf sesquiterpene fractions. The aim of the present work was to study the qualitative and quantitative compositions of leaf surface sesquiterpenes in 10 potato varieties. It was undertaken to facilitate further studies of potato−insect interactions. The quantitative results were suitable for application in chemometric analysis. We obtained information about the chemical variability of the potato cultivars. It may be possible for the structures and compositions of sesquiterpenes in potato plants to be used for the chemotaxonomy of commercial varieties, but this potential application has yet to be tested.

MATERIALS AND METHODS

Potato Plants. The potato varieties used in the studies were Aster, Drop, Lotos, Sumak, Mila, Bryza, Arkadia, Vistula, Wolfram, and Wawrzyn. The potato plants were grown on plots belonging to the Plant Breeding and Acclimatization Institute at Bonin (latitude 54° 09′ N, longitude 16° 15′ E). The potato leaves for chemical analyses were harvested 60 days after planting.

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Table 1. Kovats Retention Indices on Liquid Phases

		RTX-5		Carbowax		EC-1[b]		EC-1[c]	
peak[a]	compound	exptl	ref	exptl	ref	exptl	ref	exptl	ref
1	α-cubebene	1349	1351[d]	1480	1481[e]	1351		1361	1362[e]
2	α-copaene	1375	1376[d]	1519		1378	1378[e]	1390	
3	β-cubebene	1390	1390[d]	1558	1560[e]	1389		1400	1400[e]
4	β-elemene	1392	1391[d]	1606	1608[e]	1389		1400	1400[e]
5	α-gurjunene	1409	1409[d]			1410	1413[e]	1423	
6	β-caryophyllene	1418	1418[d,f]	1617	1618,[e] 1617[f]	1417	1417,[e] 1418[f]	1432	1436[e]
7	*trans*-α-bergamotene	1435	1436[d]			1431			
8	(*Z*)-β-farnesene	1442	1443,[d] 1442[f]	1650	1650[f]	1434	1433[f]	1442	
9	α-humulene		1454[d]	1680	1680[f]	1446	1447,[e] 1446[f]	1449	
10	(*E*)-β-farnesene	1457	1458,[d] 1457[f]	1663	1671,[e] 1664[f]	1446	1446[f]	1449	1426[e]
11	germacrene D	1479	1480,[d] 1479[f]	1715	1718,[e] 1714[f]	1471	1471[f]	1485	1488[e]
12	*trans*-β-bergamotene	1484	1486[g]	1688		1475		1485	
13	bicyclogermacrene	1493	1494,[d] 1493[f]	1736	1738,[e] 1736[f]	1475			
14	germacrene A	1503	1503[d]			1484			
15	β-bisabolene	1509	1509,[d] 1507[f]	1726	1726[f]	1500	1496,[e] 1500[f]		
16	δ-cadinene	1523	1524,[d] 1522[f]	1761	1761[f]	1505	1504,[e] 1508[f]		
17	β-sesquiphellandrene	1523	1524,[d] 1522[f]	1764	1764[f]	1509	1509[f]	1517	
18	germacrene D-4-ol	1573	1574,[d] 1575[f]			1553		1566	
19	sesquiterpene I	1600				1576		1592	
20	α-cadinol	1653	1654[d]			1627		1635	
21	sesquiterpene II	1685				1646		1661	
22	sesquiterpene III	1688				1653		1669	

[a] Peak numbers correspond to those in **Figure 2**. [b] Isothermal indices 130 °C. [c] Isothermal indices 150 °C. [d] Reference data (*10*). [e] Reference data (*15*). [f] Reference data (*9*). [g] Reference data (*5*).

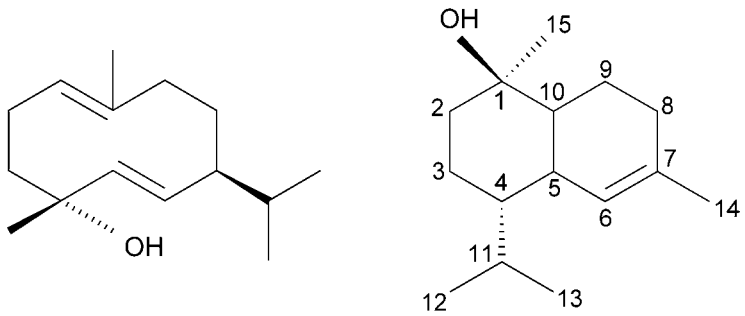

Figure 1. Sesquiterpene alcohols identified in potato leaves.

Sesquiterpene Extraction. Around 500 g of leaves from every potato variety was weighed fresh to an accuracy of 0.01 g and then extracted by being dipped and shaken for 10 s in methylene chloride. By photocopying the leaves, cutting the paper copies out and weighing them, the total surface areas of the leaves could be estimated. The extracts were fortified with an internal standard (*n*-tetradecane) for quantitative studies, dried with anhydrous sodium sulfate, and filtered. The extracts were concentrated at atmospheric pressure to a volume of ~1 mL using a 10 cm distillation column. The sesquiterpene yields for each potato variety were measured in nanograms per unit area (ng/cm^2).

Column Chromatography. Potato leaves (3 kg) of the Mila variety were steam-distilled for 1 h. The distillate was extracted three times with methylene chloride (70 mL) and the organic fraction dried with anhydrous sodium sulfate. The solution was concentrated at atmospheric pressure with a 10 cm distillation column. The concentrated sesquiterpene extract was separated on a 24 × 1 cm silica gel column. The MN-Kieselgel 60, 70−270 mesh silica gel was previously rinsed with methanol, acetone, and hexane, then dried at 160 °C for 8 h, and deactivated by the addition of 15% distilled water. The separation was carried out at a temperature of 10 °C. The three fractions collected were sequentially eluted with 50 mL of pentane (sesquiterpene hydrocarbons), 45 mL of methylene chloride (a mixture of sesquiterpene alcohols), and 12 mL of methylene chloride (single sesquiterpene alcohol). The fractions were analyzed by GC and GC-MS. The preparative procedure for separating alcohols was repeated three times. Fraction 3 was concentrated using a 10 cm distillation column, and the solvent was evaporated under a gentle stream of nitrogen. The fraction (~3 mg) was subjected to NMR analysis.

Gas Chromatography. The analyses were carried out on a GC 8000 TOP (CE Instruments) gas chromatograph equipped with a capillary column with a split ratio of 1:30 for the injection port and a direct connection to a FID. Argon was used as carrier gas at a pressure of 90 kPa. The temperatures of the injector and the detector were 220 °C. The 30 m × 0.25 mm i.d., film thickness = 0.25 μm, columns used were RTX-5 (Restek), EC-1 (Alltech), and Carbowax (Alltech). Gas chromatograms and retention times were obtained using Chrom-Card. Kovats retention indices were determined on the Carbowax column at a temperature of 130 °C and on the EC-1 column at temperatures of 130 and 150 °C. Kovats retention indices were obtained from temperature-programmed runs under the conditions (initial and program temperatures, linear gas flow) for which the distances between the sequential *n*-alkanes were the same. The oven temperature was programmed to rise from 60 to 220 °C at a rate of 3 °C/min for the determination of Kovats retention indices on the RTX-5 column. The oven temperature was programmed to rise from 40 to 200 °C at a rate of 4 °C/min for the quantitative analysis on the EC-1 and Carbowax columns. Chromatographic analysis was performed in five replicates.

Table 2. Mass Spectra of α-Cadinol and Tentatively Identified Sesquiterpenes

peak[a]	MW EI/MS	EI mass spectra *m/z* (rel intensity %)	suggested structure
19	222	41 (79), 43 (100), 55 (60), 69 (72), 81 (65), 93 (59), 95 (55), 105 (60), 107 (57), 109 (90), 121 (60), 122 (67), 133 (33), 147 (42), 161 (62), 175 (9), 189 (30), 204 (32), 222 (8)	$C_{15}H_{26}O$
20	222	41 (47), 43 (92), 55 (31), 71 (36), 79 (47), 95 (100), 105 (45), 121 (72), 137 (18),149 (9), 161 (68), 164 (27), 179 (3), 189 (11), 204 (29), 205 (22), 222 (4)	α-cadinol
21	222	41 (55), 43 (100), 55 (35), 69 (62), 71 (27), 79 (26), 93 (35), 95 (32), 109 (55),119 (42), 121 (22), 134 (5), 147 (4), 161 (8), 189 (4), 204 (12), 222 (1)	$C_{15}H_{26}O$
22	222	41 (67), 55 (54), 69 (32), 81 (68), 84 (100), 93 (40), 105 (50), 109 (45), 119 (41), 121(39), 123 (15), 134 (15), 137 (15), 147 (8), 161 (77), 179 (5), 189 (6), 204 (15), 222 (3)	$C_{15}H_{26}O$

[a] Peak numbers correspond to those in **Figure 2**.

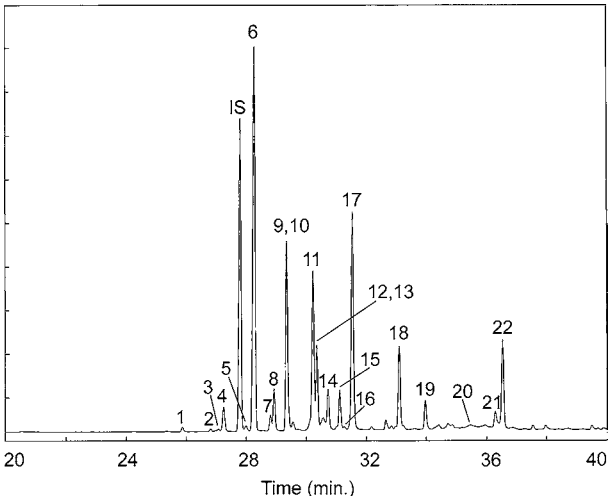

Figure 2. Gas chromatogram of potato leaf sesquiterpenes of the Wolfram variety obtained on the EC-1 capillary column. Labeling is as in **Table 1**.

Identification of some sesquiterpenes was confirmed by co-injections with standards or natural substances on the Carbowax column, as reported previously (*9*). The standard sesquiterpenes used were *β*-caryophyllene and α-humullene (Sigma). Quantitative sesquiterpene contents were determined by comparison of GC peak areas to the peak area of the internal standard (*n*-tetradecane). The relative standard deviation of the quantitative results was <5%. The recovery rate was 95%.

Mass Spectrometry. Mass spectra (70 eV) were recorded on a TRIO-2000 quadrupole mass spectrometer. The samples were introduced through a Hewlett-Packard 5890 gas chromatograph equipped with the same columns and under the same chromatographic conditions as for the GC analysis. The carrier gas was helium. The ion source was maintained at 220 °C. The spectra recorded were compared with those in the literature (*10, 11*).

Nuclear Magnetic Resonance Spectra. NMR spectra were recorded for 3 mg of sesquiterpene in 0.7 mL of deuterium chloroform on a Varian Mercury 400 MHz spectrometer. TMS was used as an internal standard. Proton ^{1}H spectra were recorded at 295 K with a 5998.8 Hz spectral width. Proton-decoupled ^{13}C spectra, 100 MHz, were recorded with a pulse of 72.9° and a 25000 Hz width for 3000 repetitions. Proton homonuclear-correlated two-dimensional spectra were performed for 256 (gCOSY) and 2 × 256 (TOCSY) increments with an accumulation of 32 scans for each step over a 3229.6 Hz sweep width. The NOESY spectrum was measured with a 3186.4 Hz sweep width in both dimensions for 32 repetitions and 2 × 256 increments. The mixing time was 0.3 s. For the 2D ^{1}H−^{13}C heteronuclear shift correlation (coupled and decoupled) 32 scans in 2 × 128 increments over 3229.6 Hz (^{1}H) and 16112.8 Hz (^{13}C) sweeps were collected. For the 2D ^{1}H−^{13}C long-range heteronuclear shift correlation (gHMBC), 400 experiments were performed with an accumulation of 64 repetitions for 2467.1 Hz (^{1}H) and 24169.2 Hz (^{13}C) sweep widths.

Chemometric Analysis. The quantitative sesquiterpene compositions, expressed in ng/cm^2 of leaf surfaces, were arranged in a matrix and analyzed by chemometric methods after autoscaling for equal significance of variances (*12*). Cluster analysis was used to find similar varieties based on their closeness in the multidimensional space spanned by the variables (*13*). The measure of similarity used here was the squared Euclidean distance. The potato varieties were also classified according to principal component analysis (PCA) (*14*).

Table 3. ^{13}C and ^{1}H NMR Chemical Shifts of α-Cadinol

carbon no.[a]	δ ^{13}C[b]	$^1J_{C-H}$ (Hz)	δ ^{1}H	$^3J_{H,H}$ (Hz)	carbon group
C-1	72.661				C
C-2	42.402	128	H_e, 1.8	ddd; 12.5, 3.5, 3.5	CH_2
			H_a, 1.4	ddd; 12.5, 12.5, 3.5	
C-3	22.150	120	H_e, 1.6	dddd; 12.5, 3.5, 3.5, 3.5	CH_2
			H_a, 1.1	m	
C-4	46.895	126	H_a, 1.04	br m	CH
C-5	40.061	128	H_a, 1.7	m	CH
C-6	122.529	160	5.5	br s	CH
C-7	135.211				C
C-8	31.136	128	1.99		CH_2
C-9	22.875	128	H_e, 1.98	br m	CH_2
			H_a, 1.22	m	
C-10	50.213	120	H_a, 1.2	m	CH
C-11	26.181	120	2.16	dqq; 7, 7, 3.5	CH
C-12	15.332	128	0.7	d; 6.8	CH_3
C-13	21.741	126	0.9	d; 6.8	CH_3
C-14	24.075	128	1.67	br s	CH_3
C-15	20.991	128	1.107	s	CH_3

[a] Carbon numbers correspond to those in **Figure 1**. [b] Chemical shifts relative to internal standard $CDCl_3$ (77.230 ppm).

Table 4. Carbon–Carbon Connectivity Assigned from the gHMBC Experiment

H–C		C
H (0.7 ppm)–C-12	→	C-4 (46.895 ppm), C-11 (26.181 ppm), C-13 (21.741 ppm)
H (0.9 ppm)–C-13	→	C-4 (46.895 ppm), C-11 (26.181 ppm), C-12 (15.332 ppm)
H (1.1 ppm)–C-15	→	C-1 (72.661 ppm), C-2 (42.402 ppm), C-10 (50.213 ppm)
H (1.67 ppm)–C-14	→	C-6 (122.529 ppm), C-7 (135.211 ppm), C-8 (31.136 ppm)

RESULTS AND DISCUSSION

Structural Studies. The compositions of sesquiterpene hydrocarbons and alcohols in 10 Polish varieties of *S. tuberosum* are reported here. The structures of the sesquiterpenes in the potato foliage extracts were identified on the basis of mass spectra matched with literature data (*10, 11*) and from comparisons of retention indices on three different capillary columns (polar and nonpolar), which were determined relative to the retention time of *n*-alkane series with linear interpolation for

Table 5. Sesquiterpene Contents[a] of Leaves from Polish Varieties of *S. tuberosum*

compound	Aster	Drop	Lotos	Sumak	Mila	Bryza	Arkadia	Vistula	Wolfram	Wawrzyn
α-cubebene	0.4	1.0	0.1	0.8	3.2	0.6	0.8	3.6	0.8	0.4
α-copaene	0.2	0.4	0.1	0.6	1.8	0.4	0.6	2.6	0.4	0.4
β-cubebene	0.2	1.0	0.1	0.4	2.0	0.8	0.6	1.4	0.4	0.4
β-elemene	1.0	3.8	1.2	4.6	23.6	5.2	3.0	12.0	3.8	3.4
α-gurjunene	0.1	1.6	1.4	1.2	4.6	1.2	3.6	0.6	1.0	2.4
β-caryophyllene	9.4	43.6	23.6	38.4	57.2	31.8	148.0	264.0	39.0	39.2
trans-α-bergamotene	0.1	1.0	1.2	1.6	7.0	1.2	2.6	2.8	2.2	2.6
(*Z*)-β-farnesene	0.2	2.6	1.4	4.6	15.2	3.8	6.8	8.2	5.9	5.4
α-humulene	0.1	1.6	1.0	1.6	4.6	5.6	5.0	41.2	2.4	1.4
(*E*)-β-farnesene	0.3	1.0	7.2	1.4	53.4	6.4	2.4	2.8	25.0	18.2
germacrene D	10.6	37.4	2.2	39.2	112.4	24.4	45.6	187.4	24.4	21.0
trans-β-bergamotene	1.4	7.0	6.0	9.2	32.6	7.4	16.0	3.2	11.8	15.2
bicyclogermacrene	tr[b]	0.4	0.4	0.8	1.8	0.8	0.8	4.6	0.4	0.6
germacrene A	0.6	4.0	1.8	6.4	6.6	3.4	12.2	18.4	6.0	5.0
β-bisabolene	0.1	0.1	2.8	0.4	4.0	0.4	0.2	0.4	5.8	0.6
δ-cadinene	tr	tr	tr	tr	tr	0.8	tr	tr	tr	tr
β-sesquiphellandrene	0.8	10.4	10.8	17.8	61.2	14.6	27.8	30.0	34.2	18.8
germacrene D-4-ol	0.4	16.6	9.4	13.8	81.2	28.0	30.8	103.6	13.0	18.6
sesquiterpene I	0.6	6.6	4.0	4.0	35.4	6.2	10.3	7.2	4.4	7.2
α-cadinol	tr	tr	tr	tr	2.0	0.8	tr	1.4	tr	tr
sesquiterpene II	1.2	3.2	0.6	3.6	32.0	2.2	4.2	16.6	3.0	1.6
seesquiterpene III	0.2	19.0	11.4	13.6	93.6	16.6	37.4	6.2	13.6	24.2
hydrocarbons	25.5	116.9	61.2	129.0	391.2	108.8	276.0	583.2	163.5	135.0
sesquiterpenoids	2.4	45.4	25.4	35.0	244.2	53.8	82.7	135.0	34.0	51.6
total	27.9	162.3	86.6	164.0	635.4	162.6	358.7	718.2	197.5	186.6

[a] Sesquiterpene contents (ng/cm^2) were calculated from the peak areas determined on the Carbowax and EC-1 columns; FID response factors were not determined. [b] Trace.

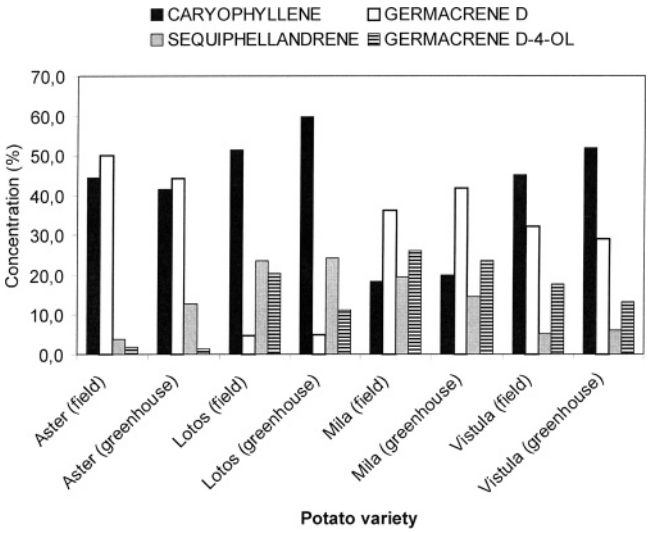

Figure 3. Comparison of sesquiterpene contents of potato leaves grown in different conditions. The content of the four main potato sesquiterpenes (β-caryophyllene, germacrene D, β-sesquiphellandrene, germacrene D-4-ol) is expressed as 100%.

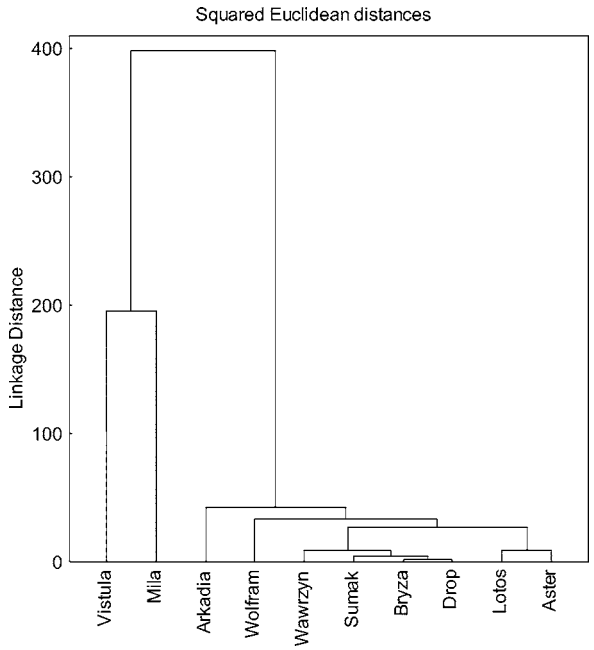

Figure 4. Cluster analysis of potato varieties based on sesquiterpene compositions.

the temperature program or the logarithmic calculation for isothermal runs and co-injections. The experimental and reference data of the gas chromatographic analyses of the components are given in **Table 1**. The chemical structures of sesquiterpene alcohols are shown in **Figure 1**. No GC analysis on a single column can separate all of the potato leaf sesquiterpenes. Thus, for unequivocal GC identification of these compounds, analyses on three separate capillary columns have to be performed. α-Humulene and (*E*)-β-farnesene overlapped on the EC-1 capillary column (**Table 1**), whereas β-sesquiphellandrene and δ-cadinene were not separated on the RTX-5 column. The best separation of α-humulene and (*E*)-β-farnesene was achieved on the Carbowax column. Sesquiterpene alcohols were analyzed on the nonpolar EC-1 and RTX-5 columns. A typical GC analysis of potato sesquiterpenes on the EC-1 column is presented in **Figure 2**. The gas chromatogram obtained on the Carbowax column was reported in our previous study (*9*).

One sesquiterpene alcohol (GC peak 20) displayed a mass spectrum similar to that of cadinol (**Table 2**). Thus, for unequivocal structure assignment, the NMR spectrum had to be recorded. Therefore, a preparative procedure for separating alcohols was used, which consisted of water vapor distillation of the leaves followed by column liquid chromatography separations of the organic fraction. Fortunately, the sesquiterpene alcohol was easily separated in sufficient quantity and quality so that the NMR spectra could be obtained for structural

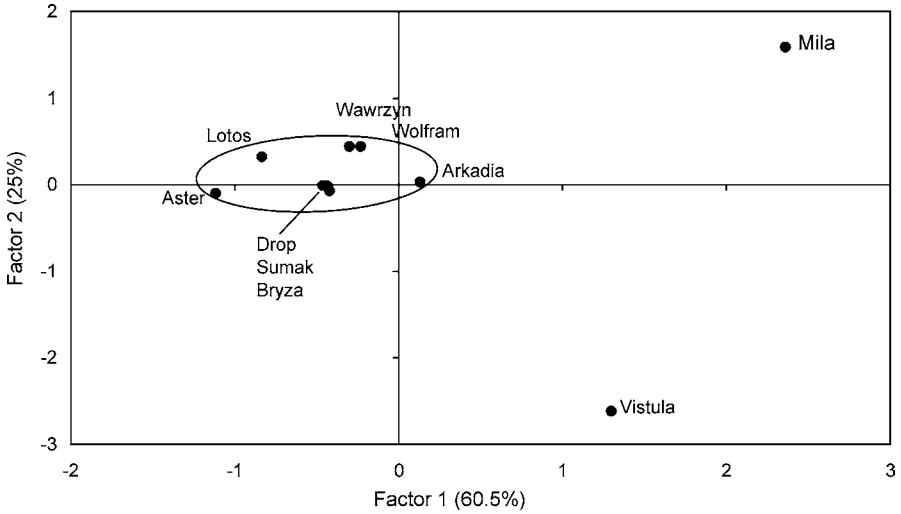

Figure 5. Classification of potato varieties in factors 1 and 2.

assignment. On the basis of proton chemical shifts, multiplicities, as well as HSQC and HMBC correlations, the α-cadinol structure was assigned to this component. The results are presented in **Table 3** (^{1}H and ^{13}C NMR spectra) and **Table 4** (proton and carbon linkages). The NMR spectra were compared with those in the literature (*16*, *17*).

As yet it has not been possible to identify the structures of the sesquiterpenes labeled I, II, and III (**Table 1**), but the mass spectra (**Table 2**) of compounds I, II, and III clearly revealed a group structure allowing them to be classified as sesquiterpene alcohols. Comparison of the mass spectra and retention indices with the literature data (*10*) was of no help with regard to assigning the full structures. These compounds were found in our earlier studies with different potato varieties. Their structures will be assigned later.

Of the 22 compounds listed in **Table 1**, 19 sesquiterpenes (17 hydrocarbons and 2 sesquiterpene alcohols) were identified, more than in our previous studies (*9*). The identity of the major components conformed well with the observations of Moede (*8*), our previous results (*9*), and those found in headspace analyses of potato plants (*5*). The structures of the identified sesquiterpene alcohols are presented in **Figure 1**. The compositions of sesquiterpenes from six potato varieties are reported for the first time.

The quantitative results of the potato sesquiterpene analyses are presented in **Table 5**. During the development of the analytical procedures, special care was taken to find the conditions permitting quantitative analyses of the mixtures without any loss of volatile compounds such as sesquiterpenes. Potato varieties vary in their sesquiterpene composition (**Table 5**). The main components were found to be sesquiterpene hydrocarbons such as β-caryophyllene, germacrene D, and β-sesquiphellandrene. These were present in almost all samples at appreciable levels. Among the alcohols, germacrene D-4-ol and the tentatively identified sesquiterpene alcohol III were present in significant amounts. A few compounds were always present in small quantities: α-cubebene, α-copaene, β-cubebene, δ-cadinene, and α-cadinol. The yield of the potato sesquiterpenes varied from 28 ng/cm^2 (Aster variety) to 718 ng/cm^2 (Vistula variety). The hydrocarbon fraction was a few times larger than the oxygenated one.

Generally speaking, the differences in sesquiterpene compositions can be attributed to two major factors: genetic factors and environmental factors such as soil, agricultural practices, weather, and length of daylight (*1*). Here, we carried out a real comparative study of potato sesquiterpene compositions, because all of the varieties were grown under the same conditions on adjacent plots. The leaves were harvested at the same time, and the extraction procedures were identical for all samples. Hence, the differences in sesquiterpene content are probably characteristic of these potato varieties. Moreover, the relative composition of the principal components (the total contents of β-caryophyllene, germacrene D, β-sesquiphellandrene, and germacrene D-4-ol expressed as 100%) calculated from the present data conform well with our earlier results (*9*) for those four varieties, which were the same in both experiments (**Figure 3**). During the previous experiment, potato plants were grown in a greenhouse, so the relative compositions of the principal sesquiterpenes were similar for potato plants grown under different conditions. The quantities of the major volatile chemicals emitted by intact potato plants were subjected to diurnal rhythm in the experiment by Agelopoulos et al. (*6*), but the ratios of compounds remained stable. Although the analysis of the emitted compounds cannot be compared directly with that of the potato foliage extract, it does have to be emphasized that the ratios of the main sesquiterpenes in the extracts were also similar between plants grown in different conditions.

Chemometric Analysis. Inspection of the sesquiterpene contents of the potato leaves (**Table 5**) reveals some regularities among the variables, which may be used for variety characterization and classification. The Ward classification (cluster analysis) of potato varieties based on sesquiterpenes is presented in **Figure 4**. The result of the clustering is a branching-tree diagram that distinguishes two potato varieties, Mila and Vistula, from the others.

Although Ward classifications of the sesquiterpene compositions discriminate the potato varieties, no direct conclusion can be drawn about the compounds involved in the classification. For this, the correlation methods of *n*-variables in *n*-dimensional space are needed. PCA was carried out to detect the data structure and to determine the relationships between samples (potato varieties) and original variables (sesquiterpene contents). In this analysis, new variables, called principal components (or factors), are calculated as a linear combination of the original variables in such a way that the first factors take up as much as possible the variances of the original variables. The procedure reduced the dimension of the space to three, covering >90% of the variances. Similar techniques are commonly applied in essential oil studies. Three predictive factors, factors 1, 2, and 3, which accounted for 90.5% of total variances, were computed

and used. Potato varieties and variables were projected onto the factorial planes formed by the first and second factors (**Figure 5**). Factor 1 separates the Vistula and Mila varieties from the main cluster. In conclusion, applying PCA to sesquiterpene data enabled the potato varieties to be separated into three chemotypes, a central cluster containing numerous cultivars, and two separate varieties, Mila and Vistula. The main chemotaxonomic potato cluster covers the varieties containing many sesquiterpenes at moderate levels, none of which are present in extreme concentrations (low level of variances). The eight samples of the main cluster were dominated by β-caryophyllene (9−148 ng/cm^2), germacrene D (2−46 ng/cm^2), germacrene D-4-ol (0.4−31 ng/cm^2), β-sesquiphellandrene (1−34 ng/cm^2), and sesquiterpene alcohol III (0.2−37 ng/cm^2). The Vistula variety is distinguished from the others by its high contents of β-caryophyllene (264 ng/cm^2), α-humulene (41 ng/cm^2), germacrene D (187 ng/cm^2), and germacrene D-4-ol (104 ng/cm^2) and the Mila by its contents of β-elemene (24 ng/cm^2), *trans*-α-bergamotene (7 ng/cm^2), (*Z*)-β-farnesene (15 ng/cm^2), (*E*)-β-farnesene (53 ng/cm^2), *trans*-β-bergamotene (33 ng/cm^2), β-sesquiphellandrene (61 ng/cm^2), sesquiterpene I (35 ng/cm^2), sesquiterpene II (32 ng/cm^2), and sesquiterpene III (94 ng/cm^2).

In conclusion, our qualitative and quantitative analyses of potato leaf sesquiterpenes, along with the chemometric data, provide new insights into the chemical variability of these compounds in different commercial potato varieties. This work arose from a need to characterize the potato varieties used in farming practice and to understand more fully the ecological role of potato sesquiterpenes.

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