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Volatile Constituents of *Festuca nigrescens*, *Phleum alpinum* and *Poa alpina* from N.W. Italian Alpine Pastures

Aldo Tava*[a], Roberto Cecotti[a], Maris Grecchi[a], Luca Falchero[b], Mauro Coppa[b] and Giampiero Lombardi[b]

[a]*CRA-FLC Centro di Ricerca per le Produzioni Foraggere e Lattiero Casearie, viale Piacenza 29, 26900 Lodi, Italy*

[b]*Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, Università di Torino, via L. da Vinci 44, 10095 Grugliasco (TO), Italy*

aldo.tava@entecra.it

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The composition of the volatile fractions of three important grasses from sub-alpine N.W. Italian pastures, namely *Festuca nigrescens* Lam. non Gaudin (chewing fescue), *Phleum alpinum* L. (alpine timothy) and *Poa alpina* L. (alpine bluegrass) was investigated. The fresh aerial parts were collected at the flowering stage during the summer season. The volatile oils obtained from green tissues by steam distillation in a Clevenger-type apparatus, were analyzed by GC/FID and GC/MS. The oil yield was 0.04 ± 0.01% weight/fresh weight bases for each of the investigated species. Several classes of compounds were found in the volatile fractions, including aldehydes, alcohols, acids, hydrocarbons, esters, ketones, terpenes, and phenolics. Qualitative and quantitative differences were observed.

Keywords: *Festuca nigrescens*, *Phleum alpinum*, *Poa alpina,* Essential oil composition, GC/MS, Alpine Grasses.

The volatile compounds have been analyzed of three of the most widespread grasses growing at high altitude on the western Italian Alps, namely *Festuca nigrescens* Lam. non Gaudin (chewing fescue), *Phleum alpinum* L. (alpine timothy) and *Poa alpina* L. (alpine bluegrass). From an ecological perspective the investigated plant species are the dominant monocotyledons growing on intermediate soils of the Western Alps [1] from early to mid season, and were selected both for their high relative abundance in the examined area and their high palatability index to cattle [2]. The area of collection, stretching between the upper sub-alpine and the alpine plain (2250-2350 m a.s.l.), is indeed subject to managed cattle grazing in the summer season for the production of milk and typical mountain cheese. In this regard, it is well known that the chemical and flavor patterns of milk and cheese can be influenced by the vegetation biodiversity of pastureland [3a-3g], especially rich in alpine marginal ecosystems.

Although there are several reports on the volatile composition of some species of *Festuca* [4a-4d], to our knowledge there are none about the chemistry of *F. nigrescens*. Little is known about the secondary metabolites of the other two genera, *Poa* [5a-5b] and *Phleum* [6a-6b], which have been extensively studied for their allergenic properties instead [7]. As for *F. nigrescens*, to our knowledge the volatile compounds of *P. alpina* and *P. alpinum* have never been investigated before.

Festuca nigrescens is a circumboreal perennial species growing on mountain-subalpine slopes in intermediate ecological conditions, with an altitudinal range spanning between 1200 and 2400 m a.s.l., as regards the European Alps. It grows on mature soils, rich in organic matter and acidified on the upper layer. It is often associated with *Agrostis tenuis*, and, at higher altitude, to *Poa alpina*.

Phleum alpinum is a perennial plant widely distributed all over the world. In the northern hemisphere it is circumboreal, with an ample altitudinal range. The growing conditions, including soil, temperature and sunlight requirements are very close to those of *F. nigrescens*. The two species are often associated, with *P. alpinum* being more abundant whenever nitrogen is more available in the soil [8].

Poa alpina is a circumboreal perennial species, growing on subalpine and alpine environments from 1500 to 2700 metres a.s.l., and occasionally reaching up to 3600m in the European Alps, in intermediate ecological conditions. In this region, *P. alpina* features a specific pastureland ecotype, on mature soils subject to prolonged snow-covering in the cold season, while extensively exploited in the summer by cattle [1]. Possibly due to its low requirements in terms of temperature and sunlight [2], it is more precocious and can thrive at higher elevations than *F. nigrescens* and *P. alpinum*.

The fresh aerial parts of the three species underwent steam distillation in a Clevenger-type apparatus for the isolation of the volatile fractions, each of which consisted of a pale yellow oil. The oil yield was 0.04 ± 0.01% weight/fresh weight bases for each of the investigated species. The GC/FID and GC/MS analyses of samples allowed the identification and quantification of several compounds, as reported in Table 1, grouped according to their chemical class and listed in order of elution on an Elite-5 column.

Aldehydes resulted to be the most abundant class of compounds in *P. alpinum* and *P. alpina*, with a total of 91.7 ± 21.9 μg/g fresh weight and 115.7 ± 26.3 μg/g fresh weight, respectively, corresponding to 30.7 ± 8.3% and 24.2 ± 6.7% of the volatile fractions, respectively. Aldehydes were quite abundant in *F. nigrescens* too, totaling 54.1 ± 17.6 μg/g fresh weight, second only to acids, with an average of 16.4 ± 6.6% of the volatiles. *P. alpinum* was rich in aromatic aldehydes (i.e. phenylacetaldehyde and benzaldehyde, which made up about 50% of the total aldehydes) whereas in the other two species, aliphatic aldehydes exceed 85.0% of the total of this class. Among the aliphatic aldehydes the best represented was nonanal in all species, with a percentage close to 6.0% of the total volatiles in each of them. According to some authors, this compound originates from the breakdown of unsaturated C_{18} fatty acids like the C_6 green leaf volatiles (i.e. hexanal, (*E*)-2-hexenal, (*Z*)-3-hexenol, (*E*)-2-hexenol, hexanol and (*Z*)-3-hexenyl-acetate), and may play some role in protecting wounded plants from fungal infections [9a,9b].

Alcohols were quantified as 44.7 ± 14.4 μg/g (14.2 ± 5.3%), 40.8 ± 16.9 μg/g (8.8 ± 3.9%) and 25.9 ± 7.3 μg/g (11.1 ± 3.4%), of the total volatile fraction for *F. nigrescens, P. alpina* and *P. alpinum*, respectively. Aliphatic alcohols were predominant in all species, accounting for about 95% of total alcohols in *F. nigrescens*, 76% in *P. alpina* and 73% in *P. alpinum.* In both *F. nigrescens* and *P. alpinum* the most represented alcohol was the unsaturated (*Z*)-3-hexenol (known as 'leaf-alcohol' derived from the breakdown of linolenic acid), with 14.4 ± 3.4 μg/g and 5.4 ± 0.9 μg/g fresh weight, respectively. On the other hand, hexanol (7.7 ± 4.8 μg/g) and 2-phenylethanol (7.8 ± 2.5 μg/g) topped the list of alcohols in *P. alpina*.

Long-chain aliphatic acids are expressed quite variably in plants, sometimes according to seasonal factors [10]. In *F. nigrescens* oil they represented the major class of compounds, quantified as 76.8 ± 16.0 μg/g fresh weight (22.5 ± 5.1 %) of the total volatiles. Organic acids were still well represented in *P. alpina*, with 76.5 ± 25.8 μg/g fresh weight (18.2 ± 7.7%). However, they were quite low in *P. alpinum* forming 19.9 ± 4.6 μg/g fresh weight (7.0 ± 1.5 %). Among them, palmitic acid (*n*-C_{16}) was the most abundant acid in all species, accounting for about 50% of the total acids in all species. On the other hand, unsaturated linoleic and linolenic acids made up nearly 10% of the total acids in *F. nigrescens* and *P. alpina,* while in *P. alpinum* they accounted for a more consistent 33% of the total acidic fraction.

Hydrocarbons were well represented in *P. alpina* forming 19.3 ± 5.4 % of the total volatiles, while they accounted for about 10% in the other two species (see Table 1). This class of compounds is almost entirely represented by odd-numbered long chain aliphatic substances. Among them tricosane and pentacosane are the most abundant components, accounting for about 50% of the total hydrocarbons in *F. nigrescens*, about 60% in *P. alpinum*, and about 70% in *P. alpina*.

Esters were relatively abundant in *F. nigrescens* with 31.6 ± 9.3 μg/g fresh weight accounting for 9.0 ± 3.8% of the total oil. Equivalent values were 16.8 ± 7.9 μg/g fresh weight (3.4 ± 1.8%) for *P. alpina*, and 8.6 ± 3.4 μg/g fresh weight (3.0 ± 1.3%) for *P. alpinum*. (*Z*)-3-hexenyl acetate was the most abundant ester in *F. nigrescens* and *P. alpinum*, while *P. alpina* yielded mostly methyl linoleate and methyl palmitate (Table 1).

The content of ketones was quite low in all species; hexahydrofarnesylacetone was predominant in *F. nigrescens* and *P. alpinum*, while tridecan-2-one was the most abundant in *P. alpina*. The former, known also as phytone, was reported to be a degradation product of chlorophyll [11].

Among terpenes, monoterpenes were rather scarce in all three species; linalool was detected in both *F. nigrescens* (0.6 ± 0.5 μg/g fresh weight, 0.2 ± 0.2%) and *P. alpina* (1.0 ± 0.2 μg/g fresh weight, 0.2 ± 0.0%), while α-terpineol and carvacrol were present in *P. alpinum* (1.9 ± 0.7 μg/g fresh weight, 1.2 ± 0.7%). As for higher terpenes, phytols were detected in all species as the most abundant compounds of this class, ranging from about 2.2% to 7.5% of the total oils.

Phenolics made up a remarkable 10% of volatile compounds in *P. alpinum*, yielding more than 22.6 ± 4.1 μg/g fresh weight of eugenol, and 1.7 ± 0.6 μg/g fresh weight of chavicol. On the other hand, *F. nigrescens* and *P. alpina* lacked both mentioned compounds, and yielded *p*-vinylguaiacol as the sole phenolic, accounting for 1.6 ± 1.2 μg/g fresh weight and 1.7 ± 0.4 μg/g fresh weight, respectively, of the total volatile fractions.

Unlike the other two species, *P. alpinum* yielded coumarin (11.9 ± 0.4 μg/g fresh weight, 2.8 ± 0.1%), within the miscellaneous compounds. On the other hand, 2,3 dihydrobenzofuran and β-ionone were the most abundant miscellaneous compounds in both *F. nigrescens* and *P. alpina*.

Table 1: Composition of the volatile oils of *Festuca nigrescens*, *Phleum alpinum* and *Poa alpina*.

Compounds[a]	K_i[b]	*Festuca nigrescens*		*Phleum alpinum*		*Poa alpina*	
		%	μg/g	%	μg/g	%	μg/g
Aldehydes		**16.4 ± 6.6**	**54.1 ± 17.6**	**30.7 ± 8.3**	**91.7 ± 21.9**	**24.2 ± 6.7**	**115.7 ± 26.3**
2-Methyl butanal	640	0.5 ± 0.3	1.4 ± 0.8	-	-	0.4 ± 0.3	1.8 ± 0.7
3-Methyl butanal	649	0.4 ± 0.3	1.4 ± 0.9	-	-	-	-
Pentanal	688	0.1 ± 0.0	0.3 ± 0.1	tr	tr	0.4 ± 0.3	1.7 ± 0.6
(*E*)-2-Pentenal	748	0.1 ± 0.1	0.4 ± 0.2	-	-	tr	tr
Hexanal	801	0.6 ± 0.4	1.9 ± 1.1	0.4 ± 0.1	0.9 ± 0.3	0.2 ± 0.1	0.9 ± 0.3
Furfural	832	0.2 ± 0.1	0.5 ± 0.3	-	-	0.2 ± 0.0	1.7 ± 0.1
(*E*)-2-Hexenal	852	tr	tr	0.2 ± 0.0	0.5 ± 0.0	3.0 ± 0.8	15.9 ± 2.1
Heptanal	895	1.5 ± 0.8	5.1 ± 2.1	1.1 ± 0.5	3.4 ± 1.6	2.9 ± 0.6	14.5 ± 3.1
3-Methylthiopropanal	907	tr	tr	0.4 ± 0.2	1.2 ± 0.7	-	-
Benzaldehyde	954	0.3 ± 0.2	1.2 ± 0.7	4.6 ± 1.3	13.7 ± 3.9	0.2 ± 0.1	0.8 ± 0.3
(*Z,E*)-2,4-Heptadienal	997	-	-	0.3 ± 0.2	1.4 ± 0.7	-	-
Octanal	999	0.1 ± 0.1	0.2 ± 0.1	tr	tr	1.6 ± 0.7	8.0 ± 2.4
(*E,E*)-2,4-Heptadienal	1005	-	-	0.3 ± 0.2	1.1 ± 0.7	tr	tr
Phenylacetaldehyde	1036	2.0 ± 0.9	7.0 ± 2.6	12.2 ± 2.0	38.1 ± 5.5	1.6 ± 0.8	7.1 ± 2.9
Nonanal	1096	6.0 ± 1.7	20.9 ± 4.8	6.3 ± 1.6	18.6 ± 4.2	5.3 ± 1.0	24.5 ± 4.3
Decanal	1197	0.3 ± 0.1	0.8 ± 0.2	0.3 ± 0.1	0.7 ± 0.3	0.4 ± 0.1	1.6 ± 0.6
β-Cyclocitral	1217	-	-	0.3 ± 0.1	1.0 ± 0.3	-	-
Undecanal	1301	2.8 ± 0.9	8.2 ± 1.7	0.4 ± 0.1	1.0 ± 0.4	2.3 ± 0.6	11.1 ± 3.1
Vanillin	1384	0.4 ± 0.1	1.4 ± 0.4	-	-	-	-
Dodecanal	1398	0.2 ± 0.0	0.6 ± 0.0	-	-	tr	tr
Tetradecanal	1600	0.2 ± 0.1	0.7 ± 0.3	0.3 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	1.5 ± 0.5
Hexadecanal	1802	0.3 ± 0.2	1.0 ± 0.6	0.6 ± 0.3	1.5 ± 0.8	4.1 ± 1.0	18.7 ± 4.5
Octadecanal	2003	0.4 ± 0.3	1.1 ± 0.7	0.7 ± 0.4	1.7 ± 0.8	1.3 ± 0.2	5.9 ± 0.8
Docosanal	2421	-	-	0.4 ± 0.2	1.1 ± 0.5	tr	tr
Hexacosanal	2836	-	-	2.2 ± 0.9	5.2 ± 1.0	-	-
Alcohols		**14.2 ± 5.3**	**44.7 ± 14.4**	**11.1 ± 3.4**	**25.9 ± 7.3**	**8.8 ± 3.9**	**40.8 ± 16.9**
3-Methyl-3-buten-2-one	653	0.1 ± 0.0	0.2 ± 0.1	tr	tr	tr	tr
Pent-1-en-3-ol	670	0.5 ± 0.1	1.7 ± 0.3	tr	tr	tr	tr
Pentanol	771	0.3 ± 0.2	1.2 ± 0.6	2.3 ± 1.4	3.1 ± 0.2	tr	tr
cis-2-Pentenol	775	0.3 ± 0.1	0.5 ± 0.1	-	-	0.3 ± 0.1	1.2 ± 0.8
2-Methyl-3-penten-1-ol	793	0.6 ± 0.3	1.6 ± 0.6	tr	tr	0.6 ± 0.3	3.1 ± 1.2
(*Z*)-3-Hexenol	859	4.1 ± 1.0	14.4 ± 3.4	2.0 ± 0.1	5.4 ± 0.9	0.4 ± 0.1	0.9 ± 0.3
(*E*)-2-Hexenol	863	1.9 ± 0.8	5.2 ± 2.0	0.4 ± 0.0	0.9 ± 0.1	0.1 ± 0.0	0.4 ± 0.2
Hexanol	871	0.9 ± 0.3	2.6 ± 0.8	0.2 ± 0.0	0.6 ± 0.2	1.2 ± 0.9	7.7 ± 4.8
3-Methyl-3-heptanol	942	0.1 ± 0.1	0.4 ± 0.1	-	-	0.2 ± 0.1	0.7 ± 0.4
Heptanol	962	0.1 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	0.7 ± 0.4	0.5 ± 0.2	2.4 ± 0.7
Oct-1-en-3-ol	973	0.7 ± 0.4	2.4 ± 1.4	0.2 ± 0.1	0.8 ± 0.3	0.9 ± 0.2	4.2 ± 0.8
Benzyl alcohol	1026	0.4 ± 0.2	1.4 ± 0.6	1.1 ± 0.5	3.0 ± 1.2	0.4 ± 0.2	2.1 ± 0.8
2-Phenylethanol	1120	0.3 ± 0.1	1.2 ± 0.4	1.9 ± 0.1	4.0 ± 1.7	1.7 ± 0.7	7.8 ± 2.5
Dodecanol	1475	0.5 ± 0.3	1.3 ± 0.6	0.3 ± 0.1	1.0 ± 0.3	0.3 ± 0.1	1.4 ± 0.5
Tetradecanol	1677	0.7 ± 0.4	2.2 ± 1.2	tr	tr	0.6 ± 0.3	2.7 ± 1.2
Hexadecanol	1879	1.1 ± 0.4	3.5 ± 1.3	0.2 ± 0.1	0.5 ± 0.1	0.7 ± 0.3	2.9 ± 1.3
Octadecanol	2086	1.6 ± 0.6	4.5 ± 0.9	0.5 ± 0.3	1.0 ± 0.4	0.9 ± 0.4	3.3 ± 1.4
Hexacosanol	2875	tr	tr	1.8 ± 0.6	4.9 ± 1.5	-	-
Acids		**22.5 ± 5.1**	**76.8 ± 16.0**	**7.0 ± 1.5**	**19.9 ± 4.6**	**18.2 ± 7.7**	**76.5 ± 25.8**
Nonanoic acid	1257	0.4 ± 0.1	1.2 ± 0.4	0.3 ± 0.2	1.0 ± 0.5	0.3 ± 0.1	1.0 ± 0.4
Dodecanoic acid	1547	0.9 ± 0.3	2.9 ± 1.1	0.2 ± 0.1	0.4 ± 0.2	1.0 ± 0.8	3.9 ± 2.3
Tetradecanoic acid	1747	2.4 ± 1.0	7.4 ± 2.1	0.3 ± 0.0	0.8 ± 0.1	5.7 ± 1.9	22.1 ± 6.8
Hexadecanoic acid	1952	13.7 ± 2.3	49.1 ± 8.5	4.1 ± 0.9	11.2 ± 2.4	6.7 ± 2.7	33.5 ± 13.6
Linoleic acid	2101	0.9 ± 0.2	2.9 ± 0.6	0.6 ± 0.1	1.7 ± 0.8	0.9 ± 0.5	3.4 ± 1.2
Linolenic acid	2108	1.2 ± 0.5	3.4 ± 1.3	1.5 ± 0.2	4.8 ± 0.6	1.1 ± 0.6	3.8 ± 0.3
Octadecanoic acid	2161	3.0 ± 0.7	9.9 ± 2.0	tr	tr	2.5 ± 1.1	8.8 ± 1.2
Hydrocarbons		**10.1 ± 3.0**	**32.1 ± 8.0**	**8.8 ± 3.5**	**24.3 ± 9.2**	**19.3 ± 5.4**	**94.7 ± 25.5**
Heptadecane	1700	0.5 ± 0.3	1.4 ± 0.4	0.2 ± 0.0	0.8 ± 0.1	tr	tr
Nonadecane	1900	0.9 ± 0.7	2.6 ± 1.3	0.4 ± 0.2	1.0 ± 0.6	0.4 ± 0.0	2.9 ± 0.2
Heneicosane	2100	1.4 ± 0.3	4.4 ± 0.8	1.2 ± 0.8	3.4 ± 2.1	2.5 ± 1.0	11.8 ± 4.5
Tricosane	2300	3.1 ± 0.6	9.8 ± 1.9	2.7 ± 0.5	8.1 ± 1.6	8.3 ± 2.1	39.9 ± 8.9
Pentacosane	2500	2.9 ± 0.7	9.2 ± 2.1	3.4 ± 1.6	8.7 ± 3.8	5.9 ± 1.5	29.3 ± 7.3
Heptacosane	2700	1.0 ± 0.3	3.7 ± 1.0	0.7 ± 0.3	1.6 ± 0.7	1.9 ± 0.7	9.5 ± 3.7
Squalene	2831	0.1 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.7 ± 0.3	tr	tr
Nonacosane	2900	0.2 ± 0.1	0.8 ± 0.4	tr	tr	0.3 ± 0.1	1.3 ± 0.9
Esters		**9.0 ± 3.8**	**31.6 ± 9.3**	**3.0 ± 1.3**	**8.6 ± 3.4**	**3.4 ± 1.8**	**16.8 ± 7.9**
(*Z*)-3-Hexenyl acetate	1002	3.1 ± 1.0	10.7 ± 3.5	2.0 ± 0.8	4.5 ± 1.5	0.4 ± 0.1	1.3 ± 0.5
Methyl salicylate	1189	0.2 ± 0.1	0.6 ± 0.1	-	-	0.1 ± 0.1	0.6 ± 0.3
Methyl tetradecanoate	1709	1.3 ± 0.6	4.4 ± 1.8	tr	tr	1.0 ± 0.7	4.6 ± 2.7
Methyl hexadecanoate	1922	1.2 ± 0.5	3.5 ± 0.8	0.2 ± 0.2	0.8 ± 0.5	1.4 ± 0.7	7.0 ± 3.6
Methyl linoleate	2073	0.8 ± 0.5	3.2 ± 1.9	0.2 ± 0.1	0.7 ± 0.4	0.1 ± 0.0	1.0 ± 0.2
Methyl linolenate	2079	2.2 ± 1.0	8.6 ± 1.1	0.6 ± 0.2	2.6 ± 1.0	0.4 ± 0.2	2.3 ± 0.6
Methyl octadecanoate	2118	0.2 ± 0.1	0.6 ± 0.1	tr	tr	tr	tr
Ketones		**2.9 ± 0.9**	**10.4 ± 3.0**	**1.6 ± 1.1**	**4.1 ± 1.8**	**1.0 ± 0.1**	**5.6 ± 0.6**
Octan-3-one	982	0.3 ± 0.1	1.2 ± 0.4	-	-	0.2 ± 0.1	1.0 ± 0.5
3,5-Octadien-2-one	1063	-	-	0.5 ± 0.3	1.7 ± 0.8	-	-
Tridecan-2-one	1483	0.2 ± 0.2	0.6 ± 0.4	-	-	0.8 ± 0.0	4.6 ± 0.1
Hexahydrofarnesylacetone	1840	2.4 ± 0.6	8.6 ± 2.2	1.1 ± 0.8	2.4 ± 1.0	tr	tr

Table 1: Continued.

Terpenes		**2.4 ± 1.1**	**7.1 ± 3.1**	**7.3 ± 2.6**	**20.8 ± 6.1**	**7.7 ± 3.2**	**33.3 ± 13.9**
Linalool	1094	0.2 ± 0.2	0.6 ± 0.5	-	-	0.2 ± 0.0	1.0 ± 0.2
α-Terpineol	1186	-	-	1.1 ± 0.7	1.6 ± 0.7	-	-
Carvacrol	1298	-	-	0.1 ± 0.0	0.3 ± 0.0	-	-
(*Z*)-Phytol	1948	1.4 ± 0.6	3.9 ± 1.7	-	-	5.4 ± 1.9	20.5 ± 6.1
(*E*)-Phytol	2122	0.8 ± 0.3	2.6 ± 0.9	4.5 ± 1.4	14.3 ± 4.2	2.1 ± 1.3	11.8 ± 7.6
(*E*)-Phytol acetate	2218	-	-	1.6 ± 0.5	4.6 ± 1.2	tr	tr
Phenolics		**0.4 ± 0.3**	**1.6 ± 1.2**	**9.9 ± 1.6**	**24.7 ± 4.8**	**0.4 ± 0.2**	**1.7 ± 0.4**
Chavicol	1247	-	-	0.7 ± 0.3	1.7 ± 0.6	-	-
p-Vinylguaiacol	1307	0.4 ± 0.3	1.6 ± 1.2	0.1 ± 0.0	0.4 ± 0.1	0.4 ± 0.2	1.7 ± 0.4
Eugenol	1356	-	-	9.1 ± 1.3	22.6 ± 4.1	-	-
Miscellaneous		**5.0 ± 1.6**	**15.2 ± 2.7**	**3.6 ± 0.3**	**14.1 ± 1.2**	**1.5 ± 0.7**	**8.1 ± 3.3**
Diethyl disulfide	925	0.3 ± 0.1	1.2 ± 0.2	-	-	0.2 ± 0.1	1.2 ± 0.4
2,3-Dihydrobenzofuran	1206	2.5 ± 0.7	7.8 ± 1.3	tr	tr	1.0 ± 0.4	5.4 ± 2.3
Coumarin	1435	-	-	2.8 ± 0.1	11.9 ± 0.4	-	-
β-Ionone	1468	2.2 ± 0.8	6.2 ± 1.2	0.6 ± 0.2	1.8 ± 0.7	0.3 ± 0.2	1.5 ± 0.6
β-Ionol	1472	-	-	0.2 ± 0.0	0.4 ± 0.1	-	-
Total		**82.9 ± 27.7**	**273.6 ± 75.**	**83.0 ± 23.6**	**234.1 ± 60.3**	**84.5 ± 29.7**	**393.2 ± 120.6**

[a]Compounds grouped according to chemical class and listed in order of elution on Elite-5 column. [b]K_i retention index determined on Elite-5 column using the homologous series of *n*-hydrocarbons.

Considering the features of each single species, a distinctive trait in *P. alpinum* was the exclusive presence of eugenol and coumarin, in addition to the relative abundance of other aromatic compounds, such as benzaldehyde, phenylacetaldehyde, 2-phenylethanol, and benzyl alcohol. A large prevalence of acids and their methylesters was detected instead in *F. nigrescens*, while a considerable amount of hydrocarbons was detected in *P. alpina*. Moreover the reported findings confirmed a trend, previously observed in Poaceae, in which low terpene levels were reported [4a-4d].

Further studies would be needed to determine whether for the volatile fraction the prevalence of the phenylpropanoid rather than the acyl-CoA metabolic pathway, may hold some taxonomic significance within the Poaceae, and whether environmental factors related to altitude play any role in this issue.

Experimental

Plant material: *Festuca nigrescens* Lam. non Gaudin, *Phleum alpinum* L. and *Poa alpina* L. were identified according to Pignatti [12]. Aerial parts were collected at the flowering stage along the slopes of Alpe Valcavera, located within the Valle Stura di Demonte in the province of Cuneo (2250 to 2350 m. a.s.l.); as a climatological reference, the mean annual rainfall of the site is 1040 mm, while its mean annual temperature is 2.6°C. Collection was carried out in homogeneous areas about 100 m^2 wide. Plants were cut at about 1 cm height above ground to avoid soil impurities; that is also the minimum height at which plants are grazed by cattle. Samples were placed into refrigerated vessels, taken to the laboratory within the day, and immediately steam-distilled. Three samples were collected of each species, which were separately distilled and analysed by GC/FID and GC/MS. Voucher specimens were deposited at the Herbarium Pedemontanum (TO-HP), Dept. of Plant Biology, University of Turin.

Isolation of the oils: Plant material (100 g) and 0.34 mg of 3-methyl cyclohexanone, added as an internal standard, were steam distilled with odor-free water in a Clevenger-type apparatus for 1 h. The distillate was saturated with NaCl, extracted with freshly distilled Et_2O (3 × 100 mL), dried over anhydrous Na_2SO_4 and conc in a rotary evaporator to give a pale-yellow oil. The obtained volatile oils were diluted in Et_2O and separately analysed by GC/FID and GC/MS.

Gas chromatography and gas chromatography-mass spectrometry: GC/FID analyses were carried out using a Perkin Elmer model 8500 GC equipped with a 30 m × 0.32 mm i.d. Elite-5MS (5% phenyl methylpolysiloxane) capillary column (0.32 μm film thickness). Samples (0.5 μL) were injected in "split" mode (1:30) with a column temperature programme of 40°C for 5 min, then increased to 280°C at 4°C/min and finally held at this last temperature for 10 min. Injector and detector were set at 250 and 300°C, respectively; the carrier gas was He with a head pressure of 12.0 psi.

GC/MS analysis was carried out using a Perkin Elmer Clarus 500 GC equipped with a Clarus 500 mass spectrometer using the same capillary column and chromatographic conditions as for the GC/FID analysis. MS were acquired over 40-500 amu range at 1 scan/sec with an ionizing electron energy of 70 eV, and ion source at 200°C. Transfer line was set at 300°C, and the carrier gas was He at 1.0 mL/min.

Identification and quantification of the essential oil components: Identification of the volatile oil components was performed based on their retention indices (K_i) and MS, by comparison with a NIST database mass spectral library [13], as from literature data [14a, 14b]. Authentic reference compounds purchased from Sigma-Aldrich were also used. Retention indices were calculated using a *n*-alkane series (C_6-C_{32}) under the same GC conditions used for the samples. The relative amount of each individual component of the oils was expressed as the percent peak relative to the total peak area from the GC/FID analysis of the whole extract. Quantitative data were obtained from

GC/FID analysis by the internal standard method using 3-methylcyclohexanone as internal reference and assuming an equal response factor for all detected compounds. For each species, results were expressed as mean of 3 independent determinations ± standard deviation.

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