

Harvest Regimen Optimization and Essential Oil Production in Five Tansy (*Tanacetum vulgare* L.) Genotypes under a Northern Climate

STEINAR DRAGLAND,[†] JENS ROHLOFF,^{*,§} RUTH MORDAL,[†] AND
TOR-HENNING IVERSEN[§]

Apelsvoll Research Centre, Division Kise, The Norwegian Crop Research Institute (Planteforsk), N-2350 Nes på Hedmark, Norway, and The Plant Biocentre, Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway

Tansy (*Tanacetum vulgare* L.) was cultivated at the Norwegian Crop Research Institute at the Apelsvoll Research Centre, Division Kise, in the period from 2000 to 2001. The study focused on different harvesting regimens for high biomass production and essential oil (EO) yield and quality. Two tansy genotypes from Canada (Richters and Goldsticks) and three Norwegian genotypes (Steinvikholmen, Alvdal, and Brumunddal) were studied. The Canadian genotypes reached a height of 130–145 cm and showed a higher dry weight of aerial plant parts compared to the Norwegian plants in 2000. Similar oil yields could be observed for the Canadian types and genotype Steinvikholmen in the range of 30.8–34.6 L/ha when the plants were harvested twice during budding and before flowering after regrowth (year 2001). In contrast, single harvesting at the full bloom stage resulted in higher oil yields, between 42.1 and 44.5 L/ha (Canadian genotypes), whereas 21.0–38.4 L/ha was obtained from the Norwegian types. Tansy genotypes could be grouped into the following chemotypes: the mixed chemotypes Steinvikholmen (thujone–camphor), Alvdal (thujone–camphor–borneol), Goldsticks (thujone–camphor–chrysanthenyl type), and Brumunddal (thujone–camphor–1,8-cineole–bornyl acetate/borneol– α -terpineol) and the distinct chemotype Richters, with average concentrations of (*E*)-chrysanthenyl acetate >40% in both leaf and flower EO.

KEYWORDS: Tansy; *Tanacetum vulgare*; biomass production; chemotypes; essential oil (EO); GC-MS; harvest regimen; hydrodistillation; plant developmental stage

INTRODUCTION

Tansy (*Tanacetum vulgare* L.) is an aromatic plant of the Asteraceae family mainly spread in the northern hemisphere in Europe, Asia, and North America. The plant has finely divided, fernlike leaves and yellow, button-like flowers (Figure 1). Due to its strong scent derived from the essential oil (EO) containing glands in leaves and flowers, the plants have traditionally been used as a repellent and deterrent against flies and other insects. Herbal preparations of tansy exert strong biological and medicinal activities, and extracts have been widely applied against intestinal worms, kidney disease, and respiratory infections and as an abortivum. Additionally, tansy has also been shown to serve as a good source for natural antioxidants (1). Besides secondary metabolites such as polysaccharides, sesquiterpene lactones, sterols, phenolics, coumarins, and alkaloids [reviewed by Dragland (2)], the EO of tansy comprises a large number of monoterpene and sesquiterpene structures. Tansy

populations show high variability with regard to the EO composition, and more than 15 distinct chemotypes have been described from Scandinavia and the Baltic so far: thujone, camphor, artemisia ketone, 1,8-cineole, yomogi alcohol, and (*E*)-chrysanthenyl acetate/chrysanthenone from Norway (3), in addition to tricyclene/myrcene, sabinene, borneol, isocamphone, camphenol, germacrene D, umbellulone, and davanone from Finland (4–6) and a myrtenol chemotype from Lithuania (7). Other noteworthy chemotypes have been reported from The Netherlands and Hungary (lyratol and campholenol; 8, 9) and from Canadian tansy populations (dihydrocarvone; 10). Despite the great EO variability in tansy, the thujone, camphor, cineole, chrysanthenyl, artemisia, and umbellulone types are the most common in Europe, but thujone-rich genotypes have also been reported from Brazil (11).

To establish successful cultivation of oil-rich tansy genotypes (or provenances), one might select early-flowering plants with a high number of single flowers in the flower heads, because generative plant organs show higher contents of EO compared to the leaves (reviewed in ref 12). However, morphological traits such as plant height, number of branches, leaf shape, and flower

* Corresponding author (telephone 0047 73590174; fax 0047 73590177; e-mail jens.rohloff@bio.ntnu.no).

[†] The Norwegian Crop Research Institute (Planteforsk).

[§] Norwegian University of Science and Technology.



Figure 1. Tansy (*T. vulgare* L.) cultivated at Planteforsk, Apelsvoll Research Centre, Division Kise.

Table 1. Plant Development and Percentage of Dry Matter in Different Parts of Tansy Harvested on September 12th in Trial Year 2000

parameter	genotype					LSD _{5%} ^a
	Steinvikholmen	Alvdal	Brumunddal	Richters	Goldsticks	
plant height (cm)	114	111	127	145	130	15
flowering stage (1–9) ^b	5.3	7.0	6.0	6.0	5.3	0.6
dry matter in flowers (%)	22.9	24.4	23.2	23.3	23.3	ns ^c
dry matter in leaves (%)	21.6	21.3	22.9	20.4	21.1	ns
dry matter in stems (%)	35.9	39.0	38.6	35.9	37.6	ns

^a LSD, least significant difference ($\alpha = 0.05$). ^b Flower developmental stage was visually assessed by grouping into the following categories: 1–3 = green buds; 4–6 = yellow flowers; 7–9 = late flowering/brownish flowers. ^c ns, no significant difference.

and biomass production might be directly related to chemotypical variation (13–15). The cultivation of distinct chemotypes rather than oil-rich genotypes might be more important for productive purposes. Keskitalo (12) pointed out that the following tansy chemotypes seem to be the most important with regard to commercial and biotechnological aspects: artemisia ketone, camphor, (*E*)-chrysanthenyl acetate, 1,8-cineole, da-vanone, and thujone.

To obtain well-defined, chemotypical oils for commercial purposes with regard to EO quality and compositional standardization, field trials with five tansy genotypes (three provenances from Norway and two genotypes from Canada) were carried out by investigating morphological traits, biomass, and EO production. Our study was aimed at answering questions about the optimal harvest regimen to obtain high EO yields with special focus on the differences of EO accumulation in leaves and flowers. As a completion of chemotaxonomical analyses of Norwegian tansy collections from wild populations (3), the present investigation mainly focuses on agricultural aspects of tansy herb and essential oil production.

MATERIALS AND METHODS

Plant Material and Cultivation. Five different genotypes of tansy (*T. vulgare* L.) were used in the study: three Norwegian genotypes of wild populations, Steinvikholmen (Nord-Trøndelag county), Alvdal, and Brumunddal (both from Hedmark county), which showed vigorous growth in earlier studies, and two Canadian genotypes, Richters and

Goldsticks, from the seed company Richters (Goodwood, ON, Canada). Seeds were sown in fertilized soil (L. O. G. Gartnerjord; 1.2 kg of NPK 15–4–12 and 0.2 kg of micronutrients per m³) in growth trays (2 g of seeds/tray) at Planteforsk, Apelsvoll Research Centre, Division Kise, in March 2000, and kept in a cold room at 0–2 °C for 4 weeks before the trays were moved to a greenhouse for germination. The young plants were transferred to plug trays (40 × 60 cm; 77 cells) with fertilized soil and grown in a greenhouse (night, 12 °C, day, 15–25 °C) for 8 weeks. When reaching an average height of 10 cm, the plants were established in the trial field area on gleyed melanic brunisol soil type on June 5, 2000.

Tansy plants were planted on a biodegradable mulch film (Mater-Agro) in rows with 50 cm of space between the rows and 25 cm of within-row space, that is, 80000 plants/ha. Plants were arranged in a randomized complete block design (RCBD) with four replications. Each plot (block) covered an area of 6 m² (4 × 1.5 m) and comprised 48 plants. The trial field dimension was 180 m² (20 × 9 m), and replicates were separated by 100 cm of extra space. The plants were fertilized in 2001 with 500 kg of 15–4–12 (Hydro), that is, 75 kg of N, 20 kg of P, and 60 kg of K per hectare.

Harvest Regimen. In trial year 2000, 10 different plants for each genotype from single plots (replicate 4) were harvested randomly four times (July 6, August 9, September 12, and October 2). Additionally, plant material from half plots (each 3 m²; three replicates) was harvested on September 12 to describe the statistical variation of plant growth parameters among the investigated genotypes (Tables 1 and 2A). In general, plants were cut 10 cm above the ground. Both plant height and fresh (FW) and dry weight (DW) of stems, leaves, and flowers were recorded. The flowers of the remaining plants (not sampled) were

Table 2. Dry Weight and Biomass of Tansy

parameter	genotype					LSD _{5%} ^a
	Steinvik-holmen	Alvdal	Brumunddal	Richters	Goldsticks	
(A) Dry Weight of Tansy Harvested Sept 12, 2000						
leaf (g/m ²)	179	127	203	177	217	ns ^b
stem (g/m ²)	195	192	260	398	370	90
flower (g/m ²)	70	120	83	177	121	34
total wt (g/m ²)	444	438	546	752	708	178
(B) Tansy Biomass Produced at Three Harvest Dates in Trial Year 2000 ^c						
leaf						
Aug 9	56	38	52	34	41	
Sept 12	40	29	37	24	31	
Oct 2	39	27	36	20	27	
av	45a	31b	42c	26d	33be	
stem						
Aug 9	39	45	43	55	51	
Sept 12	44	44	48	53	52	
Oct 2	41	38	41	50	46	
av	41a	42a	44a	53b	50b	
flower						
Aug 9	5	17	5	11	8	
Sept 12	16	27	15	23	17	
Oct 2	20	35	23	30	27	
av	14a	26b	14a	21c	17a	

^a LSD, least significant difference ($\alpha = 0.05$). ^b ns, no significant difference.

^c Data represent average values from 10 plants. Statistical analysis was done by Student's pairwise *t* test; different letters in rows indicate significant differences.

detached to avoid seed dispersal in the field; remaining stems were removed in early spring. Sampled plant material was dried at 35–40 °C in drying chambers prior to distillation and chemical analyses at The Plant Biocentre at NTNU, Trondheim.

In 2001, the half plots (3 m²) not treated in 2000 (three replicates) were divided into two sections. Half (1.5 m² ≈ 12 plants) was harvested twice with a first cut between June 18 and July 3 right before budding, and, after regrowth, a second cut between August 16 and September 5 at the early bloom stage. Both leaves and buds/flowers were harvested together without separation. The other half of the plots (1.5 m² ≈ 12 plants) was harvested only once at the full bloom stage in the period of August 6–14 by separately collecting leaves and flowers. Finally, the plant raw material was dried and further handled as described above.

Hydrodistillation of EO. The dried plant material was coarsely crushed by hand prior to hydrodistillation. The distillation apparatus consisted of a heating mantle, a 5 L distillation bottle, a 3 mL graduated receiver (Clevenger type), and a condenser (jacketed coil). H₂O (2.5 L) was used, and the distillation was carried out for 1.5 h after the mixture had reached the boiling point. Finally, the volume of the collected EO was recorded (mL/100 g of DW). Ten microliters of each EO sample was dissolved in 1 mL of EtOH, and 1 μL was analyzed using an automatic GC injector.

Gas Chromatography–Mass Spectrometry Analysis (GC-MS). A Varian Star 3400 CX gas chromatograph coupled with a Varian Saturn 3 mass spectrometer were used for all analyses. The GC was equipped with a fused silica capillary column: Chrompack CP-Wax 52CB (30 m × 0.32 mm i.d. with a film thickness of 0.25 μm). The carrier gas was He (5 psi) at 50 mL/min through the injector (split mode).

The injector temperature was 220 °C for all of the analyses done. The GC temperature program was ramped from 60 to 210 °C at a rate of 2 °C/min with a final hold at 210 °C for 5 min. The MS detector was set at 170 °C, and a mass range of *m/z* 40–300 was recorded. All mass spectra were acquired in EI mode. The compounds were identified by the use of a combination of mass spectrum database search (IMS Terpene Library, 1989; NIST MS, 1998), Kovats retention indices based on a series of *n*-alkanes (C₁₀–C₂₄), and comparison of mass spectra found in the literature. Quantitative analysis (in percent) was performed by peak area normalization measurements [total ion current (TIC)].

Table 3. Biomass Production of Tansy Leaves and Flowers (g/m² DW) under Different Harvest Regimes in Trial Year 2001 (Two Cuts, One in June/July and One in August/September, or One Cut in August)

date	dry wt	genotype					LSD _{5%} ^a
		Steinvik-holmen	Alvdal	Brumunddal	Richters	Goldsticks	
June/July	leaf	379	292	325	390	333	58
Aug/Sept	leaf	88	135	114	106	147	21
	flower		32	7	18	25	7
	sum, leaf	467	427	439	496	480	
Aug	sum, flower		32	7	18	25	
	sum, total	467	459	446	514	505	
	leaf	316	269	315	344	292	ns ^b
Aug	flower	334	388	358	346	455	ns
	sum, total	650	657	673	690	747	

^a LSD, least significant difference ($\alpha = 0.05$). ^b ns, no significant difference.

Table 4. EO Content and Yield of Tansy Leaves and Flowers^a

date		genotype				
		Steinvik-holmen	Alvdal	Brumunddal	Richters	Goldsticks
(A) EO Content (mL/100 g of DW)						
July 6	leaf	0.70	0.20	0.30	0.30	0.10
Aug 9	leaf	0.30	0.10	0.10	0.10	0.23
	flower	1.10	0.38	0.27	0.68	0.83
Sept 12	leaf	0.48	0.20	0.06	0.23	0.34
	flower	0.99	0.28	0.68	0.60	0.73
Oct 2	leaf	0.30	0.08	0.08	0.30	0.30
	flower	0.23	0.38	1.43	0.08	0.30
	av, leaf	0.45a	0.15b	0.14bc	0.23abc	0.24abc
	av, flower	0.77ac	0.35abc	0.79bc	0.45ab	0.62c
	av, sum	1.22	0.50	0.93	0.68	0.86
(B) EO Yield (L/ha)						
Aug 9	leaf	7.5	1.4	1.9	1.7	4.3
	flower	2.6	2.4	0.5	3.9	2.9
	sum	10.1	3.8	2.3	5.7	7.2
Sept 12	leaf	8.6	2.5	1.2	4.1	7.4
	flower	6.9	3.4	5.6	10.6	8.8
	sum	15.5	5.9	6.9	14.7	16.2
Oct 2	leaf	11.3	1.5	2.1	6.4	9.5
	flower	4.5	9.4	24.7	2.6	9.5
	sum	15.8	10.9	26.9	9.0	19.0
	av, leaf	9.1a	1.8b	1.7bc	4.1bd	7.0a
	av, flower	4.7ns	5.1ns	10.3ns	5.7ns	7.1ns
	av, sum	13.8	6.9	12.0	9.8	14.1

^a Data represent average values from 10 plants. Statistical analysis was done by Student's pairwise *t* test; different letters in rows indicate significant differences. ns, no significant difference.

Statistical Analyses. Data from biomass, EO production, and EO composition were subjected to statistical analysis by one-way analysis of variance (ANOVA) with least significance difference (LSD) testing ($\alpha = 0.05$). Additionally, Student's *t* test ($\alpha = 0.05$) was applied on successive sample data from trial year 2000 (Tables 2B and 4).

RESULTS AND DISCUSSION

Field Trials in 2000. Plant Growth and Harvest Regimen. After field establishment in early June 2000, the five tansy genotypes showed variations in their biomass production and the development of vegetative and reproductive plant organs

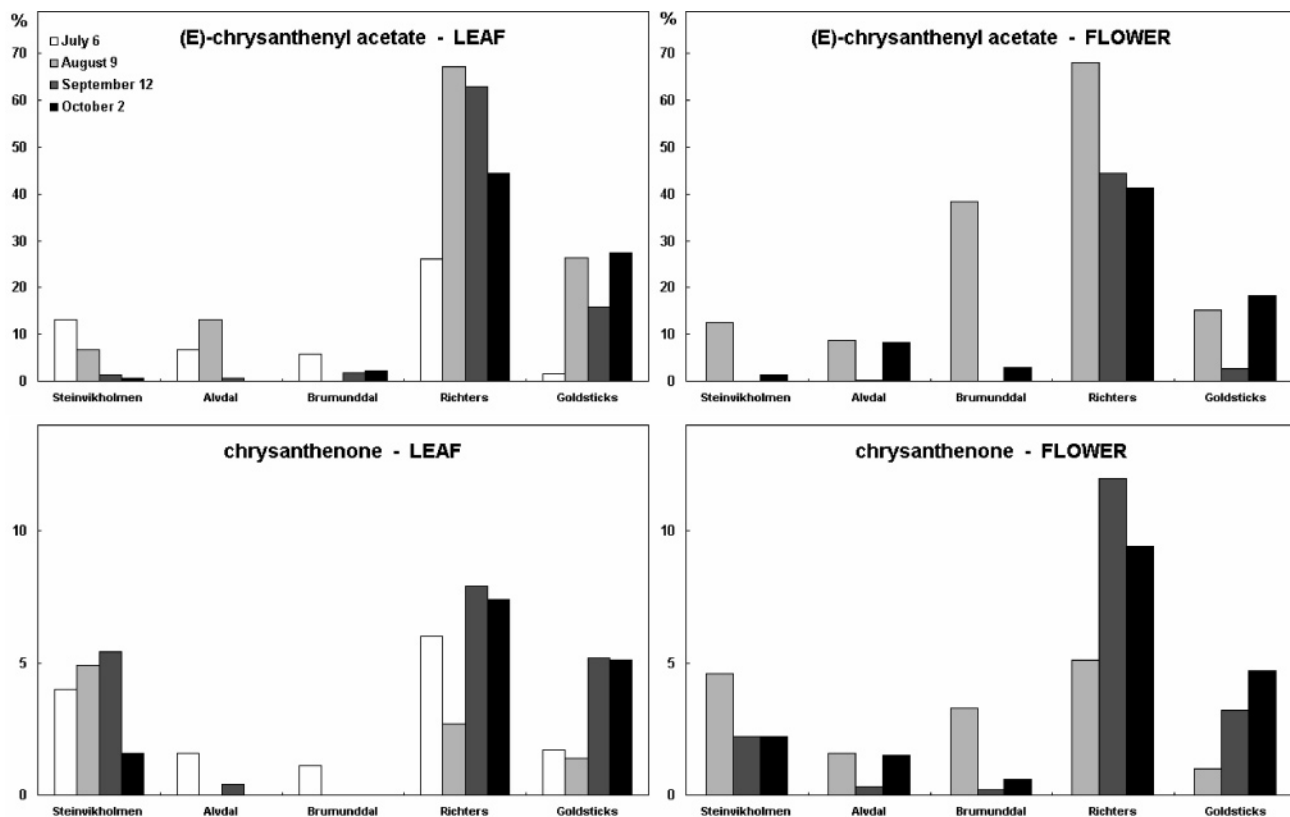


Figure 2. Variation of (E)-chrysanthenyl acetate and chrysanthenone (peak area percent) detected in leaves and flowers of tansy from three harvest dates in trial year 2000. Data represent average values from 10 plants.

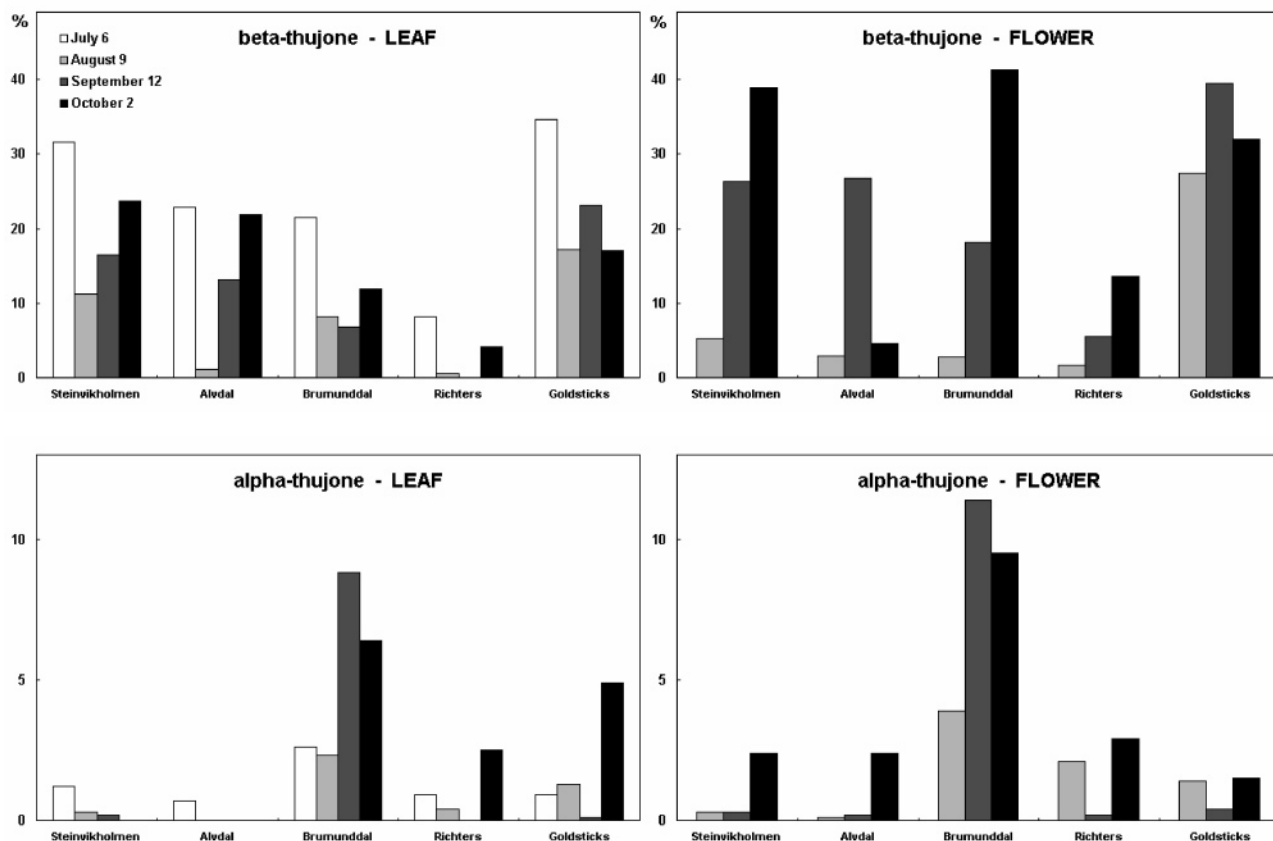


Figure 3. Variation of α - and β -thujone (peak area percent) detected in leaves and flowers of tansy from three harvest dates in trial year 2000. Data represent average values from 10 plants.

(Tables 1 and 2). First, in September, the Canadian genotypes (Richters, 130 cm;

Goldsticks, 145 cm) compared to the Norwegian genotypes (111–127 cm). The genotype Alvdal flowered earlier than all

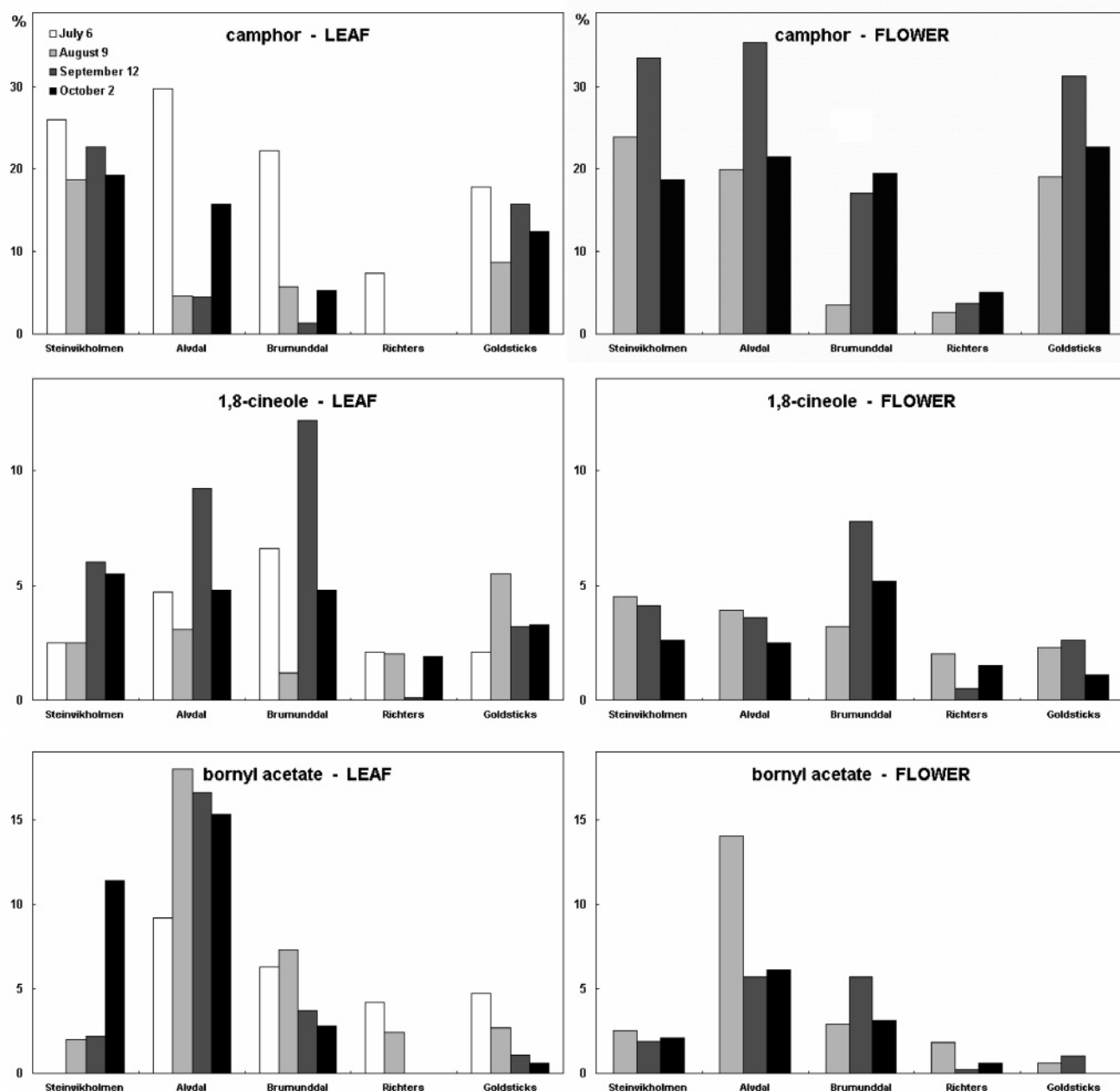


Figure 4. Variation of camphor, 1,8-cineole, and bornyl acetate (peak area percent) detected in leaves and flowers of tansy from three harvest dates in trial year 2000. Data represent average values from 10 plants.

other types at this time point (**Table 1**), whereas vigorous, yellow flowers were still observed for Steinvikholmen, Brumunddal, and Goldsticks in early October. Significantly higher biomass production was recorded for the Canadian genotypes (**Table 2A**). Due to their plant height, relative stem portions >50% were observed in the period from August to September (**Table 2B**). In contrast, the relative portions (sum) of leaves and flowers in the Norwegian genotypes showed higher levels between 52 and 62% compared to the Canadian genotypes (45–54%). Leaf portions generally decreased from August to October and, vice versa, flower portions increased as an effect of plant aging. The relative portions of the stems remained quite stable throughout the season, thus underscoring the importance of solely leaves and flowers for the overall EO production.

EO Yield. The EO content of tansy leaves and flowers was recorded at four harvest dates throughout the 2000 season (**Table 4A**). In the Norwegian genotypes, higher EO levels in leaves were observed in July, whereas the Canadian genotypes showed

increased EO levels in September and October. Highest EO contents were measured in leaves of the genotype Steinvikholmen, with 0.70 mL/100 g of DW. About 3 times higher EO levels were recorded for flowerheads compared to leaves in August and September (all genotypes), with highest EO contents in the Brumunddal genotype. Except for the genotypes Alvdal and Brumunddal, tansy plants showed decreasing EO levels in flowers from August to October. EO accumulation in tansy is limited to the leaves and especially the flowers, whereas stems produce negligible amounts (16), which is reflected in **Table 4B**. Although the Canadian genotypes showed by far the highest biomass production on September 12 (**Table 2A**), the recorded EO yield from leaves and flowers showed similar levels in all genotypes. All genotypes except for Steinvikholmen had on average higher EO yields from flowers compared to the leaves, thus underscoring that high EO levels in leaves might compensate for a lack of biomass production when leaf portions are relatively high and, simultaneously, stem portions are low.

Table 5. EO Content and Yield of Tansy Leaves and Flowers in 2001

harvest		genotype					LSD _{5%} ^a
		Steinvikholmen	Alvdal	Brumunddal	Richters	Goldsticks	
		(A) EO Content (mL/100 g of DW) from First and Second Cuts					
June/July	first cut	0.67	0.37	0.38	0.68	0.58	0.26
Aug/Sept	second cut	0.89	0.59	0.81	0.80	0.79	0.13
	sum, total	1.56	0.98	1.19	1.48	1.37	
Aug	one cut, leaves	0.71	0.44	0.51	0.73	0.57	0.15
	one cut, flowers	0.49	0.22	0.47	0.49	0.60	ns ^b
	sum, total	1.20	0.66	0.98	1.22	1.17	
		(B) EO Yield (L/ha) from First and Second Cuts and When Harvested Only Once at Full Bloom					
June/July	first cut	25.1	10.7	12.1	26.1	19.3	0.84
Aug/Sept	second cut	7.8	7.9	9.1	8.5	11.6	0.17
	sum, total	32.9	18.6	21.3	34.6	30.8	0.80
Aug	one cut, leaves	22.4	11.8	15.9	24.8	16.7	0.64
	one cut, flowers	15.9	9.2	16.9	17.4	27.8	1.10
	sum, total	38.4	21.0	32.9	42.1	44.5	1.58

^a LSD, least significant difference ($\alpha = 0.05$). ^b ns, no significant difference.

Table 6. Distribution of the Most Abundant EO Compounds (Peak Area Percent) of Tansy Leaves and Flowers When Harvested Only Once (at Full Bloom in August) in Trial Year 2001^a

KI ^c	compound	genotype										LSD _{5%} ^b	
		Steinvikholmen		Alvdal		Brumunddal		Richters		Goldsticks		leaf	flower
		leaf	flower	leaf	flower	leaf	flower	leaf	flower	leaf	flower	leaf	flower
1032	α -pinene	1.5	0.9	6.6	2.8	5.4	5.3	1.4	1.0	1.4	1.2	4.4	1.3
1083	camphene	3.5	3.1	3.3	4.2	2.0	4.8	0.1	0.2	4.3	2.9	1.4	2.7
1226	1,8-cineole	7.3	2.4	10.1	5.1	16.4	8.3	0.3	— ^d	3.9	1.0	4.2	2.8
1410	artemisia ketone	—	—	—	—	—	—	8.0	20.7	—	—	—	—
1446	α -thujone	—	—	0.8	—	9.2	11.4	—	—	—	—	—	—
1451	β -thujone	16.8	23.7	9.6	11.1	8.5	9.0	1.1	—	21.9	28.2	ns ^e	ns
1522	chrysanthenone	1.6	1.7	—	—	—	0.3	12.7	11.6	5.0	5.2	—	—
1529	camphor	26.8	33.6	8.4	34.3	0.2	18.2	1.6	0.6	32.3	40.7	9.3	11.3
1565	(<i>E</i>)-chrysanthenyl acetate	—	—	—	—	3.2	—	54.0	58.3	3.0	3.6	—	—
1675	(<i>E</i>)-verbenol	4.6	5.6	0.9	1.1	2.8	2.2	0.8	0.8	1.9	1.9	ns	ns
1680	borneol	8.3	2.3	26.4	15.7	10.4	8.0	0.3	—	0.8	—	5.0	3.8
1690	α -terpineol	1.1	0.3	3.3	2.1	10.1	5.7	0.2	—	0.3	—	4.0	3.1
2205	thymol	—	—	—	—	0.5	—	6.9	0.7	3.3	0.7	—	—

^a Data represent average values from three replications. ^b LSD, least significant difference ($\alpha = 0.05$). ^c Kovats indices on a polar column (CP-Wax 52 CB). ^d —, not detected or not calculated (LSD_{5%}). ^e ns, no significant difference.

EO Composition. The composition of EO obtained from the five tansy genotypes in 2000 showed chemotypical variation (see **Figures 2–4**). All types contained β -thujone as one of the major compounds, with highest average amounts detected in Steinvikholmen, Brumunddal, and Goldsticks (3–6), being also characterized by distinct levels of camphor. Plants from Alvdal and Brumunddal had more complex oil matrices (**Table 6**), and several monoterpenes (α -pinene, camphene) and oxygenated structures [1,8-cineole, bornyl acetate, borneol, and (*E*)-verbenol] could be detected in appreciable amounts. Brumunddal especially contained high average amounts of α -thujone as reported earlier from other genotypes (3, 11). Richters showed high levels of (*E*)-chrysanthenyl acetate, $\geq 50\%$, in both leaf and flower material (3, 4, 6, 8).

The variation of major monoterpene compounds is presented in the **Figures 2–4**. The chrysanthenyl-type compounds of the Canadian genotypes showed especially high concentrations in August, September, and October (**Figure 2**), which is also true for those Norwegian genotypes containing appreciable amounts of these structures. With the exception of early sampling in July (leaves), thujone structures reached highest levels in flowers in

September and October (**Figure 3**) in accordance with earlier reports (7, 11), whereas α -thujone concentrations (Brumunddal) did not exceed 12%. The third main EO constituent, camphor (**Figure 4**), showed high concentrations in the leaves when harvested in July (up to 30%), whereas highest amounts in flowers ($\geq 30\%$) were detected in September in accordance with Czuba and co-workers (17). All camphor-rich genotypes showed also appreciable amounts of 1,8-cineole, reaching levels of over 12 and 7% in leaves and flowers (Brumunddal genotype) in September.

Field Trials in 2001. Plant Growth and Harvest Regimen. In the second trial year, two harvest regimens were investigated to meet conditions of a short summer season typical for Scandinavian agricultural systems. The leaf and flower production was distinctly higher when tansy plants were harvested only once in full bloom (August), compared to two cuts in June/July (budding) and August/September (early bloom) (**Table 3**). Plant raw material from two cuts comprised mainly leaves, with a distinctly lower yield for the second cut (regrowth). In contrast, flower/leaf ratios of ≥ 1 could be observed in all genotypes when harvested only once, with highest flower portions in the

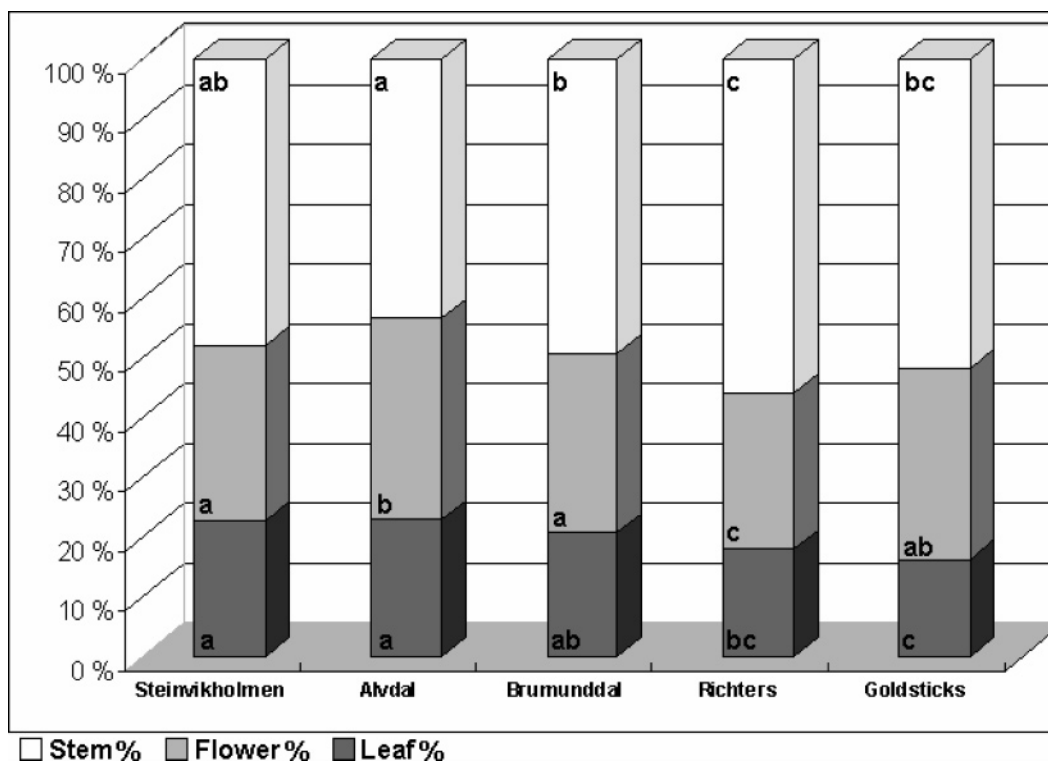


Figure 5. Distribution of FW of stems, flowers, and leaves (percent portion) when harvested only once (at full bloom stage) in trial year 2001. Statistical analysis was done by ANOVA testing; different letters indicate significant differences between the samples ($\alpha = 0.05$).

genotypes Alvdal and Goldsticks (Figure 5). Again, the Canadian genotypes had highest stem portions and, vice versa, lowest leaf portions in the harvested plant material.

EO Yield. Although the EO content increased from the first to the second cut, the EO yield from the second cut was decreased due to a weak regrowth (Table 5). Steinvikholmen, Richters, and Goldsticks were the most EO-productive genotypes under both harvest regimens (Table 5B). Because the biomass production of the Norwegian genotypes showed little variation in 2001 (Table 3), the total EO yield was determined by the EO content of the plant raw material.

EO Composition. Similar chemotypical patterns in EO composition as in 2000 could also be observed under the new harvest regimens in trial year 2001. The genotype Richters showed distinct average amounts of the irregular monoterpene (*E*)-chrysanthenyl acetate and also high levels of the irregular monoterpene artemisia ketone (3, 4, 18–20); up to 21% was detected in our study (Table 6). The genotypes Steinvikholmen, Alvdal, and Goldsticks were characterized by similar levels of β -thujone and camphor. Higher average camphor levels could be detected in the flowers compared to the leaves, and high borneol concentrations could be measured in leaves and flowers of the genotypes Alvdal and Brumunddal. Results from 2001 emphasize the EO characteristics of the investigated genotypes, which can be grouped into the following chemotypes: mixed chemotypes (3, 4, 10) such as Steinvikholmen (thujone–camphor), Alvdal (thujone–camphor–borneol), and Goldsticks (thujone–camphor–chrysanthenyl type) and the most complex EO of the genotype Brumunddal (thujone–camphor–1,8-cineole–bornyl acetate/borneol– α -terpineol). The genotype Richters can be classified as a typical strong chemotype based on the occurrence of a single compound in average concentrations in the leaf and flower, EO $\geq 40\%$ (chrysanthenyl type; 3, 4, 6–8). Although the chemotypical, dominating compounds were detected in both plant organs, varying EO profiles of flower

and leaf oils (Figures 2–4; Table 6) were observed, which is in accordance with results obtained by Holopainen (21).

In conclusion, harvest date and regimen should be based on high biomass production and EO content of leaves and flowers. Variability in tansy EO composition from different plant developmental stages and under different harvest regimens greatly depends on the individual chemotype. Chemotype-determining compounds were detected in leaves and flowers, thus favoring the use of both plant organs for EO production. In contrast to earlier reports (16), a harvest regimen with only one cut in full bloom favors highest EO yield, independent of the chosen genotype under the given environmental conditions, whereas the Canadian genotypes showed highest EO yield. To obtain a standardized oil, one has to rely on the genotypical determination of terpene accumulation when cultivating and harvesting tansy for EO production purposes.

LITERATURE CITED

- (1) Dragland, S.; Senoo, H.; Wake, K.; Holte, K.; Blomhoff, R. Several culinary and medicinal herbs are important sources of dietary antioxidants. *J. Nutr.* **2003**, *133*, 1286–1290.
- (2) Dragland, S. *Reinfann—botanikk, innholdsstoff og dyrking (Tansy—botany, active compounds and cultivation)*; Grønn Forskning 07/2000; Norwegian Crop Research Institute: Kise, Norway, 2000; 22 pp.
- (3) Rohloff, J.; Mordal, R.; Dragland, S. Chemotypical variation of tansy (*Tanacetum vulgare* L.) from 40 different locations in Norway. *J. Agric. Food Chem.* **2004**, *52*, 1742–1748.
- (4) Keskitalo, M.; Pehu, E.; Simon, J. E. Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes. *Biochem. System. Ecol.* **2001**, *29*, 267–285.
- (5) Holopainen, M.; Kauppinen, V. Antimicrobial activity of essential oils of different chemotypes of tansy (*Tanacetum vulgare* L.). *Acta Pharm. Fenn.* **1989**, *98*, 213–219.
- (6) Forsén, K.; Von Schantz, M. Chemotypes of *Chrysanthemum vulgare*. *Nobel Symp.* **1974**, *25*, 145–152.

- (7) Mockute, D.; Judzentiene, A. The myrtenol chemotype of essential oil of *Tanacetum vulgare* L. var. *vulgare* (tansy) growing wild in the Vilnius region. *Chemija* **2003**, *14*, 103–107.
- (8) Hendriks, H.; Van Der Elst, D. J. D.; Van Putten, F. M. S.; Bos, R. The essential oil of Dutch tansy (*Tanacetum vulgare* L.). *J. Essent. Oil Res.* **1990**, *2*, 155–162.
- (9) Héthelyi, É.; Tétényi, P.; Kettens v. d. Bosch, J. J.; Salemink, C. A.; Heerma, W.; Versluis, C.; Kloosterman, J.; Sipma, G. Essential oils of five *Tanacetum vulgare* genotypes. *Phytochemistry* **1981**, *20*, 1847–1850.
- (10) Collin, G. J.; Deslauriers, H.; Pageau, N.; Gagnon, M. Essential oil of tansy (*Tanacetum vulgare* L.) of Canadian origin. *J. Essent. Oil Res.* **1993**, *5*, 629–638.
- (11) Siqueira, N. C. S.; Silva, G. A. A. B.; Bauer, L.; Alice, C. B.; Pinto, A. D. Classification of *Tanacetum vulgare* L. essential oil from Rio Grande Do Sul. *Rev. Brasil. Farm.* **1988**, *69*, 42–45.
- (12) Keskitalo, M. K. Exploring biodiversity to enhance bioactivity in the genus *Tanacetum* through protoplast fusion. Dissertation (Publication 53), University of Helsinki, Department of Plant Production, Section of Crop Husbandry: Helsinki, Finland, 1999; 113 pp.
- (13) Keskitalo, M. K.; Linden, A.; Valkonen, J. P. T. Genetic and morphological diversity of Finnish tansy (*Tanacetum vulgare* L., Asteraceae). *Theor. Appl. Genet.* **1998**, *96*, 1141–1150.
- (14) Németh, É. Z.; Héthelyi, É.; Bernath, J. Comparison studies on *Tanacetum vulgare* L. chemotypes. *J. Herbs Spices Med. Plants* **1994**, *2*, 85–92.
- (15) Bernath, J. *Vadon termö és termesztett gyógynövények*; Mezőgazda Kiadó: Budapest, Hungary, 1993; 566 pp (ISBN 963 816059 4).
- (16) Dobos, J.; Földesi, D.; Zámboi-Németh, É. Experiments for determination the optimum harvesting time of *Tanacetum vulgare* L. *Acta Hort.* **1992**, *306*, 319–323.
- (17) Czuba, W.; Bankowski, C.; Ganszer, W. Variations in the composition of the volatile oil from *Tanacetum vulgare* (tansy) during a year growing time. *Dissert. Pharm. Pharmacol.* **1967**, *19*, 281–288.
- (18) Umlauf, D.; Zapp, J.; Becker, H.; Adam, K. P. Biosynthesis of the irregular monoterpene artemisia ketone, the sesquiterpene germacrene D and other isoprenoids in *Tanacetum vulgare* L. (Asteraceae) *Phytochemistry* **2004**, *65*, 2463–2470.
- (19) Héthelyi, E.; Koczka, I.; Bernáth, J. Chemotaxonomical varieties of *Tanacetum vulgare* L. in Hungary and Canada. *Olaj, Szappan, Kozmetika* **2000**, *49*, 143–148.
- (20) Stahl, E.; Scheu, D. Artemisiaketon als Hauptbestandteil des ätherischen Öles einer neuen Rainfarn-Rasse. *Naturwissenschaften* **1965**, *52*, 394.
- (21) Holopainen, M. Comparison between flowerhead and leaf essential oils in tansy (*Tanacetum vulgare* L.). *Acta Pharm. Fenn.* **1989**, *98*, 101–107.

Received for review December 23, 2004. Revised manuscript received April 9, 2005. Accepted April 10, 2005. Financial funding for the project “Grunnlag for lokal foredling og produktutvikling med norske urter som råvare” (Regional Processing and Product Development based on Norwegian Aromatic Plants) by the Norwegian Industrial and Regional Development Fund (SND) and Research Allocations from the National Agricultural Agreement in the period from 1999 to 2002 is gratefully acknowledged.

JF047817M