Foliar and Emission Composition of Essential Oil in Two Carrot Varieties

P. Kainulainen,*,† J. Tarhanen,‡ K. Tiilikkala,§ and J. K. Holopainen§

Department of Ecology and Environmental Science and Department of Environmental Sciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland, and Agricultural Research Centre of Finland, Plant Protection, FIN-31600 Jokioinen, Finland

The compositions of leaf essential oil of two carrot varieties growing in different growth conditions were studied. About 50 monoterpenoids, sesquiterpenoids, and propenylbenzenes were found. The essential oil compositions, for example, the relative proportions of sabinene and limonene, were significantly different between the varieties. Young leaves contained more propenylbenzenes, methylisoeugenol, and α -asarone than old leaves. There were also significant differences in essential oil composition between leaflets and petioles. Within varieties variation of essential oil composition in individual carrots was detected, which suggests genetical impurity within breeding lines of varieties. There were no significant differences between composition and concentration of compounds in carrot leaves grown in a greenhouse and in an open field. Headspace volatiles collected from carrot leaves contained the same compounds as in leaf extracts, but proportions were different due to different volatilization rates of compounds. These results suggest that there is a high variation in secondary compound composition among different carrot varieties and that the relative abundance of compounds changes during growth, which might have important value in host selection to carrot pests.

Keywords: Daucus carota; terpenoids; propenylbenzenes; plant volatiles; insect-host plant interactions

INTRODUCTION

Volatile constituents are important cues when insect pests orient to crop plants (Bernays and Chapman, 1994). Species-specific combination of volatile terpenes has been identified as an essential host recognition cue for the most important carrot pests, the carrot psyllid (Trioza apicalis Förster) (Nehlin et al., 1996) and the carrot fly (Psila rosae F.) (Guerin, 1983). In addition to the presence of foliar volatiles, leaf color and shape are mostly responsible for orientation to the host plant by the carrot fly (Städler, 1992). The composition of the volatiles released by the Apiaceae species are speciesspecific, and in oviposition tests carrot psyllids deposited few eggs on plants other than carrot (Nehlin et al., 1996). Concentrations of secondary compounds in the leaf surface determine oviposition host selection of the carrot fly (Städler and Buser, 1984). Current knowledge of carrot leaf volatiles and the variation caused by varietal differences and growth condition is rather limited (Senalik and Simon, 1987; Kilibarda et al., 1996). Volatiles of carrot roots (Buttery et al., 1968; Linko et al., 1978; Simon et al., 1980; Simon, 1982a,b; Yoo et al., 1997) and seeds (Seifert et al., 1968) are better known.

The purpose of this paper is to find out how leaf content and emissions of mono- and sesquiterpenoids and propenylbenzenes vary between carrot varieties and how leaf essential oil content varies by plant age between greenhouse-grown and field-grown plants and between different parts of the leaf. The answers to these questions are important to know in the development of pest control methods based on disturbance of insect orientation behavior, because experimental work is mostly conducted with greenhouse-grown plants.

MATERIALS AND METHODS

Plant Material. Two commercial carrot (Daucus carota L. subsp. sativus) varieties (seed from Frökompaniet i Landskrona AB, Sweden), Flakkeer 2 (Grosa) and Nantes 3 Express OE20, were studied. In an open field, carrot seeds were sown on May 18, in a sandy loam soil fertilized with Puutarhan yleislannos (100 kg ha⁻¹, 8:4:14 N:P:K). In a greenhouse carrot seeds were sown in plastic pots (volume = 1.1 L) in a mixture of fertilized peat and sand (2:1, v:v) in May and July, and four carrot seedlings were allowed to grow in each pot. In the greenhouse carrots were fertilized weekly with 0.1% 9-Superex (19:5:20 N:P:K) and watered with tap water as needed. The maximum photon flux density in the shaded greenhouse was \sim 250 μ mol m⁻² s⁻¹, while in the open field photon flux density exceeded 1000 μ mol m⁻² s⁻¹ on sunny days. Carrots in the greenhouse were grown in the natural light cycle of June and July ($\sim 22/2$ h light/dark cycle).

Chemical Analysis of Leaves. Leaflets were collected from 8-week-old carrots grown in a greenhouse and an open field to compare the essential oil composition of two carrot varieties grown in different conditions (seeds sown in May). To compare essential oil composition in younger carrot leaflet and also petiole, samples were collected from 5-week-old carrots grown in a greenhouse (seeds sown in July). Fresh leaf material taken from the same position of the plant was extracted with *n*-hexane for 2 h at room temperature. 1-Chloro-

^{*} Author to whom correspondence should be addressed (telephone +358 17 163193; fax +358 17 163230; e-mail pirjo.kainulainen@uku.fi).

[†] Department of Ecology and Environmental Science.

[‡] Department of Environmental Sciences.

[§] Agricultural Research Centre of Finland.

octane was used as an internal standard. Extracts were analyzed by GC/MS (Hewlett-Packard GC type 6890, MSD 5973) using a 30-m-long HP-5MS (0.25 mm i.d., 0.25 μ m film thickness, Hewlett-Packard) capillary column. Column temperature was maintained at 50 $^\circ\mathrm{C}$ for 2 min and then programmed at 10 °C/min from 50 to 110 °C, at 5 °C/min from 110 to 150 °C, and at 30 °C/min from 150 to 270 °C and held at 270 °C for 5 min. Helium was used as the carrier gas. Mass numbers from m/z 30 to 300 were recorded. The identification of unknown compounds was determined by comparing spectra with those of authentic reference compounds (mostly monoterpenes). When reference material for confirmation was unavailable, compounds were identified by comparison of mass spectra with those in the Wiley and NBS Libraries and by comparison of retention indices based on authentic samples in the literature (Davies, 1990; Elias et al., 1997). The proportional composition of the essential oil of carrots was calculated from the peak areas. For quantitation of some terpenes, calibrations were made using known amounts of available pure terpenes relative to known amounts of the internal standard.

Collection and Analysis of Carrot Volatiles. Carrot volatiles released from foliage were collected from 7- (seeds sown in July) and 12-week-old (seeds sown in May) carrots grown in a greenhouse. After collection of volatiles, the essential oil composition of leaf samples (leaflet and petiole) of each carrot was extracted and analyzed by GC/MS as for other extracts. For volatile sampling, leaves of an individual carrot growing in a pot were surrounded by a plastic bag. Volatiles released from undamaged foliage were collected on Tenax absorbant (150 mg) packed in a glass tube. Airflow was ~90 mL/min, and sampling time was 60 min for 7-week-old carrots and 20 min for 12-week-old carrots. The Tenax tubes were analyzed within 1 week. Compounds absorbed were first released from the Tenax by thermodesorption at 250 °C for 12 min with a nitrogen flow. Desorbed compounds were cryofocused in a cold trap at -50 °C and subsequently analyzed on a 30-m-long HP-5MS (0.25 mm i.d., 0.25 μ m film thickness, Hewlett-Packard) capillary column by GC/MS (Hewlett-Packard GC type 5890, MSD 5970). The temperature program began at 40 °C (2 min) and was raised to 240 °C at 8 °C/min. Mass numbers from m/z 34 to 230 were recorded. The identification of compounds was determined by comparison of mass spectra with those in the Wiley and NBS Libraries.

RESULTS AND DISCUSSION

Carrot leaves contained a complex mixture of monoand sesquiterpenes and propenylbenzenes. Major compounds in leaflets were myrcene, sabinene, (E)- β ocimene, limonene, germacrene D, and (E)- β -caryophyllene (Table 1). Younger leaves contained more propenylbenzenes, methylisoeugenol, and α -asarone than older leaves (Table 1, Figure 1); these compounds were nearly absent in old leaves. This demonstrates that large changes in secondary compound composition occur during the growth of carrots, which might have important value in host selection to carrot pests.

Overall, the composition of carrot leaf secondary compounds has been poorly studied (Senalik and Simon, 1987; Kilibarda et al., 1996). The essential oil composition was significantly different between varieties, but there was also variation in essential oil composition of individual carrots within a variety, which suggests genetical impurity, probably indicating different inbred lines within a variety. However, the Flakkeer variety contained significantly higher proportions of sabinene and lower amounts of limonene than the Nantes variety (Tables 1 and 2). The essential oil composition of wild carrots was significantly different from that of the cultivated carrots reported in the present study. In leaves of wild carrots high proportions of α -pinene,

 γ -terpinene, and camphene and low proportions of myrcene were reported (Kilibarda et al., 1996), whereas in the present study myrcene was the main compound in both cultivated carrot varieties. Similarly, Senalik and Simon (1987) observed significant variation of volatile terpenoids in different genetic stocks of carrots; for example, myrcene was a common compound in two genetic stocks of carrots, but it was nearly absent in two other stocks. Earlier studies have reported only a few sesquiterpenoids in carrot leaves. In the present study several sesquiterpenoids were found and some of them have not previously been reported as constituents of carrot leaves (Senalik and Simon, 1987; Kilibarda et al., 1996) and roots (Buttery et al., 1968; Simon et al., 1980; Yoo et al., 1997). Germacrene D (molecular ion 204; major ions 161, 105, 91, 119, and 81) was identified to be among the most common sesquiterpenes in carrot leafs.

The essential oil profiles were significantly different between leaflets and petioles (Table 3). The essential oil from young petioles contained >50% methylisoeugenol, whereas in leaflets myrcene was the dominating compound. It has been reported that there is a high variation in terpenoid composition and concentration in different parts of carrots (Senalik and Simon, 1987). According to a study by Senalik and Simon (1987), the concentration of myrcene was higher in carrot foliage than in roots and higher concentrations of myrcene was found in petioles than in leaflets. However, we found higher concentrations of myrcene in leaflets (53 μ g/g of fresh weight) than in petioles (26 μ g/g of fresh weight).

There were no significant differences between composition and concentration (Tables 1 and 2) of secondary compounds in carrot leaflets grown in a greenhouse and in an open field. This was slightly unexpected, since secondary metabolites often increase in plant foliage with better light availability (Jansen and Stamp, 1997; Kainulainen et al., 1998). Due to this lack of changes in secondary compound concentrations, the orientation behavior of pest insects could be determined with greenhouse-grown plants and those results would be comparable with plants grown in an open field.

Our data of carrot leaves suggest that the most common compounds found in hexane-extracted leaf samples were among the most common in headspace samples (Figure 1). However, some compounds, such as α -pinene, appeared to be more volatile than others, whereas, for example, sesquiterpenes and methylisoeugenol were not so volatile. The volatilization rate of monoterpenes is dependent on the vapor pressure of terpenes, air humidity, and amount of oil in the plant. Nehlin et al. (1996) reported high proportions of sabinene in headspace samples of an unknown cultivated carrot variety and relatively low concentrations of myrcene, which was the most common compound in the leaf and air samples of carrot varieties studied by us. Sabinene was also one of the main compounds in leaf extracts and headspace samples in the Flakkeer variety (Figure 1). Methylisoeugenol was found only from extracts of young carrot leaves and also in headspace samples; in older carrots it was not found (Figure 1). This observation suggests that other compounds such as propenylbenzenes are responsible for pest and pathogen resistance of older carrots.

Our results suggest that there is a high variation in secondary compound composition among different carrot varieties, which might have important value in host

Table 1. Relative Proportions (Percent) of Mono- and Sesquiterpenes and Propenylbenzenes in Leaflets of Carrots^{*a*} Grown in a Greenhouse (n = 12) and in an Open Field (n = 10)

		greenhouse			4	ïeld	
	5-week-old 8-week-old		eek-old	8-week-old			
compound	\mathbf{RRT}^{b}	F	Ν	F	N	F	Ν
monoterpenes							
α -pinene (C ₁₀ H ₁₆)	0.736	2.0	1.9	3.9	4.6	3.3	5.3
unknown C ₁₀ H ₁₆	0.746	_ <i>c</i>	_	tr^d	tr	0.1	tr
unknown $C_{10}H_{16}$	0.756	_	_	tr	0.1	0.4	0.5
camphene ($C_{10}H_{16}$)	0.769	0.2	0.2	0.4	0.4	0.3	0.3
sabinene $(C_{10}H_{16})$	0.820	4.5	3.5	12.7	6.9	13.5	2.4^{**e}
β -pinene (C ₁₀ H ₁₆)	0.829	0.6	0.7	1.6	1.2	1.3	0.7**
myrcene ($C_{10}H_{16}$)	0.854	35.8	28.6	25.7	44.6***	35.4	35.3
limonene ($C_{10}H_{16}$)	0.936	4.8	5.3	3.6	8*	3.8	6.9
unknown ($C_{10}H_{16}$)	0.947	0.1	tr	tr	0.1	$^{-}_{2.1}$	_ 2 0
(Z)- β -ocimene (C ₁₀ H ₁₆) ^f (E)- β -ocimene (C ₁₀ H ₁₆) ^f	0.951 0.973	1.9 11.3	1.2 8.2	1.4 9.2	1.6 8.0	2.1 10.8	2.0 10.8
(E) - β -oclimente $(C_{10}H_{16})^{\beta}$ unknown $C_{10}H_{16}$	0.985	-	0.2 _	9.2 tr	0.2	10.8	10.8
unknown $C_{10}H_{16}$ unknown $C_{10}H_{16}$	1.060	1.4	tr	0.8	0.2	_	_
α -terpinolene (C ₁₀ H ₁₆)	1.074	0.3	0.1	tr	0.3	0.8	0.6
linalool ($C_{10}H_{16}$)	1.080	tr	tr	0.1	0.1	0.3	0.0
allo-ocimene ($C_{10}H_{16}$)	1.145	0.3	0.2	0.1	0.1	0.4	0.3
bornyl acetate $(C_{10}T_{16})$	1.532	0.3	0.2	0.1	0.1	0.4	0.3
α -terpinene (C ₁₀ H ₁₆) ^{<i>f</i>}	1.664	tr	tr	0.3	0.1	0.2	tr
sesquiterpenes and propenylbenzenes	1.001	ti -	CI .	0.2	0.1	0.1	CI .
δ -elemene (C ₁₅ H ₂₄) ^{<i>f</i>}	1.670	0.4	0.4	0.4	_	1.6	0.5
α -copaene (C ₁₅ H ₂₄)	1.778	tr	tr	tr	_	0.2	0.1
unknown $C_{15}H_{24}$	1.816	tr	tr	tr	tr	0.2	0.1
unknown $C_{15}H_{24}$	1.819	tr	tr	tr	tr	tr	tr
methyleugenol $(C_{11}H_{14}O_2)^f$	1.841	1.2	tr	2.1	tr	_	tr
unknown C ₁₅ H ₂₄	1.852	0.1	tr	tr	_	tr	0.4
unknown C ₁₅ H ₂₄	1.866	tr	tr	tr	tr	_	_
β -bergamotene (C ₁₅ H ₂₄) ^f	1.879	0.8	0.8	0.7	0.3*	0.4	0.4
(<i>E</i>)- β -caryophyllene (C ₁₅ H ₂₄)	1.900	4.8	5.6	5.4	3.7	3.9	8.0**
β -cubebene (C ₁₅ H ₂₄)	1.925	tr	tr	0.2	tr	0.3	0.3
α -bergamotene (C ₁₅ H ₂₄) ^{<i>f</i>}	1.934	0.9	1.0	0.9	0.5	0.5	0.5
unknown C ₁₅ H ₂₄	1.954	0.2	0.4	0.2	_	0.2	0.2
unknown C ₁₅ H ₂₄	1.964	tr	tr	0.1	_	0.2	0.2
unknown C ₁₅ H ₂₄	1.991	1.8	1.9	2	0.9*	1.1	1.5
β -farnesene (C ₁₅ H ₂₄) ^{<i>f</i>}	1.999	1.5	1.8	1.6	0.8	0.8	0.8
unknown C ₁₅ H ₂₄	2.019	_	_	tr	-	0.1	tr
germacrene D $(C_{15}H_{24})^{f}$	2.061	3.6	3.2	12.4	8.3	9.8	10.9
β -selinene (C ₁₅ H ₂₄) ^{<i>f</i>}	2.072	3.0	tr**	4.1	tr**	0.8	tr
(<i>E</i>)-methylisoeugenol $(C_{11}H_{14}O_2)^f$	2.083	7.2	23.3*	3.3	0.6	2.2	3.8
α -selinene $(C_{15}H_{24})^f$	2.089 2.095	1.9	0.4	2 tr	1.9 0.8	0.6 0.3	0.2 0.1
unknown C ₁₅ H ₂₄ unknown C ₁₅ H ₂₄	2.101	0.1 0.1	tr 0.8*	tr tr	0.8	0.3	0.1
β -bisabolene (C ₁₅ H ₂₄) ^{<i>f</i>}	2.101	2.9	2.0	tr 1.2	0.5	0.3	0.5
unknown $C_{15}H_{24}$	2.100	0.1	0.1	tr	0.5	tr	tr
unknown $C_{15}H_{24}$ unknown $C_{15}H_{24}$	2.127	0.1	0.1	0.4	0.2	0.3	0.3
γ -bisabolene (C ₁₅ H ₂₄) ^{<i>f</i>}	2.145	tr	tr	0.1	tr	0.1	0.1
(Z) - α -bisabolene $(C_{15}H_{24})^{f}$	2.145	2.2	1.4	1.5	2	1.6	0.7
unknown $C_{15}H_{24}$	2.192	tr	tr	tr	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.1	0.1
unknown $C_{15}H_{26}O$	2.208	0.2	tr	0.1	0.1	0.1	tr
longiborneol $(C_{15}H_{24})^f$	2.237	tr	1.9**	tr	2.4*	0.1	2.0
β -asarone (C ₁₂ H ₁₆ O ₃) ^{<i>f</i>}	2.254	tr	tr	tr	tr	tr	tr
unknown $C_{15}H_{24}$	2.278	0.2	tr	0.1	0.1	0.1	0.1
α -asarone $(C_{12}H_{16}O_3)^f$	2.306	0.9	3.1	0.4	0.3	0.1	0.2
α -bisabolol (C ₁₅ H ₂₆ O) ^f	2.314	0.4	0.4	0.6	0.4	0.4	0.5
	-						

*^a*Varieties: F, Flakkeer 2; N, Nantes 3 Express. *^b* RRT, relative retention time. *^c* –, not found. *^d* tr, trace compound (<0.1%). *^e**, *p* < 0.05; **, *p* < 0.01; ***, *p* < 0.001 (*t* test). *^f* Identified by comparison to Wiley and NBS Libraries and by comparison of retention indices based on authentic samples in the literature.

selection to carrot pests. Oviposition preference of the carrot rust fly to carrot varieties varies greatly. Kettunen et al. (1988) proposed that low numbers of eggs laid on the variety Sytan may be due to lower amounts of oviposition stimulants, such as methylisoeugenol (Städler and Buser, 1984), emanating from this slowgrowing cultivar. The carrot psyllid (*Trioza apicalis*) is attracted to Apiacea species that release large amounts of α -pinene and sabinene, whereas plants releasing high amounts of limonene had low preference (Valterova et al., 1997). Sabinene was typical to the Flakkeer variety, whereas the Nantes variety contained more limonene. In addition to the terpenoids and propenylbenzenes, other secondary compounds such as phenolic compounds might have important value in pest attractivity and resistance of carrots. Cole (1985) observed that chlorogenic acid was the main phenolic compound in the peel of carrot roots. Concentrations were higher in cultivars that were more susceptible to carrot fly larval damage.

Attractive and deterring terpenoids may serve as a guide for the creative design of safe, environmentally compatible control methods for destructive pests of many agricultural crops as concluded by Binder and

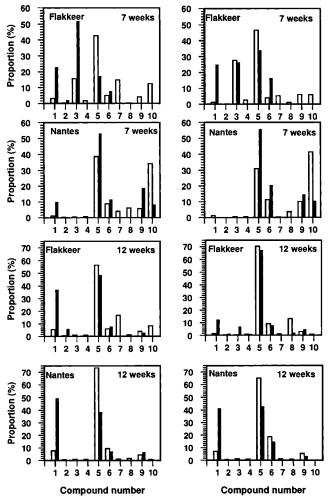


Figure 1. Relative proportions of most common secondary compounds in hexane extracts of carrot leaves (white bars) and in headspace samples (black bars) of the same carrot plant. The variety and plant age are given in each panel. 1, α -pinene; 2, camphene; 3, sabinene; 4, β -pinene; 5, myrcene; 6, limonene; 7, (*E*)- β -ocimene; 8, α -terpinolene; 9, (*E*)- α -caryophyllene; 10, methylisoeugenol.

Table 2. Concentrations (Micrograms per Gram of Fresh Weight) of Some Mono- and Sesquiterpenes in Leaflets of Carrot^a Grown in a Greenhouse (n = 12) and in a Field (n = 10)

	greenhouse				field	
	5-week-old		8-week-old		8-week-old	
compound	F	Ν	F	Ν	F	Ν
monoterpenes						
α-pinene	3	4	7	10	9	19
sabinene	7	14	38	17* ^b	56	14**
β -pinene	1	2	1	3	2	1
myrcene	62	57	71	97	103	92
limonene	10	12	11	20	14	14
total	84	90	131	146	185	141
sesquiterpene						
(E) - β -caryophyllene	6	7	11	6	7	15*

 a Varieties: F, Flakkeer 2; N, Nantes 3 Express. b Significance: *, p < 0.05; **, p < 0.01 (t test).

Robbins (1997). Crop plant varieties that release compounds that do not attract pest species but do attract their natural enemies might be the best suitable for cultivation. Push-pull strategy (Pickett et al., 1997) might be done also by planting attractive plants in the field surroundings or spraying certain deterring compounds on crop plants. One problem is the different

Table 3. Relative Proportions (Percent) of Mono- and Sesquiterpenes and Propenylbenzenes in Leaflets and in Petioles of 5-Week-Old Carrots (Nantes 3 Express) (n = 5)

compound	RRT ^a	leaflet	petiole
monoterpenes			
α-pinene	0.736	1.8	1.8
camphene	0.769	0.3	0.1* ^b
sabinene	0.820	3.2	3.1
β -pinene	0.829	0.6	0.5
myrcene	0.854	26.5	9.9*
limonene	0.936	4.4	5.1
(Z)- β -ocimene	0.951	1.7	tr*** <i>c</i>
(E)- β -ocimene	0.973	11.6	0.6***
α-terpinolene	1.074	tr	0.1
allo-ocimene	1.145	0.3	tr***
bornylacetate	1.532	0.9	0.4
sesquiterpenes and propeny	lbenzenes		
δ -elemene	1.670	0.1	tr
unknown C ₁₅ H ₂₄	1.852	tr	0.1
β -bergamotene	1.879	0.8	1.3
(E)-α-caryophyllene	1.900	6.6	4.0*
α-bergamotene	1.934	1.0	1.8
unknown C ₁₅ H ₂₄	1.954	0.5	0.6
unknown C ₁₅ H ₂₄	1.964	1.7	3.4
β -farnesene	1.999	1.9	3.3
germacrene D	2.061	3.2	0.7
methylisoeugenol	2.083	19.7	55.3**
α-selinene	2.089	0.1	tr
unknown C ₁₅ H ₂₄	2.095	tr	0.3
unknown C ₁₅ H ₂₄	2.101	1.2	1.5
β -bisabolene	2.106	1.9	2.5
unknown C ₁₅ H ₂₄	2.127	0.1	0.1
unknown C ₁₅ H ₂₄	2.132	0.4	0.8*
γ -bisabolene	2.145	tr	0.1
(Z)-α-bisabolene	2.158	1.2	1.0
longiborneol	2.237	1.4	0.2
β -asarone	2.254	0.3	0.1
unknown C ₁₅ H ₂₄	2.278	0.1	tr
α-asarone	2.306	5.8	0.7*
α-bisabolol	2.314	0.5	0.7
a RRT = relative retetion	time ^b Signifi	cance [,] * <i>n</i>	< 0.05. **

^{*a*} RRT = relative retetion time. ^{*b*} Significance: *, p < 0.05; **, p < 0.01; ***, p < 0.001 (*t* test). ^{*c*} tr, trace compound, <0.1%.

host selection strategy of the specialist and generalist insects (van der Meijden, 1996). The same compounds that act as defense compounds for many polyphagous pests usually serve as feeding and oviposition attractants for specialist herbivores of the same plant species (Städler and Buser, 1984; Nehlin et al., 1996). On the other hand, changing the ratio of attractive and deterring secondary metabolites in carrots by plant breeding may lead to reduced quality of carrots for consumers due to their harsh flavor (Yoo et al., 1997).

ACKNOWLEDGMENT

We thank the staff of the Research Garden of the University of Kuopio for growing the carrots and Pertti Pasanen for the advice in analyses of carrot volatiles. This publication is a part of the research program of Biologically Active Molecules coordinated by Agricultural Research Centre of Finland.

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Received for review February 5, 1998. Revised manuscript received May 11, 1998. Accepted June 2, 1998.

JF980108M