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COMPARISON OF PHENOLIC COMPOUNDS FROM GALLS AND SHOOTS OF *PICEA GLAUCA*

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Key Word Index—*Picea glauca*; Pinaceae; white spruce; *Adelges abietis*; pineapple gall; quantification; GC-MS; phenolics; glucosides; lignans.

Abstract—The influence of the gall aphid *Adelges abietis* on the metabolism of phenolic compounds was studied by comparing extracts of spruce shoots and galls of the same tree during one vegetation period. Phenolics and phenolic glucosides, 47 in total, were identified and quantified as their trimethylsilylated derivatives by GC-mass spectrometry. Shoots contained two to ten times more free phenolics than galls. Their amount increased over the vegetation period. The main compounds, beside cinnamic acids, were acetophenone derivatives in shoots and 4-hydroxyphenylpropionic acid in galls. The amount of phenolic glucosides in shoots exceeded that in galls by a factor of 60 to 100. Shoots contained preferentially acetophenone glucosides. Derivatives of cinnamic alcohol glucosides prevailed in galls. An accumulation of lignans and a decrease of cinnamic alcohol glucosides started in galls when larvae began hatching. In shoots lignans were present only in small amounts. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Biotic or abiotic stress is known to increase the production of phenolic compounds in plants [1]. Phenolics are known to be involved in defence reactions, probably due to their toxic and tanning properties. In addition the *de novo* synthesis of phytoalexins, a large number of which are phenolics, is an important response of plants to stress [2].

In spruce, one of the major classes of secondary metabolites is phenolic compounds. The effect of stress on the metabolism of some of these substances has already been investigated. In most studies piceol (**1**) and picein (**1a**), the main components of the phenolic fraction in spruce, were quantified [3, 4]. The ratio of **1**:**1a** was proposed as a chemical indicator for plant stress [5]. Richter and Wild [6] reported a significantly higher level of catechin, epicatechin and piceatannol-glucoside in needles of damaged trees compared to those of healthy ones. Accumulation of lignans was observed in sapwood of *Picea abies* infected by the fungus *Fomes annosus* [7].

In the studies mentioned above stress was achieved by infection with fungi, infection with bacteria or by fumigation with ozone. In contrast, galls are local responses of plants to well defined gall formers.

The eastern spruce gall aphid *Adelges abietis* initiates the formation of pineapple galls in spruce. Microscopic investigations [8] revealed that a hypersensitive response in combination with an accumulation of phenolics and lignin is not triggered by the feeding activity of the larvae. Only the distal parts of the gall became enriched with phenolic compounds and the tissue along the shoot and leaf axes becomes lignified [9]. These observations describe visible changes of gall cells, but up to this point the phenolic constituents of spruce galls have not been identified.

In order to obtain a better understanding of the changes in plant metabolism following gall formation, we have investigated and quantified the phenolic compounds and their glucosides in extracts of galls and shoots of *Picea glauca*.

RESULTS AND DISCUSSION

Since galls of *A. abietis* consist of modified needles, bases of needles and cortical tissue [9], extracts of galls were compared with those of complete shoots. Samples were harvested throughout one vegetation period, taking the start of the curving of the new shoots, which indicates the formation of a gall, as zero time. The phenolic compounds of galls and shoots were obtained from the EtOAc extracts. The glucosides were present in the *n*-BuOH extracts. Their aglycones were analysed after enzymic hydrolysis with β-glucosidase [10].

A number of the phenolics were identified by measurement of reference compounds (**1**–**13** and **19**). The

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phenolics **16–18** were identified by comparing the mass spectra of their trimethylsilylated derivatives with those reported in the literature [11]. Identical mass spectra together with identical retention indices on GC were regarded as proof of structure.

The mass spectra of lignans **20–23**, **26** and **27** were published earlier by Ekman [12], and Kraus and Spiteller [11].

Structures of the compounds **14**, **15**, **24**, **25** and **28–30** were deduced from their mass spectra by comparing their fragmentation patterns with those of known compounds by use of Biemann's shift rule [13].

The lignan **24** is a side chain hydrogenated derivative of **23**. Consequently, the molecular ion of its trimethylsilylated derivative was increased by 2 amu compared to that of trimethylsilylated **23**. All peaks corresponding to the 3-methoxy-4-trimethylsilyloxyphenyl ring (e.g. the ion $m/z = 209$) were found at identical masses. The saturated side chain favoured, in comparison to **23**, a loss of the $-CH_2OTMSi$ group, leading to the ion $m/z = 473$ $[M - 103]^+$ which is more intensive than the corresponding fragment at $m/z = 471$ in **23**.

Compound **25** differed from **24** by replacement of a $-OCH_3$ group with an –OTMSi group. The ion $m/z = 209$ indicated the presence of a 3-methoxy-4-trimethylsilyloxy substitution at the phenyl ring. Therefore the second –OTMSi group is localized in the dihydrobenzofuran moiety.

The trimethylsilylated lignan aglycones **29** (Fig. 1) and **30** showed in their mass spectra a base peak at $m/z = 209$, generated by benzylic cleavage. Another important ion was found at $m/z = 324$. It is generated by elimination of part B in a McLafferty type rearrangement by hydrogen shift with the charge remaining in part A of the lignan. In addition, the cleavage of the β-arylether bond combined with transfer of a $-SiMe_3$ group to part B ($m/z = 326$; **29** and $m/z = 384$; **30**) indicated the structure of part B.

An analogous fragmentation was observed in the mass spectrum of the trimethylsilylated aglycon **28**. The shift of the ion $m/z = 209$ to $m/z = 297$ indicated the presence of a trimethylsilyloxy group at the benzylic position of ring A. Again the ion at $m/z = 384$ established the substituents in the B ring system.

The EI-mass spectra of trimethylsilylated phenolic glucosides usually show molecular ions and fragments indicating the glucose residue ($m/z = 450$, 361, 271, 217, 204, 147 and 103 [14]). In addition, the spectra are characterized by an ion corresponding to the mass of the trimethylsilylated aglycon. Specific fragmentation of the aglycon is rarely observed.

Therefore, a part of the glucoside fraction, that did not contain any free aglycones, was subjected to enzymic hydrolysis with β-glucosidase, an enzyme that exhibits a high degree selective for the cleavage of β-glucosides. The aglycones were then identified as mentioned above.

The position of the β-glucose residue in the aglycones was determined in an additional experiment. The glucosides were methylated with CD_3I/Ag_2O [15]. Thus, the free hydroxyl groups were converted to deuteromethyl ethers. Subsequent hydrolysis of the glucosidic bond with 2 N TFA produced a molecule containing a hydroxyl group at the former position of the glucose residue. After trimethylsilylation, the position of $-OCD_3$ and –OTMSi functions became evident by an appropriate shift in the mass spectra with respect to the non-methylated samples (examples are given in the Experimental section). This allowed the location of the –OTMSi function, indicating the position of the glucose residue in either the aromatic or the aliphatic part of the aglycon.

Although **3a** and **3b** showed identical mass spectra after this experiment, a distinction was possible by measuring the GC R_I values, as the retention indices of 3-methoxy-4-trimethylsilyloxyacetophenone and 3-[d_3]-methoxy-4-trimethylsilyloxyacetophenone are very close, whereas the retention index of 4-[d_3]-methoxy-3-trimethylsilyloxy diverged from it.

In Table 1, the structures of identified phenolics and lignans are listed by compound classes. A great number of these compounds have already been detected in

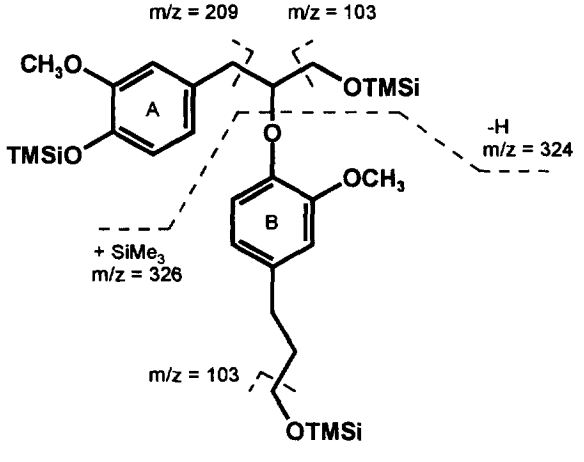


Fig. 1. Fragmentations in the mass spectrum of **29**.

various species of *Picea*: **1–3** [16]; **4, 5, 7, 10, 11, 13** [17]; **6, 9, 12** [18]; **18a, 19a, 22, 26, 27** [19]; **20, 24, 24a, 25, 25a, 28a** [20]; **21, 26** [21]; **23** [22].

Qualitative differences between the identified compounds in galls and shoots were not found, with the exception of some lignans (**21–23**) which were only found in mature galls.

3-Hydroxy-4-methoxybenzy alcohol, absent in spruce, was used as internal standard for the quantification of phenolics. Glucosides were quantified after enzymic hydrolysis relative to salicin. These measurements revealed large differences in the content of free phenolic (Table 2) and phenolic glucosides (Table 3) in galls and shoots.

In shoots, the content of most phenolics increased continuously over the vegetation period. This corresponds to studies made by Strack *et al.* [23], who observed an analogous accumulation pattern of phenolics in needles of *P. abies*. Along with the amount of free phenolics, the content of phenolic glucosides increased over the investigated period in shoots. The ratio of glucosides to free phenolics turned out to be on average about 60:1.

In addition to the total amount of phenolics and their glucosides the percentage of each class of compound is indicated in Tables 2 and 3.

Acetophenones are the most important phenolics in spruce shoots. During the vegetation period their amounts, especially that of piceol (**1**), increased rapidly. By mid-August almost 70% of the phenolics in shoots were acetophenones. Acetophenone glucosides were the main constituents in the glucosidic fraction of shoots (90%). Surprisingly not **1a** (picein) but **3a**, a further ring hydroxylated product, was the most abundant acetophenone glucoside present.

Cinnamic acid and its derivatives, the precursors of the compounds discussed in this paper, were the main components in shoots at the beginning of the vegetation period ($>90\%$). The percentage of cinnamic acids decreased later in the year in favour of other phenolic compounds. Dihydrocinnamic acids, dihydocinnamic alcohols, cinnamic alcohols, benzoic acids and benzaldehydes as well as their glucosides contributed only very little to the total phenolic and glucoside content in shoots (Tables 2 and 3).

Lignans, dimeric products of cinnamic alcohols, were present in shoots only in trace amounts until mid-June (5 weeks; Table 4). Then a slight increase of compounds **24–27** was noticed. The amount of lignan glucosides increased continuously in shoots and was 120–270 times higher than that of free lignans.

Comparison of the accumulation pattern of phenolics and their glucosides in spruce shoots with that in spruce galls revealed remarkable differences: Starting from week 4 the total amount of phenolics in shoots exceeded that in galls by a factor of two to ten. In contrast to shoots the amount of phenolics remained nearly constant at this low level in galls over the vegetation period. When larvae had left the gall (after the 17th week) the content of phenolics in the galls increased considerably, too. Variations in the time-dependent measurements of phenolics in galls might be due to the fact that galls were collected from sites all over the tree, whereas shoots were taken always from the same branch. It is known from the literature [24] that the total content of phenolics depends on the part of the tree from which the needles are taken.

Quantification of the phenolic glucosides in galls and shoots revealed even more striking differences than the comparison of the free phenolic aglycones (Table 3). At the beginning of the sampling period the total amount of phenolic glucosides in shoots already exceeded that in galls by a factor of 30. This difference increased over time, since the content of glucosides increased faster in shoots than in galls. When the larvae had left the gall (week 16) a sudden drop in the total content of phenolic glucosides in galls occurred. At this time the amount in galls was only 1/100 of that in shoots.

In addition, the ratio glucosides to free phenolics was lower in galls (about 4:1) compared to shoots (about 60:1). We are not able to distinguish whether this is due to an inhibited conjugation reaction in galls or whether hydrolytic enzymes in the saliva of larvae [25] cleave glucosides.

The data presented in Tables 2 and 3 indicate not only considerable differences in the total content of phenolics and glucosides but also a remarkable shift in the percentage of compound classes in galls and shoots: The main phenolic in galls was 4-hydroxyphenylpropionic acid (**14**) next to the derivatives of cinnamic acids. The presence of **14** in *P. glauca* has not been described so far. In shoots, this compound occurred in negligible amounts. When galls turned yellow and chambers were opening the content of **14** dropped suddenly.

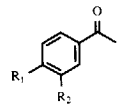
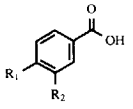
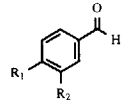
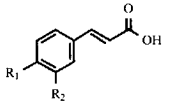
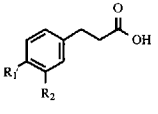
When galls were inhabited and growing the portion of acetophenones was always lower than 5%, whereas these compounds were the main phenolics in shoots (up to 70%). When larvae had left the galls a sudden rise of the proportion of acetophenones was observed. The proportion of acetophenone glucosides in galls decreased continuously from 70 to 30% of total glucosides. This is also in contrast to shoots.

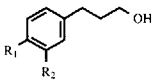
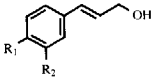
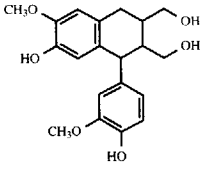
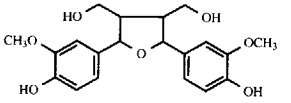
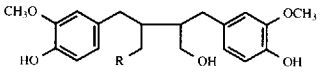
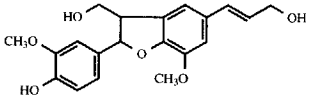
About 50% of total phenolic glucosides of mature galls consisted of cinnamic alcohols **18a** and **19a** (shoots: lower than 3%). During and after hatching, the portion dropped again to 30%. The decrease in the amount of total phenolic glucosides in galls after the 16th week was caused mainly by this decline in **18a** and **19a**.

As cinnamic alcohols which were not found as free aglycones, and their glucosides are precursors of lignans this result is in accordance with the strong accumulation of lignans. Compounds **20**, **21**, **23** and **26** were found only as free lignan aglycones, whereas **28a–30a** were present only as lignan glucosides.

In galls, the content of lignans increased rapidly. Mature galls contained about 17 times more dilignols than shoots. An even higher level of lignans was

Table 1. Identified phenolics, lignans and their glucosides from shoots and galls of *P. glauca*

Compound		No.
Acetophenones		
	$R_1 = OH, R_2 = H$	**1**
	$R_1 = OGluc, R_2 = H$	**1a**
	$R_1 = OH, R_2 = OCH_3$	**2**
	$R_1 = OGluc, R_2 = OCH_3$	**2a**
	$R_1 = R_2 = OH$	**3**
	$R_1 = OGluc, R_2 = OH$	**3a**
	$R_1 = OH, R_2 = OGluc$	**3b**
Benzoic acids		
	$R_1 = R_2 = H$	**4**
	$R_1 = OH, R_2 = H$	**5**
	$R_1 = OGluc, R_2 = H$	**5a**
	$R_1 = OH, R_2 = OCH_3$	**6**
	$R_1 = OGluc, R_2 = OCH_3$	**6a**
	$R_1 = R_2 = OH$	**7**
	$R_1 = OGluc, R_2 = OH$	**7a**
Benzaldehydes		
	$R_1 = OH, R_2 = H$	**8**
	$R_1 = OH, R_2 = OCH_3$	**9**
Cinnamic acids		
	$R_1 = R_2 = H$	**10**
	$R_1 = OH, R_2 = H$	**11**
	$R_1 = OGluc, R_2 = H$	**11a**
	$R_1 = OH, R_2 = OCH_3$	**12**
	$R_1 = OGluc, R_2 = OCH_3$	**12a**
	$R_1 = OGluc, R_2 = OH$	**13a**
Dihydrocinnamic acids		
	$R_1 = OH, R_2 = H$	**14**
	$R_1 = OGluc, R_2 = H$	**14a**
	$R_1 = OH, R_2 = OCH_3$	**15**
	$R_1 = OGluc, R_2 = OCH_3$	**15a**

Compound		No.
Dihydrocinnamic alcohols		
	$R_1 = OGluc, R_2 = H$	**16a**
	$R_1 = OH, R_2 = OCH_3$	**17**
	$R_1 = OGluc, R_2 = OCH_3$	**17a**
Cinnamic alcohols		
	$R_1 = OGluc, R_2 = H$	**18a**
	$R_1 = OGluc, R_2 = OCH_3$	**19a**
Lignans		
		**20**
		**21**
	R = OH	**22**
	R = OGluc	**22a**
		**23**

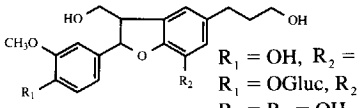
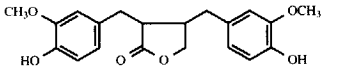
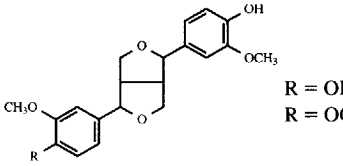
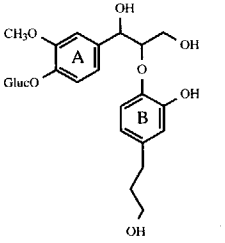
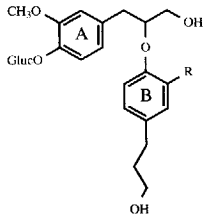
Compound		No.
	$R_1 = OH, R_2 = OCH_3$	**24**
	$R_1 = OGluc, R_2 = OCH_3$	**24a**
	$R_1 = R_2 = OH$	**25**
	$R_1 = OGluc, R_2 = OH$	**25a**
		**26**
	R = OH	**27**
	R = OGluc	**27a**
		**28a**
	$R = OCH_3$	**29a**
	R = OH	**30a**

Table 2. Amounts of phenolics (nmol g^{-1} dry wt) from shoots and galls of *P. glauca*

	Age of galls (weeks)													
	1		4		5		7		11		16		17	
Compound	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall yellow**	Shoot	**Gall left**
Acetophenones (%)	(—*)	**(—)**	*(10.8)*	***(4.6)***	*(12.8)*	***(2.0)***	*(15.3)*	***(2.9)***	*(19.0)*	***(3.1)***	*(72.0)*	***(3.7)***	*(71.5)*	***(36.9)***
1	tr.†	**tr.**	4	**0.5**	7	**0.5**	10	**0.4**	15	**0.6**	224	**0.4**	228	**27**
2	—‡	—	0.4	**0.1**	1	**0.1**	1	**0.2**	2	**0.1**	17	**0.2**	18	**0.7**
3	tr.	**tr.**	1	**0.2**	2	**0.3**	2	**0.5**	4	**0.4**	35	**0.5**	36	**12**
Benzoic acids (%)	*(4.6)*	***(0.7)***	*(2.0)*	***(12.6)***	*(1.7)*	***(4.5)***	*(3.8)*	***(5.0)***	*(4.5)*	***(9.8)***	*(3.7)*	***(15.0)***	*(4.4)*	***(23.4)***
4	0.1	**0.2**	0.4	**0.7**	1	**0.7**	2	**0.6**	3	**2**	9	**3**	10	**20**
5	tr.	**tr.**	0.2	**tr.**	0.1	**0.1**	0.6	**0.1**	1	**0.3**	4	**0.4**	5	**3**
6	0.8	**0.2**	0.3	**0.9**	0.1	**0.6**	0.4	**0.7**	0.3	**0.4**	1	**0.5**	2	**2**
7	tr.	**tr.**	0.1	**0.6**	0.1	**0.6**	0.2	**0.5**	0.7	**0.7**	0.2	**0.6**	0.2	**0.2**
Benzaldehydes (%)	*(3.1)*	***(0.9)***	*(1.0)*	***(2.9)***	*(0.6)*	***(1.4)***	*(1.4)*	***(1.3)***	*(1.1)*	***(2.6)***	*(0.3)*	***(3.6)***	*(0.3)*	***(4.1)***
8	tr.	**tr.**	tr.	**tr.**	tr.	**tr.**	0.3	**tr.**	0.2	**0.4**	0.3	**0.3**	0.2	**0.4**
9	0.6	**0.5**	0.5	**0.5**	0.5	**0.6**	0.9	**0.5**	1	**0.5**	0.8	**0.8**	0.8	**4**
Cinnamic acids (%)	*(90.3)*	***(95.0)***	*(85.6)*	***(62.6)***	*(84.5)*	***(83.1)***	*(78.6)*	***(58.4)***	*(74.1)*	***(60.9)***	*(23.5)*	***(74.1)***	*(23.3)*	***(34.1)***
10	tr.	**1**	2	**0.5**	3	**0.5**	3	**0.3**	3	**0.2**	15	**0.3**	16	**0.7**
11	17	**54**	35	**10**	55	**36**	56	**21**	71	**20**	67	**21**	68	**34**
12	0.7	**0.7**	6	**0.4**	8	**0.4**	8	**0.9**	8	**1**	8	**1**	8	**2**
Dihydrocinnamic acids (%)	*(2.0)*	***(3.4)***	*(0.6)*	***(17.2)***	*(0.4)*	***(9.0)***	*(0.7)*	***(31.9)***	*(1.0)*	***(23.3)***	*(0.3)*	***(3.0)***	*(0.3)*	***(0.9)***
14	0.4	**2**	0.3	**3**	0.3	**4**	0.6	**12**	0.9	**8**	0.8	**0.8**	0.9	**0.8**
15	tr.	**tr.**	tr.	**tr.**	tr.	**tr.**	tr.	**0.1**	0.2	**0.1**	0.2	**0.1**	0.2	**0.2**
Dihydrocinnamic alcohols (%)	*(—)*	***(—)***	*(—)*	***(—)***	*(—)*	***(—)***	*(0.2)*	***(0.5)***	*(0.3)*	***(0.3)***	*(0.2)*	***(0.6)***	*(0.2)*	***(0.6)***
17	tr.	**tr.**	tr.	**tr.**	tr.	**tr.**	0.2	**0.2**	0.3	**0.1**	0.7	**0.2**	0.7	**0.6**
Total phenolics (100%)	19.6	**58.6**	50.2	**17.4**	78.1	**44.4**	85.2	**38.0**	110.6	**34.8**	383.0	**30.1**	394.0	**107.6**

* = less than 0.1%.

† = less than 0.1 nmol g^{-1} dry wt.

‡ = not detected.

Table 3. Amounts of phenolic glucosides (aglycones–μmol g^{-1} dry wt) from shoots and galls of *P. glauca*

	Age of galls (weeks)											
	4		5		7		11		16		17	
Compound	Shoot	**Gall**	Shoot	Gall	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall yellow**	Shoot	**Gall left**
Acetophenones (%)	*(93.8)*	***(70.8)***	*(94.4)*	***(67.3)***	*(93.8)*	***(45.8)***	*(93.0)*	***(25.6)***	*(89.9)*	***(23.1)***	*(89.9)*	***(38.9)***
1a	**79**	**1**	**160**	**1**	**266**	**2**	**405**	**2**	**472**	**2**	**472**	**2**
2a	**3**	**tr.***	**5**	**tr.**	**5**	**0.1**	**5**	**0.3**	**5**	**0.3**	5	**1**
3a	112	**0.3**	260	**3**	379	**3**	509	**3**	601	**2**	606	**1**
3b	1	**50**	29	**0.3**	42	**0.3**	56	**0.4**	74	**0.2**	67	**0.1**
Benzoic acids (%)	*(2.4)*	***(8.3)***	*(2.1)*	***(9.4)***	*(2.2)*	***(7.6)***	*(2.2)*	***(6.3)***	*(4.9)*	***(6.7)***	*(4.9)*	***(13.0)***
5a	4	**0.3**	9	**0.3**	10	**0.6**	12	**0.8**	44	**0.8**	44	**1**
6a	0.2	**tr.**	0.2	**0.1**	0.5	**0.1**	0.8	**0.2**	1	**0.2**	1	**0.2**
7a	0.8	**0.1**	1	**0.2**	6	**0.2**	10	**0.4**	18	**0.3**	18	**0.2**
Cinnamic acids (%)	*(2.0)*	***(8.3)***	*(1.5)*	***(7.8)***	*(1.1)*	***(5.9)***	*(1.1)*	***(3.6)***	*(1.7)*	***(3.1)***	*(1.7)*	***(4.6)***
11a	1	**0.3**	2	**0.4**	2	**0.5**	4	**0.5**	10	**0.3**	10	**0.2**
12a	1	**0.1**	1	**0.1**	1	**0.1**	1	**0.2**	2	**0.1**	2	**0.1**
13a	2	**tr.**	4	**tr.**	5	**0.1**	7	**0.1**	10	**0.2**	10	**0.2**
Dihydrocinnamic acids (%)	(—†)	***(2.1)***	*(—)*	***(3.1)***	*(0.2)*	***(6.8)***	*(0.3)*	***(4.0)***	*(0.5)*	***(3.1)***	*(0.5)*	***(3.7)***
14a	tr.	**tr.**	tr.	**tr.**	1	**0.6**	2	**0.4**	4	**0.2**	4	**0.2**
15a	0.1	**0.1**	0.3	**0.2**	0.3	**0.2**	1	**0.5**	2	**0.4**	2	**0.2**
Dihydrocinnamic alcohols (%)	*(1.8)*	***(4.2)***	*(0.8)*	***(6.2)***	*(1.2)*	***(8.5)***	*(1.4)*	***(11.2)***	*(2.0)*	***(12.8)***	*(2.0)*	***(12.0)***
16a	3	**0.1**	3	**0.3**	3	**0.4**	3	**0.5**	0.4	**0.5**	0.3	**0.4**
17a	0.7	**0.1**	1	**0.1**	6	**0.6**	12	**2**	25	**2**	25	**0.9**
Cinnamic alcohols (%)	*(—)*	***(6.3)***	*(1.2)*	***(6.2)***	*(1.5)*	***(25.4)***	*(2.0)*	***(49.3)***	*(1.0)*	***(51.2)***	*(1.0)*	***(27.8)***
18a	tr.	**0.1**	2	**0.1**	2	**1**	2	**3**	0.7	**2**	0.7	**1**
19a	tr.	**0.2**	4	**0.3**	9	**2**	19	**8**	12	**8**	12	**2**
Total glucosides (100%)	207.8	**4.8**	481.5	**6.4**	737.8	**11.8**	1048,0	**22.3**	1281.1	**19.5**	1279.0	**10.8**

* = less than 0.1 μmol g^{-1} dry wt.
† = less than 0.1%.

Table 4. Amounts of lignans and lignan glucosides (aglycones–μmol $\times 10^{-1}$ g $^{-1}$ dry wt) from shoots and galls of *P. glauca*

	Age of galls (weeks)													
	1		4		5		7		11		16		17	
Compound	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall**	Shoot	**Gall yellow**	Shoot	**Gall left**
Lignans														
20	—*	—	—	tr.†	—	**tr.**	tr.	**2**	tr.	**3**	tr.	**3**	tr.	**14**
21	—	—	—	—	—	—	—	—	—	**0.1**	—	**0.2**	—	**0.6**
22	—	—	—	—	—	—	—	—	—	**0.8**	—	**1**	—	**2**
23	—	—	—	**tr.**	—	**tr.**	—	**tr.**	—	**0.2**	—	**0.9**	—	**0.9**
24	tr.	**tr.**	tr.	**tr.**	0.1	**0.2**	0.7	**0.8**	0.8	**2**	1	**4**	1	**10**
25	—	**tr.**	tr.	**tr.**	0.1	**0.1**	0.4	**1**	0.6	**2**	0.8	**4**	0.8	**6**
26	tr.	**tr.**	tr.	**0.2**	tr.	**1**	tr.	**4**	tr.	**9**	tr.	**11**	0.1	**40**
27	tr.	**tr.**	0.3	**2**	0.3	**2**	0.3	**8**	0.2	**11**	0.1	**22**	0.1	**76**
Total lignans	tr.	**tr.**	0.3	**2.2**	0.5	**3.3**	1.4	**15.8**	1.6	**28.1**	1.9	**46.1**	2.0	**149.5**
Lignan glucosides														
22a			14	**tr.**	20	**tr.**	47	**0.7**	57	**7**	65	**5**	65	**2**
24a			3	**tr.**	5	**5**	46	**20**	47	**46**	47	**27**	47	**16**
25a			49	**tr.**	90	**3**	91	**17**	102	**56**	105	**40**	105	**17**
27a			0.4	**tr.**	2	**1**	3	**3**	5	**3**	7	**3**	7	**3**
28a			0.9	**tr.**	6	**0.9**	16	**2**	44	**27**	31	**21**	31	**7**
29a			5	**tr.**	6	**0.1**	3	**0.2**	6	**1**	5	**1**	6	**0.8**
30a			5	**tr.**	8	**0.5**	9	**1**	9	**3**	10	**2**	10	**1**
Total lignan glucosides			77.3	**tr.**	137.0	**10.5**	215.0	**43.9**	270.0	**143.0**	270.0	**99.0**	271.0	**46.8**

* = not detected.

† = less than 0.1 nmol/g dry wt.

observed when the larvae had left the gall. Then almost 70 times more lignans were found in galls than in shoots. The main components were matairesinol (**26**) and pinoresinol (**27**).

A different trend was observed for lignan glucosides. In galls their content increased until the 11th week. Then their level decreased following the pattern of cinnamic alcohol glucosides. The ratio of lignan glucosides to free lignans (2:1–5:1) was remarkable lower than that observed for shoots (120:1–170:1). When galls were left even more free lignans than glucosides were found in galls.

In conclusion, the formation of galls causes a different accumulation pattern of phenolics than reported in response to different kinds of plant stress [1]. An accumulation of phenolics in galls was not detected. Remarkable is the extreme low content of glucosides in galls compared to shoots. The shift in the ratio free aglycones to glucosides from 60:1 (shoots) to 4:1 (galls) indicates a stress response.

EXPERIMENTAL

General. GC-MS: DB-1 fused silica capillary column (l: 30 m; i.d.: 0.32 mm; film thickness: 0.1 μm; carrier gas: H_2 2 ml min^{-1}; temp. prog.: 80–280° at 3° min^{-1}) coupled to a double focussing mass spectrometer running under EI conditions at 70 eV.

GC. Column as above. Detector: FID; injector temp: 270°; detector temp: 290°; split ratio 1:30. Retention indices (R_I) were calculated according to Kováts [26] with *n*-alkanes C_{10}–C_{36} as ref. compounds.

Trimethylsilylation. 0.3 mg sample were dissolved in 10 μl THF (purified and dried) and 20 μl MSTFA added. The mixt. was allowed to stand at room temp for 12 hr. 1 μl of the mixt. was then subjected to GC and GC-MS.

Plant material. Shoots and galls were harvested from the beginning of May till mid-August 1995 from one *P. glauca* tree grown in the Botanical Garden of the University of Bayreuth, Germany. The tree was about 6 m tall. The age of galls was determined by taking the time when attacked shoots started curving as zero time. The size of the galls increased from 0.5 cm (age: one week) to 2–3 cm (age: from week 9 on). The size of the shoots increased over the same time from 3–12 cm.

Isolation of phenolics. Shoots or galls (10 g fr. wt) were homogenized in 100 ml MeOH with an Ultra Turrax homogenizer at room temp. The soln was allowed to stand for 4 hr and then solid material was removed by centrifugation. The residue was re-extracted ($\times 2$) with 30 ml MeOH–H_2O (1:1). The combined supernatants were evapd to dryness, resuspended in 60 ml MeOH–H_2O (9:1) and extracted with 2×25 ml cyclohexane to remove nonpolar compounds. The MeOH–H_2O layer was evapd to dryness, resuspended in 60 ml H_2O and extracted with 3×30 ml EtOAc. The organic layer was evapd to dryness, dissolved in 80 ml Et_2O and extracted with 3×25 ml dil. KOH (2%, aq.). The combined KOH layers were immediately acidified with 2M HCl (pH 4) and re-extracted with 3×20 ml EtOAc. The organic layers were washed with 2×10 ml H_2O, dried over Na_2SO_4 and concentrated *in vacuo*. Phenolics were identified and quantified after trimethylsilylation by GC-MS.

Isolation of glycosides. The remaining aq. layer after extraction with cyclohexane and EtOAc was extracted with 3×15 ml *n*-BuOH. The organic layers were washed with 10 ml H_2O and concd *in vacuo*.

Enzymic hydrolysis of glucosides [10]. 10 mg of the residue of the glucoside fraction were dissolved in 15 ml acetate buffer (pH 5.0) then about 5 mg β-glucosidase (Sigma, Germany) were added. The mixt. was incubated overnight at 37° and extracted with 3×10 ml EtOAc. The organic layers were washed, dried over Na_2SO_4 and concd *in vacuo*.

Deuteromethylation of glucosides [15]. Reaction was performed in the dark. The glucoside fraction (sums) was dissolved in 0.5 ml dry DMF. About 100 mg Ag_2O were added and the mixt. stirred for 10 min. After addition of 0.3 ml CD_3I (Fluka, Germany) the suspension was stirred overnight. A part of DMF and remaining CD_3I were removed in a N_2 stream, and the residue was suspended in EtOAc and filtered over 5 g silica gel to remove Ag_2O and unmethylated glucosides. The solvent was removed *in vacuo*.

Acid hydrolysis of glucosides. The methylated glucosides (10 mg) were dissolved in 0.5 ml 2M TFA and kept at 120° for 80 min. The mixt. was then diluted with 5 ml H_2O, extracted with 3×2 ml EtOAc. The organic layers were washed with 1 ml H_2O. The solvent was removed *in vacuo*.

Quantification. The absolute content of the phenolics and glucosides in shoots and galls was determined by GC of the TMSi derivatives of EtOAc and hydrolysed *n*-BuOH extracts, respectively. 4-Methoxy-3-hydroxybenzylic alcohol ($R_I = 1625$) and salicin (aglycon: $R_I = 1430$) were added as int. standards to the plant extracts immediately after homogenization. Peak areas were integrated. Quantifications were performed at least $\times 3$ to ensure reproducibility.

Data from mass spectra after trimethylsilylation. **14**: GC: $R_I = 1752$; GC-MS 70 eV, *m/z* (rel. int.): 310 $[M]^+$ (26), 295 $[M - Me]^+$ (7), 193 $[M - CO_2TMSi]^+$ (12), 192 $[M - HCO_2TMSi]^+$ (65), 179 (100), 177 (15). **15**: GC: $R_I = 1889$; GC-MS 70 eV, *m/z* (rel. int.): 340 $[M]^+$ (100), 325 $[M - Me]^+$ (32), 310 $[M - CH_2O]^+$ (30), 223 $[M - CO_2TMSi]^+$ (20), 209 (97), 193 (14), 192 (43), 179 (26). **24**: GC: $R_I = 3095$; GC-MS 70 eV, *m/z* (rel. int.): 576 $[M]^+$ (22), 561 $[M - Me]^+$ (7), 546 $[M - CH_2O]^+$ (11), 487 $[M - TMSiO]^+$ (22), 486 $[M - TMSiOH]^+$ (100), 473 $[M - CH_2OTMSi]^+$ (8), 355 (10), 310 (9), 209 (8), 147 (7), 103 (6). **25**: GC: $R_I = 3027$; GC-MS 70 eV, *m/z* (rel. int.): 634 $[M]^+$ (26), 619 $[M - Me]^+$ (7), 604 $[M - CH_2O]^+$ (9), 545 $[M - TMSiO]^+$ (32), 544 $[M - TMSiOH]^+$ (100), 531 (9), 413 (12), 310 (8), 209 (11), 147 (6), 103 (7). **28**: GC: $R_I = 2901$; GC-MS 70 eV, *m/z* (rel. int.): 723 $[M - Me]^+$ (7), 633 $[M - Me - TMSiOH]^+$ (5), 543 (3), 384 (8), 323 (8), 298 (20), 297 (100), 267 (6), 209

(8), 147 (9), 103 (8). **29**: GC: $R_I = 3014$; GC-MS 70 eV, *m/z* (rel. int.): 578 $[M]^+$ (8), 489 (3), 326 (9), 324 (13), 311 (8), 235 (17), 209 (100), 147 (12), 103 (8). **30**: GC: $R_I = 2982$; GC-MS 70 eV, *m/z* (rel. int.): 636 $[M]^+$ (8), 547 (11), 546 (14), 443 (13), 384 (8), 324 (9), 235 (11), 210 (18), 209 (100), 179 (16), 147 (13), 103 (6).

Shift of characteristic ions in the mass spectra of deuteromethylated and trimethylsilylated phenolics compared to their non-deuteromethylated analogues. **3**, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 296 $[M]^+$ (59), 281 $[M - Me]^+$ (100), 208 $[M - SiMe_3]^+$ (8), 193 $[TMSiO - C_6H_4 - CO]^+$ (10), 179 $[TMSiO - C_7H_6]^+$ (4). **3a**, deuteromethylated, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 241 $[M]^+$ (55), 226 $[M - Me]^+$ (100), 208 $[M - CD_2OH]^+$ (62), 193 $[TMSiO - C_6H_4 - CO]^+$ (82), 179 $[TMSiO - C_7H_6]^+$ (4). **5**, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 282 $[M]^+$ (42), 267 $[M - Me]^+$ (100), 193 $[TMSiO - C_6H_4 - CO]^+$ (50). **5a**, deuteromethylated, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 227 $[M]^+$ (62), 212 $[M - Me]^+$ (100), 193 $[TMSiO - C_6H_4 - CO]^+$ (22). **11**, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 308 $[M]^+$ (89), 293 $[M - Me]^+$ (100), 249 $[M - Me - CO_2]^+$ (42), 219 $[M - TMSiO]^+$ (78), 179 $[TMSiO - C_7H_6]^+$ (4). **11a**, deuteromethylated, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 253 $[M]^+$ (100), 238 $[M - Me]^+$ (71), 219 $[M - CD_3O]^+$ (27), 179 $[TMSiO - C_7H_6]^+$ (6). **16**, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 296 $[M]^+$ (13), 281 $[M - Me]^+$ (8), 206 $[M - TMSiOH]^+$ (100), 191 $[M - TMSiOH - Me]^+$ (46), 179 $[TMSiO - C_7H_6]^+$ (20), 103 $[CH_2 = O^+TMSi]$ (12). **16a**, deuteromethylated, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.: 241 $[M]^+$ (44), 226 $[M - Me]^+$ (12), 206 $[M - TMSiOH]^+$ (76), 191 $[M - TMSiOH - Me]^+$ (70), 179 $[TMSiO - C_7H_6]^+$ (100), 48 $[CH_2 = O^+CD_3]$ (15). **24**, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 576 $[M]^+$ (22), 561 $[M - Me]^+$ (7), 487 $[M - TMSiO]^+$ (22), 486 $[M - TMSiOH]^+$ (100), 473 $[M - CH_2OTMSi]^+$ (8), 209 $[(TMSiO, OCD_3), C_7H_6]^+$ (8), 103 $[CH_2 = O^+TMSi]$ (6). **24a**, deuteromethylated, trimethylsilylated: GC-MS 70 eV, *m/z* (rel. int.): 466 $[M]^+$ (18), 451 $[M - Me]^+$ (6), 432 $[M - CD_3O]^+$ (37), 431 $[M - CD_3OH]^+$ (100), 418 $[M - CH_2OCD_3]^+$ (12), 209 $[(TMSiO, OCD_3), C_7H_6]^+$ (21), 48 $[CH_2 = O^+CD_3]$ (12).

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