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Glucosinolates, flea beetle resistance, and leaf pubescence as taxonomic characters in the genus *Barbarea* (Brassicaceae)

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Abstract

Glucosinolate content of leaves and roots, diversity in leaf pubescence, and resistance to two near-isogenic lines of the flea beetle *Phyllotreta nemorum* with or without an R-gene, were determined for 27 accessions of 7 *Barbarea* taxa, i.e. *B. stricta*, *B. orthoceras*, *B. intermedia*, *B. verna*, *B. vulgaris* var. *vulgaris*, the G-type of *B. vulgaris* var. *arcuata* and the P-type of *B. vulgaris* var. *arcuata*. Four variable glucosinolate biosynthetic characters were deduced. For (formally) homophenylalanine-derived glucosinolates: (1) Presence or absence of 2-hydroxylation, and if present, *R*- or *S*-configuration of 2-hydroxylation; (2) presence or absence of *p*-hydroxylation; and for tryptophan-derived glucosinolates: (3) presence or absence of *N*-methoxyglucobrassicin; and (4) presence or absence of 1,4-dimethoxyglucobrassicin. Three phenotypes of leaf-pubescence were observed; (1) glabrous to glabrate leaves; (2) glabrous to glabrate leaves with hairs along the edge; (3) pubescent leaves. The hairs were characterized as simple by scanning electron microscopy. Full resistance to a flea beetle line (ST) was found in *B. vulgaris* var. *vulgaris* and in the G-type of var. *arcuata*; partial resistance was found in *B. verna* and *B. intermedia*, while the remaining taxa were fully susceptible to the ST line. All investigated *Barbarea* taxa were susceptible to larvae from another line containing an R-gene, indicating a similar flea beetle resistance mechanism in the three resistant species. Most *Barbarea* taxa could be characterized by a particular combination of the investigated characters. The most aberrant was the P-type of *B. vulgaris* var. *arcuata*, and the taxonomic status of this type should be reconsidered.

Keywords: *Barbarea*; Brassicaceae; *Phyllotreta nemorum*; Coleoptera; Chrysomelidae; Alticinae; Biodiversity; Chemotaxonomy; Glucosinolates; Trichomes; Insect resistance

1. Introduction

Barbarea R. Br. (Wintercress) is a relatively small genus in the Brassicaceae comprising about 20 biennial or perennial taxa/species distributed mainly in the temperate regions of Eurasia (10 species) and North America (Al-Shehbaz, 1988; Mabberly, 1997). However, a few species are also found in subtropical regions in East Asia, e.g. Taiwan (Al-Shehbaz and Peng, 2000), and in Australia (Hewson, 1982). The type species *Barbarea vulgaris* R.Br. has a wide native distribution area (Eurasia) and is furthermore introduced to North America, Africa, and Australia (Hegi, 1958) where it appear as a noxious weed. It is a variable species, for which taxonomic delimitation has been and still is discussed (Bush, 1939; Hegi, 1958; Rich, 1987; MacDonald and Cavers, 1991). Today many floras include only *B. vulgaris* (e.g., Ball, 1993; Rich, 1987; Stace, 1991), still others generally accept two varieties (or subspecies), i.e. *B. vulgaris* var. *vulgaris* and *B. vulgaris* var. *arcuata* (Opiz.) Fries. These two varieties are mainly distinguished by the orientation of pedicels and fruits, which are straight in var. *vulgaris* and arcuate in var. *arcuata*. Var. *arcuata* is far more common than var. *vulgaris* in e.g. the former Soviet Union (Bush, 1939), in Canada (Mac Donald and Cavers, 1991) and in Scandinavia (Lange, 1937; Jensen, 1985; Nielsen, unpublished). *Barbaraea* (sic)

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Beck. *arcuata* Rchb. var. *pubescens* N. Busch was described by Bush (sic) (1939) with "stem, leaves and petioles more or less pubescent."

Recently two types (G- and P-type) of *B. vulgaris* var. *arcuata* [syn. *B. vulgaris* ssp. *arcuata* (Opiz) Simkovics] were described, appearing in wild populations from eastern Denmark (Nielsen, 1997a; Agerbirk et al., 2001a). The two types differ in leaf pubescence, in resistance to the flea beetle *Phyllotreta nemorum*, and in the glucosinolate content. *P. nemorum* L. (Coleoptera: Chrysomelidae: Alticinae) will be referred to as 'flea beetle' in the rest of this paper, although it is only one of several flea beetle species. The G-type has **G**labrous leaves, is resistant to the flea beetle, and the leaf glucosinolate profile is dominated by **2*S*** (Fig. 1). The P-type has **P**ubescent rosette leaves, is susceptible to the flea beetle, and the leaf glucosinolate profile is dominated by **2*R***. Two additional glucosinolates, **3*R*** and **7**, are present in the P-type but absent from the G-type (Agerbirk et al., 2001a, b).

The chemical basis of flea beetle resistance in the G-type of *B. vulgaris* var. *arcuata* is unknown, but resistance is not correlated to glucosinolates or glucosinolate levels (Agerbirk et al., 2001a). Resistance may be due to the occurrence of a triterpenoid saponin in *B. vulgaris* (Shinoda et al., 2002), since this saponin is responsible for resistance against another crucifer specialist, *Plutella xylostella* (Lepidoptera: Plutellidae). A polymorphism of one or more "R-genes", which render the flea beetles immune to the defences of the G-type, was previously reported (Nielsen, 1997b; de Jong et al., 2000). The R-gene, which in one flea beetle line was inherited as a Y-linked locus, provided close to 100% survival of flea beetle larvae feeding on the G-type of *B. vulgaris* var. *arcuata*.

The R-gene had no effect when flea beetle larvae were fed with non-*Barbarea* crucifers, regardless of the plants being flea beetle resistant or susceptible (Nielsen, 1999). Thus, the R-gene has a high specificity for the chemical resistance-mechanism of the G-type of *B. vulgaris* var. *arcuata*. By comparing the frequency of survival of near-isogenic flea beetle larvae with and without the R-gene, a specific bioassay for the flea beetle resistance mechanism in the G-type was carried out. Thereby the presence or absence of the same (or a chemically similar) insect resistance mechanism in several species could be measured experimentally, allowing a chemotaxonomic study even though the chemical nature of the resistance mechanism was unknown.

Variation in leaf pubescence is common within crucifers (e.g., in *B. intermedia*; Ball, 1993), and therefore usually not considered a character of taxonomic value. However, it turned out that the presence or absence of leaf pubescence in *B. vulgaris* var. *arcuata* seemed to be correlated with several chemical and biological characters (Nielsen, 1997a; Agerbirk et al., 2001a,b), which

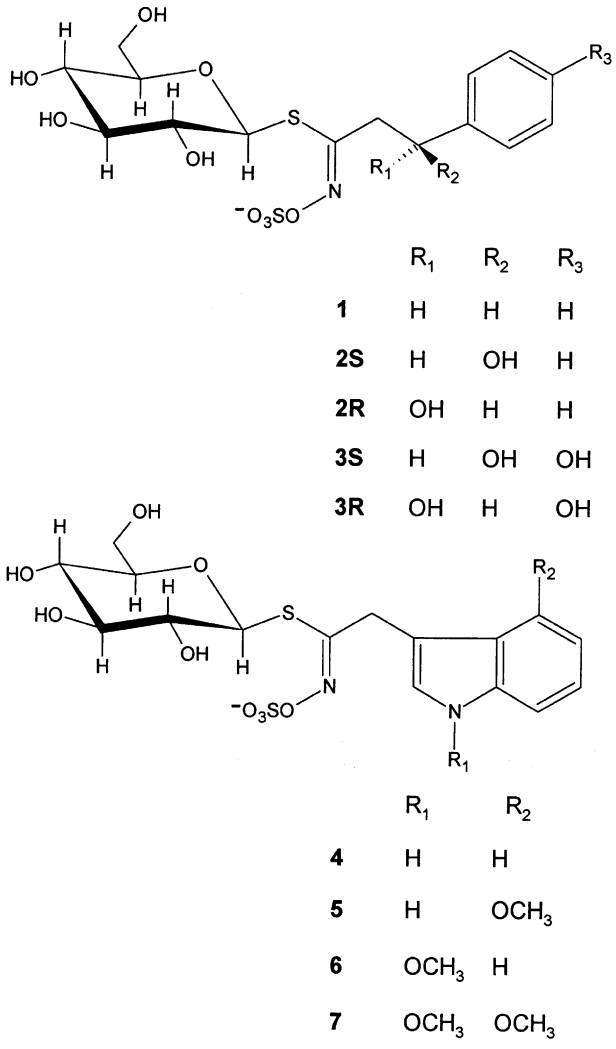

Fig. 1. Glucosinolates (gls) detected in leaves or roots of the investigated *Barbarea* species. Systematic and common names (in parentheses) are: **1**, 2-phenylethylgls (gluconasturtiin); **2*S***, (*S*)-2-hydroxy-2-phenylethylgls (glucobarbarin); **2*R***, (*R*)-2-hydroxy-2-phenylethylgls (glucosibarin); **3*S***, (*S*)-2-hydroxy-2-(4-hydroxyphenyl)ethylgls (*p*-hydroxyglucobarbarin); **3*R***, (*R*)-2-hydroxy-2-(4-hydroxyphenyl)ethylgls (*p*-hydroxyglucosibarin); **4**, indol-3-ylmethylgls (glucobrassicin); **5**, 4-methoxyindol-3-ylmethylgls (4-methoxyglucobrassicin); **6**, *N*-methoxyindol-3-ylmethylgls (neoglucobrassicin); **7**, 1,4-dimethoxyindol-3-ylmethylgls (1,4-dimethoxyglucobrassicin).

differed significantly between the two types. These findings suggested a greater taxonomic distance between the two types of *B. vulgaris* var. *arcuata*, but the variability of these characters among presently recognised *Barbarea* taxa was poorly known.

The aims of the present work were: (1) to register chemical, morphological, and ecological (insect resistance) diversity in additional accessions of the P- and G-types of *B. vulgaris* var. *arcuata*, and in *B. vulgaris* var. *vulgaris* and four other presently recognized *Barbarea* species, and (2) to investigate the value of these characters for unravelling the taxonomy and phylogeny of the genus.

2. Results

2.1. Glucosinolates

The observed glucosinolates comprised structures that can formally be derived from the amino acids homophenylalanine (homo-Phe) (**1**, **2*R***, **2*S***, **3*R***, **3*S***) and tryptophan (Trp) (**4**, **5**, **6**, **7**) (Fig. 1). The glucosinolate profiles of roots and leaves were different (Table 2): Accumulation of substituted Trp-derived glucosinolates (**5**, **6**, **7**) was mainly seen in roots, while the un-substituted glucosinolate **4** was by far the dominating Trp-derived glucosinolate in leaves. The homo-Phe-derived glucosinolate **1** was a major constituent in roots of all accessions, while most species (except *B. verna*) accumulated only trace amounts of **1** in the leaves. Finally, the ratio between the two epimers **2*R*** and **2*S*** (see below) was in all accessions more or equally extreme in the leaves than in the roots, meaning that ratios between these two glucosinolates in the leaves could be expected to be more taxonomically informative.

The 2-hydroxylated glucosinolates **2*R*** and **2*S*** were absent in *B. verna*, but present in all the remaining taxa although in variable proportions. The *R*-epimer predominated in the P-type of *B. vulgaris* var. *arcuata* while the *S*-epimer predominated in all other taxa (Table 2). The relative proportions of the two compounds in leaves was nearly constant among accessions within each taxon, although relatively high proportions of the *S*-epimer (11.2 and 26.2%) was found in pooled leaf samples from two accessions of the P-type of *B. vulgaris* var. *arcuata* (B13 and B20). However, most individual plants from B13 and B20 had a very low content of **2*S*** relative to **2*R*** (Table 2, footnote c and d), and the present results confirm that the typical pattern for the P-type of *B. vulgaris* var. *arcuata* is a low proportion of the **2*S*** compared to **2*R***. The *para*-hydroxylated glucosinolates **3*R*** and **3*S*** had a restricted occurrence. **3*S*** was found in *B. orthoceras* and in some accessions of *B. stricta*, and **3*R*** was found in the P-type of *B. vulgaris* var. *arcuata* (Table 2).

The glucosinolate analyses demonstrated important similarities as well as differences among the investigated taxa. All taxa contained the Trp-derived glucosinolates **4** and **5**, while **6** was absent from *B. intermedia* and from the P-type of *B. vulgaris* var. *arcuata* (Table 2). The P-type of *B. vulgaris* var. *arcuata* was further characterised by the low proportion of **2*S*** compared to **2*R***, the presence of **3*R*** in leaves and roots, and **7** in the roots. *B. verna* was characterised by the occurrence of **1** and the absence of the 2-hydroxylated glucosinolates **2*R*** and **2*S***. There were no consistent differences in glucosinolates between *B. stricta*, the G-type of *B. vulgaris* var. *arcuata* and *B. vulgaris* var. *arcuata*, and the analysis of only a single accession of *B. orthoceras* precludes definite conclusions about chemical similarities or differences between this American species and its European relatives.

Table 1
Barbarea accessions investigated. Danish accessions were all collected as seeds at natural growth sites by JKN, from Zealand (Z), Jutland (J) or Falster (F) as indicated

Species, accession	Origin of seeds
B. stricta Andrz.	
B8	Tissø (55° 36′ N; 11° 18′ E), Z, DK, 1997
B40	Madesø (55 37′ N; 11° 19′ E), Z, DK, 1999
B41	Strøby Egede (55° 25′ N; 12° 14′ E), Z, DK, 1999
B42	Lydersholm (54° 55′ N; 9° 01′ E), J, DK, 1999
B43	Sølsted (55° 02′ N; 8° 50′ E), J, DK, 1999
B. orthoceras Ledeb.	
B26	Wolcott (44° 40 N; 72° 26′ W), Vermont, USA, 1994
B. verna (Miller) Ascherson	
B16	Copenhagen Bot. Gd. 1997 No. 4851-4B
B36	"Richters - The Herb Specialist", Cat. No. Bien 2032, Goodwood, Ontario, Canada
B. intermedia Boreau	
B7	Klampenborg Station (55° 47′ N; 12° 35′ E), Z, DK, 1998
B37	Mols Bjerge (56° 13′ 30″ N; 10° 33′ E), J, DK, 1999
B38	Eskebjerg (55° 43′ N; 11° 17′ E), Z, DK, 1999
B39	Goulier-Neige, The Pyrenees, France (42° 44′ N; 1° 34′ E), 1999.
B. vulgaris R. Br. var. *vulgaris*	
B5	Risby (55° 41′ N; 12° 21′ E), Z, DK, 1996
B6	Fabriksparken, Glostrup (55° 41′ N; 12° 24′ E), Z, DK, 1997.
B. vulgaris R. Br. var. *arcuata* (Opiz.) Fries, G-type	
B1	Herlev (55° 44′ N; 12° 33′ E), Z, DK, 1994
B2	Gl. Svebølle (55° 39′ N; 11° 20′ E), Z, DK, 1998
B15	Ithaca (41° 13′ N; 76° 29′ W), NY, USA, 1994
B21	Skagen (57° 43′ N; 10° 33′ E), J, DK, 1998.
B23	Termestrup (56° 21′ N; 10° 21′ E), J, DK, 1998
B29	Morup Mølle (56° 49′ N; 8° 21′ E), J, DK, 1998
B. vulgaris R. Br. var. *arcuata* (Opiz.) Fries, P-type	
B3	Tissø (55° 36′ N; 11° 18′ E), Z, DK, 1998
B4	Trundholm Mose (55° 53′ N; 11° 34′ E), Z, DK, 1997
B13	Skellingsted (55° 35′ N; 11° 29′ E), J, DK, 1998
B20	Store Vildmose (57° 16′ N; 9° 51′ E), J, DK, 1998
B22	Manna/Ryå (57° 17′ N; 9° 51′ E), J, DK, 1998
B24	Hannenov Skov (54° 51′ N; 11° 58′ E), F, DK, 1996
B25	Risby (55° 41′ N; 12° 20′ E), Z, DK, 1996

B15, B26 and B39 were collected as seeds at natural growth sites by J.A.A. Renwick, F.S. Chew and J.K.N., respectively.

2.2. Pubescence

All accessions of the P-type of *B. vulgaris* var. *arcuata* were characterized by having rather dense, simple hairs along the margins, and on both the upper and lower surfaces of the rosette leaves. Accessions of *B. vulgaris* var. *arcuata* could easily be attributed to either the P-type (most leaves had more than 20 hairs on the basal fourth of the rosette leaf margin) or the G-type (most leaves had less than five hairs on the basal fourth of the rosette leaf margin) (Table 3). Indeed, the mean number of hairs on the basal fourth of the leaf margin was

Table 2
Glucosinolates in leaves and roots of young rosette plants of the *Barbarea* accessions

Species, accession	Glucosinolate									100×[2*S*]/([2*R*]+[2*S*]) (S.D.)
	1	**2*R***	**2*S***	**3*R***	**3*S***	**4**	**5**	**6**	**7**	
B. stricta										
B8	+	+	+	n.d.	n.d.[b]	+	+	+	n.d.	99.1[a]
B40	+	+	+	n.d.	+	+	+	+	n.d.	99.1[a]
B41	+	+	+	n.d.	+	+	+	+	n.d.	98.8[a]
B42	+	+	+	n.d.	n.d.	+	+	+	n.d.	99.0[a]
Roots, mean (S.D.)	26 (11)	3 (2)	69 (13)	–	2 (3)	44 (8)	43 (15)	14 (10)	–	95.7 (3.0)
Leaves, mean (S.D.)	0	0.9 (0.1)	95 (5)	–	4 (5)	98 (2)	2 (2)	0	–	99.0 (0.1)
B. orthoceras										
(B26)	+	+	+	n.d.	+	+	+	+	n.d.	99.1[a]
Roots	29	3	65	0	3	58	20	21	–	95.8[a]
Leaves	0	0.9	95	0	4	100	0	0	–	99.1[a]
B. verna										
B12	+	n.d.	n.d.	n.d.	n.d.	+	+	+	n.d.	–
B16	+	n.d.	n.d.	n.d.	n.d.[b]	+	+	+	n.d.	–
B36	+	n.d.	n.d.	n.d.	n.d.[b]	+	+	+	n.d.	–
Roots, mean (S.D.)	100 (0)	–	–	–	–	30 (5)	42 (15)	28 (10)	–	–
Leaves, mean (S.D.)	100 (0)	–	–	–	–	70 (18)	30 (18)	0	–	–
B. intermedia										
B7	+	+	+	n.d.	n.d.[b]	+	+	n.d.	n.d.	98.7[b]
B37	+	+	+	n.d.	n.d.[b]	+	+	n.d.	n.d.	99.0[a]
B38	+	+	+	n.d.	n.d.[b]	+	+	n.d.	n.d.	97.9[a]
B39	+	+	+	n.d.	n.d.[b]	+	+	n.d.	n.d.	99.0[a]
Roots, mean (S.D.)	54 (9)	0.6 (0.4)	45 (9)	–	–	96 (3)	4 (3)	–	–	98.8 (0.6)
Leaves, mean (S.D.)	1 (1)	1 (0.1)	98 (1)	–	–	100 (0)	0	–	–	99.0 (0.2)
B. vulgaris var. *vulgaris*										
B5	+	+	+	n.d.	n.d.[b]	+	+	+	n.d.	97.6[a]
B6	+	+	+	n.d.	n.d.[b]	+	+	+	n.d.	97.8[a]
Roots, mean (S.D.)	30 (2)	5 (0.1)	64 (3)	–	–	49 (8)	32 (4)	19 (4)	–	92.3 (0.1)
Leaves, mean (S.D.)	3 (2)	2 (0.1)	95 (2)	–	–	98 (3)	2 (3)	0	–	97.7 (0.2)
B. vulgaris var. *arcuata*, G-type										
B1	+	+	+	n.d.	n.d.[b]	+	+	+	n.d.	99.0[a]
B2	+	+	+	n.d.	n.d.	+	+	+	n.d.	98.4[a]
B15	+	+	+	n.d.	n.d.	+	+	+	n.d.	99.1[a]
B21	+	+	+	n.d.	n.d.	+	+	+	n.d.	97.2[a]
B23	+	+	+	n.d.	n.d.	+	+	+	n.d.	98.4[a]
B29	+	+	+	n.d.	n.d.	+	+	+	n.d.	98.3[a]
Roots, mean (S.D.)	50 (15)	3 (2)	46 (15)	–	–	39 (10)	32 (15)	29 (13)	0	92.3 (3.4)
Leaves, mean (S.D.)	0.5 (0.8)	2 (0.7)	98 (1)	–	–	94 (5)	6 (5)	0	0	98.4 (0.7)
B. vulgaris var. *arcuata*, P-type										
B3	+	+	+	+[b]	n.d.	+	+	n.d.	+	7.4[a]
B4	+	+	+	+[b]	n.d.	+	+	n.d.	+	1.5[a]
B13	+	+	+	+	n.d.	+	+	tr.	+	11.2[a,c]
B20	+	+	+	+	n.d.	+	+	n.d.	+	26.2[a,d]
B22	+	+	+	+	n.d.	+	+	n.d.	+	1.0[a]
B24	+	+	+	+	n.d.	+	+	n.d.	+	1.9[a]
B25	+	+	+	+	n.d.	+	+	n.d.	+	4.9[a]
Roots, mean[e] (S.D.)	30 (13)	67 (13)	1 (0.6)	1 (0.2)	–	19 (11)	35 (4)	0	46 (8)	2.2 (0.9)
Leaves, mean[e] (S.D.)	0.3 (0.5)	94 (5)	3 (2)	3 (3)	–	99 (2)	1 (2)	0	0	3.3 (2.7)

For each accession, presence or absence of each glucosinolate is given (+ = detected, n.d. = not detected). For each taxon, quantitative results are given as mol% of homophenylalanine-derived glucosinolates (**1**, **2*R***, **2*S***, **3*R***, **3*S***) and mol% of tryptophan-derived glucosinolates (**4**, **5**, **6**, **7**). S.D. = Standard deviation. The *B. stricta* accession B43 was not analyzed for glucosinolates.

[a] In leaves.

[b] Presence or absence of *p*-hydroxylation confirmed in November for plants grown outdoor.

[c] Variable; subsequent analysis of three individual plants gave the ratios 1.1; 51.0; 1.6.

[d] Variable; subsequent analysis of three individual plants gave the ratios 2.1; 3.8; 1.0.

[e] Accessions B13 and B20 not included.

Table 3
Distribution of rosette leaves from *Barbarea* accessions in classes according to the number of hairs counted on the basal fourth of the leaf margin, starting from the petiole

	Numbers of leaves with different numbers of hairs				
	0–5	6–10	11–15	16–20	>20
B. stricta					
B8	38	10	1	1	0
B40	15	14	10	1	0
B41	38	7	3	1	0
B42	27	15	6	2	0
B43	37	3	0	0	0
B. orthoceras					
B26	39	10	2	0	0
B. verna					
B16	90	0	0	0	0
B36	60	0	0	0	0
B. intermedia					
B7	28	44	33	12	3
B37	1	5	10	14	50
B38	0	0	0	5	85
B39	16	16	15	15	18
B. vulgaris var. *vulgaris*					
B5	60	0	0	0	0
B6	50	0	0	0	0
B. vulgaris var. *arcuata*, G-type					
B1	50	0	0	0	0
B2	40	0	0	0	0
B15	38	2	0	0	0
B21	71	4	2	1	0
B23	65	7	2	0	0
B29	97	7	5	0	1
B. vulgaris var. *arcuata*, P-type					
B3	0	0	0	0	40
B4	0	2	3	2	33
B13	1	0	0	2	37
B20	0	2	3	8	87
B22	0	0	2	8	70
B24	0	1	2	4	23
B25	1	3	2	2	27

The leaves were from plants grown in a growth chamber.

between 42 and 85 for the seven accessions of the P-type investigated. Accessions of the G-type of *B. vulgaris* var. *arcuata* collected in western Denmark (B21, B23, B29) tended to have slightly more hairs on the basal fourth of the leaf margin than accessions collected in eastern Denmark (B1, B2).

Two phenotypes were observed in *B. intermedia*: The glabrate phenotype (B7 and B39) had simple hairs along the leaf margin, but was glabrous to glabrate on the remaining leaf surface. The pubescent phenotype (B37 and B38) had simple hairs along the leaf margin and was pubescent on the upper (adaxial) surface of the rosette leaves (Fig. 2). Within *B. intermedia* there were statistically significant differences among accessions in the number of hairs along the basal fourth of the leaf margin (Kruskal Wallis Test; $P<0.0001$) (Table 3). This difference was obviously due to generally higher numbers of hairs along the basal fourth of the leaf margin in the pubescent accessions B37 (mean 27) and B38 (mean 48), compared to the glabrate accessions B7 (mean 9) and B39 (mean 14). The remaining taxa, *B. stricta*, *B. orthoceras*, *B. verna* and *B. vulgaris* var. *vulgaris,* had glabrous or glabrate rosette leaves.

The hairs of the P-type of *B. vulgaris* var. *arcuata* and of *B. intermedia* were examined by light microscopy and by SEM. The hairs seemed to be unicellular. A swollen base of the hairs on the upper surface of a pubescent accession of *B. intermedia* (accession B38) may indicate some kind of glandular activity (Fig. 2). Further studies are needed to determine whether the hairs of the P-type of *B. vulgaris* var. *arcuata* and of *B. intermedia* are homologous structures.

2.3. Flea beetle resistance

The survival rate of larvae from the susceptible ST line of the flea beetle *P. nemorum* was very low on all accessions of *B. vulgaris* var. *vulgaris* and the G-type of *B. vulgaris* var. *arcuata*, whereas it was high on all accessions of *B. stricta*, *B. orthoceras* and the P-type of *B. vulgaris* var. *arcuata* (Table 4). Survival rates of the ST-line on *B. verna* and *B. intermedia* were intermediate.

All accessions of *B. stricta*, *B. orthoceras* and the P-type of *B. vulgaris* var. *arcuata* were fully acceptable also to the YE-line and there were no significant differences between responses of the two flea beetle lines on any accession from these plant taxa (Table 4). Survival rates of larvae from the YE-line were close to 50% in all accessions of *B. vulgaris* var. *vulgaris* and the G-type of *B. vulgaris* var. *arcuata*. This result was expected since it is known that approximately 50% of the larvae—the males—from the YE-line contain a Y-linked R-gene that enable them to survive on the G-type of *B. vulgaris* var. *arcuata*, while the remaining 50%—the females—are isogenic with the ST-line (Nielsen, 1999). Survival rates of larvae from the YE-line were higher than those of the ST-line on all accessions of *B. verna*, *B. intermedia* (except B39), *B. vulgaris* var. *vulgaris* and the G-type of *B. vulgaris* var. *arcuata* (Table 4). The R-gene in the YE-line is therefore effective against defences found in all these plant taxa, and this result suggests that the defence mechanisms found in *B. verna* and *B. intermedia* are similar to those found in *B. vulgaris* var. *vulgaris* and the G-type of *B. vulgaris* var. *arcuata*.

3. Discussion

3.1. Glucosinolates

We assume that the biosynthesis of glucosinolates in *Barbarea* starts with either homo-Phe (derived from

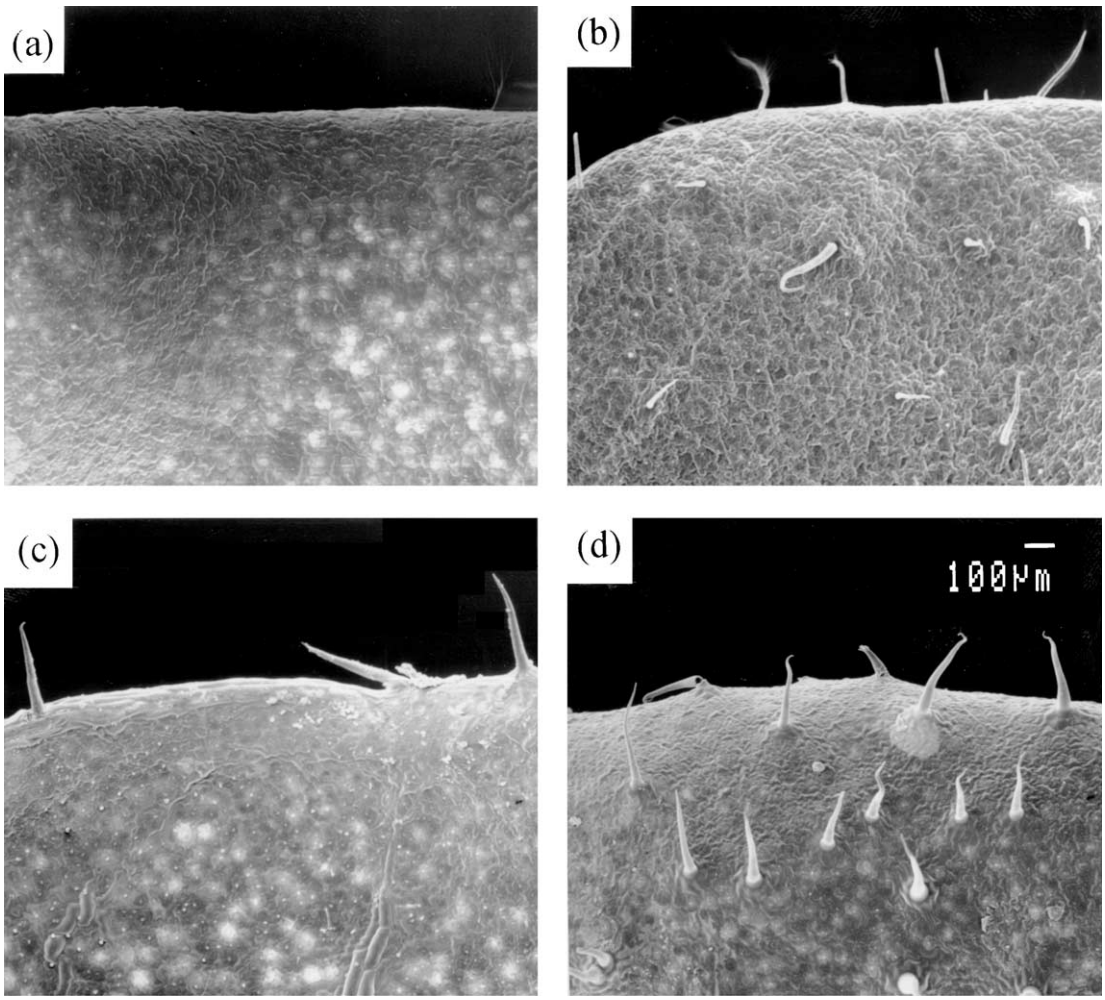

Fig. 2. Scanning electron micrograph (×43) of pubescence on the adaxial surfaces and leaf edges of *B. vulgaris* and *B. intermedia* rosette leaves: (a) G-type of *B. vulgaris* var. *arcuata* (B1). (b) P-type of *B. vulgaris* var. *arcuata* (B4). (c) *B. intermedia* accession B7, with hairs along leaf edge but not on adaxial surface. (d) *B. intermedia* accession B38 with hairs both along leaf edge and on adaxial surface. The 100 μm bar in (d) indicates the scale on all four micrographs.

Phe) or Trp, which are converted to desulfoglucosinolates and glucosinolates in several steps, and at some point modified to give the observed side chain variations. This assumption is in accordance with knowledge of glucosinolate biosynthesis in other species (Underhill and Kirkland, 1972; Rossiter and James, 1990; Mikkelsen et al., 2002). All of the investigated *Barbarea* species were able to biosynthesise glucosinolates apparently derived from these two amino acids, while glucosinolates derived from other amino acids were not detected. The differences in glucosinolate content could be rationalised by assuming variation in four biosynthetic characters (Table 5):

(1) 2-Hydroxylation in glucosinolates derived from homo-Phe. *B. verna* was deficient in 2-hydroxylation. All other taxa had a high level of stereospecific 2-hydroxylation, with **2*R*** being the predominating epimer in the P-type of *B. vulgaris* var. *arcuata*, and with **2*S*** being the predominating epimer in the remaining taxa. Surprisingly, in several taxa (*B. stricta*, *B. orthoceras*, *B. vulgaris* var. *vulgaris* and the G-type of *B. vulgaris* var. *arcuata*, the 2-hydroxylation reaction was apparently less stereo specific in roots than in leaves, as the relative level of the minor epimer was higher in roots than in leaves (Table 2).

(2) *Para*-hydroxylation in glucosinolates derived from homo-Phe. *Para*-hydroxylation was detected in *B. orthoceras*, some accessions of *B. stricta*, and all accessions of the P-type of *B. vulgaris* var. *arcuata*. Previous results suggested that the hypothetical enzyme conferring *para*-hydroxylation does not show absolute substrate specificity, but is able to hydroxylate both **2*R*** and **2*S*** (Agerbirk et al., 2001a). For this reason, presence or absence of *para*-hydroxylation was considered for comparison between species (Table 5), regardless of the actual *para*-hydroxylated glucosinolate found. It was previously reported that the extent of *para*-hydroxylation in the P-type was correlated with season,

Table 4
Survival of two flea beetle lines on rosette leaves of *Barbarea* accessions grown in a growth chamber

	ST-line		YE-line		*P*
	N	*S* (%)	*N*	*S* (%)	
B. stricta					
B8	186	88.2	170	88.2	ns
B40	40	92.5	40	92.5	ns
B41	50	84.0	60	90.0	ns
B42	50	96.0	40	90.0	ns
B43	40	90.0	40	95.0	ns
B. orthoceras					
B26	40	92.5	10	100	ns
B. verna					
B16	205	22.0	159	38.4	<0.001
B36	144	15.3	122	39.3	<0.001
B. intermedia					
B7	266	45.1	242	71.9	<0.001
B37	90	31.1	135	62.2	<0.001
B38	90	21.1	90	43.3	<0.01
B39	80	60.0	90	66.7	ns
B. vulgaris var. *vulgaris*					
B5	88	0	141	30.5	<0.001
B6	60	0	60	45.0	<0.001
B. vulgaris var. *arcuata*, G-type					
B1	257	0	268	44.9	<0.001
B2	90	0	90	34.4	<0.001
B15	40	0	16	50.0	<0.001
B21	80	0	40	50.0	<0.001
B23	75	2.7	65	47.7	<0.001
B29	110	0	70	47.1	<0.001
B. vulgaris var. *arcuata*, P-type					
B3	80	92.5	90	83.3	ns
B4	120	90.0	95	89.5	ns
B13	85	89.4	85	89.4	ns
B20	100	83.0	50	96.0	ns
B22	80	95.0	60	91.7	ns
B24	110	90.0	95	87.4	ns
B25	130	89.2	105	92.4	ns

N is the number of larvae tested. *S* is the number of surviving larvae after 3–4 days in%. *P* indicates the degree of significance for a given difference between the survival of the two lines, ns: not significant ($P > 0.05$).

being high late in the season (Agerbirk et al., 2001a). This was also the case for the potted plants grown outdoor for vernalisation (data not shown). An alternative explanation for occurrence of the *p*-hydroxylated glucosinolates **3*R*** and **3*S*** would be that they were derived from homotyrosine rather than from homo-Phe (Table 5, footnote a; Agerbirk et al., 2001a).

(3) Absence or presence of 1,4-dimethoxyglucobrassicin (**7**). This glucosinolate was found in the P-type of *B. vulgaris* var. *arcuata*, but not in any other *Barbarea*, *Arabidopsis* or *Brassica* taxon investigated (Table 5; Agerbirk et al., 2001b).

(4) Absence or presence of *N*-methoxyglucobrassicin (**6**). This glucosinolate was not detected in *B. intermedia*, and was not detected in the P-type of *B. vulgaris* var. *arcuata* in this study, exept for a trace in accession B13 which may have included G-type×P-type hybrids (see below). In a previous paper, we reported occasional occurrence of **6** in root material of the P-type (Agerbirk et al., 2001a). In the remaining taxa, **6** was abundant in roots. It is possible that the absence or irregular occurrence of **6** in the P-type is connected to the presence of **7**, since the two compounds are assumed to have a common precursor. The relative amounts of the two compounds may be dependent on the growing conditions. In contrast, *N*-methoxylation has not been detected at all in *B. intermedia*.

The relative content of **2*S*** relative to **2*R*** in pooled leaves of the accessions B13 and B20 was higher than found for other P-types. To test whether the higher relative content of **2*S*** was a general feature of these two accessions, three individual plants of each accession were tested. One individual from B13 showed a ratio close to 50%, as has previously been described for apparent natural hybrids between the G- and the P-types (Agerbirk et al., 2001a). The remaining individuals showed the usual low content of **2*S***, as found in other P-types. The higher content of **2*S*** in pooled leaves from some accessions of the P-type may thus be due to occasional plants with a hybrid-like glucosinolate profile. This may be an indication of occasional gene transfer between the P- and G-types in nature.

Previous reports of the glucosinolates of the genus, reviewed by Fahey et al. (2001), are to some extent in agreement with the results reported here. The absolute stereochemistry for **2*S*** and **2*R*** used in this report relies on studies by Kjær and Gmelin (1957) and Sørensen (1990), as discussed elsewhere (Agerbirk et al., 2001a). The report of **2*S*** as the dominating glucosinolate in leaves of *B. vulgaris* (Huang et al., 1994) is confirmed by our results, except for the P-type. The isolation of (*R*)-5-phenyl-2-oxazolidinethione from leaves of *B. orthoceras* (Seo et al., 1999) is in agreement with our finding of **2*S*** as a major component in this species (due to the priority rules of the *R*-*S* nomenclature, hydrolysis of **2*S*** yields the *R*-enantiomer of the resulting substituted oxazolidine-2-thione).

Hydrolysis products corresponding to two homomethionine- (homo-Met) derived glucosinolates, allylglucosinolate and 3-methylthiopropylglucosinolate, and to a valine- (Val) derived glucosinolate, iso-propylglucosinolate, have been reported from *B. intermedia*, *B. stricta* and *B. vulgaris* (Cole, 1976; Daxenbichler et al., 1991). The desulfoglucosinolate method can in general be assumed to produce quantitatively correct glucosinolate profiles of a given tissue (Sang et al., 1984), since the method is free from the complexities of myrosinase-catalysed hydrolysis of glucosinolates. The

Table 5
Summary of the distribution of characters among *Barbarea* species

Species	No. of accessions	Flea beetle resistance	Pubescence	Glucosinolate biosynthesis			
				2-OH	*p*-OH[a]	**6**	**7**
B. stricta	5	−	−	*S*	±	+	−
B. orthoceras	1	−	−	*S*	+	+	−
B. verna	2	+	−	–	−	+	−
B. intermedia	4	+	±	*S*	−	−	−
B. vulgaris var. *vulgaris*	2	++	−	*S*	−	+	−
B. vulgaris var. *arcuata*, G-type	6	++	−	*S*	−	+	−
B. vulgaris var. *arcuata*, P-type	7	−	+	*R*[b]	+	−[c]	+

Flea beetle resistance: Full (++), partial (+) or no (−) resistance to *Phyllotreta nemorum* without R-genes. Pubescence: The pubescent leaf phenotype, with young leaves densely covered by hairs (+), or the glabrous to glabrate phenotype (−). Glucosinolate biosynthesis, characters deduced from the accumulated glucosinolates in roots and leaves. 2-OH: 2-hydroxylation, predominantly in *R*-configuration (*R*), or in the *S*-configuration (*S*), or no 2-hydroxylation (–). *p*-OH: *para*-hydroxylation of homophenylalanine-derived glucosinolates (+) or no *para*-hydroxylation (−). **6**: Occurrence of *N*-methoxyglucobrassicin (+) or no occurrence of this glucosinolate (−). **7**: Occurrence of 1,4-dimethoxyglucobrassicin (+), or no occurrence of this glucosinolate (−).

[a] Detection of *p*-hydroxylated glucosinolates may alternatively be interpreted as glucosinolates derived from homotyrosine (+) or no glucosinolates derived from homotyrosine (−).

[b] Two accessions included some atypical plants which accumulated both the *R*- and *S*-stereo isomer in appreciable amounts, possibly due to hybridisation with the G-type.

[c] Occasional occurrence of this glucosinolate reported in a previous paper (Agerbirk et al., 2001b).

results presented here and chromatograms presented elsewhere (Huang et al., 1994; Müller et al., in press) clearly show that homo-Met- or Val-derived glucosinolates are not quantitatively important in rosette leaves and roots of the investigated *Barbarea* species. But our results do not exclude the possibility of presence of low amounts of such glucosinolates in the genus. The glucosinolate contents of seeds of *B. vulgaris*, *B. verna* and *B. orthoceras* were reported by Daxenbichler et al. (1991) and for the two former species also by Andersson et al. (1999), and are not in conflict with the biosynthetic characters for vegetative plant parts deduced in this report. The unpublished results showing dominance of **2*R*** relative to **2*S*** in seeds of *B. intermedia* (Andersson et al., 1999), probably referring to Jensen (1990), would appear to be in contrast to the results reported here for leaves and roots (Table 2), unless the ratio of **2*R*** to **2*S*** is opposite in seeds relative to roots in *B. intermedia*. Unless the results of Jensen (1990) can be repeated with properly identified plant material, we suggest that the experiments were in reality carried out with seeds from a misnamed specimen of the P-type of *B. vulgaris* var. *arcuata*, since the ratio of **2*R*** to **2*S*** in seeds of the P-type is similar to the ratio in leaves and roots (Agerbirk et al., 2001a). Indeed, the P-type is known from several of the localities listed by Jensen (1990), while *B. intermedia*, which is rare in Denmark, is not known from these localities (Nielsen, unpublished). It is stated in the review by Fahey et al. (2001) that Daxenbichler et al. (1991) reported the presence of *p*-hydroxybenzylglucosinolate in *B. verna*. However, Daxenbichler et al. (1991) did not report this glucosinolate from *B. verna*: The presence of thiocyanate ion after myrosinase-catalyzed hydrolysis was reported, which is indicative of *either* *p*-hydroxybenzylglucosinolate *or* any of the Trp-derived glucosinolates.

As pointed out by Sørensen (1990) and Andersson et al. (1999), acyl conjugates of **2*S*** occur in seeds of *B. vulgaris* and can be detected with suitable methods (Michaelsen et al., 1992), but this aspect of the glucosinolate biosynthesis in *Barbarea* was not investigated in the present study. Apart from acyl-substituted glucosinolates, the present report appears to be the most complete survey of homo-Phe and Trp derived glucosinolates in the genus *Barbarea*. However, as some glucosinolates are specific for particular tissues (e.g., roots), there may still be more *Barbarea* glucosinolates to be discovered, e.g. in the floral parts.

While **1** is one of the most widespread glucosinolates in Brassicaceae, only few genera are known to contain 2-hydroxylated derivatives of **1**. Fahey et al. (2001) made the simplification to list distributions of 2-hydroxylated glucosinolates irrespective of the absolute configurations, so that occurrence of **2*R*** *or* **2*S*** was listed, etc. However, from the original litterature, the absolute configuration can be concluded in most cases (Gmelin et al., 1970; Kjær and Schuster, 1972; Jensen, 1990; Andersson et al., 1999; Agerbirk et al., 2001a), except where hydrolysis products have been analysed by gas chromatography (Daxenbichler et al., 1991), which does not distinguish between the enantiomeric substituted oxazolidine-2-thiones. The predominant 2-hydroxylated glucosinolate is usually found together with small amounts of the epimer (Jensen, 1990; Andersson et al., 1999; Agerbirk et al., 2001a; Table 2), probably due to a less than 100% stereospecificity of the

2-hydroxylating biosynthetic step. For this reason, it is mainly the predominant epimer that is chemotaxonomically interesting, and the minor epimer will be ignored in the following status of the present knowledge of the chemotaxonomy of 2-hydroxylated, formally homo-Phe derived glucosinolates: Within Brassicaceae, **2*S*** is known from *Barbarea* (Agerbirk et al., 2001a, and references cited therein; Table 2). In some cases, **2*S*** is accompanied by **3*S*** (Agerbirk et al., 2001a; Table 2). An independent occurrence of **2*S*** is in Resedaceae (Jensen, 1990). **2*R*** is known from *Sibara virginica* (Gmelin et al., 1970), the *p*-methoxy derivative of **2*R*** is known from *Arabis hirsuta* (Kjær and Schuster, 1972), and **2*R*** and **3*R*** are known from the P-type of *B. vulgaris* var. *arcuata* (Agerbirk et al., 2001a; Table 2). Either **2*S*** or **2*R*** is known from *Selenia auria* and the *p*-methoxy derivative of either **2*R*** or **2*S*** is known from some *Arabis* species (Daxenbichler et al., 1991). In future chemotaxonomic studies, the absolute configuration of 2-hydroxylation should preferably be determined.

3.2. Taxonomic value of characters

The reported evidence for a common flea beetle resistance mechanism in *B. verna*, *B. intermedia*, and the previously known resistant taxa *B. vulgaris* var. *vulgaris* and the G-type of var. *arcuata*, expands previous investigations, and demonstrates an effect of the R-gene on two additional species. Comparative investigations have previously been unable to detect any effects of the R-gene when the larvae are feeding on cruciferous plants outside the genus *Barbarea* (Nielsen, 1999). The defences in the four *Barbarea* taxa are therefore more similar to each other than they are to any defences found in other genera of the Cruciferae. The most simple explanation is that the resistance mechanisms in these *Barbarea* taxa are homologous. This may indicate a monophyletic origin of flea beetle resistance, before the divergence of the species *B. vulgaris*, *B. intermedia* and *B. verna* from a common ancestor, but after the divergence of *B. stricta* and *B. orthoceras*. The distinction between these two groups of species is also reasonable due to morphological similarities. The lack of flea beetle resistance in the P-type of *B. vulgaris* var. *arcuata* may then be due to a secondary loss. The close morphological similarity of the P-type and the G-type, supports the hypothesis of a secondary loss of resistance. Further speculations on this matter should await elucidation of the chemical basis of flea beetle resistance in the three resistant species (Shinoda et al., 2002).

Within *B. intermedia*, there was variation in leaf pubescence (see Section 2.2). Pubescence in *B. intermedia* was not correlated with differences in glucosinolate content in any way. Specifically, pubescent as well as glabrate accessions contained the same dominant epimer (**2*S***) of 2-hydroxy-2-phenylethylglucosinolate (Table 2). One glabrate accession was not significantly more resistant to the ST-line than to the YE line (Table 4), but this is likely to be a chance effect of the low sample number, and of the generally weak phenotype of resistance in this species. So our results do not imply a need for a separation of the species *B. intermedia* into a pubescent and a glabrous taxon, in contrast to the situation for *B. vulgaris* var. *arcuata*.

The glucosinolate contents of *B. vulgaris* var. *vulgaris* and the G-type of *B. vulgaris* var. *arcuata* were identical, and the remaining *Barbarea* taxa except the P-type of *B. vulgaris* var. *arcuata* differed in no more than a single biosynthetic character (Table 5). When all characters measured in this paper were combined, all of the species except *B. stricta* and *B. orthoceras* contained a unique combination. From inspection of Table 5, it is obvious that the characters were in no way correlated in the genus, i.e. there was no correlation between presence of a particular glucosinolate, pubescence, or resistance. The wide distribution of **2*S*** suggests that this glucosinolate was present in the common ancestor of the genus, and the trivial name of **2*S***: glucobarbarin, is indeed appropriate. From a similar argument, the common ancestor was apparently devoid of flea beetle resistance and pubescence, i.e. a combination of characters much like todays *B. stricta*.

3.3. Taxonomic status of the P- and G-types of *B. vulgaris var. arcuata*

Compared to the generally minor interspecific variation in the genus, the differences in glucosinolate biosynthesis between the two types of *B. vulgaris* var. *arcuata* were remarkable. Also in other respects, namely presence or absence of flea beetle resistance and leaf pubescence, the differences between the two types were significant (Table 5). A cytological description of most of the *Barbarea* accessions used here, including the P- and G-type of *B. vulgaris* var. *arcuata*, will be published elsewhere (Ørgaard et al., in prep.). Our data clearly confirm the previously proposed distinction of the two types of *B. vulgaris* var. *arcuata* among Danish accessions, originally based mainly on the difference in flea beetle resistance (Nielsen, 1997a). The significant chemical differences between the two types, compared to the failure to detect any differences between var. *vulgaris* and the G-type of var. *arcuata*, raises the question whether the P-type belongs to the *B. vulgaris* complex at all. However, the results presented here and elsewhere (Agerbirk et al., 2001a), are provisional indications that there is at least a limited gene flow in geographical areas where the two types co-occur.

Since *B. vulgaris* has a very large geographical distribution, and is considered to be an extremely variable species, accessions obtained from a larger geographical area should be investigated, to determine whether the

differences found in this report between glabrous and pubescent types of var. *arcuata* s.l. are generally valid. For practical reasons, we recommend to continue the present taxonomic affiliation of the P-type and G-type as chemo types of *B. vulgaris* var. *arcuata* s. l., until further evidence is available. It seems quite likely that the variety named *Barbaraea arcuata* var. *pubescens* in Bush (1939) is an older synonym for the P-type, which may eventually replace the provisional designation "P-type".

Leaf pubescence on first year rosette plants is the only definite field character for the distinction of the P-type. Rich (1987) considered sparse leaf pubescence to be highly dependent on the growth conditions, and thus unfit for use as a field character. However, the extent of leaf pubescence reported here for plants grown in a growth chamber is in approximate agreement with the previously reported quantitative results for leaves collected in nature (Agerbirk et al., 2001a). Thus, a convinient field character for rosette leaves of the G-type would be: Usually less than 10 hairs along the basal fourth of the leaf margin, starting from the petiole, and glabrous to glabrate leaf surface. The corresponding character for the P-type would be: Usually more than 20 hairs (often 40–100 or more, Agerbirk et al., 2001a) along the basal fourth of the leaf margin, pubescent leaf surface. [The rosette leaves should be from the same year, and not collected late in the fall, since the hairs are gradually lost during the fall and winter (Agerbirk et al., 2001a)]. An additional convenient character is the ratio of **2*S*** to **2*R***, which can be measured in a single, dried, field-collected leaf of both first year and second year plants, and probably even in quite old herbarium specimens. If a type-specimen of *B. arcuata* var. *pubescens* N. Busch exists, glucosinolate analysis of leaf material from the type would be an obvious test to decide whether "P-type" is synonymous for var. *pubescens* N. Busch, since the high concentration of **2*R*** relative to **2*S*** in leaves of the P-type is not known for any other *Barbarea* taxon.

4. Experimental

4.1. Plants

The accessions investigated were collected as seeds from wild populations of the various species, except for *B. verna*, which was obtained as seeds from the Botanical Garden at the University of Copenhagen and from a commercial supplier (Table 1). The accession B1 has previously been named "accession 3" in a number of studies from one of our laboratories (e.g. Nielsen, 1997a, 1999; de Jong et al., 2000). The accession B26 (*B. orthoceras*) is the same as previously used (Agerbirk et al., 2001a). The accessions B1, B4, B5, B7, B8, and B16, are the same as previously used (Agerbirk et al., 2001b). Plants were grown from the seeds under identical conditions in a growth chamber (Nielsen, 1999). For each accession, a "lawn" consisting of dozens of individuals was grown, and leaves for tests of resistance and pubescence were picked randomly. Experiments were performed in several blocks from 1996 to 2000. Most accessions were tested for resistance and pubescence in several blocks, and in those cases the data were pooled since they were not statistically different. First year rosette plants, 3–12 weeks old, were used for all experiments.

After establishment in the growth chamber, several plants of each accession were potted and placed outdoor (at RVAU, Copenhagen, Denmark) for further growth, vernalisation during the winter, and flowering, to produce herbarium material of floral plant parts. The taxonomic identity of the material was controlled based on the resulting flowering plants. The identification of accession B26 as *B. orthoceras* was based on the lenght of the petals, which is given as the key character distinguishing *B. orthoceras* and *B. stricta* by Bush (1939). However, in general morphology, accession B26 was very similar to the five investigated *B. stricta* accessions. The *B. stricta* accessions (B8, B40, B41, B42 and B43), were identified as *B. stricta* rather than *B. orthoceras* based on their origin from Denmark, where *B. orthoceras* is not known to occur (Ball, 1993). Voucher specimens of both first-year rosette leaves and of second-year flowering plants from Danish accessions collected in the wild were deposited at the Herbarium of Danish Vascular Plants, Botanical Museum, University of Copenhagen. Voucher specimens of the remaining few accessions, not collected in the wild or collected from the rest of the world, are kept in the herbarium of the Department of Ecology, Section for Botany, RVAU.

4.2. Glucosinolate analysis

Freshly picked leaves (including petioles) and fresh, gently washed roots were freeze dried without previous freezing. Pooled leaves and roots, originating from several individuals, were used for the extraction unless otherwise stated (Table 2, footnote c and d). Glucosinolate (gls) analysis was performed as previously described (Agerbirk et al., 2001a), a representative chromatogram has been published elsewhere (Müller et al., in press). The calculated mean gls profiles of each taxon were based on one analysis of leaves and one of roots, but presence or absence of a particular gls in an accession was usually confirmed by additional analyses (Table 2, footnote b and unpublished). Peak identification was based on retention times and on-column diode array UV spectra, compared to authentic desulfogls standards. Desulfoderivatives of **2*R***, **2*S***, **3*R***, **3*S***, **4**, **5** and **7** were previously isolated from selected *Barbarea* species, and identified by spectroscopic means (Agerbirk

et al., 2001a, b). **1** was isolated as the desulfoderivative after sulfatase treatment of gls from roots of the P-type of *B. vulgaris* var. *arcuata* and identified by MS and ^{1}H NMR (data not shown). Desulfo **6** was identified by comparison with the compound known from broccoli (*Brassica oleracea* var. *italica*) (Agerbirk et al., 1998). Calculation of gls profiles as mol% of homo-Phe-derived gls (**1**, **2*R***, **2*S***, **3*R***, **3*S***) and mol% of Trp-derived gls (**4**, **6**, **5**, **7**) was done from the HPLC peak areas, assuming that all homo-Phe-derived gls had the same response factor, and assuming the previously used response factors for indole-gls (Agerbirk et al., 2001b). The ratio of **2*S*** to **2*S*** + **2*R*** was defined as 100×[**2*S***]/([**2*R***] + [**2*S***]), and calculated directly from the HPLC areas.

4.3. Flea beetle resistance

The near isogenic *Phyllotreta nemorum* lines ST and YE were described elsewhere (Nielsen, 1999). 3-Day resistance assays with neonate *P. nemorum* larvae were carried out as described elsewhere (Nielsen, 1999).

4.4. Leaf pubescence

Leaf pubescence was determined for first year rosette plants grown at identical conditions in growth chambers. The number of hairs along one quarter of the end lobe margin was counted, starting at the petiole, as described elsewhere (Agerbirk et al., 2001a). The four first true leaves were not included. Leaf tissue for the SEM study was dehydrated in a graded series of acetone, dried in a critical point dryer, sputter-coated and examined in a Jeol JSM-840A microscope.

4.5. Statistical analysis

The Kruskal Wallis Test was performed with the NPAR1WAY procedure in SAS (SAS Institute, 1990). Chi-square tests were performed as described for a two-sample case by Daniel (1995).

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References

Agerbirk, N., Olsen, C.E., Sørensen, H., 1998. Initial and final products, nitriles, and ascorbigens produced in myrosinase-catalyzed hydrolysis of indole glucosinolates. J. Agric. Food Chem. 46, 1563–1571.

Agerbirk, N., Olsen, C.E., Nielsen, J.K., 2001a. Seasonal variation of leaf glucosinolates and insect resistance in two types of *Barbarea vulgaris* ssp *arcuata*. Phytochemistry 58, 91–100.

Agerbirk, N., Petersen, B.L., Olsen, C.E., Halkier, B.A., Nielsen, J.K., 2001b. 1,4-Dimethoxyglucobrassicin in *Barbarea* and 4-hydroxyglucobrassicin in *Arabidopsis* and *Brassica*. J. Agric. Food Chem. 49, 1502–1507.

Al-Shehbaz, I.A., 1988. The genera of Arabideae (Cruciferae; Brassicaceae) in the Southeastern United States. J. Arnold Arboretum 69, 85–166.

Al-Shehbaz, I.A., Peng, C.-I., 2000. The genus *Barbarea* (Brassicaceae) in Taiwan. Bot. Bull. Acad. Sin. 41, 237–242.

Andersson, A.A.M., Merker, A., Nilsson, P., Sørensen, H., Åman, P., 1999. Chemical composition of the potential new oilseed crops *Barbarea vulgaris*, *Barbarea verna* and *Lepidium campestre*. J. Sci. Food Agric. 79, 179–186.

Ball, P.W., 1993. *Barbarea* R. Br. In: Tutin, T.G., Burges, N.A., Chater, A.O., Edmondson, J.R., Heywood, V.H., Moore, D.M., Valentine, D.H., Walters, S.M., Webb, D.A. (Eds.), Flora Europaea, Vol. 1, 2nd ed. Cambridge University Press, Cambridge, pp. 342–343.

Bush, N.A., 1939. *Barbaraea* Beck. In: Komarov, V.L. (Ed.), Flora SSSR. Moskva. English version: Flora of the U.S.S.R., Vol. 8. Israel Program for Scientific Translations, Jerusalem, 1970, pp. 99–102.

Cole, R.A., 1976. Isothiocyanates, nitriles and thiocyanates as products of autolysis of glucosinolates in cruciferae. Phytochemistry 15, 759–762.

Daniel, W.W., 1995. Biostatistics, 6th edn. John Wiley, New York.

Daxenbichler, M.E., Spencer, G.F., Carlson, D.G., Rose, G.B., Brinker, A.M., Powell, R.G., 1991. Glucosinolate composition of seeds from 297 species of wild plants. Phytochemistry 30, 2623–2638.

Fahey, J.W., Zalcmann, A.T., Talalay, P., 2001. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. Phytochemistry 56, 5–51.

Gmelin, R., Kjær, A., Schuster, A., 1970. Glucosinolates in seeds of *Sibara virginica* (L.) Rollins: two new glucosinolates. Acta Chem. Scand. 24, 3031–3037.

Hegi, G., 1958. Illustrierte Flora von Mittel-Europa. Carl Hanser Verlag, München.

Huang, X., Renwick, J.A.A., Sachdev-Gupta, K., 1994. Oviposition stimulants in *Barbarea vulgaris* for *Pieris rapae* and *P. napi oleracea*: isolation, identification and differential activity. J. Chem. Ecol. 20, 423–438.

Hewson, H.J., 1982. Brassicaceae. In: Briggs, B.G., Barlow, B.A., Eichler, H., Pedley, L., Ross, J.H., Symon, D.E., Wilson, P.G., McCusker, A., George, A.S. (Eds.), Flora of Australia, Vol. 8. Australian Government Publishing Service, Canberra, pp. 231–357.

Jensen, J., 1985. Brassicaceae. In: Hansen, K. (Ed.), Dansk Feltflora. Gyldendal, Copenhagen, pp. 225–252.

Jensen, S.K., 1990. Characterization and properties of epimeric 2-hydroxy substituted glucosinolates. In: Biochemical and Physiological Investigations of the Meal and Syrup Fractions from Aqueous Enzymatic Rapeseed Processing. PhD Thesis, Royal Veterinary and Agricultural University, Denmark, pp. 57–71.

de Jong, P.W., Frandsen, H.O., Rasmussen, L., Nielsen, J.K., 2000. Genetics of resistance against defences of the host plant *Barbarea vulgaris* in a Danish flea beetle population. Proc. R. Soc. Lond. B 267, 1663–1670.

Kjær, A., Gmelin, R., 1957. Isothiocyanates XXVIII. A new isothiocyanate glucoside (glucobarbarin) furnishing (-)-5-phenyl-2-

oxazolidinethione upon enzymic hydrolysis. Acta Chem. Scand. 11, 906–907.

Kjær, A., Schuster, A., 1972. Glucosinolates in seeds of *Arabis hirsuta* (L.) Scop.: some new, naturally derived isothiocyanates. Acta Chem. Scand. 26, 8–14.

Lange, T., 1937. Sveriges *Barbarea*-arter. Botaniska Notitser 1937, 216–230.

Mabberly, D.J., 1997. The Plant-Book. Cambridge University Press, Cambridge.

MacDonald, M.A., Cavers, P.B., 1991. The biology of Canadian weeds 97. *Barbarea vulgaris* R. Br. Can. J. Plant Sci. 71, 149–166.

Michaelsen, S., Møller, P., Sørensen, H., 1992. Factors influencing the separation and quantitation of intact glucosinolates and desulphoglucosinolates by micellar electrokinetic capillary chromatography. J. Chromatogr. 608, 363–374.

Mikkelsen, M.D., Petersen, B.L., Olsen, C.E., Halkier, B.A., 2002. Biosynthesis and metabolic engineering of glucosinolates. Amino Acids 22, 279–295.

Müller, C., Agerbirk, N., Olsen, C.E. Lack of sequestration of host plant glucosinolates in *Pieris rapae* and *P. brassicae*. Chemoecology (in press).

Nielsen, J.K., 1997a. Variation in defences of the plant *Barbarea vulgaris* and in counteradaptations by the flea beetle *Phyllotreta nemorum*. Ent. Exp. Appl. 82, 25–35.

Nielsen, J.K., 1997b. Genetics of the ability of *Phyllotreta nemorum* larvae to survive in an atypical host plant, *Barbarea vulgaris* ssp. *arcuata*. Ent. Exp. Appl 82, 37–44.

Nielsen, J.K., 1999. Specificity of a Y-linked gene in the flea beetle *Phyllotreta nemorum* for defences in *Barbarea vulgaris*. Ent. Exp. Appl. 91, 359–368.

Rich, T.C.G., 1987. The genus *Barbarea* R. Br. (Cruciferae) in Britain and Ireland. Watsonia 16, 389–396.

Rossiter, J.T., James, D.C., 1990. Biosynthesis of (*R*)-2-hydroxybut-3-enylglucosinolate (progoitrin) from [3,4-^{3}H]but-3-enylglucosinolate in *Brassica napus*. J. Chem. Soc. Perkin Trans 1, 1909–1913.

Sang, J.P., Minchinton, I.R., Johnstone, P.K., Truscott, R.J.W., 1984. Glucosinolate profiles in the seed, root, and leaf tissue of cabbage, mustard, radish and swede. Can. J. Plant Sci. 64, 77–93.

SAS Institute Inc., 1990. SAS/STAT Users Guide, Vols. 1 and 2, Version 6, 4th ed. SAS Institute Inc., Cary, USA.

Seo, B., Yun, J., Lee, S., Kim, M., Hwang, K., Kim, J., Min, K.R., Kim, Y., Moon, D., 1999. Barbarin as a new tyrosinase inhibitor from *Barbarea orthocerus*. Planta Med. 65, 683–686.

Shinoda, T., Nagao, T., Nakayama, M., Serizawa, H., Koshioka, M., Okabe, H., Kawai, A., 2002. Identification of a triterpenoid saponin from a crucifer, *Barbarea vulgaris*, as a feeding deterrent to the diamondback moth, *Plutella xylostella*. J. Chem. Ecol. 28, 587–599.

Stace, C., 1991. New Flora of the British Isles. Cambridge University Press, Cambridge.

Sørensen, H., 1990. Glucosinolates: structure—properties—function. In: Shahidi, F. (Ed.), Canola and Rapeseed. Production, Chemistry, Nutrition and Processing Technology. Van Nostrand Reinhold, New York, pp. 149–172.

Underhill, E.W., Kirkland, D.F., 1972. L-2-Amino-4-phenylbutyric acid and 2-phenylethylglucosinolate, precursors of 2-hydroxy-2-phenylethylglucosinolate. Phytochemistry 11, 1973–1979.