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Factors Affecting the Glucosinolate Content of Kale (*Brassica oleracea acephala* Group)

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Kales (*Brassica oleracea acephala* group) are important vegetable crops in traditional farming systems in the Iberian Peninsula. They are grown throughout the year to harvest their leaves and flower buds. The glucosinolate content of kales is dependent upon the environmental factors, plant part examined, phenological stage of plant growth, and level of insect damage. The objectives of this study were to evaluate the changes in the total and individual glucosinolate concentrations during plant development and to determine if significant variation of glucosinolate levels can be explained by insect pests attack and other environmental factors in four locations in northwestern Spain. The total glucosinolate concentration in leaves of *B. oleracea* increased with plant age from seedling to early flowering stages. At that stage, the aliphatic glucosinolate content in leaves of *B. oleracea* declined drastically over time as the content in the flower buds increased. The highest contents of indolyl glucosinolate (glucobrassicin) and of the aromatic glucosinolate occurred in leaves harvested at the optimum consumption stage while flower buds contained the highest concentration of aliphatic glucosinolates, especially sinigrin. Sinigrin is reported to have anticarcinogenic properties. There appears to be a loss of total and individual glucosinolate concentrations related to pest attack. Leaves damaged by lepidopterous pests contained a lower total glucosinolate content (25.8 μmol g^{-1} dw) than undamaged leaves (41 μmol g^{-1} dw). The amounts of sinigrin, glucoiberin, and glucobrassicin were also lowest in insect-damaged leaves. Environmental factors such as soil properties and temperature appear to influence the glucosinolate content in leaves although more research on this subject is needed.

KEYWORDS: ***Brassica oleracea*; environmental factors; development stages; glucosinolates; kale; lepidopterous pests; sinigrin**

INTRODUCTION

Brassica species are important crops in traditional farming systems in the Iberian Peninsula. In particular, kales (*Brassica oleracea acephala* group) are grown throughout the year for their leaves and flower buds. Leaves are harvested by "picking over" during plant growth, using the most tender ones for human consumption and the inferior ones for animal feed (*1*). The consumption of kales in northwestern Spain is high, and in the coldest regions of Portugal and northern Spain, they constitute an important vegetable during the winter. Kales are biannual crops; hence, the leaf consumption period starts 3 months after transplanting until almost 1 year afterward. At this stage, green flower buds are used for human consumption, being eaten as a boiled vegetable in a similar way as turnip tops.

Glucosinolates (GSs) are the main class of secondary metabolites found in cruciferous crops. The GS consists of a β-thioglucose moiety, a sulfonated oxime moiety, and a variable side chain derived from an amino acid. GSs and their breakdown products (isothiocynates, thiocynates, nitriles, and epithionitriles) have been shown to adversely affect human health, reduce or stimulate insect herbivory, and inhibit the growth of nematodes, fungi, and other microorganisms and the growth of neighboring plants, and they have a chemoprotective effect against certain cancers in humans (*2*−*5*). In vitro and in vivo studies have reported that isothiocyanates affect many steps of cancer development including modulation of phase I and II detoxification enzymes (*6*−*9*).

Variation on the amount and pattern of GSs has been attributed to genetic and environmental factors, including plant age, temperature, water stress, and soil type (*2*, *10*−*13*). Distribution of the GSs varies depending on plant part, with both quantitative and qualitative differences among roots, leaves, stems, and seeds. Agronomic factors, such as soil type, moisture, and mineral nutrient availability, are known to exert a significant effect on GS content. Soil fertility has significant effects on levels of specific GSs in the growing plants (*2*). Total and indole GS concentrations have been correlated with climatic factors in several crops of *B. oleracea* (*14*, *15*). Winter seasons seem

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to induce lower GS levels due to short days and cool temperatures accompanied by frost (*2*).

In addition, pest attack can markedly alter GS levels in leaves. The role of GSs in plant−herbivore interactions has been discussed (*16*−*19*). Herbivore attack, particularly by chewing insects, causes tissue disruption, thereby bringing GSs into contact with myrosinase, and resulting in the production of a variety of toxic degradation products. The role of the GS myrosinase system in plant interactions with insect herbivores has been recently discussed (*20*).

At the Misión Biológica de Galicia (Spanish Council for Scientific Research), a *B. oleracea* collection of landraces is kept as part of the *Brassica* genus germplasm bank. The nutritional content of this collection has been recently studied (unpublished data). In that study, the average GS concentration in the leaves was 27 μm g^{-1} of dry tissue, with sinigrin (34%), glucoiberin (28%), and glucobrassicin (25%) as the predominant GSs. In addition to other *B. oleracea* crops, kale crops could play an important role in human alimentation because they act as antioxidants, they reduce cholesterol levels, and they have anticarcinogenic properties. The study of GS levels through plant development will determine which plant parts and stages of the plant cycle contain the highest concentrations of these beneficial compounds for human health. Because GSs are important in human nutrition, the objectives of this study were to evaluate the changes in the total and individual GS concentrations during plant development and to determine if significant variation of GS levels can be explained by insect pests attack and other environmental factors.

MATERIALS AND METHODS

Plant Material. A local variety of kale, MBG-BRS0103, kept at the germplasm bank at the Misión Biológica de Galicia and representative of kale germplasm grown in this area, was chosen for this study. Plots were sowed with the same local variety, and seeds were planted in multipot trays. Seedlings were then transplanted into the field at the five- or six-leaves stage. Each plot consisted of 20 rows (80 cm between rows) with about 20 plants per row (60 cm between plants). Sowing of seeds was made in March, and the transplantation was performed in mid-April. The study was carried out in four locations in the northwest of Spain: Pontevedra (42° 24′ N, 8° 38′ W, 20 m above the sea level), Lalín (42° 37′ N, 8° 8′ W, 490 m above the sea level), Pontecaldelas (42° 23′ N, 8° 32′ W, 300 m above the sea level), and Cotobade (42° 28′ N, 8° 28′ W, 450 m above the sea level). The soil type was acid sandy loam. For all locations, the same cultural operations, fertilization, and weed controls were made according to local practices in the north of Spain.

Sampling Times and Plant Parts. Several samplings were carried out between sowing and maturity periods. Samples were taken at five critical phases in the life cycle of this winter biannual crop: 1L, the initial plant stage before transplanting at the five- or six-leaves stage, 30 days after sowing (DAS); 2L, the vegetative phase at the first optimum consumption stage, 90 DAS; 3L, the vegetative phase at the second optimum consumption stage, 180 DAS; 4L, the vegetative−reproductive phase transition, at the last consumption stage 300 DAS; and 5, the reproductive phase before bolting, 390 DAS. Because flower buds are highly appreciated in human consumption, in that last phase, samples were taken on two plant parts: on leaves (5L) and on flower buds (5B). Samples in stage 1 were taken in seedlings at the greenhouse while samples in stages from 2 to 5 were taken in the four locations. A sample of five healthy and fresh leaves was randomly chosen on each sampling date from 20 to 30 plants from each plot.

GS Analysis. Samples were frozen in situ in liquid N_2, and they were taken immediately into the laboratory where they were stored at −80 °C. Then, the green material was ground in liquid N_2, freeze-dried, and milled to a fine powder for the GS extraction. The GS composition of the leaves was determined by high-performance liquid chromatography according to ref *21* with minor modifications. The type and amount of GSs were estimated in comparison to certified GS levels of a standard rapeseed reference material developed by the Commission of the European Community Bureau of References (Brussels, Belgium) (*22*). All GS contents are given as the concentration in dry weight (μmol g^{-1} dw).

Insect Pests. To relate the changes on the GS concentration caused by insect pests, the incidence and leaf damage caused by lepidopterous pests were evaluated throughout the plant cycle in the four locations mentioned above. Fifty plants were randomly sampled from June to November at 2 week intervals in each plot. Leaf damage and the abundance of larvae of several species were evaluated. Leaf damage caused by larval feeding was determined by using a visual damage rating (1−9 scoring, from 1, more than 70% of leaf area was damaged, to 9, no damage) over each individual plant. Plants were examined for the presence of larvae of the major insect pests previously described in our environmental conditions by ref *23*. At plant stage 3L, damaged leaves and undamaged leaves from 20 to 30 plants were collected at each location. In order to relate GS content with lepidopterous incidence and damage, the qualitative and quantitative GS variation of leaves was analyzed. The difference on GS content between the two damage stages was in this work called "GS loss percentage" and was expressed as 1 − (GS content on damage leaves/GS content on undamaged leaves).

Soil Analyses and Climate Data. Soil samples were collected in July 2005 at the four locations above-mentioned. Samplings were carried out using a hollow cylindrical corer with an internal diameter of 7 cm. Six subsamples, each 25 cm deep, were taken following a zigzag path across the center of each plot. Subsamples were mixed to obtain homogeneous samples, about 500−1000 g, to be analyzed. The aim of this sampling was to study the influence of soil properties on the GS content. Soil samples were air-dried and passed through a 2 mm sieve. The soil properties examined were soil pH, percentage of organic matter, available phosphorus, available potassium, exchangeable magnesium, exchangeable cations (Ca, Mg, Na, K, and Al), and cation exchange capacity. Soil analyses were performed at "Estación Fitopatológica do Areeiro" (Pontevedra, Spain). The pH values in water and in KCl were determined using a w/v ratio of 1:2.5. Extractable P was analyzed using a modification of Olsen's method (*24*). Exchangeable cations were extracted using w/v ratios of 5:100 soil:1 M NH_4Cl (*25*), and determinations were made of Al using colorimetry with pyrocatechol violet (*26*), Ca and Mg by atomic absorption spectrophotometry, and Na and K by emission spectrophotometry.

Climatic data (temperature and precipitation) were obtained from meteorological stations located close to the experimental fields. All locations were situated in northwestern Spain and had a humid climate with an annual rainfall of about 1600 mm.

Statistical Analysis. Individual and combined analyses of variance over locations and plant stages were performed for each GS according to a completely randomized design. Stages and locations were considered as fixed factors. Analyses were carried out using the GLM procedure of SAS (*27*). Comparisons of means among plant stages and locations were performed for each trait by Fisher's protected least significant difference at $P = 0.05$ (*28*).

RESULTS AND DISCUSSION

Stage of Development and GS Content and Profile. Nine GSs were identified in the edible parts of vegetable kales (leaves and flower buds), belonging to the three chemical classes: five aliphatic, three indolyl, and one aromatic (**Table 1**). Aliphatic GSs were predominant, representing 78% of the total GS content, followed by indolyl GSs (20.4%) and aromatic GSs (1.6%).

Most GSs, except gluconapin and neoglucobrassicin showed a significant plant stage × location interaction (**Table 2**). Differences among locations were significant ($P \leq 0.01$) for total GS content, sinigrin, glucoiberin, 4-OH-glucobrassicin, and gluconasurtiin (**Table 2**). Plant development stages showed significant differences ($P \leq 0.01$) for the total GS content as

Table 1. GSs Found in Leaves and Flower Buds of Kales

chemical class	common name	chemical name
aliphatic GSs (derived from methionine)	sinigrin	2-propenyl
	glucoiberin	3-methylsulfinylpropyl
	progoitrin	2-(*R*)-2-hydroxy-3-butenyl
	epiprogoitrin	2-(*S*)-2-hydroxy-3-butenyl
	gluconapin	3-butenyl
indolyl GSs (derived from tryptophan)	glucobrassicin	indol-3-ylmethyl
	neoglucobrassicin	1-methoxyindol-3-ylmethyl
	4-hydroxyglucobrassicin	4-hydroxyindol-3-ylmethyl
aromatic GSs (derived from phenylalanine)	gluconasturtiin	2-phenylethyl

well as for all of the individual GSs. This agrees with other works that showed that GS content is dependent upon the agronomic factors associated with its growth stage and the plant part examined (*2*, *10*, *11*). In spite of the fact that the plant stage × location interaction was significant for most GSs, all compounds showed a similar pattern through the plant cycle (**Figure 1**) in the different locations. Therefore, to make the

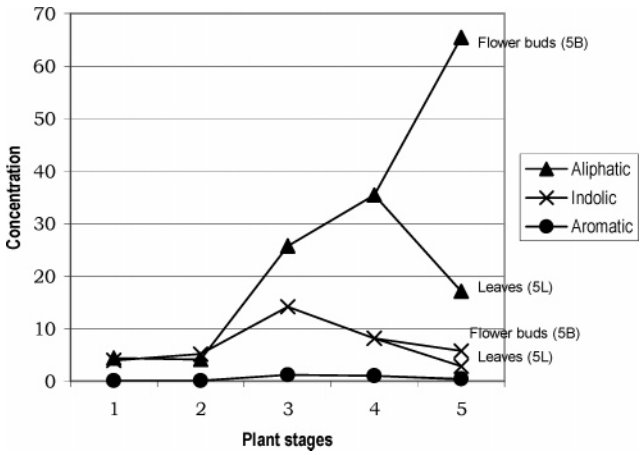

Figure 1. Mean concentration (μmol g^{-1} dw) for the three classes of GSs through plant growth.

presentation clear, means for different plant stages will be presented across locations (**Table 3**). Later, to discuss the differences in GS content among locations, we will focus on two representative development stages: leaves harvested at 180 DAS (stage 3) and flower buds harvested at the reproductive stage before bolting (stage 5B) (**Table 4**). Plants in these two stages are preferably accepted for human consumption.

Sinigrin was the major GS followed by glucoiberin and glucobrassicin (**Table 3**). In flower buds (5B), it represented 67% of the total GSs, while in leaves its percentage varied from 27% in 2L to 59% in 5L (**Table 3**). Isothiocyanates derived from sinigrin have been related to anticarcinogenic properties (*13*). Moreover, it is known that isothiocyanates derived from sinigrin can cause a reduction in the cholesterol levels in mice (*30*). Another beneficial effect attributed to sinigrin is its role as a suppressor of the growth of nematodes, fungi, and other soil microorganisms (*2*), although this GS also contributes, as well as glucoiberin and gluconasturtiin, to the presence of some specialist pests (*17*). The second GS in abundance was glucobrassicin. This GS represented 41% of total GS content in 1L, and it was decreasing until 5B (7% of the GS content). Glucobrassicin is the parent compound of indole-3-carbinol, which has also been reported along with the sulforaphane as the most potent anticancer compounds found in cruciferous vegetables. Smaller amounts of other minor GSs such as progoitrin, gluconasturtiin, and neoglucobrassicin were also detected. Most previous studies have shown a similar GLS pattern in leaves of kales and cabbages where the sinigrin was the dominant GS (*7*, *11*, *29*).

Because in this species the amounts of the progoitrin, described as a GS, potentially goitrogenic, are very low, there does not appear to be a health risk associated with the consumption of these vegetables. This result agrees with previously reported levels of progoitrin in leaves of edible cabbage and kale (*29*). Because sinigrin and glucobrassicin are important for cancer chemoprotection, plant stages and plant parts should be considered when planning harvest or when making breeding selections for GS concentrations.

The GS pattern in the flower buds (5B) (67.3% of sinigrin, 21.4% of glucoiberin, and 6.8% of glucobrassicin of total GS content) was similar to leaves collected at the same stage (5L) (59.0% of sinigrin, 24.1% of glucoiberin, and 12.2% of glucobrassicin of total GS content) (**Table 3**). However, the GS content varies greatly during plant development (**Figure 1**). Flower buds had a higher total GS content and aliphatic GS content than leaves collected at any plant stage (**Table 3**). Leaves harvested at the periods of optimum consumption (180 and 300 DAS) had more total GS content than leaves harvested at the first and at the end of the growing season. The pattern on the first plant stages (until 180 DAS) was characterized by high amounts of indolyl GSs, mainly glucobrassicin (**Table 3**), while aliphatic GSs were predominant in flower buds and in leaves taken 300 DAS, at the end of vegetative stage.

Flower buds were the plant part with the maximum GS content (71.6 μmol g^{-1} dw), whereas seedlings at 30 DAS (stage 1) and leaves taken at 90 DAS (stage 2) showed the minimum GS concentration (8.5 and 9.3 μmol g^{-1} dw, respectively) (**Table 3**). The GS concentration in leaves increased with plant age from seedling to early flowering stage. At that stage, the aliphatic GS content in leaves of *B. oleracea* declined drastically over time as the content in the flower buds increased. The results agreed with the findings from Fieldsen et al. (*31*), who found that total GS and most of individual GS content in rapeseed increased from vegetative to reproductive stages and maturity. Rosa and Heaney (*11*) indicated that the GS content in leaves increased over time in contrast to what happens with indolyl GS concentration.

Aliphatic and indolyl GSs showed unlike variation through the plant development. The highest contents for all aliphatic GSs, including sinigrin and glucoiberin, were noted in flower

Table 2. Mean Squares of the Combined Analysis of Variance for the GS Content[a]

	DF	total GS	sinigrin	glucoiberin	progoitrin	epiprogoitrin	gluconapin	glucobrassicin	OHglucobrassicin	neoglucobrassicin	gluconasturtiin
location (L)	3	3541.60**	1106.01**	450.47**	1.51	0.09	0.06	36.42	0.22*	0.95	2.96**
plant stage (PS)	4	47877.44**	23753.80**	2901.13**	22.97**	1.75**	0.17**	916.31**	1.34**	8.19**	20.85**
L × PS	12	1389.35**	352.60*	310.50**	2.86**	0.32**	0.04	100.27**	0.51**	0.50	0.53*
error	535	324.99	165.82	31.67	1.18	0.07	0.05	16.69	0.07	0.65	0.23

[a] *,**Significant at $P < 0.05$ and 0.01, respectively. DF, degrees of freedom.

Table 3. Mean (μmol g^{-1} dw), Standard Errors, and percentage for Total GS Content and GS Compounds through Plant Growth[a]

leaves	sinigrin	%	glucoiberin	%	progoitrin	%	epiprogoitrin	%
1 (30 DAS)	2.73 (0.77)	32.3	1.68 (0.50)	20.0	0.00 (0.00)	0.0	0.00 (0.00)	0.00
2 (90 DAS)	2.52 (0.24)	27.1	1.49 (0.20)	16.0	0.08 (0.02)	0.9	0.00 (0.00)	0.00
3 (180 DAS)	15.30 (1.09)	37.3	9.90 (0.92)	24.1	0.48 (0.11)	1.2	0.05 (0.03)	0.12
4 (300DAS)	24.28 (1.18)	54.4	10.45 (0.58)	23.4	0.63 (0.10)	1.4	0.00 (0.00)	0.00
5L (390 DAS)	11.94 (1.53)	59.0	4.87 (0.78)	24.1	0.25 (0.07)	1.2	0.02 (0.01)	0.10
				flower buds				
5B (390 DAS)	48.14 (2.81)	67.3	15.34 (1.02)	21.4	1.55 (0.24)	2.2	0.34 (0.07)	0.50
LSD (5%)	4.13		1.81		0.35		0.08	

leaves	gluconapin	%	glucobrassicin	%	OH glucobrassicin	%	neo glucobrassicin	%
1 (30 DAS)	0.00 (0.00)	0.00	3.45 (0.40)	40.8	0.00 (0.00)	0.0	0.47 (0.09)	5.6
2 (90 DAS)	0.00 (0.00)	0.00	4.20 (0.31)	45.1	0.03 (0.01)	0.3	0.91 (0.11)	9.8
3 (180 DAS)	0.00 (0.00)	0.00	12.62 (0.93)	30.8	0.26 (0.04)	0.6	1.24 (0.14)	3.0
4 (300DAS)	0.08 (0.04)	0.20	7.48 (0.30)	16.8	0.12 (0.02)	0.3	0.57 (0.04)	1.3
5L (390 DAS)	0.01 (0.01)	0.05	2.46 (0.25)	12.2	0.13 (0.07)	0.6	0.26 (0.04)	1.3
				flower buds				
5B (390 DAS)	0.07 (0.02)	0.10	4.90 (0.27)	6.8	0.41 (0.03)	0.6	0.41 (0.04)	0.6
LSD (5%)	0.07		1.31		0.08		0.26	

leaves	GNAS	% GNAS	total	% aliphatic	% indolyl	% aromatic
1 (30 DAS)	0.11 (0.04)	1.30	8.45 (1.31)	52.3	46.4	1.3
2 (90 DAS)	0.09 (0.02)	0.95	9.31 (0.65)	43.9	55.2	0.9
3 (180 DAS)	1.18 (0.07)	2.90	41.03 (2.53)	62.7	34.4	2.9
4 (300DAS)	1.02 (0.07)	2.30	44.63 (1.63)	79.4	18.3	2.3
5L (390 DAS)	0.32 (0.04)	1.50	20.24 (2.39)	84.4	14.1	1.5
			flower buds			
5B (390 DAS)	0.39 (0.04)	0.50	71.55 (3.47)	91.5	8.0	0.5
LSD (5%)	0.15		5.78			

[a] $N = 30$ for stage 1 (30 DAS) and between 80 and 110 for the other stages. Standard errors are inside the parentheses.

Table 4. Mean (μmol g^{-1} dw) and Standard Errors for Total GS Content and GS Compounds of Two Plant Growth Stages (Leaves at 180 DAS and Flower Buds at 390 DAS) in Four Locations of Northwestern Spain[a]

	total	sinigrin	glucoiberin	progoitrin	epiprogoitrin	gluconapin	glucobrassicin
				leaves (180 DAS)			
Pontevedra	46.54 (4.39)	15.79 (1.65)	9.88 (1.22)	0.55 (0.17)	0.01 (0.01)	0.00 (0.00)	17.17 (2.00)
Lalín	45.92 (4.77)	17.97 (2.45)	11.31 (1.86)	1.06 (0.34)	0.20 (0.10)	0.00 (0.00)	12.76 (1.62)
Pontecaldelas	23.04 (3.20)	9.38 (1.73)	3.49 (1.01)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	8.16 (0.82)
Cotobade	50.48 (5.72)	19.71 (2.81)	17.83 (2.40)	0.27 (0.21)	0.00 (0.00)	0.00 (0.00)	9.84 (1.19)
LSD (5%)	13.17	6.00	4.47	0.61	0.14	0.00	4.90
				buds (390 DAS)			
Pontevedra	93.90 (7.33)	61.66 (6.74)	24.94 (2.38)	1.58 (0.25)	0.59 (0.38)	0.09 (0.04)	3.85 (0.44)
Lalín	62.32 (4.91)	41.98 (3.94)	12.05 (1.52)	1.08 (0.26)	0.02 (0.01)	0.01 (0.00)	5.98 (0.68)
Pontecaldelas	72.93 (5.71)	47.18 (4.39)	16.86 (1.72)	1.33 (0.20)	0.39 (0.08)	0.05 (0.02)	5.62 (0.48)
Cotobade	66.15 (7.60)	46.93 (6.51)	11.63 (1.59)	2.14 (0.69)	0.43 (0.09)	0.13 (0.06)	3.91 (0.26)
LSD (5%)	19.09	16.02	5.04	1.35	0.41	0.12	1.41

	OHglucobrassicin	neoglucobrassicin	gluconasturtiin	aliphatic	indolyl	aromatic
			leaves (180 DAS)			
Pontevedra	0.43 (0.06)	1.62 (0.36)	1.11 (0.13)	26.23	19.21	1.11
Lalín	0.47 (0.08)	0.92 (0.13)	1.22 (0.14)	30.55	14.15	1.22
Pontecaldelas	0.00 (0.00)	1.17 (0.18)	0.85 (0.07)	12.87	9.32	0.85
Cotobade	0.04 (0.03)	1.02 (0.24)	1.78 (0.14)	37.80	10.90	1.78
LSD (5%)	0.16	0.83	0.37			
			buds (390 DAS)			
Pontevedra	0.37 (0.03)	0.37 (0.08)	0.46 (0.07)	88.85	4.60	0.46
Lalín	0.41 (0.03)	0.36 (0.05)	0.44 (0.04)	55.13	6.75	0.44
Pontecaldelas	0.58 (0.10)	0.58 (0.12)	0.33 (0.05)	65.81	6.78	0.33
Cotobade	0.29 (0.04)	0.33 (0.05)	0.37 (0.05)	61.26	4.53	0.37
LSD (5%)	0.18	0.23	0.16			

[a] N between 20 and 30 in each location and stage.

buds while the highest levels for indolyl GSs, as glucobrassicin, and the aromatic GS levels were observed in leaves harvested at the optimum consumption stage at 180 DAS (stage 3) (**Table 3**) (**Figure 1**). The lowest total GS content was found from leaves collected at 30 DAS, at the onset of vegetative growth. The increase in total GS content indicated de novo synthesis of GSs and, in particular, in sinigrin in these plants. The increase was most pronounced in Pontecaldelas where the total GSs and

Table 5. Main Lepidoptera Pests Found in Four Locations at Northwestern Spain, Larvae Incidence ($n = 600$ Plants) from June to November, and Means for Damage Traits for Kale Population Evaluated under Natural Infestation

location	*Mamestra brassicae*	*Pieris rapae*	*Pieris brassicae*	*Plutella xylostella*	*Autographa gamma*	total	damaged plants (%)	rating scale (1–9)
Pontevedra	210	29	1	66	4	310	67.5	8.05
Lalín	322	116	0	187	3	628	81.1	7.30
Pontecaldelas	93	67	17	54	34	265	78.9	7.80
Cotobade	435	81	0	587	12	1115	88.8	6.90

Table 6. Mean (μmol g^{-1} dw) and Standard Errors for Total GLS Content and GLS Compounds for Damaged and Undamaged Leaves and Percentage of GS Loss at Each Location[a]

stage	total	sinigrin	glucoiberin	progoitrin	epiprogoitrin	gluconapin	glucobrassicin
undamaged leaves	41.03 (2.53)	15.30 (1.09)	9.90 (0.92)	0.48 (0.11)	0.05 (0.03)	0.00 (0.00)	12.62 (0.93)
damaged leaves	25.76 (1.89)	9.38 (0.91)	6.69 (0.75)	0.12 (0.05)	0.00 (0.00)	0.00 (0.00)	7.12 (0.59)
LSD (5%)	5.67	2.67	1.99	0.23	0.05	0.00	2.05
			location				
	%	%	%	%	%	%	%
Pontevedra	41.36	31.93	38.83	37.64	0.00	0.00	55.05
Lalín	48.92	54.08	43.67	95.67	0.00	0.00	50.09
Pontecaldelas	28.23	38.97	21.09	0.00	0.00	0.00	26.02
Cotobade	27.75	35.35	29.88	0.00	0.00	0.00	16.43

stage	OHglucobrassicin	neoglucobrassicin	gluconasturtiin	aliphatic	indolyl	aromatic
undamaged leaves	0.26 (0.04)	1.24 (0.14)	1.18 (0.07)	25.73	14.12	1.18
damaged leaves	0.13 (0.03)	1.18 (0.09)	1.14 (0.06)	16.18	8.44	1.14
LSD (5%)	0.07	0.34	0.17			
			location			
	%	%	%	%	%	%
Pontevedra	38.39	18.55	21.61	34.68	51.61	21.61
Lalín	64.56	–3.21	–6.55	51.98	47.10	–6.55
Pontecaldelas	0.00	12.36	–13.24	34.12	23.80	–13.24
Cotobade	62.55	–36.60	10.08	33.22	11.61	10.08

[a] *N* between 80 and 110 for the other stages. Standard errors are inside the parentheses.

sinigrin content in buds increased 3.2- and 5.0-fold, respectively, from leaves to buds. These results strongly suggest a partial translocation of sinigrin from leaves into flower buds and agree with the findings of Rangkalidok et al. (*12*) who reported changes in sinigrin concentration from the vegetative to mature stages in three *Brassica* species.

In Pontecaldelas, leaves harvested at 180 DAS showed the lowest concentration for both total GSs and most of the individual GSs (**Table 4**). Leaves collected in Cotobade contained approximately two times the concentration of total GSs and sinigrin as compared to samples from Pontecaldelas. The indolyl GS, glucobrassicin, had different behavior with respect to aliphatic ones, and the highest GS concentration was found in Salcedo. In flower buds, the highest content of total GSs (93.9 μmol g^{-1} dw) and of glucoiberin (24.9 μmol g^{-1} dw) occurred in Salcedo. No significant differences among locations were found for sinigrin at this period.

Considering the chemical classes, previous studies have suggested that indolyl GSs are more susceptible to environmental effects than aliphatic GSs (*29*, *32*, *33*). Our current study confirms this founding since sinigrin had the same pattern in the four locations while the glucobrassicin content varied according the location and plant stage, suggesting that environmental factors are more important than genetic factors for the content of this GS.

As it was mentioned above, leaf GS variation among locations occurred in our study. That GS variation could be attributed to several environmental factors such as pest incidence, soil properties, or climatic conditions. Discussion will be focused on these three features.

Effect on GS Content by Insect Predation. Besides plant age and the plant part, biotic factors as feeding damage by insect pests have also been implicated in altering levels and proportions of individual GSs. Five lepidopterous species were found through the vegetative growing cycle. *Mamestra brassicae* was the most abundant followed by *Plutella xylostella* and *Pieris rapae*. Proportions of each insect fluctuated over locations (**Table 5**). *M. brassicae* was most abundant in all locations, with the exception of Cotobade where *P. xylostella* was the principal pest. The relation between the insect species found in the current study and the changes on the GS content has been previously studied. The role of GSs and their hydrolysis products in insect−plant resistance has been the subject of considerable research (*16*, *17*, *34*). Special attention has been paid to the concentration of short-chain aliphatic GSs, as attractants of adult lepidopterous, and to indolyl GSs, as egg-laying stimulants (*16*).

There were significant differences ($P \leq 0.01$) among locations and between damage and undamaged plants for the total GS content and for sinigrin, glucoiberin, and glucobrassicin (data not shown). In contrast, the damage stage × location interaction was only significant ($P \leq 0.01$) for glucobrassicin and some minor GSs, suggesting that influence of other environmental factors on GS content is less pronounced than variation caused by pest attack.

Leaves damaged by lepidopterous pests contained significantly lower total GS content (25.8 μmol g^{-1} dw) than undamaged leaves (41 μmol g^{-1} dw) (**Table 6** and **Figure 2**). The amounts of sinigrin, glucoiberin, and glucobrassicin were also lower in damaged leaves, suggesting that either larvae attack clearly lead to a disruption of the leaf tissue and consequently

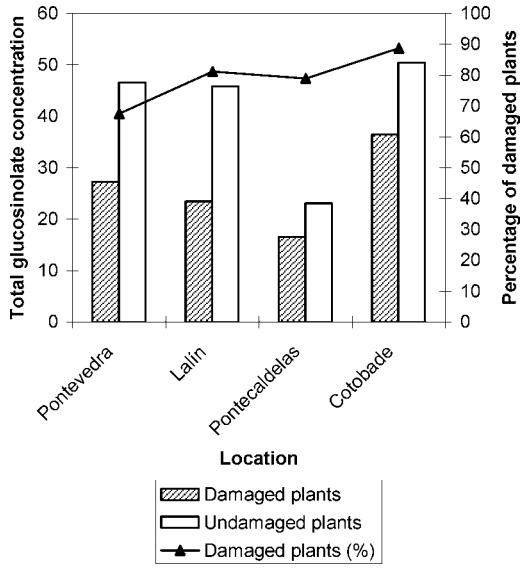

Figure 2. Mean (μmol g^{-1} dw) for total GS content for damaged and undamaged leaves and percentage of damaged plants.

the GS degradation or that leaves with the highest content of sinigrin, glucoiberin, and glucobrassicin are less preferred by Lepidoptera larvae. The pungency of isothyocyanates could also explain, in part, the insect-repellent properties of certain GSs (*10*). This experiment was performed on random sampling of plants in each location. For this reason, plants were not individually marked and it is not possible to state whether plants lost GSs due to the attack of different pests or if plants with low levels or GSs were preferred for larval feeding.

The relation between the isothyocianate concentration from aliphatic GSs in leaves and the survival and growth of several specialists lepidopterous species has been described by various authors (*17*−*19*).

In this study, a possible relation between pest damage and GS content could be present. Cotobade with the highest content of total GSs and highest contents of two aliphatic compounds (sinigrin and glucoiberin) at 180 DAS (**Table 4**) showed the highest larvae incidence and leaf damage (**Table 5**), suggesting that these compounds may act as attractants for specialized insects as it has been previously reported (*16*, *17*). Damages and larvae abundance were less important in Pontecaldelas (**Table 5**), and leaves from that location had the lowest GS content (**Table 4**).

Two locations (Lalín and Cotobade) showed the highest number of lepidopterous larvae (two larvae per plant at each location) and the highest percentage of leaf damage (91 and 98% of damaged plants, respectively). The modifications on GS content followed a different pattern in these two locations. Lalín showed a GS loss percentage of about 50%, and this decrease affected both aliphatic and indolyl GSs while Cotobade showed the minor reduction for total GS content between damages and undamaged leaves (less than 30% of GS loss) (**Table 6**). In Lalín, *M. brassicae* was the main pest whereas in Cotobade the main pest was *P. xylostella*, indicating that the response could vary according the crucifer-specialist Lepidoptera. Larvae of *P. xylostella* are less voracious than *M. brassicae* larvae, and attack results in less damaged leaf area. This could explain the lowest loss of GS content found at that last location.

Salcedo, with an important decrease on GS content (about 41% of GS loss), was the location with least number of damaged plants. In this last location, only indolyl GSs were drastically reduced and aliphatic GSs were relatively unmodified, suggesting that individual GSs may be affected in different ways by pest attack. In summary, with our data, it is difficult to confirm a relation between GS content loss in leaves and feeding damage by insect pests, suggesting that other environmental factors could be also implicated.

Location Effect on Total and Individual GS Content. Differences in the soil parameters were proved by edaphic analyses. The main characteristic of soils used in this study was their high acidity, with an average pH value of 5.3 (**Table 7**). The average cation exchange capacity was deficient in four sites. Soils were rich in organic matter with an average content ranging from 4.5% in Pontevedra to 7.8% in Cotobade. Available phosphorus was also found to be high in all plots. The available potassium was high in Lalín, medium in Cotobade and Pontevedra, and very low in Pontecaldelas. Because of their acidity, the aluminum appears under the toxic form for the plants (Al^{+3}), mainly in Cotobade, Pontecaldelas, and Pontevedra. Moreover, this acidity could affect soil microorganisms and consequently, problems on the process of humification, mineralization, and fixation of atmosphere nitrogenous could occur in these soils. Kim et al. (*33*) found that the GS content from edible parts of *B. rapa* is strongly affected by nitrogen and sulfur applications. The environmental effect in the hydroxylation step that links gluconapin and progoitrin in the aliphatic pathway was reported by Zhao et al. (*35*) who demonstrated that sulfur deficiency reduces the aliphatic GS concentration and increasing nitrogen results in higher proportions of progoitrin, suggesting that the hydroxylation step is favored. However, soil differences across locations could be the cause of the significant differences between locations and stage × location interaction for most GSs, as the response to soil effect. The highest GS content occurred in locations with the highest soil pH, suggesting some type of relation between them.

It is generally thought that high temperatures induce higher GS levels (*14*) although Rosa et al. (*2*) reported that temperature and GS variability are not related. In this study, the coldest locations (Lalín and Cotobade) had a reduction on total GS content from stage 3 (September) (46 and 50 μm g^{-1} dry tissue, respectively) to stage 4 (January) (41 and 45 μm g^{-1} dry tissue, respectively), possibly due to cool temperatures accompanied by frost effects, which can cause a tissue degradation on leaves and, consequently, a notable loss on GS content as was already reported by Rosa et al. (*2*). Therefore, total GS levels would be lower in cold seasons than in spring and summer (*14*, *36*). However, an inverse relation has been reported for myrosinase

Table 7. pH and Minerals in the Soils of the Four Locations

location	pH (H_2O) (1:2.5)	pH (KCL) (1:2.5)	organic matter (%)	ppm P	ppm K	ppm Mg	Ca	Mg	Na	K^+	Al	CiCe	Ca/Mg	K/Mg	Ca:Mg:K
Pontevedra	5.3	4.2	4.5	131	158	45	3.36	0.36	0.09	0.4	1.2	5.4	9	1.1	82:09:10
Lalín	5.4	4.8	7.4	69	512	222	12.31	1.77	0.12	1.28	0.4	15.88	7	0.7	80:12:08
Pontecaldelas	5.2	4.2	5.6	104	141	32	2.36	0.26	0.09	0.35	1.4	4.46	9	1.4	80:09:12
Cotobade	4.5	4	7.8	120	335	82	3.09	0.65	0.2	0.84	1.8	6.59	5	1.3	67:14:18

activity, which increases its activity in the fall season (*15*). Total and aliphatic GS contents were significantly affected by the temperature and they increased with temperature. Leaves collected in September at 180 DAS (stage 3) registered the highest increase of average GS content. Our data suggest that high temperatures through spring could have a positive effect on GS biosynthesis. By contrast, leaves sampled in January, which correspond to the lowest temperatures, had a low increase on total GS concentration and did not differ from the preceding sampling.

Data of our study suggest that temperature could play a positive effect on the GS concentration through the vegetative cycle of the plants as low temperatures caused a reduction on GS content. A clear relationship between the GS content and several climatic factors at the different locations used in this work was not found, and more research on this subject would be necessary.

In conclusion, the GS pattern was stable among locations and plant parts but not among plant stages. Flower buds and leaves harvested from 5 months after transplanting contain the highest concentration of GSs, and in particular sinigrin, which it was already pointed out is responsible for anticarcinogenic properties of crucifers. The high concentrations of sinigrin in kales prove its potential value as a crop with substantial health benefits. Aliphatic GSs were less susceptible to environmental effects than indolyl GSs. The relationship between the variation on the GS concentration in leaves and the damage caused by several specialists lepidopterous species appears to occur, although it is difficult to confirm a direct relation between the GS content loss in leaves and pest damage. Environmental factors as pH soil and temperature appear to have some influence in the GS variability.

ABBREVIATONS USED

GS, glucosinolates; dw, dry weight; DAS, days after sowing.

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