Journal of Plant Nutrition, 34:258–271, 2011

ISSN: 0190-4167 print / 1532-4087 online
DOI: 10.1080/01904167.2011.533326

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EXPERIMENTAL LEACHING OF MACRONUTRIENTS FROM *BRASSICA NAPUS* L. INCREASES WITH LEAF AGE AND GROWTH STAGE

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□ *It was the aim of the present study to determine the mobile nutrient content in leaves and pods of oilseed rape in relation to leaf age and growth stage and to develop an instrument to estimate the possible significance for interpretation of plant analytical data. From older leaves an increasing amount of sulfur (S), potassium (K), magnesium (Mg), calcium (Ca), and phosphorus (P) was leached, while no N was extracted after 24 h of leaching. In younger leaves the potential for nutrient leaching was below 10% for all investigated nutrients while in older leaves this value increased to 58% for S, 28% for P, 21% for Mg, 18% for Ca and 16% for K. Generally the potential for nutrient leaching from leaves and pods increased with growth stage. Until BBCH 83 nutrient leaching from pods was very low with less than 5% for the investigated elements, except S, but increased with further ripening drastically.*

Keywords: oilseed rape, remobilization, senescence, potassium, sulfur, phosphorus, calcium, magnesium

INTRODUCTION

During their life cycle plants take up several nutrients and plant analysis aims at determining critical values for all nutrients at defined growth stages to enable scientists and farmers to characterize the nutrient supply of a crop at any developmental stage (Barker and Pilbeam, 2007). Many different methods such as the determination of total contents, the available fraction, nutrient ratios and others are used to characterize the nutrient supply of a crop. One problem in this context is that senescence starts in many annual crops long before harvest and this process remobilizes nutrients

Received 19 May 2009; accepted 9 November 2009.

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such as nitrogen (N), sulfur (S), phosphorus (P) and potassium (K), which are transported to young vegetative and generative tissues (Himelblau and Amasino, 2001). Senescence and cell death are important processes in the plant life cycle and cell death may occur selectively in a few cells and specific organs such as leaves (Nooden et al., 1997). The process of senescence seems to be internally programmed rather than a product of nutrient limitation (Nooden et al., 1997) though senescence is closely associated with reproductive development. The process operates under the active control of genes but is known to be modulated by environmental signals such as daylength (Levey and Wingler, 2005) extremes in temperature, light intensity, moisture, mineral balance, hormones, symbiotic interactions, pathogens and oxidative stress (Buchanan-Wollaston, 1997; Lim et al., 2006; Munne-Bosch and Alegre, 2004; Zimmermann and Zentgraf, 2005). During senescence a broad syndrome of metabolic changes occurs (Smart, 1994). Many genes are down-regulated while specific senescence-associated genes are up-regulated (Biswal and Biswal, 1999; Buchanan-Wollaston et al., 2005; Gregersen et al., 2008; Lim and Nam, 2007). More than 800 senescence-associated genes have been identified illustrating the dramatic changes during leaf senescence (Buchanan-Wollaston et al., 2005; Lim and Nam, 2007). The degradation of macromolecules involves the *de novo* synthesis of RNA and proteins (Buchanan-Wollaston, 1997; Smart, 1994) underlining that senescing leaves are still viable and metabolically active. One important aspect of the senescence syndrome is the breakdown of chloroplasts and the loss of chlorophyll (Ougham et al., 2008), which is the reason why senescence can be quantified by measuring the chlorophyll content (Nooden et al., 1997). With the breakdown of chloroplasts a large proportion of plant resources is remobilized and re-used elsewhere.

Other symptoms of senescence are degradation of proteins and nucleic acid, and loss of membrane structure with concomitant increase in permeability to inorganic and organic solutes (Buchanan-Wollaston, 1997; Smart, 1994). Loss of selective permeability of membranes is an early event in the senescence cascade caused by de-esterification of membrane lipids (Hopkins et al., 2007). These changes occur before visible symptoms of senescence occur. As a result nutrients that are remobilized for re-utilization are not only transported within the plant but they are also prone to wash-out by precipitation and dew.

Cell death is ultimately manifested in the loss of integrity of the cell membrane and the ability of the cell to maintain homeostasis (Nooden et al., 1997). No data are assumedly available about the potential of nutrient losses from a living canopy during crop development.

Generally for the evaluation of the nutrient status of a crop leaves are sampled and analyzed for their nutrient content which is affected by several biotic and abiotic factors and experimental conditions. In addition the nutrient status is related to growth stage, plant part and developmental stage

of the leaves (Smith and Loneragan, 1997). As a result it is only possible to define ranges for critical nutrient values and it is more or less impossible to compare results from different experiments (Haneklaus et al., 2007). The present investigation was conducted to investigate the potential for nutrient losses from leaves and pods of different age and growth stage in order to quantify the possible influence of senescence on plant analytical data from field experimentation.

MATERIALS AND METHODS

Field trials with winter oilseed rape (*Brassica napus* L.) were conducted in Braunschweig (52°18′ N, 10°27′ E) on a sandy loam (Cambisol). In 2000 two different varieties of winter oilseed rape, one single low variety (Jet Neuf) and one double low variety (Ceres) were grown in plots of 45 m^2 which were sufficiently supplied with all essential macro- and micronutrients. The varieties Ceres and Jet Neuf differed mainly in their glucosinolate content and therefore in their S demand (Bloem et al., 2007). At harvest Jet Neuf realized a seed glucosinolate content of 111.3 μmol g^{-1} in air dried seeds (8–9% H_2O) while a seed glucosinolate content of 35.4 μmol g^{-1} was determined for Ceres.

Experimental Setup of the Leaching Experiment

A leaching experiment was conducted with leaves and pods of different age and growth stage of oilseed rape. Remobilization of nutrients and senescence of leaves start with the development of generative organs. Therefore, the first sampling was conducted when 50% of pods had reached their final size [BBCH 75 according to BBA (2001)]. About 40 leaves of the same age without any damage by herbivores or pathogens were collected from single plants and pooled to obtain a sufficient amount of material for nutrient analyses. Half of the material was used to determine the total nutrient content and the other half was used for the leaching experiment. An overview of sampling procedure and analyses is given in Figure 1. Leaves from six different leaf positions reflecting different leaf ages were collected (Figure 1) and sampling was conducted twice in 2000 on the 24 May and the 6 June [BBCH 75 and 81 according to BBA (2001)].

Pods were collected first at the 24 May and then weekly from the 6 June on until the 4 July when the pods were close to maturity and nutrient contents were determined in whole pods. Leaf samples and pods were immersed in 300 mL and 100 mL of stagnant distilled water at room temperature, respectively. Their lesions from dissection were carefully placed outside the water. Daily for in total three days an aliquot of 50 or 20 mL was removed.

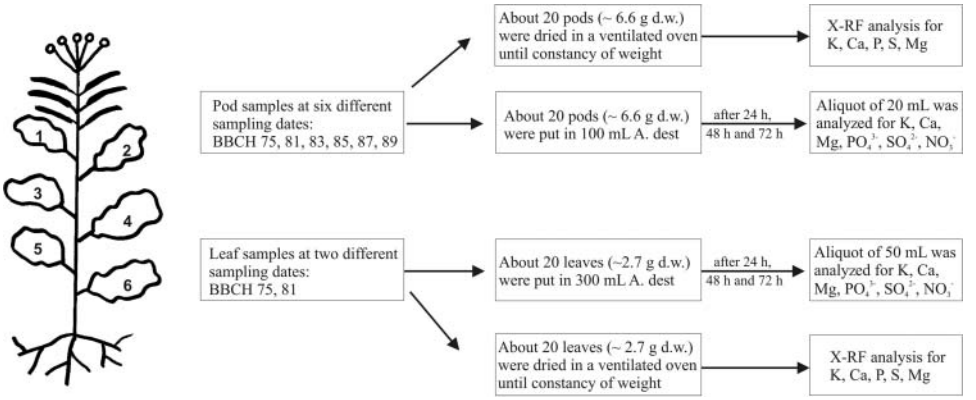

FIGURE 1 Sampling and preparation procedure of oilseed rape leaves and pods for quantification of nutrient losses by leaching (1–6: 1 youngest fully developed leaf; 6 oldest leaves without macroscopic symptoms of senescence).

Investigation Parameters and Analyses

The aliquots of the leaching experiment were filtered and analyzed for K and calcium (Ca) by flame photometer (Eppendorf, Elex 6361, Hamburg, Germany); magnesium (Mg) by atomic absorption spectroscopy (Unicam 929 AA spectrometer, ATI Unicam, Cambridge, UK); for phosphate, sulfate, and nitrate by ion chromatography (Methrom 761 Compact IC, Filderstadt, Germany) equipped with a Metrosep Anion Dual 2 column (Methrom, Filderstadt, Germany). All analyses were carried out in three-fold repetition.

As a measure for the decomposition status of the leaf and pod material the color of the extracts was determined visually by using the Munsell code. The extract color is of central information for the degree of decomposition. The Munsell color chart results were transformed into numeric data for statistical analysis. A decimal code with 0 matched with a colorless clear solution, 10 with a dark yellow one and 20 matched with a brown solution were attributed to the three values (hue, value and chroma) of the different colors. After 24 h of leaching all values were below 4 indicating a slightly coloring of the solutions. Higher values were reached after 48 and 72 h of elution.

For mineral analysis by X-ray fluorescence spectroscopy (PW 1400 with Rb tube, Philips, Hamburg, Germany) samples were dried at 60°C in a ventilated oven until constancy of weight and finely ground (<0.12 mm) using a RETSCH ultra-centrifugal mill. 1.1 g of plant material was mixed with 4.4 g of HOECHST wax C. The analysis was carried out according to Schnug and Haneklaus (1999).

For statistical analysis the ANOVA procedure of the CoHort software package (CoHort, Minneapolis, MN, USA) was used to segregate different sources of variation.

RESULTS AND DISCUSSION

The nutrient status of leaves (Table 1) and the potential nutrient leaching (Table 2) was determined twice during crop development in order to quantify the influence of growth stage and leaf age on changes in nutrient mobility.

Mineral Composition of Leaves and Pods of Oilseed Rape

In Table 1 the nutrient status of leaves and pods of oilseed rape in relation to leaf age, growth stage and variety of oilseed rape is shown.

Elements such as K, Mg, and P belong to the mobile elements in the plant while S and Ca are more immobile (Marschner, 1995). The nutrient status of the plants (Table 1) was slightly below the optimum level of 3.4–6.9 mg g^{-1} for P and 2.0–6.2 mg g^{-1} for Mg, but in the optimum range for S and Ca (Barker and Pilbeam, 2007; Reuter and Robinson, 1997). In older leaves (Table 1) significantly more K and less S and Mg was determined than in younger leaves while P and Ca remained more or less constant. A lower K

TABLE 1 Nutrient status of leaves and pods of *Brassica napus* in relation to leaf age, growth stage and variety

		K	Mg	P	S	Ca
		Mobile element			Immobile element	
		mg g^{-1} (d.w.)				
	Leaves					
Leaf position	1 (youngest leaf)	22.2	1.50	2.66	10.9	44.0
	2	22.8	1.35	2.61	10.9	44.3
	3	24.6	1.43	2.68	10.7	46.4
	4	25.1	1.30	2.61	10.0	45.9
	5	26.0	1.32	2.67	9.8	46.1
	6 (oldest leaf)	26.7	1.23	2.64	8.9	47.4
$LSD_{5\%}$		3.17	0.25	0.14	1.47	3.66
Growth stage	75	25.1	1.03	2.49	8.5	39.1
(BBCH)	81	24.1	1.67	2.79	11.8	52.1
$LSD_{5\%}$		1.82	0.14	0.08	0.85	2.11
Variety	Jet Neuf	25.7	1.29	2.66	7.8	45.6
	Ceres	23.6	1.41	2.63	12.5	45.9
$LSD_{5\%}$		1.83	0.14	0.08	0.85	2.11
	Pods					
Growth stage	75	13.9	0.83	3.03	4.47	12.1
(BBCH)	81	15.6	1.13	3.40	5.93	14.0
	83	15.3	1.09	3.48	5.73	13.4
	85	11.7	1.10	3.54	5.63	15.1
	87	12.9	1.09	3.49	6.19	18.3
	89	10.1	1.16	3.60	6.32	16.8
$LSD_{5\%}$		0.90	0.14	0.19	0.68	1.39
Variety	Jet Neuf	13.4	1.01	3.43	6.07	15.9
	Ceres	12.9	1.12	3.43	5.36	14.1
$LSD_{5\%}$		0.52	0.08	0.11	0.39	0.80

TABLE 2 Leaching potential of macro-nutrients from leaves and pods of *Brassica napus* in relation to leaf position, growth stage and variety

		K	Mg	P	S	Ca	Color code (Munsell)
		Mobile element			Immobile element		
		mg g^{-1} (d.w.)					
	Leaves[1]						
Leaf position	1 (youngest leaf)	0.58	0.10	0.17	0.68	0.76	0.4
	2	0.69	0.11	0.09	0.64	0.70	0.3
	3	0.69	0.16	0.06	0.92	1.12	0.6
	4	1.06	0.13	0.23	1.17	1.84	1.3
	5	1.89	0.22	0.23	2.05	3.59	1.6
	6 (oldest leaf)	2.62	0.19	0.42	3.05	5.44	3.0
$LSD_{5\%}$		0.82	0.07	0.30	1.27	1.97	1.1
Growth stage	75	0.50	0.07	0.04	0.31	0.32	0.6
(BBCH)	81	2.01	0.23	0.36	2.51	4.16	1.8
$LSD_{5\%}$		0.48	0.04	0.17	0.73	1.14	0.6
Variety	Jet Neuf	1.57	0.18	0.30	1.16	2.71	1.5
	Ceres	0.94	0.12	0.09	1.70	1.78	0.9
$LSD_{5\%}$		0.48	0.04	0.17	0.73	1.14	0.6
	Pods[1]						
Growth stage	75	0.26	0.02	0.12	0.13	0.01	0.0
(BBCH)	81	0.19	0.01	0.01	0.13	0.05	2.8
	83	0.21	0.02	0.08	0.98	0.10	0.5
	85	2.00	0.09	0.79	1.52	1.46	2.2
	87	0.57	0.05	0.27	0.96	1.08	3.0
	89	2.64	0.16	0.65	2.89	3.20	3.3
$LSD_{5\%}$		0.42	0.02	0.28	0.82	0.34	0.6
Variety	Jet Neuf	1.04	0.06	0.40	0.86	0.99	2.1
	Ceres	0.92	0.05	0.24	1.34	0.98	1.8
$LSD_{5\%}$		0.24	0.01	0.17	0.47	0.20	0.3

[1]Plant material was immersed for 24 h in distilled water.

content in leaves of position 1 and 2 when compared to leaves at position 3–6 indicates that a concentration of about 23 mg g^{-1} was sufficient for plant growth even though Mengel (2007) found a critical value of 28–50 mg K g^{-1} (d.w.) for oilseed rape.

Sulfur belongs to the relatively immobile elements within plants; S needs to be mobilized before being translocated from senescent to younger leaves (Biswal and Biswal, 1999). The significantly lower S and Mg contents in older leaves indicate translocation processes from older to younger leaves. Himelblau and Amasino (2001) determined a drastic decrease in the levels of K (85.3%), N (85.4%), P (78.4%), and S (66.6%) during senescence and suggested that these macronutrients were mobilized from senescing leaves in *Arabidopsis* while the levels of Ca, Mg and sodium (Na) remained constant in their study.

Unlike other essential nutrients, such as N and S, K is not incorporated into organic matter and does not become part of the chemical structure of plants but is present in unbound form in the cytoplasm where K is highly

mobile (Marschner, 1995) and K ions cycle via xylem from roots to upper plant parts and via phloem from leaves to roots following the physiological demand (Mengel, 2007). The function of K in plant metabolism is mainly based on ionic action, activation of enzymes, regulation of protein biosynthesis, and the synthesis of carbohydrates. Young developing vegetative plant parts have a high demand for K as do fruits which are rich in water (Mengel, 2007). In contrast generative organs with low water contents such as seeds do not require K in high amounts (Mengel, 2007).

Other highly mobile nutrients within the plant are Mg and P. Magnesium displays the central atom of chlorophyll and functions in activation of enzymatic reactions (Marschner, 1995) while P plays an important role in energy storage (ATP and NADP) and as a constituent of nucleotides, nucleic acid and phospholipids. In contrast Ca and S display a lower mobility in plants. A high proportion of Ca is located in the cell wall and S is bound to proteins (Marschner, 1995).

From BBCH 75 to 81 the nutrient content of all investigated elements significantly increased in the leaves (with exception of K). The same was observed for the pods during maturation (BBCH 75–89). Only the K content of pods decreased from 13.9 to 10.1 mg g^{-1} (d.w.) during this period.

The variety of oilseed rape plays an important role for the S storage in leaves and pods as both cultivars differ in S because of an altered glucosinolate biosynthesis in single low and double low oilseed rape varieties. Single low and double low varieties take up comparable amounts of S but while single low varieties convert S effectively into glucosinolates double low varieties accumulate S in vegetative plant parts (Bloem et al., 2007; Haneklaus et al., 2007). The differences in the glucosinolate biosynthesis result in a higher S demand of double low varieties because S from glucosinolates can be remobilized while protein-S is more immobile. Therefore double low varieties display symptoms of S deficiency at a S content below 3.5 mg g^{-1} (d.w.) while single low varieties show symptoms when the S content in younger leaves drops below 3 mg g^{-1} (d.w.) (Haneklaus et al., 2007).

Nutrient Leaching from Leaves and Pods of Oilseed Rape

The nutrient leaching from leaves and pods, which were immersed in water for 24 h is shown in Table 2.

The nitrate content was consistently below the limit of detection (data not shown). A similar result was observed by Dezzeo et al. (1998) who investigated nutrient losses of terrestrial green leaves and small branches from different living trees under constant water-logging. They observed that the N content of leaves remained relatively unchanged during the first month of decomposition while they found high losses of K (93%) and Mg (82%) and a slower release of Ca (39% loss) and P (53% loss). For all investigated nutrients, except N, the potential for leaching increased with leaf age

(Table 2) as well as with growth stage. The variety was of minor relevance for the potential of nutrient leaching.

The color code of the extract was chosen as a measure for the degree of disintegration of leaves and pods. At maximum a value of 3.3 (Table 2) was determined after 24 h of leaching revealing only a slightly yellow coloring of the solutions. In younger leaves and pods hardly any coloring was determined. This reflects an intact cell structure with a very low degree of disintegration. When leaves and pods were kept longer than 24 h in water, processes started which resulted in an intensive coloring of the extracts. The pod extracts showed no coloring up to BBCH 83, but from BBCH 85 onwards an intensive coloring with values of up to 13 was determined when pods were leached for more than two days. It can be concluded that the nutrient release of leaves and pods within 24 h seems to be a good measure for the potential of nutrient leaching from a living canopy while longer leaching periods induce decomposition processes of leaf material rather than reflect the leaching of nutrients.

When calculating the percentage of nutrient elution of the youngest leaf in relation to the oldest leaf the values are a good indicator for the different mobility of the nutrients: Ca (14.0%) < K (22.1%) < S (22.3%) < P (40.5%) < Mg (52.6%). Only K revealed a low value despite of the high mobility of this element.

Proportion of Nutrient Leaching from Total Mineral Contents

In Figures 2 and 3 the percentage of nutrient leaching after 24 h in relation to the total content (Table 1) is shown for leaves and pods. In younger leaves nutrient losses are more or less constant and for most nutrients below 10%. In older leaves the percentage of nutrients which are prone to leaching increased significantly, especially S.

In younger pods the potential for nutrient leaching was very low but it increased with ripening (Figure 3). Senescence is generally accompanied by a decline in the structural and functional integrity of cellular membranes as a result of enhanced catabolism of membrane lipids (Buchanan-Wollaston, 1997; Thompson et al., 1998) and it can be assumed that with catabolism of membranes the potential for nutrient leaching increases. Especially S showed a high potential for leaching in older pods. The different varieties Ceres and Jet Neuf showed significant differences in their potential for S leaching: the single low variety Jet Neuf revealed a significantly higher S leaching from BBCH 85 onwards when 50% of the pods were ripe.

Interactions between Macronutrient Contents and Mineral Leaching

Interactions between macro-nutrients are known for several elements (Barker and Pilbeam, 2007). In Figures 4 and 5 correlation matrices for the

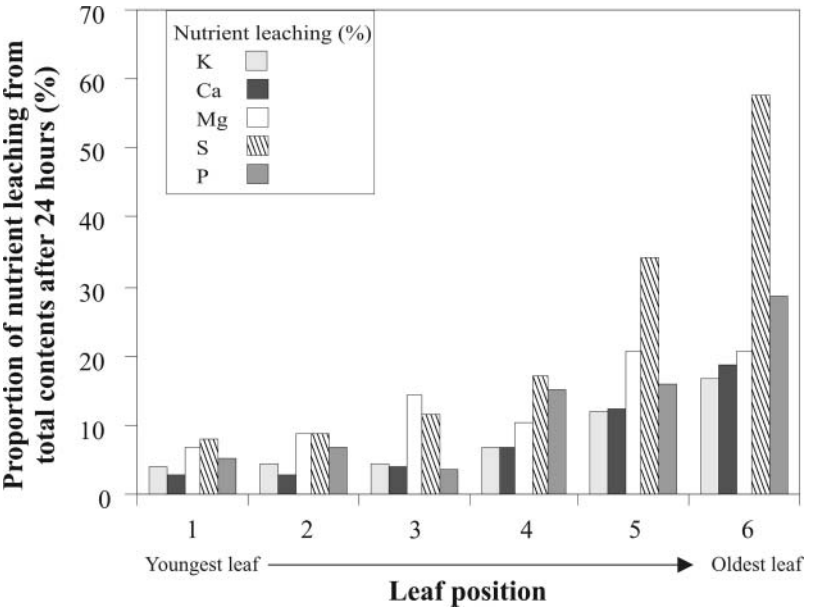

FIGURE 2 Percentage of nutrient leaching from the total nutrient content in leaves of *Brassica napus* L. (leaves of different age were immersed in water for 24 h on 7 June 2000).

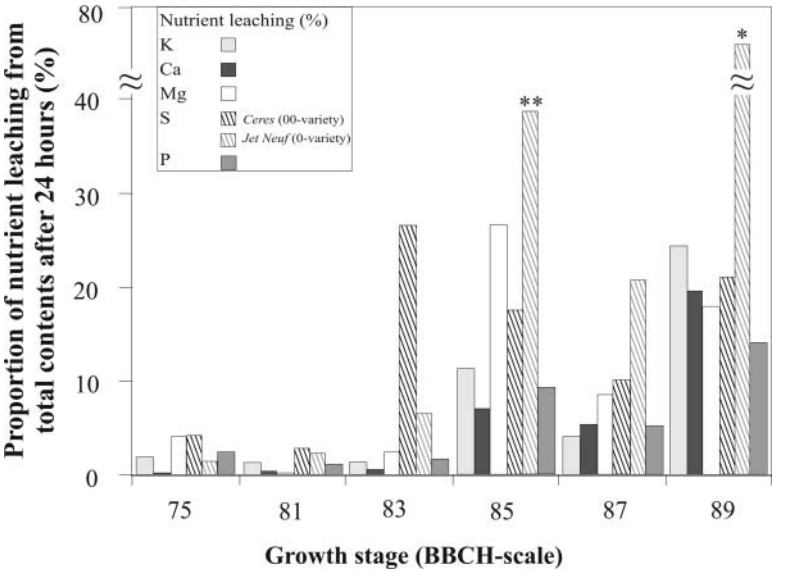

FIGURE 3 Percentage of nutrient leaching from the total nutrient content in pods of *Brassica napus* L. in dependence on growth stage (pods were immersed in water for 24 h; BBCH scale according to BBA, 2001; for all nutrients with exception of S the data from the varieties *Ceres* and *Jet Neuf* were averaged as no statistical differences were observed; asterix marks statistical significant differences at $p < 0.05$ (*) and $p < 0.01$ (**).

Leaves[1] (r)		Total nutrient content (mg g^{-1})					Nutrient leaching (mg g^{-1})					
		K	Ca	P	S	Mg	K	Ca	P	S	Mg	Munsell Code
Total nutrient content (mg g^{-1})	K		ns	0.28 *	ns	ns	ns	ns	ns	ns	ns	ns
	Ca			0.66 ***	0.44 ***	0.74 ***	0.54 ***	0.57 ***	0.44 ***	0.48 ***	0.50 ***	0.47 ***
	P				0.34 **	0.86 ***	0.36 **	0.35 **	0.42 ***	0.28 *	0.54 ***	ns
	S					0.53 ***	ns	ns	ns	ns	ns	ns
	Mg						0.27 *	0.27 *	0.26 *	0.26 *	0.45 ***	ns
Nutrient leaching (mg g^{-1})	K							0.96 ***	0.66 ***	0.77 ***	0.65 ***	0.82 ***
	Ca								0.65 ***	0.85 ***	0.65 ***	0.82 ***
	P									0.52 ***	0.51 ***	0.63 ***
	S										0.56 ***	0.64 ***
	Mg											0.53 ***

FIGURE 4 Correlation coefficients (r) between total nutrient contents and leaching of nutrients in leaves of *Brassica napus* L. ([1]Leaf material was immersed for 24 h in distilled water).

Pods[1] (r)		Total nutrient content (mg g^{-1})					Nutrient leaching (mg g^{-1})					
		K	Ca	P	S	Mg	K	Ca	P	S	Mg	Munsell Code
Total nutrient content (mg g^{-1})	K		-0.35 *	-0.35 *	ns	ns	-0.80 ***	-0.82 ***	-0.64 ***	-0.52 **	-0.81 ***	-0.47 **
	Ca			0.45 **	0.66 ***	ns	0.40 *	0.56 ***	0.35 *	ns	0.50 **	0.75 ***
	P				0.72 ***	0.78 ***	0.48 **	0.52 **	0.35 *	ns	0.46 **	0.52 **
	S					0.51 **	ns	0.43 *	ns	ns	0.38 *	0.56 ***
	Mg						ns	0.37 *	ns	ns	ns	0.49 **
Nutrient leaching (mg g^{-1})	K							0.92 ***	0.80 ***	0.64 ***	0.96 ***	0.51 **
	Ca								0.69 ***	0.71 ***	0.98 ***	0.63 ***
	P									0.43 **	0.74 ***	0.37 *
	S										0.68 ***	0.38 *
	Mg											0.55 ***

FIGURE 5 Correlation coefficients (r) between total nutrient contents and leaching of nutrients in pods of *Brassica napus* L. ([1]Pod material was immersed for 24 h in distilled water).

investigated nutrients as well as leached nutrients are presented. The total P content in leaves correlated significantly with all other investigated nutrients. Ca correlated with S and Mg, and S with Mg. Only the K content seemed to be independent from the other nutrients. For *Brassica* species also in literature several interactions between macro-nutrients were reported: Jaggi and Sharma (1999) showed a positive interaction between S and P for Indian mustard on a soil deficient in both elements and positive interactions were also reported between P and Mg and also S and Mg in pot culture experiments with mustard (Krishnakumari et al., 1999; Purakayastha and Nad, 1998). It is important to note that such interactions differ in relation to nutrient supply and fertilizer application rates and other factors that control nutrient uptake. Shankaralingappa et al. (2000) observed a significant synergistic effect of a combined application of S and P on K uptake of pigeon pea up to a rate of 50 kg P_2O_5 and 40 kg S ha^{-1}; with a higher rate of P of 75 kg P_2O_5 an antagonistic effect on the N, P, and K uptake was found.

Generally a strong relationship was found between the leaching potential of the investigated macro-nutrients in leaves (Figure 4) and pods (Figure 5). A strong correlation was also determined between nutrient leaching and the coloring of the extracts. The decomposition of chloroplasts (Chandlee, 2001) is most likely the reason for the increasing coloring of extracts of older leaves. The eluted amount of Ca, P, and Mg from leaves was strongly correlated with the total nutrient content, while the eluted amount of K and S was not related to their total contents.

For pods (Figure 5) different relationships were determined: the total K content was highly negative correlated with the leaching of all investigated macro-nutrients while the other nutrients showed only weak (Ca, P), or extremely poor (S, Mg) correlations with nutrient leaching.

The strong negative correlation between the total K content and K, Ca, P, Mg, and S leaching in pods might indicate that K in pods is involved in seed development processes and translocation of nutrients into seeds.

As there is no interaction of nutrients during leaching the strong correlation coefficients between leached nutrients confirm that re-mobilization and translocation processes are genetically determined by starting of senescence and only secondarily by nutritional factors. Güsewell (2005) found similar results for the N and P resorption efficiency during leaf senescence in wetland graminoids which was highly correlated independent of nutritional conditions.

The data have shown that nutrient contents can differ significantly in relation to leaf position (K, Mg, S) and growth stage (Mg, P, Ca, S) (Table 1). The differences with growth stage were even stronger: Within only two weeks nutrient contents changed significantly. A significant increase was observed in case of Mg by >60%, S by >38%, Ca by >33%, P by >12% and a non-significant decrease in case of K by 4%. In case of Mg, S and Ca nutrient contents can be easily misinterpreted when samples are not taken exactly at

TABLE 3 Comparison of the leaching potential of macro-nutrients in leaves of different age and in leaves and pods of oilseed rape in relation to growth stage

	Range of relative nutrient leaching [%] from total content	Leaching potential in increasing order
Leaves		
Youngest leaf	1.7–2.6	Ca < K < S < P < Mg
Oldest leaf	9.8–34.3	K < Ca < Mg < P < S
BBCH 75[1]	0.8–6.8	Ca < P < K < S < Mg
BBCH 81[1]	8.0–21.3	Ca < K < P < Mg < S
Pods		
BBCH 75	0.1–4.0	Ca < K < Mg < S < P
BBCH 89	13.8–45.7	Mg < P < Ca < K < S

[1]Values over all leaf ages.

the defined growth stage. This problem potentiates with view to individual nutrients because of differences in the leaching potential in relation to leaf position and growth stage of the crop (Table 3).

The percentage of leaching of macro-nutrients from leaves and pods increased distinctly with growth, especially for S and Mg. At BBCH 81 the percentage of S leaching from the total S content accounted for 21.3% in leaves and for 45.7% in pods at BBCH 89 (Table 3). The results reveal that a significant amount of nutrients may be washed out before ripening due to unfavorable climatic conditions. Moreover nutrient losses are also enhanced if the epidermis and leaf parts are damaged by herbivores and pathogens (Smart, 1994). Such events may also change nutrient ratios significantly so that interpretation of plant analytical data is hampered. The N/S ratio is often used to characterize the S nutritional status of a crop because plants require S and N in proportional quantities for the biosynthesis of amino acids resulting in typical ratios (Calvo et al., 2008). Deviations from the typical N/S ratio were proposed as an indicator for S or N deficiency (Spencer et al., 1984). The present results show that N was not prone to leaching even from older leaves and pods while a great proportion of S was rapidly eluted in 24 h. This result reflects the basic uncertainties related to nutrient ratios as a tool to assess the nutrient status of a crop.

CONCLUSION

The phenomenon of nutrient leaching from growing plants on the one hand and the quantification of the amount of nutrients that is remobilized and translocated within the plant on the other hand are difficult to quantify because of its complex nature and the interaction with numerous processes. These imply leaf senescence, environmental conditions such as rain events and temperature, damage by pests and pathogens, species differences in tissue composition and nutrient content and differences in nutrient mobility.

The present data provides figures about the amount of mobile nutrients in relation to leaf age and growth stage. The data show that with increasing leaf age and growth stage the potential for nutrient leaching increased significantly and with strict, element-specific differences. Significant correlations were determined for most nutrients (Ca, P, S, and Mg) with respect to their total as well as mobile contents. K reacted differently, most likely because of its function which is mainly ionic action and not as a constituent of the organic matter.

In addition the results of this investigation show that for crops such as oilseed rape the mineralization of nutrients and here in particular that of S starts with early senescence of the leaves and with respect to environmental conditions, nutrient losses might be completed over a short period as it took only 72 h to leach more than 50% of the total leaf S.

REFERENCES

Barker, A. V., and D. J. Pilbeam. 2007. *Handbook of Plant Nutrition*. Boca Raton, FL: CRC Press.

BBA. 2001. Growth stages of mono- and dicotyledonous plants. Available at: http://www.ki.bund.de/de/startsite/veroeffentlichungen/bbch-codes.html Accessed 3 Nov 2010.

Biswal, B., and U. C. Biswal. 1999. Leaf senescence: Physiology and molecular biology. *Current Science* 77: 775–782.

Bloem, E., S. Haneklaus, and E. Schnug. 2007. Changes in the sulphur and glucosinolate content of developing pods and seeds of oilseed rape (*Brassica napus* L.) in relation to different cultivars. *Agricultural Research* 57: 297–306.

Buchanan-Wollaston, V. 1997. The molecular biology of leaf senescence. *Journal of Experimental Botany* 48: 181–199.

Buchanan-Wollaston, V., T. Page, E. Harrison, E. Breeze, P. O. Lim, H. G. Nam, J. F. Lin, S. H. Wu, J. Swidzinski, K. Ishizaki, and C. J. Leaver. 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation induced senescence in *Arabidopsis*. *Plant Journal* 42: 567–585.

Calvo, N. I. R., H. E. Echeverria, and H. S. Rozas. 2008. Usefulness of foliar nitrogen-sulfur ratio in spring red wheat. *Journal of Plant Nutrition* 31: 1612–1623.

Chandlee, J. M. 2001. Current molecular understanding of the genetically programmed process of leaf senescence. *Physiologia Plantarum* 113: 1–8.

Dezzeo, N., R. Herrera, G. Escalante, and E. Briceno. 1998. Mass and nutrient loss of fresh plant biomass in a small black-water tributary of Caura River, Venezuelan Guayana. *Biogeochemistry* 43: 197–210.

Gregersen, P. L., P. B. Holm, and K. Krupinska. 2008. Leaf senescence and nutrient remobilisation in barley and wheat. *Plant Biology* 10: 37–49.

Güsewell, S. 2005. Nutrient resorption of wetland graminoids is related to the type of nutrition limitation. *Functional Ecology* 19: 344–354.

Haneklaus, S., E. Bloem, E. Schnug, L. J. DeKok, and I. Stulen. 2007. Sulfur. In: *Handbook of Plant Nutrition*, eds. A. V. Barker and D. J. Pilbeam, pp. 183–238. Boca Raton, FL: CRC Press.

Himelblau, E., and R. M. Amasino. 2001. Nutrients mobilized from leaves of *Arabidopsis thaliana* during leaf senescence. *Journal of Plant Physiology* 158: 1317–1323.

Hopkins, M., C. Taylor, Z. Liu, F. Ma, L. McNamara, T. W. Wang, and J. E. Thompson. 2007. Regulation and execution of molecular disassembly and catabolism during senescence. *New Phytologist* 175: 201–214.

Jaggi, R. C., and R. K. Sharma. 1999. Sulphur-phosphorus interaction in raya (*Brassica juncea* var. Varuna) in acid Alfisols of western Himalaya. *Tropical Agriculture* 76: 157–163.

Krishnakumari, M., B. M. Sharma, R. K. Sharama, and S. S. Balloli. 1999. Effect of phosphorus-magnesium interaction on yield and oil content of mustard (*Brassica juncea*). *Journal of the Indian Society of Soil Science* 47: 379–380.

Levey, S., and A. Wingler. 2005. Natural variation in the regulation of leaf senescence and relation to other traits in *Arabidopsis*. *Plant Cell and Environment* 28: 223–231.

Lim, P. O., H. J. Kim, and H. G. Nam. 2006. Leaf senescence. *Annual Reviews of Plant Biology* 58: 115–136.

Lim, P. O., and H. G. Nam. 2007. Aging and senescence of the leaf organ. *Journal of Plant Biology* 50: 291–300.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. London: Harcourt Brace and Co.

Mengel, K. 2007. Potassium. In: *Handbook of Plant Nutrition*, eds. A. V. Barker and D. J. Pilbeam, pp. 91–120. Boca Raton, FL: CRC Press.

Munne-Bosch, S., and L. Alegre. 2004. Die and let live: Leaf senescence contributes to plant survival under drought stress. *Functional Plant Biology* 31: 203–216.

Nooden, L. D., J. J. Guiamet, and I. John. 1997. Senescence mechanism. *Physiologia Plantarum* 101: 746–753.

Ougham, H., S. Hörtensteiner, I. Armstead, I. Donnison, I. King, H. Thomas, and L. Mur. 2008. The control of chlorophyll catabolism and the status of yellowing as a biomarker of leaf senescence. *Plant Biology* 10: 4–14.

Purakayastha, T. J., and B. K. Nad. 1998. Effect of sulphur, magnesium and molybdenum on mustard and wheat: Yield and uptake of macronutrients. *Indian Journal of Plant Physiology* 3: 112–115.

Reuter, D. J., and Robinson, J. B. 1997. *Plant Analysis—An Interpretation Manual*. Collingwood, Australia: CSIRO Publishing.

Schnug, E., and S. Haneklaus. 1999. Diagnosis of the nutritional status and quality assessment of oilseed rape by X-ray spectroscopy. In: *Proceedings of the 10th Int. Rapeseed Congress, Canberra, Australia* [CD–ROM].

Shankaralingappa, B. C., B. Shivaraj, K. Sudhir, and K. P. Viswanatha. 2000. Interaction effect of phosphorus and sulphur on uptake of nitrogen, phosphorus, potassium and sulphur by pigeonpea (*Cajanus cajan*). *Indian Journal of Agronomy* 45: 348–352.

Smart, C. M. 1994. Gene expression during leaf senescence. *New Phytologist* 126: 419–448.

Smith, F. W., and J. F. Loneragan. 1997. Interpretation of plant analysis: concepts and principles. In *Plant Analysis—An Interpretation Manual*, eds. D. J. Reuter and J. B. Robinson, pp. 3–26. Collingwood, Australia: CSIRO Publishing.

Spencer, K., J. R. Freney, and M. B. Jones. 1984. A preliminary testing of plant analysis procedures for the assessment of the sulphur status of oilseed rape. *Australian Journal of Agricultural Research* 35: 163–175.

Thompson, J. E., C. D. Froese, E. Madey, M. D. Smith, and Y. Hong. 1998. Lipid metabolism during plant senescence. *Progress in Lipid Research* 37: 119–141.

Zimmermann, P., and U. Zentgraf. 2005. The correlation between oxidative stress and leaf senescence during plant development. *Cellular & Molecular Biology Letters* 10: 515–534.

Journal of Plant Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597277

EXPERIMENTAL LEACHING OF MACRONUTRIENTS FROM *BRASSICA NAPUS* L. INCREASES WITH LEAF AGE AND GROWTH STAGE

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Online publication date: 01 December 2010

To cite this Article Bloem, Elke , Haneklaus, Silvia and Schnug, Ewald(2011) 'EXPERIMENTAL LEACHING OF MACRONUTRIENTS FROM *BRASSICA NAPUS* L. INCREASES WITH LEAF AGE AND GROWTH STAGE', Journal of Plant Nutrition, 34: 2, 258 — 271
To link to this Article: DOI: 10.1080/01904167.2011.533326
URL: http://dx.doi.org/10.1080/01904167.2011.533326