THE BIOSYNTHESIS AND CONTENTS OF GLUCOSINOLATES IN THE COURSE OF WINTER RAPE (Brassica napus L.) ONTOGENY

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INTRODUCTION

The present high production of winter rape, which is a major raw material for the food industries and other uses, makes it necessary to utilize also the processing residues representing a valuable animal feed.

All species of the genus Cruciferae, involving also the winter rape (Brassica napus L.), contain the glucosinolates (Fenwick, Heaney 1983; Sörensen 1984) the hydrolytic products of which are toxic and limit this its value and application in animal feeding (Sones et al. 1984).

Attention has been thus given both to the inclusion of the rape defatted meal in mixed feeds and to the role of rape as a cover crop to be used in livestock nutrition. These include unfortunately various amounts of glucosinolates in the dry or green matter, the toxicity of which constrains greatly their feeding applications.

To obtain a comprehensive information on the whole problem of glucosinolate biosynthesis and levels, we studied their dynamics in the "0" Jet Neuf and "00" Darmor varieties in the course of ontogeny, giving particular attention to winter rape application in crop improvement and animal feeding.

MATERIALS AND METHODS

The biosynthesis and contents of glucosinolates throughout winter rape ontogeny were studied in its French varieties currently certified in Czechoslovakia - "0" Jet Neuf and "00" Darmor, and the respective field experiments were carried out in 1987-1988 at the School Farm of the Agricultural University Prague. This is a sugarbeet-wheat growing region.

The trial pattern and timing were as follows: the plot was first under the winter wheat and the rape seeding took place on August 29, 1987 at a rate of 6 kg/ha. No fertilizer was applied in the fall but there were two spring nitrogen applications (April 3 - 70 kg/ha, LAV) and (78 kg/ha, DAM), and the no-dessication harvesting was done on July 1, 1988 and gave a yield of 4,59 tons/ha for the Jet Neuf variety, and 4,50 tons/ha for the Darmor one, respectively.

The plant samples (10) had been taken weekly since spring regeneration stage (April 6, 1988) to the green maturity (June 1, 1988), and average samples had been made by combining the leaf, stalk, and root samples. The seed samples had been taken simultaneously throughout the green to full ripeness period.

QUANTITATIVE GLUCOSINOLATE ANALYSIS

Vegetative tissues were extracted and cleaned using the

method described by Heaney and Fenwick (1980). Isolated glucosinolates were desulfated and silylated by trimethylchlorsilane (TMCS) and N,O- bis (trimethylsilyl) acetamide.

The desulphotrimethylsilylderivatives of glucosinolates were determined by gas chromatography using the internal standard method, the latter being the sinigrine. Chromatographic analysis of derivatized glucosinolates was performed on the Hewlett-Packard 5890 chromatograph with a capillary column HP-5, the respective carrier gas being nitrogen flowing at 12 ml/min. Thermal programming had an initial temperature of 220°C, the increase of which at 10-minute intervals was 10°C up to the final temperature of 280°C. The injector and detector temperatureswere 250°C and 300°C, respectively.

The seed samples were also analysed by chromatography (Zukalová, Vašák 1978).

RESULTS AND DISCUSSION

The dynamics of glucosinolate biosynthesis was studied in the "0" Jet Neuf and "00" Darmor rape varieties, and the results obtained (Fig. 1) showed that these are present throughout the whole plant.

The glucosinolate content in the roots at the stage of regeneration is six time more in either variety, and at the stage of butonization more than two times in a relative comparison with the leaf and stalk contents, the leaf one being less than the stalk level.

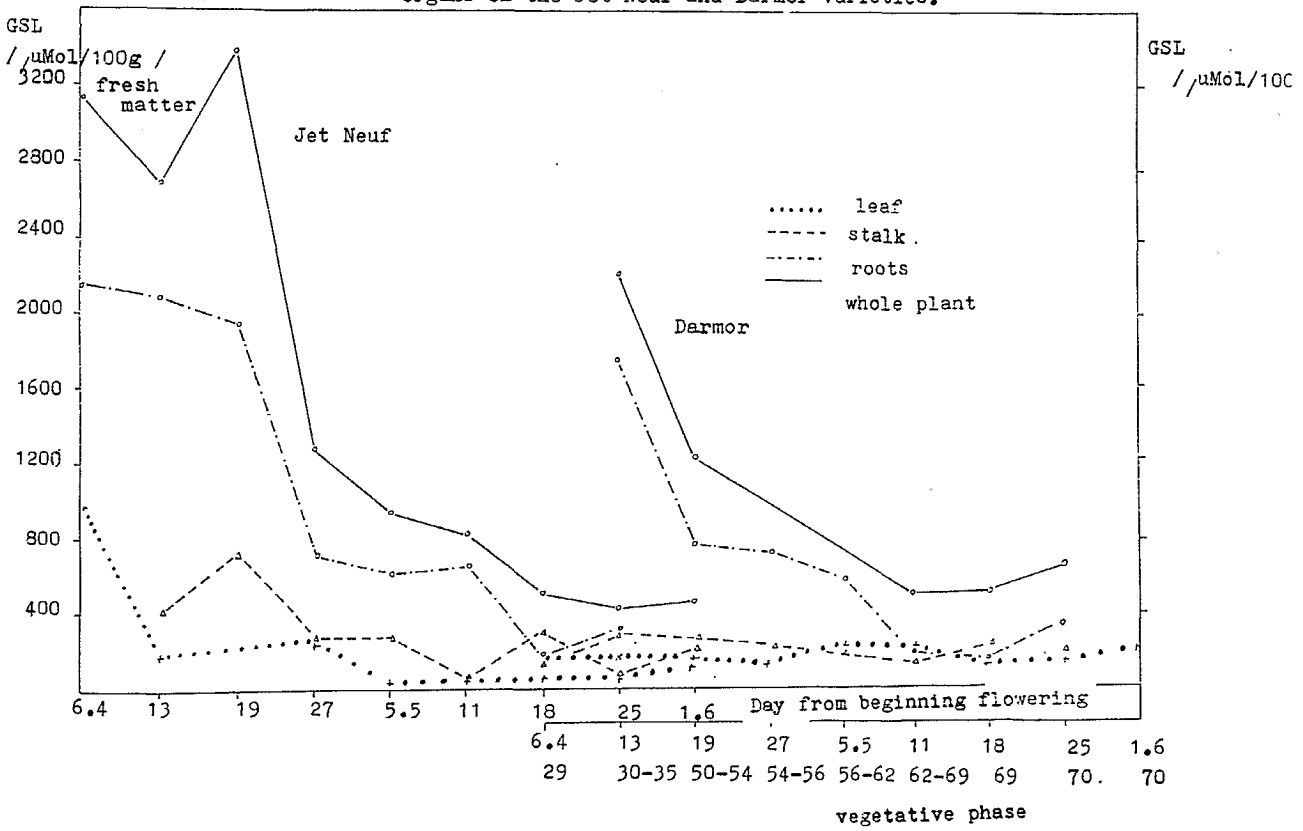
The highest glucosinolate levels were found in young meristematic tissues and were declining in the course of the growth (Kutáček et al. 1957) which seems to be in line with the generally valid knowledge of changes in the content of undesirable secondary products in the green matter of cultivated crops in the process of tissue ageing (Hackbarth 1969).

The glucosinolates present in the vegetative rape plant parts contain both alkenyle and indole glucosinolates, and the aerial parts of the Jet Neuf rape variety had on the average 14 to 19% of indole glucosinolates while its roots contained as much as 50% of them. The "00" Darmor variety had 3 to 6% of them in its above-the-ground parts, and about 33% in its roots.

Declining glucosinolate contents in vegetative plant parts in the course of growth were reported by a number of authors (Jürges 1978; McGregor 1987; Smith 1988), but their absolute levels are governed by the plant genotype and by a variety of agroecological factors. Our findings are in a line with the results published by Sörensen (oral communication 1989) who had found - apart from the alkenyle glucosinolates in the roots of rape - particularly the neoglucobrassicine of the indole ones, and the glucobrassicine in the leaves and stalks.

At the time of optimum green forage harvesting (growth stage 54-56, butonization to 56-62, inflorescence) is the glucosinolate content low, averaging some 200 µmol/100 g fresh matter in both rape varieties. Toothey (1980) reported that harmful glucosinolate concentrations to living organisms start from 0,4% in dry matter, this corresponding, when taking into account a 13% dry matter of samples, to 123,5 µmol/100 g of

Fig.1. The formation of total contents of glucosinolates in vegetative organs of the Jet Neuf and Darmor varieties.



fresh matter, which is exceeded almost by all "0" and "00" rape varieties under study , except for the glucosinolate content in the Ceres variety leaves (179,89 μmol) approaching most the abovementioned level. The glucosinolates, representing one of the factors responsible for the toxic syndrome of the Cruciferae, do cause the swelling of the thyroid gland in domestic animals only but no significant pathological changes leading sometimes to wildlife death. Apart from another toxic compound, the S-methylcysteinesulphoxide, are the game losses most likely due to imbalanced fodder (Fábry 1988).

The differences in the glucosinolate contents for the two variety types in their green matters are not as significant as in their seed (Fig. 2), and in rape seeds is the glucosinolate content of the "00" varieties only 1/10 to 1/5 of that in the "0" varieties.

In the course of ripening, "0" varieties showed a marked increase in the glucosinolate content since about the 55th day from the beginning of flowering, in contrast to the "00" varieties in which the respective biosynthetic system is obviously blocked.

The results of our observations are in agreement with the studies of biosynthesis published by Underhill et al. (1973) and with the findings on the model "00" rape variety Librador (Zukalová 1985).

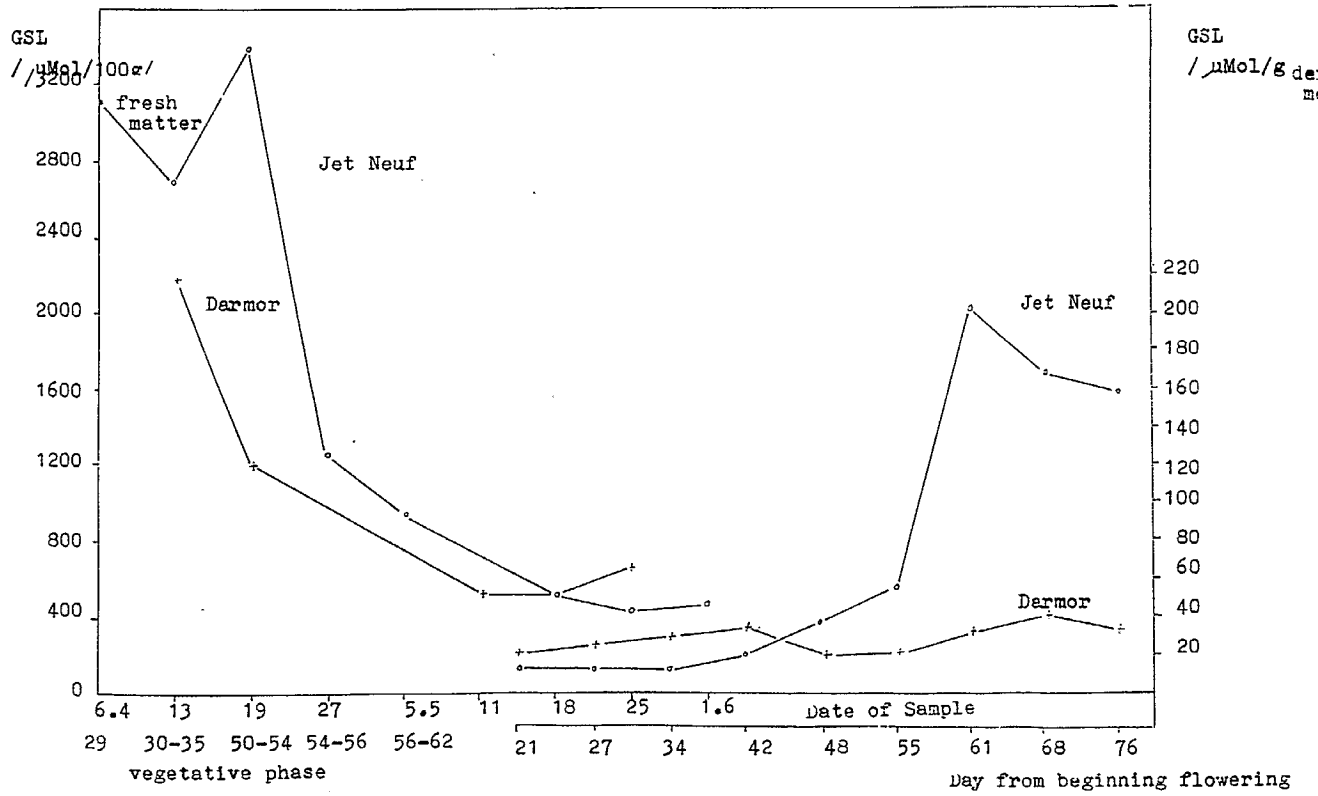
The glucosinolates would be synthesized in vivo from the aminoacide, and the key product of this synthesis was found to be oxime, and/or aldoxime (Underhill 1967). The sulphur atom in the thiogroup would be transmitted from methionine or cysteine. Glucosis would be transmitted in the process of glucosinolate synthesis in its activated form from the uridinebisphosphateglucose (UDPG), and the sulphate group from the phosphoadenosinephosphosulphate (PAPS). The knowledge of the biosynthetic processes is important in the study of the genetic blockages of glucosinolate synthesis in varieties of lowered glucosinolate content. In the "00" variety Bronowski, blockages in the oxime synthesis and in the hydroxylation processes in hydroxyderivatives synthesis were identified. As for the green matter, accumulation takes place at the beginning of the growing period and probably to a decline due to the dilution effect. The decline might be due, in addition to the dilution effect, also to the degradation of specific glucosinolates or due to different biosynthesis rate at various developmental stages (Bergmann 1970). Apart from the glucosinolates representing one of the rape anti-nutritional components, attention should be given also to substances such as sinapine, tannine, and fiber, S-methylcysteinesulpoxide, as well as to interactions of glucosinolates, phenolic substances and proteins.

CONCLUSION

- The highest glucosinolate contents were found in young meristematic tissues of vegetative rape plant organs; these were declining in the process of growth. This is why off the growth season is the harm limit surpassed.

- The lowest glucosinolate contents will be found at times of green forage harvesting, i.e. at the growth stages 54-56,

Fig.2. The formation of total contents of glucosinolates in the course of ripening of seeds of the Jet Neuf and Darmor varieties in comparison with formation of total contents of glucosinolates in the vegetative part of plant.



butonization, and 56-62, inflorescence, amounting to about 200 µmol/100 g of fresh matter.

- Most represented in the green matter are the alkenyll glucosinolates (88-92%) which are primarily responsible for the toxic syndrome, while the percentage of the indole ones is 8 to 12% only.

- The percentage of indole glucosinolates in rape roots varies around 40%.

REFERENCES

BERGMANN, F. 1970. Die Glucosinolat - Biosyntheses im Verlauf der Ontogenese von Sinapis alba L. Z.Pfl.-Physiol., 62, 4, pp. 362-375.

FÁBRY, A. 1988. Několik poznámek k tzv. úhynu lovné zvěře. Systém výroby řepky, Praha, VŠZ, Nr. 3, pp. 6-8.

FENWICK, G.R. and HEANEY, K.R. 1983. Glucosinolates and their breakdown products in food plants. CRL Crit. Rev. Fd Sci. Nutr., 18, pp. 123-201.

HACKBARTH, J. 1969. Schädliche Inhaltsstoffe bei Kulturpflanzen als Problem der Züchtung des Anbaues und der Verwertung. Fachget, Kali-Briefe, Nr. 3

HEANEY, R.K. and FENWICK, G.R. 1980. Glucosinolates in Brassica vegetables. Analysis of 22 varieties of Brussels sprout (Brassica oleracea var. gemmifera). J.Sci Fd Agric., 31, pp. 785-793.

JURGES, K. 1978. Glucosinolates in the growing and flowering plants of Brassica napus and Brassica campestris. Proc. 5th. Int. Rapskongr. Malmö, 1, pp. 57-60.

KUTÁČEK, M., VALENTA, M. and ICHA, F. 1957. Der Gehalt des Ascorbigeus in Kohlrabi (Brassica oleracea var. gongylodes) während des Vegetation und einige Beweise für den Zussammenhang des Ascorbigens mit dem Wachstum der Pflanzen aus der Familie Brassicaceae. Experientia, 13, pp. 284-285.

Mc GREGOR, D.I. and LOVE, H.K. 1987. Analysis of vegetative tissue as means of facilitating selection for seed glucosinolate composition in Brassica. Proc. 7th Int. Rapeseed Conf. Poznaň.

Mc SMITH, F.W.H. and GRIFFITHS, D.W. 1988. A time-course study of glucosinolates in the ontogeny of forage rape (Brassica napus L.). J. Fd Agric., 43, pp. 121-134.

SONES, K., HEANEY, R.K. and FENWICK, G.R. 1984. The glucosinolate content of UK vegetables - cabbage (Brassica oleracea), swede (Brassica napus) and turnip (Brassica campestris). Fd Addit. Contam., 1, pp. 289-296.

SORENSEN, H. 1984. Advances in the production and utilization of cruciferous crops. Proc. Semin. CEC Prog. Res. Pl. Protein Improv. Copenhagen, 11.-13. September, pp. 41-49.

TOOTHEY, H.L. 1980. Glucosinolates. In: Toxic constituens of plant foodstuffs. I.E. Liener (ed.). New York, Academia Press, pp. 103-142.

UNDERHILL, E.W. 1967. Biosynthesis of mustard oil glucosides: Conversion of phenylacetaldoxin and 3-phenylpropion aldoxim to glucotropaeolin and gluconasturtin. Ezrop. J. Biochem., 2, pp. 61-63.

UNDERHILL, E.W., WETTER, L.R. and CHISHOLM, M.D. 1973. Biosynthesis of glucosinolates. Biochem. Soc. Symp., 38, pp. 303-326.

ZUKALOVÁ, H. and VAŠÁK, J. 1978. Plynově chromatografické stanovení glukosinolátů u rodu Brassica (L.) metodou trimethylsilylderivátů. Rost. Výr., 24, Nr.10, PP. 1009-1017.

ZUKALOVÁ, H., VAŠÁK, J. and FÁBRY, A. 1985. Dynamika kvalitativních charakteristik dvounulových typů ozimé řepky. Rost. Výr., 31, Nr.7, pp. 685-692.