THIOCYANATE ION CONTENT OF CRUCIFEROUS VEGETABLES AS INFLUENCED BY STAGE OF DEVELOPMENT, GENOTYPE AND GRAFTING

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science

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Montreal, Canada

March 1976

Suggested short title

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Ju

ABSTRACT

M.Sc. HAK-YOON JU Horticulture

THIOCYANATE ION CONTENT OF CRUCIFEROUS VEGETABLES AS INFLUENCED BY STAGE OF DEVELOPMENT, GENOTYPE AND GRAFTING

Several commercially grown cultivars of cabbage, cauliflower, broccoli, Brussels sprouts and Chinese cabbage were tested for their content of thiocyanate ion. Significant differences in thiocyanate ion content were observed among cultivars of cabbage, cauliflower and Chinese cabbage but not among broccoli and Brussels sprouts cultivars. Brussels sprouts contained much higher amounts of thiocyanate ion than any of the other vegetables.

The average thiocyanate ion content of cabbage cultivars was much higher in 1975 than in 1974. Presumably dry conditions and high temperatures were responsible for the increased yields of thiocyanate ion in cabbage. Also for cabbage, the late maturing cultivars tended to have higher thiocyanate ion contents than early maturing cultivars.

Variations in thiocyanate ion content, during plant development, were observed in field-grown cauliflower and broccoli. For both crops, thiocyanate ion content of tissues

tended to decrease as plants aged.

The thiocyanate ion content of kale was genetically determined. The variation of thiocyanate ion content among plants of the same genotype (clones) was smaller than that among plants propagated from seeds.

A study to determine the effect of reciprocal grafts on radish thiocyanate ion content indicated that foliage had a dominant influence on overall thiocyanate ion content of the plant.

SOMMAIRE

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L'influence du stade de développement du génotype et du greffon sur le contenu en ions thiocyanate chez des crucifères

Le contenu en ions thiocyanate a été évalué chez plusieurs légumes commerciaux soit le chou, le chou-fleur, le brocoli, le chou de Bruxelles et le chou chinois. Des différences significatives dans le contenu en ions thiocyanate ont été observées chez le chou, le chou-fleur et le chou chinois mais non chez le chou de Bruxelles et le brocoli. Le chou de Bruxelles contenait plus d'ions thiocyanate que tous les autres légumes.

Le contenu moyen en ions thiocyanate chez le chou était beaucoup plus élevé en 1975 qu'en 1974. Des conditions de sécheresse et des hautes températures sont problement responsables pour ces augmentations. De plus les cultivars tardifs de chou contenaient plus d'ions thiocyanate que ceux hâtifs.

Des variations dans le contenu en ions thiocyanate pendant la croissance ont été observées chez le chou-fleur et

le brocoli cultivés en plein champs. Dans les deux cas, le contenu des tissus en ions thiocyanate semble décroître avec l'âge.

Nous avons aussi comparé le contenu en ions thiocyanate de choux frisés génétiquement differents. La variation en ions thiocyanate était plus petite parmi les plantes de même genotype (clones) que parmi les plantes issues de semences.

Enfin, une étude a été faite pour évaluer l'effet des greffes réciproques sur le contenu en ions thiocyanate chez le radis, les résultats ont montré que le feuillage avait une influence marquante sur le contenu total en ions thiocyanate de la plante.

ACKNOWLEDGEMENTS

I wish to express my deepest appreciation to Prof. B. Bible, thesis director, for his assistance, guidance and encouragement throughout the course of this study.

Special thanks are extended to Prof. C. D. Taper, Chairman of the Department of Horticulture, for permission to use the laboratory and facilities. Special thanks are also due to Dr. C. Chong for his valuable advice. Appreciation is also extended to Mrs. B. Burdett, Secretary, Mr. J. P. Laplaine and Mr. E. J. P. Gyapay, technical staff of the Department.

Appreciation is expressed to my wife for her encouragement and assistance in the typing of this thesis.

Finally, the assistance of the National Research Council of Canada, in the form of a generous grant (NRC A-9961) to Dr. Bible and a research assistantship to the writer, is gratefully acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Endemic goitre has been known for many years. The disease is more widely spread throughout the world than is generally suspected (Nutrition Canada, 1973; Greer, 1968; Clements et al., 1960; Anonymous, 1946).

Many studies have confirmed or implied that cruciferous plants as a part of the diet could be one of the contributing factors to goitre in human and animals (VanEtten and Wolff, 1973; Wright, 1958).

Most plants of the Cruciferae family (Mustard family) contain glucosinolates which can be hydrolysed by the enzyme system myrosinase, yielding isothiocyanates, glucose and sulphate. The isothiocyanate and its related products, i.e. thiocyanate ion (SCN^-), thiooxazolidone and nitrile have been found toxic and goitrogenic to human beings and animals.

Many studies have related goitrogenic toxicity of cruciferous plants to their SCN^- content (Michajlovskij and Langer, 1958; Munoz-Rodriquez, 1970). Thiocyanate ion content of cruciferous plants varies between various morphological parts, and during plant growth and development. Perhaps the consumption of cruciferous vegetables contributes to the continuing problem of goitre disease that persists in

the population in spite of the widespread use of iodized table salt.

The purpose of this study was to determine the SCN^- content of various cruciferous vegetables, and also was to study SCN^- content of cruciferous vegetables as influenced by stage of development, genotype and grafting. The result of this study should be of interest to plant breeders, toxicologists and nutritionists.

REVIEW OF LITERATURE

I. Incidence of Goitre

Goitre i.e. any abnormal enlargement of the thyroid gland which is visible as a swelling in the front of the neck in humans or animals, has been known since prehistoric times. It is believed to have been first described by the ancient Chinese, early Hindus and the Egyptians (Cooper et al., 1958). But the difficulties in language and lack of knowledge in early times about body organs leaves us uncertain as to how long this disease has been known. It was not recognized as a deficiency disease until the late 19th century (Lowenberg et al., 1974; Cooper et al., 1958).

For surveys of endemic goitre many different criteria have been used. For instance Pérez et al. (1960) outlined the following classification system: Grade* I - palpable goitres that are more than 4 to 5 times enlarged but not visible unless the head is thrown back and the neck extended; Grade II - goitres that are visible when the head is in a normal position; Grade III - goitres that are large and prominent.

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*Grade is substituted as Group in original paper.

In 1895, Baumann discovered iodine which was itself a normal constituent of the thyroid gland. Several years later iodine was found to be a curative treatment for endemic goitre. Since the discovery of the effect of iodine on the thyroid gland, the bulk of evidence has supported the hypothesis that lower concentrations of iodine exist in soil and water in areas where goitre is endemic (Kennedy and Purves, 1941; Greer, 1968). However, this disease may also be caused by inhibition of iodine uptake which may result from the effects of such natural goitrogens as thiocyanate (SCN^-), isothiocyanate and goitrin etc. (VanEtten and Wolff, 1973).

In 1917, goitre was common in humans and animals in the Pemberton valley (north of Vancouver)(Keith, 1924). European settlers suffered so severely from goitre, both in themselves and among their cattle, pigs and horses, that they almost decided to leave the valley.

In 1932, Walker reported a great deal of goitre in a strip of territory between Edmonton and Calgary in Alberta. Goitre was prevalent in the irrigated districts of the southern parts of the province and north of the Peace River territory where drinking water comes chiefly from rain and snow.

In 1932, Abbott reported goitre studies of school children made at widely separated places in Manitoba. At Dauphine,

in the west of the province, 74 per cent of the children were affected. At Morden, in the south, 21 per cent of children had goitre. In Winnipeg the incidence was 50 per cent, and in the towns of Birds Hill and Stonewall, both in the Winnipeg area, 85 per cent of children were sufferers. The Indian School at Waugh, in the extreme east of the province, was free from goitre.

In 1934, there are accounts of goitre at Saskatoon in Saskatchewan (Binning, 1935). According to Jackes (1941), goitre was also found farther south, in the country immediately surrounding the town of Regina.

In 1975, Nutrition Canada reported that goitre was observed in all groups beyond pre-school age in the Prairie provinces, British Columbia and Newfoundland. The prevalence was lower in New Brunswick, Prince Edward Island, Quebec and Ontario and among Indians. The results for Nova Scotia were intermediate between these high and low prevalence areas. Grade I goitre, classification of Pérez et al. (1960), was relatively common in both male and female; the highest prevalence (17.8 %) was among pregnant women (Nutrition Canada, 1975). Grade II and Grade III goitres occurred in a small percentage of females and were rarely seen in males. The Nutrition Canada survey indicates that goitre is still a significant problem in some parts of Canada, in spite of the mandatory iodization of table salt.

In 1960, according to World Health Organization (WHO), there were estimated to be 200 millions cases of endemic goitre in the world (Kelly and Snedden, 1960).

II. The Thyroid Gland

A. The Normal Thyroid Gland

Thyroid size varies in the adult male from 20 to 60 grams, the average being about 25 grams (Anonymous, 1946). This agrees with Marine (1922) who suggested that the normal thyroid gland should not exceed 0.35 of a gram for every kilogram of body weight. On this basis the thyroid of a man of 70 kilograms would be 24.5 grams in weight.

The thyroid gland is a ductless gland -the largest in the endocrine system- located immediately below the larynx on either side of and anterior to the trachea (Guyton, 1966). It consists of two lateral lobes connected by a narrow strip, the isthmus, from 5 mm to 2 cm in breadth (Huber, 1930). The height of the lateral lobes range from 3 cm , or less, to twice as much within normal limits. The transverse diameter of the whole organ is 6 to 7 cm.

According to Rice (1938), the average thyroid weight is 1.5 grams at birth, and it increases to 30 grams in young adults. It gradually decreases in weight to less than 20 grams at the age of 80 years. It has been observed that thyroid enlargement is common during puberty in adolescences,

and during menstruation, pregnancy and lactation in women (Greer, 1968; Marine and Lenhart, 1909).

The thyroid gland is composed of spherical, cystlike follicles 0.02 to 0.9 mm in diameter, lined with a simple epithelium and containing a gelatinous colloid (Bloom and Fawcett, 1968), that arises from the secretory activity of the epithelium. There is great variability in follicle size in man but the follicles in animals are more uniform.

These closed, saclike lobules are independent secretory units separated from each other only by fine stands of connective tissue which contain capillary blood vessels to carry the gland secretion into larger vessels embedded in the stroma and from thence into the main blood stream (Anonymous, 1946). When the gland is functioning normally,

SECTION OF THE HYPERPLASTIC GLAND
Magnified 250 diameters

all the follicles are well filled with colloid.

B. The Thyroid Secretion

The thyroid gland synthesizes, stores and secretes the thyroid hormones, which are stored as thyroid colloid in the follicles (Stanbury, 1967). The thyroid gland normally releases its hormonal secretion in the event of an appropriate stimulus. This substance was first isolated in crystalline form by Kendall (1915) who named it thyroxin* (Kendall, 1919). Harrington and Barger in 1927 established the chemical formula of thyroxine and synthesized it.

The mechanism of secretion was explained briefly by Guyton (1974). First iodine is absorbed into the follicular cells in the form of an iodide salt. The follicular cell converts the iodide to elemental iodine. At the same time the cell also secretes a protein called thyroglobulin into the follicle. The elemental iodine reacts with the thyroglobulin either before being released into the follicle or after release to convert much of the amino acid tyrosine in the molecule of the thyroglobulin into thyroxine. The chemical reaction for this process is as follows (Harrington and Barger, 1927):

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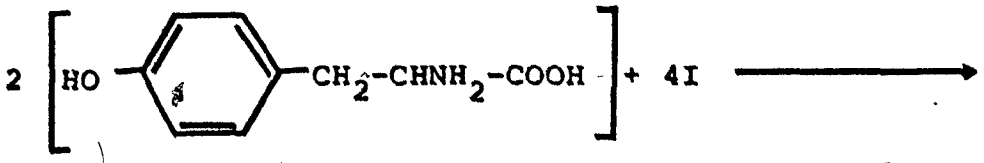
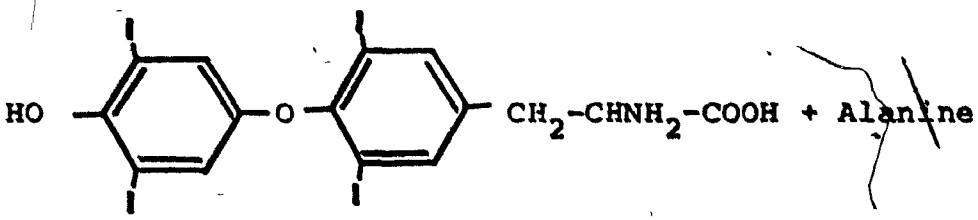
*Now usually spelled thyroxine.

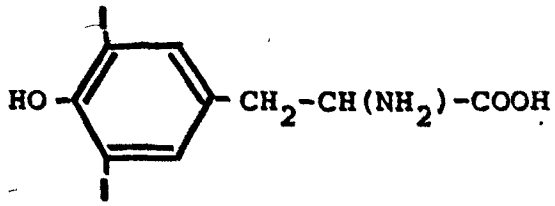


Tyrosine



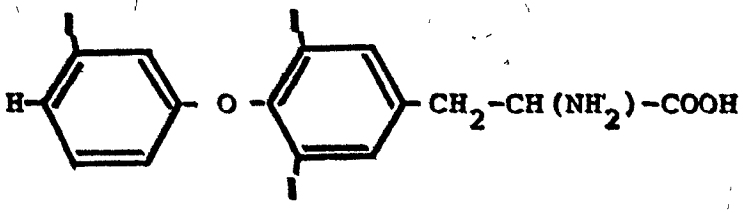
Thyroxine

According to Orten and Neuhaus (1970), much of the iodine present in the gland (about 70 %) is present as an even closer relative to tyrosine, namely, diiodotyrosine and also some monoiodotyrosine, which has little physiologic activity.



Diiodotyrosine

Since the gland converts diiodotyrosine to thyroxine, it is believed that diiodotrysine is the precursor of thyroxine. Another related compound 3,5,3' -triiodothyronine has five times the physiologic activity of thyroxine but it is present in relatively small amounts.



3,5,3' -Triiodothyronine

It is possible that neither thyroxine nor triiodothyronine is the true thyroid hormone (Orten and Neuhaus, 1970). Some authorities maintain that the true thyroid hormone is a much larger molecule, e.g., thyroglobulin or a peptide complex built around one or more of the iodine-containing compounds. Thyroglobulin has a greater activity than thyroxine in proportion to its iodine content.

However, a current study (Hamilton, 1968) indicates that thyroxine causes an increased synthesis of all forms of RNA, nuclear, ribosomal, and transfer. This may well prove to be the fundamental biochemical effect of the thyroid hormone in affecting growth and development of the body and in stimulating total metabolism.

C. The Thyroid Changes in Goitre

Marine and Lenhart (1909) observed in test animals that the strictly normal glands have the highest iodine contents and those with marked glandular hyperplasia the lowest. They indicated that the weights of thyroid glands vary directly with the degree of hyperplasia and inversely with the percentage iodine content.

However it is clear that a deficiency in iodine intake and a consequent deficiency in thyroid hormone synthesis leads to the compensatory change in the thyroid gland recognizable as hyperplastic goitre (Astwood, 1948).

According to Guyton (1966), lack of iodine prevents production of thyroxine by the thyroid gland, and, as a result, no thyroxine is available to inhibit production of thyrotropin by the adenohypophysis; this allows the adenohypophysis to secrete excessively large quantities of thyrotropin. Actually the thyrotropin causes the gland to enlarge.

Using the Brassica-seed diet which usually causes goitre in test animals, striking results have been obtained in rats given adequate iodine rations (Kennedy and Purves, 1941). They found that during the first seven days the thyroid loses the greater part of its colloid. There is considerable hypertrophy of the epithelium, the gland as a whole remaining unchanged in size. During the second week there occurs a rapid increase in the weight of the entire gland. At fourteen days advanced hypertrophy together with hyperplasia is observed, and complete loss of colloid. At the end of the third week the changes are at a maximum. Thereafter a slow thyroid growth continues, paralleling that of body growth in the rat. Colloid, absent from the fourteenth day, reappears in significant amounts in glands examined subsequent to the fifty-sixth day. Since no progressive accumulation of colloid occurs thereafter, it assumed that the thyroid at this period has attained physiologic equilibrium.

Whenever the amount of thyroid tissue becomes insufficient to supply thyroid secretion, whether from reduced iodine intake or from increased demands or following partial thyroidectomy, compensatory hypertrophy occurs (Marine, 1935). This hypertrophy appears always to be the result of direct stimulation by the thyrotropic hormone. This is characterized principally by a decrease in the stainable colloid and in the iodine content, by an increase in the blood supply and by a change in the follicular epithelium from low cuboidal and cuboidal to high cuboidal or even columnar. Then, as hyperplasia takes place, infoldings and plications of the lining epithelium are formed (Marine and Lenhart, 1909). This process is apparently an attempt to increase the epithelial surface without undue general glandular enlargement. Marine and Lenhart (1909) mentioned that there is no essential difference between the forms seen in human and in dog, sheep, ox or pig thyroids. Greer (1968) explained that the reasons for thyroid change during puberty in adolescent, and during menstruation, pregnancy and lactation in women are due to the increased demands for thyroid hormone during these periods, thus causing an augmented secretion of thyrotrophin and hence an increase in thyroid size and activity.

These hypertrophic and hyperplastic changes are in their very nature inevitably accompanied by enlargement of

the gland. The degree of enlargement will depend on the extent of the hyperplasia (Anonymous, 1946).

III. Relationship between Incidence of Goitre and Consumption of Cruciferous Vegetables and Weeds

The relationship between iodine deficiency and endemic goitre has been well established (Keith, 1924; McClure, 1937; Spencer, 1954; Clements et al., 1960), and no factor other than iodine deficiency has been as yet related to the distribution of endemic goitre in humans (Kennedy and Purves, 1941; Orten and Neuhaus, 1970).

According to Fertman and Curtis (1951), the iodine-deficiency hypothesis alone fails to explain satisfactorily several situations which are still enigmatic. Thus, there is the problem of certain isolated seacoast regions where the iodine intake is presumably high and yet simple goitre is prevalent; or the more baffling observations concerning cretinism, which over-runs certain areas endemically while shunning others.

Since the discovery of 'cabbage goitre' (Chesney et al., 1928), many studies have confirmed or implied a relationship between consumption of cruciferous plants or their products and incidence of goitre in humans and animals (VanEtten, 1969; Wright and Sinclair, 1958).

From a study of simple goitre in Winnipeg and its relation to racial incidence and to nutrition, Abbott (1932) reported that cabbage was a predominant constituent in the diet of the central European races among which thyroid enlargement was very prevalent. The recent Nutrition Canada (1975) study shows a high incidence of goitre even though people have adequate iodine.

The production of thyroid enlargement experimentally in laboratory animals caged and controlled under different conditions of diet has added greatly to our knowledge of the factors which may cause goitre in a natural environment.

Chesney et al. (1928) observed large hyperplastic goitres in rabbits maintained on cabbage diets. According to McCarrison et al. (1933), a group of rabbits fed exclusively on fresh raw cabbage had significantly heavier thyroid glands compared with another group which were fed on a stock diet of raw cabbage, carrots, bran, sprouted Bengal gram, and green grass. Stiner (1933) reported that goitre was produced in guinea pigs by the daily feeding of 10 g kohlrabi leaves (Brassica oleracea). Judina (1940) reported that addition of the wild herb Brassica rapa oleifera (140 g daily for months) to the diet of rabbits increased the thyroid weight by 25 to 65 per cent in six out of ten animals. Similarly, 150 g daily of Raphanus raphanistrum (wild

radish) for three-and-a-half months increased the thyroid weight by 45 to 125 per cent in three out of ten animals.

In 1941, Kennedy and Purves from their study of experimental goitre of rats found that diets including 45 per cent ground seeds of Brassicae such as (rape, swede, soft and hard turnip, and chou moellier) produced thyroid hyperplasia in rats which was not prevented by giving 1.3 mg of potassium iodide (i.e. 1,000 µg iodine) per rat daily. Blum (1943) reported that the goitre produced by consumption of large amounts of cabbage presents a stage preceding true exophthalmic goitre.

Often cabbage failed to cause any goitre. According to Hercus and Aiken (1933), cabbage feeding sometimes gave rise to small goitres in rabbits but often no effect was observed. Marine (1933) suggested that the reason why cabbage sometimes does and sometimes does not cause goitre depends on the relative proportions present in cabbage of antigoitrous factors (iodine and hexuronic acid) and goitrogenic factors (cyanides).

The goitrogenic chemicals in cruciferous plants appear to be transferable to the milk of cows consuming these plants and to be capable of interfering with thyroid function of humans who drink such milk (Wills, 1966). Thus, Clements and Wishart (1956) have suggested that an appreciable amount of endemic goitre in certain areas of Tasmania

(Australia) may be due to a goitrogen present in the milk of cows fed 'chou moellier'. Apart from other evidence, milk and extracts of skim milk from chou moellier-fed cows were found to depress the uptake of iodine-131 by the thyroid glands of experimental animals and human volunteers. Greene and Glascock (1958), in comparing milk from cows fed hay, chou moellier or pastures lightly contaminated with cruciferous weeds, found the latter to have the highest goitrogenic activity.

In late 1949, Greer et al. isolated a compound similar to thiouracil, 1-5-vinyl-2-thio-oxazolidone from the seeds of rutabaga, turnip, cabbage, kale and rape. This compound was thought responsible for the goitre in sheep fed on turnips (Hercus and Purves, 1936) and for the epidemic of goitre in western Europe among the peoples who had to subsist largely on brassica vegetables during world war II (Bastenie, 1947).

Kreula and Kiesvaara (1959) have obtained evidence that the goitrogenic activity in milk is unlikely to be due to 1-5-vinyl-2-thio-oxazolidone even though turnip root or other forage known to provide this compound is eaten by cows. This finding seems to indicate that the thiocyanate or some unknown goitrogenic material other than a derivative of 2-thiooxazolidone must be ingested by cows grazed on grass having an admixture of goitrogenic weeds (Wills, 1966).

Wright (1958) found that milk from goats fed on kale contained 4.6 mg per cent of thiocyanate although that from animals fed on grass pasture contained only 0.8 mg per cent.

By implication, other cruciferous weeds might be considered suspect in relationship to the general hypothesis of an association between plant thioglycosides (glucosinolate) and endemic goitre in certain circumstances (Bachelard and Trikojus, 1960).

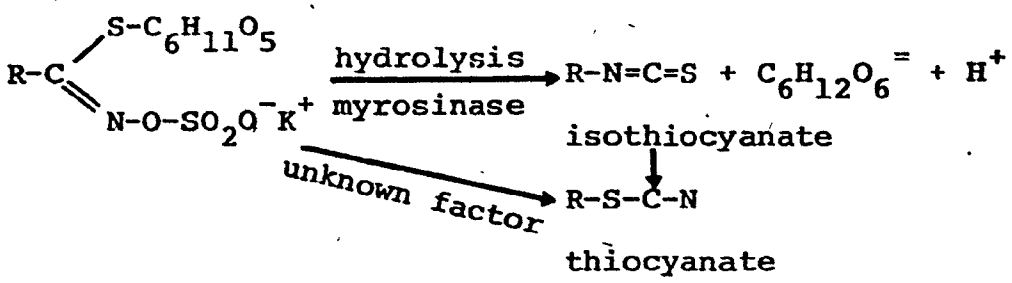
According to Astwood (1949), the incidence of goitre is strikingly reduced by increasing the iodine intake but goitre is by no means completely abolished by this procedure. This might be suggest that some other factor besides iodine deficiency is contributing to goitrogenesis. Clements and Wishart (1956) stressed that goitrogenic substances in the diet may be a significant factor in the etiology of endemic goitre. These findings give rise to the questions; what is the proper public health policy for the prevention of goitre, and what are the ramifications for agricultural practice?

IV. Natural Goitrogens and Associated Compounds

A. Chemistry and Development

1. Thiocyanate Ion

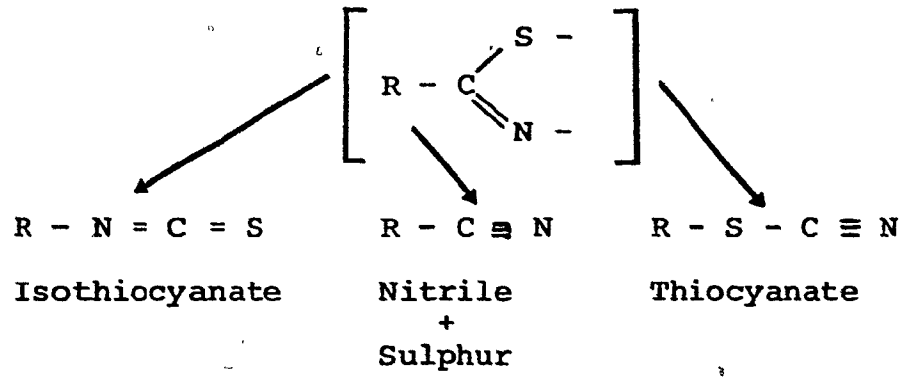
Thiocyanate ion, SCN^-, which was first observed to act as a goitrogen by Barker in 1936, is only one of several products liberated by enzyme hydrolysis of glucosinolates when plant cells are ruptured (Gmelin and Virtanen, 1960; Ettlinger and Kjaer, 1968).



Gmelin and Virtanen (1960) showed that the cleavage of glucosinolates into SCN^- by the myrosinase in the presence of an unidentified enzyme factor is common in plants of the Cruciferae.

Together with thiocyanates, related hydrolytic products such as isothiocyanates and nitriles and thiooxazolidones (a group containing very potent antithyroid compounds) are responsible for the pungent odor, taste and toxicity of plants of the Cruciferae (Friis and Kjaer, 1966; VanEtten, 1969).

According to VanEtten (1969), most of the thioglucosides (glucosinolates) have been characterized through the isothiocyanates formed by hydrolysis because their formation appears favored over the nitriles and thiocyanate as follow:



Ettinger and Thompson (1962) reported that 3-indolylmethyl and 4-hydroxybenzyl isothiocyanate readily evolve thiocyanate ion.

2. Glucosinolates

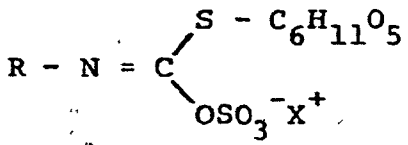
Glucosinolates are the naturally occurring precursors of organic isothiocyanates, thiocyanates, and cyanides, and of further transformation products of these. Little is known about the functions of glucosinolates in the plants that produce them (Ettlinger and Kjaer, 1968).

Sinalbin, sinigrin and progoitrin are trivial names for specific glucosinalates. As early as in 1831, Robiquet and Boutron isolated from the seeds of white mustard (Sinapis alba L.) a crystalline, sulphur-containing constituent which was later named sinalbin and recognized as a

mustard-oil producing glucosinolate. Busy (1840) isolated another glucosinolate, which has become known as sinigrin from black mustard seeds (Brassica nigra Koch). He envisaged also the enzymatic conversion of glucosinolates to isothiocyanates from his work.

Ettinger and Kjaer (1968) indicated that only eight different glucosinolates had been observed in plants until 1912. At present about 65 different glucosinolates have been isolated from plants of the Cruciferae and related families (VanEtten and Wolff, 1973).

The crystalline compounds sinigrin and sinalbin were early subjects of structural speculations. Gadamer (1897) reported the structure of sinigrin and sinalbin as follows:



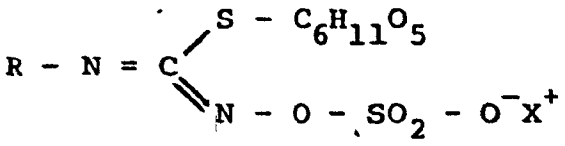
Sinigrin: $R = CH_2 = CHCH_2$ $X = K$

Sinalbin: $R = (p) - HOC_6H_4CH_2$ $X =$ sinapine

He concluded that the general structure apparently accounted for the production of isothiocyanates, glucose and sulphate on enzymic hydrolysis. Several investigators, including Gadamer, had commented upon the unexpected formation of varying amounts of nitriles concomitant with

isothiocyanates during enzymic glucosinolate hydrolysis as well as upon chemical degradation. However, the above structure (Gadamer, 1897) was generally accepted for many years.

Ettlinger and Lundeen (1956) revised the structure of sinigrin and sinalbin as follows:

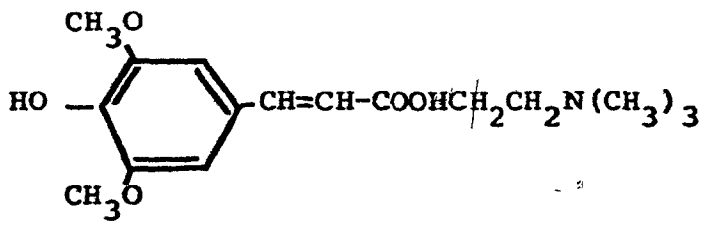


Sinigrin: $R = CH_2 = CH - CH_2$ $X = K$

Sinalbin: $R = (p)HOC_6H_4CH_2$ $X =$ sinapine

This revised structure explains the occurrence of nitriles and isothiocyanates in the same essential oil.

Like most subsequently crystallized mustard oil glucosinolates, sinigrin is obtained as a potassium salt from plant sources, in most of which ions of this metal are abundantly present. In sinalbin, however, the basic moiety consists of the aromatic choline ester sinapine (I) (Kjaer, 1961).



I

The glucosinolates in which the R group is allyl-3-butenyl-, 4-pentenyl-, benzyl-, 2-phenylethyl-, or 4-methyl-thio-3-butenyl- are the sources of the isothiocyanates that are the steam-volatile mustard oils (VanEtten and Wolff, 1973). Glucosinolates are distributed diffusely throughout the parenchymal tissue in plants (Kjaer, 1960).

These compounds invariably seem to conform to the general structure as sinigrin and sinalbin, differing only in the chemical nature of the side-chain, R (Kjaer, 1966).

3. Myrosinase

According to Kjaer (1961), the embryonic tissue of seeds contains the glucosinolates, functioning as substrates for the enzyme 'myrosinase', which is accumulated in special cells (idioblasts). Thus, it is only after disintegration and subsequent contact between glucosinolate and enzyme that enzymic hydrolysis takes place.

The enzyme was first demonstrated for crucifers by Guignard (1890). A more detailed study of the enzyme which was called 'myrosin' at that time was initiated in 1926 by Neuberg and Wagner. They adopted the term 'myrosinase' instead of 'myrosin'.

According to van Euler and Erikson (1926), the enzyme myrosinase was composed of two entities, one liberating

glucose in a fast reaction and another acting as a slow sulphatase. Subsequent work by Sandberg and Holly (1932) supported the two-enzyme system theory.

In contrast to these investigations, Nagashima and Uchiyama (1959) confirmed the conception of myrosinase as a single enzyme that hydrolyzes the glucosinolate linkage. Ettlinger and Dateo (1961) agreed with the one enzyme theory.

Several investigators have tested the stability of myrosinase by varying pH and temperature. It appears that myrosinase has a pH optimum at 6.5 - 7.5 and a temperature optimum between 30° and 40° C (Kjaer, 1960).

Appelqvist and Josefsson (1967) showed that myrosinase in seeds with a moisture content of about 8 per cent was effectively destroyed by heat treatment in a closed vessel, for 15 min. at 90° C. Recent work by Paxman and Hill (1974) and Josefsson (1975) also suggests that myrosinase can be destroyed by heat treatment.

4. Isothiocyanate

Most isothiocyanates are pungent substances, easily detected by their biting taste. In higher concentrations they posses vesicant and frequently lachrymatory properties. For these reasons many isothiocyanate-producing plants have been applied as potherbs, condiments, and remedies in folk medicine (Kjaer, 1966). However, on account of the conspi-

cuously pungent properties of many isothiocyanates (mustard oils) this group of compounds has attracted scientific interest for hundreds of years (Kjaer, 1960).

According to Challenger (1959), Portas in 1968 and Febure in 1660 in Paris observed first that the formation of a volatile oil on distillation of mustard seeds with water. A Dutch scientist Boerhaave appears to have been the first to prepare mustard oil and to describe its properties in 1732.

Isothiocyanates are not present in the free state in plants but produce on enzymic hydrolysis of precursors 'glucosinolates' (Kjaer, 1960). These glucosinolates are found in a large number of plants belonging to a small number of botanical families. Of these families, the Cruciferae is the most prominent source of isothiocyanate-producing glucosinolates.

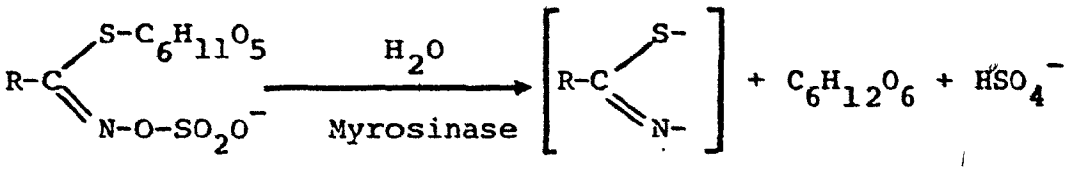
Isothiocyanates, of the general formula R-N=C=S, are considered as esters of isothionic acid, H-N=C=S (Assony, 1961). They are isomeric with the thiocyanates, R-S-C≡N. Isothiocyanates will react with ammonia to yield thiourea in the following manner (Ettlinger and Thompson, 1962):

$$R\text{-}N{=}C{=}S + NH_3^+ \longrightarrow R\text{-}NHCSNH_2$$

This reaction is the basis of several techniques for the identification and quantitative estimation of the isothio-

cyanates.

The following reaction shows the products generally formed when glucosinolates (I) are hydrolyzed by myrosinase (Florkin and Stotz, 1965). Glucose and sulphate ions are always released while the remainder of the glucosinolate molecule, the aglucon, is usually converted to thiocyanate (III), to nitriles (IV) and sulphur through a Lossen arrangement to isothiocyanate (II).



R-N=C-S R-C≡N R-S-C≡N

I II III IV

The glucosinolates in which R group is allyl-, 3-butenyl-, 4-pentenyl-, benzyl-, 2-phenylethyl-, or 4-methylthio-3-butenyl- are the sources of the isothiocyanates that are the steam-volatile mustard oils (VanEtten and Wolff, 1973). Although the mustard oils appear to be the main hydrolysis products from the organic aglucon of the glucosinolates, the nitriles may be formed instead (Challenger, 1959; Kjaer, 1960). In some instances the isothiocyanates initially formed undergo further transformation. According to Kjaer (1960) enzymic hydrolysis of sinalbin (P-hydroxybenzylglucosynolate) from white mustard gives p-hydroxylbenzyl isothiocyanate which

readily evolves SCN^-.

Ettlinger and Thompson (1962) have reported that 2-hydroxy-3-butenyl isothiocyanate naturally cyclizes to 5-vinyl-2-thiooxazolidone. These cyclic products are called goitrins because of their potent goitrogenic activity (VanEtten and Wolff, 1973).

Ettlinger and Thompson (1962) have noticed also that sulforaphene was the major isothiocyanate compound in radish (Raphanus maritimus Smith). According to them, in Brassica nigra seed is the classic source of allyl isothiocyanate. In Brassica oleracea, allyl isothiocyanate is also prominent except for broccoli, but the other compounds are notable and varied. In Brassica campestris, 3-butenyl isothiocyanate and goitrin are dominant in the seed and goitrin occurs also in turnip root but allyl isothiocyanate recedes to a trace.

It is of interest that certain compounds are not always produced in all parts of the plant. According to Neuberg and Wagner (1926), 3-indolylmethyl isothiocyanate is found in fresh plants of Brassica and Raphanus but not in the seed.

B. Effect of Thiocyanate Ion on Thyroid Gland

The first indications of the effect of thiocyanate ion upon the thyroid gland were provided by observations of Barker (1936) on patients with hypertension. It was observed that a certain number of patients experienced enlargement of the thyroid gland.

Rawson et al. (1943) implied that the mechanism of Brassica-seed goitre and that of thiocyanate caused goitres in human patients were one and the same.

Thiocyanate was found to be goitrogenic in animals, but it was soon apparent that it differed from the large groups of antithyroid compounds in being readily inhibited by increasing the iodine intake (Astwood, 1943). Thiocyanate was also set apart from other goitrogens by findings of Franklin and Chaikoff (1943) who showed that thiocyanate inhibits the uptake of iodine by surviving thyroid slices in vitro, whereas other antithyroid agents have little effect at concentrations which effectively prevent hormone synthesis.

According to Langer (1964), allyl isothiocyanate acts as a goitrogen, because the compound is metabolized to give the thiocyanate ion as one of the products. Doses of 2-4 mg of allyl isothiocyanate mixed with water and fed by stomach tube to rats caused a marked increase in thiocyanate ion in the blood plasma and a decrease in radioactive iodine uptake

by the thyroid gland. This dosage is equivalent to the amount of the compound each rat would get in eating 40 g cabbage per day.

In the presence of thiocyanate, the thyroid gland is obliged to synthesize thyroid hormone from the iodide which passively diffuses into it from the blood, when the blood iodide is very low, because of deficient ingestion of iodine, hormone synthesis is deficient and hypothyroidism and goitre ensue (Astwood, 1948). Thus thiocyanate ion acts by lowering iodine concentration in the thyroid. Its goitrogenic effect can be prevented or reduced by increasing the iodine content of the diet (VanEtten, 1969).

Rawson et al. (1943) outlined the relationship between thiocyanate and goitre in the following diagram.

From the diagram, we see that the thiocyanate ion blocks the formation of thyroid hormone by the thyroid, and that the consequent lowering of concentration of active thyroid hormone in the blood stream causes stimulation of the anterior pituitary to produce an excess of thyrotropic hormone. This in turn causes thyroid hyperplasia but, because of the block, there is no increase in output of physiologically active thyroid hormone. It is a hyperplasia of frustration. An excess of administered iodine may force the block, and cause liberation of active hormone. Administration of thyroid hormone by passes the block, and relieves the situation by substitution.

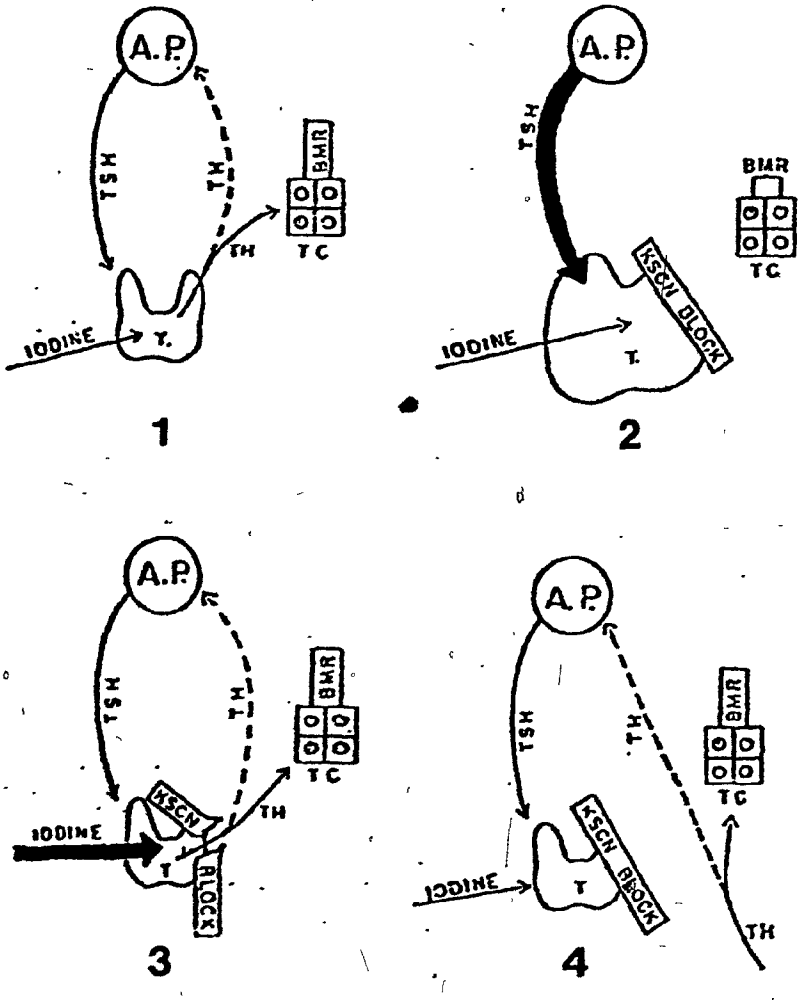


Figure 1. Diagrammatic representation of the effect of thiocyanate on the pituitary-thyroid axis.

1. Normal relationships. The anterior pituitary-AP- by means of its hormone-TSH- and in the presence of an adequate supply of iodine, stimulates the thyroid-T- to produce its hormone-TH- which stimulates the metabolism of tissue cells-TC- and inhibits AP.

2. Thiocyanate imposes an obstruction to the completion of TH. The BMR of TC therefore falls, and the uninhibited AP produces an excess of TSH. This causes hyperplasia of T, but since the block remains, no active TH is delivered to the body.

3. The supply of an excess of iodine forces the thiocyanate block so that TH is made in adequate amounts, BMR remains normal and TSH formation remains normal, and therefore no enlargement of T takes place.

4. The supply of TH from without by-passes the thiocyanate block so that BMR remains normal and TSH formation remains normal, as does the thyroid gland.

Thus, thiocyanate goitre can be reduced or prevented by prophylactic doses of iodine, and also can be relieved by the administration of thyroid hormone.

C. Occurrence of Thiocyanate Ion in Cruciferous Vegetables

The most prominent source of thiocyanate-producing glucosinolates is undoubtedly the family Cruciferae (Kjaer, 1966). On basis of the available evidence, about 300 cruciferous species have been investigated and all contain one or more glucosinolates.

Josefsson (1967) isolated glucosinolates from lamina, petiole, stem, root tissue of Brassica napus, Brassica campestris and Brassica oleracea cultivars. The highest concentration of glucosinolates has been found in mature seeds (VanEtten, 1969).

It is well known that the production of thiocyanate on tissue disintegration is dependent upon the presence of both glucosinolate and enzyme(s) (Ettlinger and Kjaer, 1968; Gmelin and Virtanen, 1960). Paxman and Hill (1974) observed

that small young kale leaves contained more than five times the amount of thiocyanate found in large, fully formed leaves, and about twice the amount present in leaves of intermediate size. Chong and Bible (1974) suggested that thiocyanate precursors or possibly their enzyme(s) of hydrolysis are synthesized in young photosynthetic tissue. They also reported the amounts of thiocyanate present in leaf and root tissues of fifteen radish cultivars.

Michjlovskij and Langer (1958) found that the edible part of cabbage and related plants contained thiocyanate ion ranging from 0.7 to 10.2 mg per 100 g fresh material. According to Paxman and Hill (1974), the thiocyanate content of small young leaves of four types of kales was about 50 mg/100 g of fresh tissue, in leaves of intermediate size about 25 mg/100 g and in large fully formed leaves about 10 mg/100g.

The factors affecting the occurrence and the level of glucosinolate compounds in plant tissue are not well known. However, Josefsson and Appelqvist (1968) indicated that environmental factors may play a role in determining glucosinolate levels in plant tissue. From their study of rape and turnip rape seed, the glucosinolate content was effected by polyploidisation and sulphur level.

GENERAL MATERIALS AND METHODS

General Procedure

All field, greenhouse, and growth chamber work was done at Macdonald College, Ste Anne de Bellevue (45° 25' North, 73° 56' West), Quebec. Seeds were purchased from Stokes Seed Ltd., Ste Catharines, Ontario.

In 1974 several commercial cultivars of cabbage, cauliflower, broccoli, Brussels sprouts and Chinese cabbage were seeded on April 30 in paper trays (24.5 x 14.5 cm) containing turface medium and kept in the greenhouse at a mean daily temperature of 22 ± 4° C. After sprouting, seedlings were watered every other day with a dilute solution of 20-20-20 fertilizer (about 0.25 g per tray); subsequently trays were thinned to 12 plants each, with three trays of plants for each cultivar. At field setting (St. Bernard clay loam soil) on May 28, seven plants per row were spaced at 0.6 m intervals in rows 0.9 m apart and plants watered in with 10-52-17 starter solution (2.5 g of 10-52-17 in 250 ml water per plant). Standard commercial recommendations were followed during growth of the crops for application of insecticides, fungicides and fertilizer; and irrigation was used occasionally to supplement rainfall.

The different crops were each set out in randomized complete block designs with three replications. The data was tested by analysis of variance where appropriate.

Thiocyanate Ion Analysis

Thiocyanate ion was determined by the colormetric method of Johnston and Jones (1966). Each sample of tissue was extracted by homogenizing or grinding in a suitable volume of distilled water, clarified, and determined in triplicate, as described by Neil and Bible (1972), but with various modifications due to different size of sample or different morphological part of sample. In all cases parallel samples were collected and used to determine per cent dry weight. For seed analysis, it was necessary to store unclarified seed extracts over night in the refrigerator (4^{o} C) before further processing (Chong and Bible, 1975). Lead acetate ($Pb(CH_3COO)_2 3H_2O$) (0.1 g) was used for the clarification of all extracts (12 ml aliquots). Extracts from red cabbage cultivars were clarified by addition of both lead acetate (0.1 g) and magnesium oxide (0.2 - 0.5 g) for the removal of red pigmentation. The clarified extract was centrifuged and the SCN^- content immediately read at 460 nm on a Coleman Model 6A Junior Spectrophotometer from aliquots (0.5 ml - 2.5 ml), each made up to a 2.5 ml volume with

distilled water and to which were added 2.5 ml of 0.4 M ferric nitrate ($Fe(NO_3)_3 9H_2O$) in 1 N nitric acid (HNO_3). To provide the reference blank for reading the sample, one drop of 5 % $HgCl_2$ was used to destroy the red ferric thiocyanate complex. Readings of the SCN^- content were corrected for the percentage moisture in each sample and are expressed as µg KSCN per gram dry weight of tissue.

Reagents

0.4 M $Fe(NO_3)9H_2O$ in 1 N HNO_3 . . . 32 ml of 15.5 N nitric acid was added to 80.8 g ferric nitrate dissolved in 200 ml distilled water and volume adjusted up to 500 ml.

5 % $HgCl_2$ . . . 5 g of mecuric chloride was dissolved in 100 ml of distilled water.

KSCN . . . 0.1 N (9.718 / 1) potassium thiocyanate solution was used for the standard curve determination.

Experiment 1

Thiocyanate ion content of cabbage, cauliflower, broccoli, Brussels sprouts and Chinese cabbage cultivars (Field)

Cabbage (Brassica oleracea var. capitata)

Fourteen cultivars of green cabbage Early Greenball, Princess, Badger Market, Canada'Kraut, Stonehead, Copenhagen Market Late, Early Round Dutch, Roundup Hybrid, Jumbo, Penn State Ballhead, Autumn Marvel, Storage Green, Evergreen Ballhead and Ultra Green and two red pigmented cultivars Storage Red and Red Acre were grown in 1974 as previously described.

For each cultivar four mature cabbages per row were selected at random, harvested and number of outer wrapper leaves, top weight, and head weight per plant recorded. Thin slices of tissue from cross-sectional cuts through the center of the head were taken from each pair of cabbages and combined into single samples that weighed about 100 g. These samples were ground with distilled water 1:2 ratio (i.e. 100 g of sample to 200 g of water) in a blender and SCN^- content determined as previously described. Each datum of SCN^- content is the mean of three samples, each analysed in triplicate. Each sample consisted of head tissue from two plants.

In 1975 essentially the same cabbage trial as 1974 was

repeated with the following exceptions: the growing medium for the transplants was 1/3 loam soil, 1/3 peat moss, and 1/3 vermiculite, and extra four and a half week old transplants of each cultivar were sampled for their SCN^- content on May 31. Three samples each consisting of leaf and stem tissue of two plants were tested for each of the 16 cultivars.

Cauliflower (Brassica oleracea var. botrytis)

Ten cultivars of cauliflower Jet Snow, Stokes Early Abundance, Whitehorse, Stokes Super Snowball, Snowball-Y, Snowball Imperial, Snowmound, Clou, Imperial 10-6 and Igloo were grown in 1974 as previously described. For each cultivar, four plants per row with mature curds were selected at random, harvested and number of leaves and weight of curd, leaf and stems recorded. Representative sections of curd, leaf and stem tissues were taken from each pair of cauliflower plants and combined into single samples for each of the respective tissues. Sample size and amount of water added for grinding of curd, leaf and stem tissues were 100 g in 250 g of water, 100 g in 250 g of water, and 20 g in 40 g of water respectively.

Broccoli (Brassica oleracea var. cauliflora)

Seven cultivars of broccoli Spartan Early, Italian Sprouting, Cleopatra, Rapine, Waltham 29, Green Mountain and

Early Purple Head were grown in 1974 as previously described. The cultivar Rapine bolted or flowered prior to reaching the mature size and therefore was not sampled. Choice of tissues sampled, size of samples and amounts of water added for grinding were the same as outlined for cauliflower.

Brussels sprouts (Brassica oleracea var. gemmifera)

Seven cultivars of Brussels sprouts Early Morn, Long Island Improved, Green Pearl, Topscore, Jade Cross, Indra and Peer Gynt were grown in 1974 as previously described. For each cultivar four plants per row were selected at random and mature sprouts on the basal one-third of the stem were harvested and their weight recorded. Samples were of two or three sprouts from each of two plants. About 200 g of water was added to each sample (about 50 g) for grinding.

Chinese cabbage (Brassica campestris [Pekinensis group])

Five cultivars of Chinese cabbage Chihli, Hybrid F-1 No.11, Michihli, Springtime and Wong Bok were grown in 1974 as previously described. Extra transplants were sampled for SCN^- content of leaves on May 31. Two of the cultivars (Springtime and Wong Bok) bolted, flowered prematurely, and therefore were not sampled. At the mature stage the three remaining cultivars were sampled like the cabbage.

Experiment 2

Variation of SCN^- content in cauliflower and broccoli plants during their life cycles (field)

Two cultivars of cauliflower Jet Snow and Igloo and two of broccoli Spartan Early and Waltham 29 were grown in the greenhouse and set in the field on May 28 as previously described.

The seeds and cotyledon stage of the four cultivars was analysed for SCN^-. Subsequently, different morphological parts (i.e. curd or head, leaves and stem) were each analysed for SCN^- content, at two week interval from the transplanting stage to the seed producing stage. Number of leaves and plant weight were recorded at each sampling. The samples were taken as previously outlined for the cauliflower and broccoli. Seed samples of 1 g were ground in 25 g of water.

Experiment 3

Relative variability of SCN^- content of kale in clonal plants versus plants from seed (field)

Dwarf Green Curled kale was seeded on August 14, 1974 and grown to maturity in the greenhouse. On April 14, 1975 one plant was selected for cloning from which several cutting

were taken and placed in perlite for rooting. At the same time seeds of Dwarf Green Curled kale were also started. Three weeks later both rooted cuttings and seedlings were transplanted to peat pots and grown in the greenhouse until field setting on May 29.

Fertilizing, pest control, and plant spacing were the same as previously discussed in the general methods, however the clones and plants from seed were alternated in each row. All plants were labelled with plastic tags to identify clones and seeded plants. On June 20, nine clonal plants and nine plants from seed were harvested and representative samples of leaf blade (excluding midrib), petiole and stem tissue were collected from each plant. Sample size and amount of water added for grinding of leaf blade, petiole and stem tissue were about 70 g in 350 g of water, 20 g in 100 g of water and 7 g in 35 g of water respectively.

The variability of SCN^- content was compared through the calculation of the standard deviation and per cent variation due to genetics. Percentage of variation due to genetics was determined according to the formula:

$$\text{Variation(\%) due to genetics} = 100\,\frac{S_1^2 - S_2^2}{S_1^2}$$

where S_1^2 is the variance of control and S_2^2 is the variance of clone.

Experiment 4

Effect of reciprocal grafts on SCN^- content of radish (growth chamber)

Grafting

Two radish cultivars (cv. White Icicle and Burpee White) were seeded on July 5, 1974 in the greenhouse. On July 17, seedlings were removed from the growing medium and carefully washed with warm (25° C) tap water. The cleaned seedlings of each cultivar were kept in a beaker of water, prior to grafting. The small leaves and growing points were removed from each rootstock seedling while cotyledons were left until after the grafts took. The apical portion of the stem was split down the middle longitudinally (about 0.4 cm) with a clean razor blade. The scion was prepared by slanted cuts on both side of the stem just below the cotyledons. The scion was inserted into the split on the rootstock stem, and tied with small pieces of masking tape. Grafted seedlings were placed in 1 cm thick styrofoam plates suspended over eight liter containers filled with tap water. On July 24 more seeds of the two cultivars were started to produce plants that could be used as non-grafted controls. The ungrafted plants were started later because grafting delayed the radish development about two and one half weeks.

For this experiment, 56 plants were grafted with a successful take of 52 per cent.

Radishes grafted on July 17, 1974

Scion cultivar	Rootstock cultivar	No. of grafts	No. of successful grafts	Per cent successful
White Icicle	Burpee White	29	19	66
Burpee White	White Icicle	27	10	37

On July 23, grafted plants were placed in a growth chamber with a photoperiod of 12 hours; thermoperiod, 20/17° C, illuminance at plant level, 6,000 lux; relative humidity, 50-60 per cent. On July 31, non-grafted control plants were placed in the growth chamber with the grafted plants. Due to poor plant growth the thermoperiod was adjusted to 21/15° C on July 31.

Plant spacing was 9 cm between plants in the plastic pot, with a maximum population of eight plants per pot. Plants were grown in solution culture using 1/2 X Hoagland's nutrient solution. The nutrient solution was renewed every week except for the first two weeks.

Nutrient	ml/8 l of nutrient solution
KNO_3 (1 Molar)	20
$Ca(NO_3)_2 \cdot 4H_2O$ (1 Molar)	20
KH_2PO_4 (1 Molar)	4
$MgSO_4$ (1 Molar)	8
Micronutrient mix	8
FeEDTA (Fe 5,000 ppm)	8

Both grafted and control plants were analysed after 50 days from seeding. For each plant, foliage, scion root (see Fig. 7) and rootstock root were weighed and samples collected and their SCN^- content determined. From control plants of both cultivars, foliage, stem tissue (hypocotyl region of root) and root tissue were also weighed and SCN^- determined. Sample size and amount of water added for grinding of foliage, stem tissue and root tissue were about 15 g in 30 g of water, 8 g in 16 g of water and 20 g in 40 g of water respectively.

The experiment was repeated again beginning December 21, 1974 and ending on February 28, 1975. Grafts were made on January 21. This time instead of using non-grafted plants as checks, grafts were made for all possible combinations.

All other procedures were the same as for the previous grafting experiment.

Radishes grafted on January 6, 1975

Scion cultivar	Rootstock cultivar	No. of grafts	No. of successful grafts	Per cent successful
White Icicle	Burpee White	31	21	68
Burpee White	White Icicle	30	13	43
White Icicle	White Icicle	28	11	39
Burpee White	Burpee White	28	11	39

RESULTS AND DISCUSSION

Experiment 1

Thiocyanate ion content of cabbage, cauliflower, broccoli, Brussels sprouts and Chinese cabbage cultivars (field)

There are many reports in the literature on the relationship between consumption of cruciferous vegetable or their products and incidence of goitre in humans and animals (Wright and Sinclair, 1958; VanEtten, 1969). Since endemic goitre continues to be of worldwide concern (Stanbury, 1969; Nutrition Canada, 1973), there is need to know the amounts of toxins, such as SCN^-, in vegetable cultivars (Kehr, 1973).

For this reason, several cultivars of the most important cruciferous vegetables were grown and their SCN^- content determined.

Cabbage (*Brassica oleracea* var. *capitata*)

There was a 4.4-fold difference in the 1974-75 average SCN^- content of marketable heads of 16 cabbage cultivars (Table 1) (Appendix 2 and 3). The red type cultivars appeared to have a lower SCN^- content on the average than the green type.

However, the anthocyanin (red pigment) may have interferred with the SCN^- readings so the data must be interpreted with caution. Among the green cultivars, the early maturing (Table 2) Early Greenball and Princess had much lower SCN^- contents than the late maturing Penn State Ballhead, Autumn Marvel and Storage Green. This evidence is indicative of a genetic control of SCN^- content of cabbage.

Generally, the greater the number of days from seeding to maturity for a cultivar, the greater its SCN^- content. This agrees with the findings of Chong and Bible (1974) for radishes and turnips. Michaljlovskij and Langer (1958) found that the head of cabbage contained SCN^- ranging from 0.7 to 10.2 mg per 100 g fresh weight. One can convert their readings to 700 to 1,020 µg/g D.W. given that a cabbage head has about 10 per cent dry weight. Their readings correspond to the highest SCN^- readings for cabbage sampled during 1975.

The average SCN^- content of cabbage was 92 per cent higher in 1975 than it was in 1974 (Table 1). The number of days from seeding to maturity, top weight and head weight were all reduced in 1975 compared to 1974 (Table 2). In Table 3, the 16 cultivars have been divided into two groups, those that received more rainfall in 1975 than 1974 and those that received less. The results indicate that those

cultivars receiving less rainfall in 1975 than 1974 had the largest per cent increase in SCN^- content in 1975 relative to 1974. The daily temperature and daily mean hours of bright sunshine were higher in 1975 than 1974 (Table 3). Perhaps warm, dry conditions and bright days stimulate accumulation of SCN^- precursors in cabbage.

Several investigators have reported increased goitrogenic potency of cabbage during periods of high rainfall (McCarrison et al., 1933; Spence et al., 1933). Bible and Chong (1975) have obtained a positive correlation between rainfall and SCN^- content in radishes grown in loam soil. However, the results of the present cabbage experiments do not agree with those reports.

Josefsson (1970) reported that the environmentally determined variation of glucosinolate content of white mustard (Sinapis alba L.) generally amounted to ± 12 per cent. But the cabbage experiments showed a 92 per cent differrence in SCN^- content between the years 1974 and 1975.

The results of this two year study with cabbage indicates that variation in SCN^- content is under genetic control but markedly influenced by climatic factors.

Table 5 shows the SCN^- contents of cabbage plants analyzed at the transplanting stage during the spring of 1975.

Table 1. Thiocyanate ion content of marketable heads of sixteen cabbage cultivars grown during 1974 and 1975

Cultivar	1974		1975		Average
	SCN^- content of head (µg/g D.W.[1])	C.V.[2]	SCN^- content of head (µg/g D.W.)	C.V.	SCN^- content of head (µg/g D.W.) 1974-1975
Green type					
Early Greenball	184 ± 48	26.1	291 ± 45	15.5	238
Princess	182 ± 44	24.2	284 ± 32	11.3	233
Badger Market	168 ± 8	4.8	451 ± 61	13.5	310
Canada 'Kraut	203 ± 18	8.9	467 ± 78	16.7	335
Stonehead	178 ± 25	14.0	596 ± 143	24.0	387
Copenhagen Market Late	276 ± 51	18.5	415 ± 93	22.4	346
Early Round Dutch	399 ± 59	14.8	660 ± 136	20.6	530
Roundup Hybrid	303 ± 35	11.6	816 ± 88	10.8	560
Jumbo	264 ± 97	36.7	573 ± 155	27.1	419
Penn State Ballhead	425 ± 111	26.1	700 ± 95	13.6	563
Autumn Marvel	425 ± 96	22.6	699 ± 156	22.3	562
Storage Green	447 ± 75	16.8	718 ± 72	10.0	583
Evergreen Ballhead	358 ± 30	8.4	649 ± 62	9.6	504
Ultra Green	306 ± 64	20.9	710 ± 69	9.7	508
Red type					
Red Acre	117 ± 28	23.9	150 ± 49	32.7	134
Storage Red	209 ± 34	16.3	352 ± 56	15.9	281
Average	278 ± 51	18.4	533 ± 87	17.2	406
Per cent change 1974-1975			+92	-6.5	

[1] Dry weight

[2] Coefficient variation

Table 2. Days to maturity, number of leaves, top weight and head weight for sixteen cabbage cultivars grown for SCN analysis during 1974 and 1975.

Cultivar	Days from seeding		No. of outer leaves		Top weight(kg)		Head weight(kg)	
	1974	1975	1974	1975	1974	1975	1974	1975
Green type								
Early Greenball	78	71	12-14	12-15	1.09	0.99	0.71	0.56
Princess	84	77	13-16	12-16	1.96	1.77	1.21	1.09
Badger Market	107	84	10-15	15-22	2.32	1.53	1.74	0.83
Canada 'Kraut	107	87	11-14	11-17	2.90	2.02	2.19	1.30
Stonehead	107	84	13-19	16-19	2.59	1.40	1.81	0.78
Copenhagen Market Late	113	93	13-16	16-22	3.71	2.43	2.48	1.46
Early Round Dutch	113	93	11-17	16-25	3.26	2.06	2.18	1.15
Roundup Hybrid	113	93	22-29	25-31	4.03	2.67	2.37	1.28
Jumbo	125	104	14-18	16-30	3.74	3.45	2.36	1.82
Penn State Ballhead	128	120	19-27	22-45	4.10	3.10	1.75	1.36
Autumn Marvel	145	123	11-17	11-17	2.89	2.34	1.59	1.25
Storage Green	145	123	19-25	24-36	3.41	2.91	1.84	1.31
Evergreen Ballhead	145	123	12-23	25-34	3.84	3.24	2.10	1.44
Ultra Green	148	120	17-34	31-48	4.44	3.49	2.59	1.49
Red type								
Red Acre	148	104	10-16	11-18	3.17	1.91	2.11	1.12
Storage Red	148	125	13-24	17-26	3.19	3.07	1.54	0.99
Average	122	102			3.17	2.40	1.91	1.20
Per cent change 1974-1975		-16%				-24%		-37%

Table 3. Relationship of rainfall accumulation[1] to per cent change from 1974 to 1975 in head SCN^- content of sixteen cabbage cultivars

Cultivar	Rainfall 1975 -rainfall 1974	Per cent increase of SCN^- content from 1974 to 1975
Penn State Ballhead	11.57- 9.19 = +2.38	65
Jumbo	9.99- 8.72 = +1.27	117
Autumn Marvel	11.61-10.14 = +1.47	64
Storage Green	11.61-10.14 = +1.47	61
Evergreen Ballhead	11.61-10.14 = +1.47	81
Storage Red	11.61-10.22 = +1.39	68
Ultra Green	11.57-10.22 = +1.35	132
Princess	5.02- 4.59 = +0.43	56
Early Greenball	4.29- 4.05 = +0.24	58
Average	+1.27	78
Red Acre	9.99-10.22 = -0.23	28
Copenhagen Market Late	7.59- 8.45 = -0.86	50
Early Round Dutch	7.59- 8.45 = -0.86	65
Roundup Hybrid	7.59- 8.45 = -0.86	169
Canada 'Kraut	6.66- 8.21 = -1.55	130
Badger Market	6.14- 8.21 = -2.07	168
Stonehead	6.14- 8.21 = -2.07	235
Average	-1.21	121

[1]Inches of rainfall accumulated between transplanting and harvest.

Table 4. Meteorological conditions in growing period of each cabbage cultivar in 1974 and 1975[1]

Cultivar	Heat unit (degree days)[2] Accumulation		Heat unit (degree days)[2] Daily mean		Rainfall (inch) Accumulation		Rainfall (inch) Daily mean		Bright sunshine hours Accumulation		Bright sunshine hours Daily mean	
	1974	1975	1974	1975	1974	1975	1974	1975	1974	1975	1974	1975
Green type												
Early Greenball	1337	1250	26.7	29.8	4.05	4.29	0.08	0.10	425.7	394.3	8.51	9.39
Princess	1507	1449	26.9	30.2	4.59	5.02	0.08	0.10	471.5	440.0	8.42	9.17
Badger Market	2179	1670	27.6	30.4	8.21	6.14	0.10	0.11	638.6	504.5	8.08	9.17
Canada 'Kraut	2179	1767	27.6	30.5	8.21	6.66	0.10	0.11	638.6	522.6	8.08	9.01
Stonehead	2179	1670	27.6	30.4	8.21	6.14	0.10	0.11	638.6	504.5	8.08	9.17
Copenhagen Market Late	2344	1917	27.6	30.4	8.45	7.59	0.10	0.12	691.9	591.6	8.14	9.39
Early Round Dutch	2344	1917	26.7	30.4	8.45	7.59	0.10	0.12	691.9	591.6	8.14	9.39
Roundup Hybrid	2344	1917	27.6	30.4	8.45	7.59	0.10	0.12	691.9	591.6	8.14	9.39
Jumbo	2698	2353	27.8	31.4	8.72	9.99	0.09	0.13	781.0	675.3	8.05	9.00
Penn State Ballhead	2743	2811	27.4	30.9	9.19	11.57	0.09	0.13	789.7	811.4	7.90	8.92
Autumn Marvel	3110	2893	26.6	30.8	10.14	11.61	0.09	0.12	885.8	833.0	7.57	8.86
Storage Green	3110	2893	26.6	30.8	10.14	11.61	0.09	0.12	885.8	833.0	7.57	8.86
Evergreen Ballhead	3110	2893	26.6	30.8	10.14	11.61	0.09	0.12	885.8	833.0	7.57	8.86
Ultra Green	3120	2811	26.4	30.9	10.22	11.57	0.09	0.13	887.9	811.4	7.52	8.92
Red type												
Red Acre	3120	2353	26.4	31.4	10.22	9.99	0.09	0.13	887.9	675.3	7.52	9.00
Storage Red	3120	2947	26.4	30.7	10.22	11.61	0.09	0.12	887.9	833.0	7.52	8.68
Average	2534	2219	27.1	30.6	8.6	8.8	0.09	0.12	736.3	652.9	7.93	9.07
Per cent change 1974-1975		-12		+13		+2		+33		-11		+14

[1]These data were carried out from transplanting to harvest of each cultivar.

[2]Heat units were calculated with 40° F as base temperature.

Table 5. Thiocyanate ion content of 4 weeks old cabbage transplants in 1975[1].

Cultivar	SCN^- content (µg/g D.W.)[2]
Green type	
Early Greenball	275 ± 26
Princess	247 ± 22
Badger Market	180 ± 16
Canada 'Kraut	257 ± 62
Stonehead	189 ± 17
Copenhagen Market Late	264 ± 80
Early Round Dutch	214 ± 54
Roundup Hybrid	225 ± 97
Jumbo	284 ± 128
Penn State Ballhead	298 ± 22
Autumn Marvel	191 ± 58
Storage Green	347 ± 20
Evergreen Ballhead	163 ± 42
Ultra Green	328 ± 61
Red type	
Red Acre	266 ± 82
Storage Red	422 ± 24
Average	259 ± 51

[1]After 30 days from seeding, each plant (4-6 leaf stage; top weight: 4-5 g) was cut just below 1st true leaf.

[2]Each value is the mean of 3 samples and 2 plants (tops) were used for each sample, ± standard error.

The SCN^- content of plants at the transplanting stage was lower than at the marketable stage except for the red type cultivars. The SCN^- content at the transplanting stage was not related to time of maturity for the cultivars. In fact, there appeared to be no relationship between SCN^- content at the transplanting stage and content at the marketable stage.

Cauliflower (Brassica oleracea L.)

Following the observation of 'cabbage goitre' by Chesney et al. (1928), it was also rapidly established that the other vegetables of genus Brassica, such as cauliflower, turnips and Brussels sprouts, have goitrogenic properties (Marine et al., 1931). Various isothiocyanate compounds were observed by Ettlinger and Thompson (1962) in different cauliflower cultivar seeds as well as in the other genus Brassica seeds. However, variety trials for SCN^- content in cauliflower have not been reported.

The curd SCN^- content of the extremely early cultivar Jet Snow was the lowest (268 µg/g D.W.) while the latest cultivar (Igloo) had the highest SCN^- content (1,435 µg/g D.W.) (Table 6). The other cultivars yielded SCN^- content in curd of about 1,000 µg/g dry weight.

The SCN^- content of leaves ranged from 120 to 300 µg/g dry weight and was generally higher than that of stems.

Table 6. Thiocyanate ion content in different tissues of ten cauliflower cultivars at the marketable stage in 1974

Cultivar	Days from seeding	Leaf stage	Weight(kg)[1]				SCN^- (µg/g D.W.)[2]		
			Top	Curd	Leaf	Stem	Curd	Leaf	Stem
Jet Snow	79	15-22	0.80	0.26	0.50	0.04	268± 91	121± 61	159±14
Stokes Early Abundance	85	21-28	1.23	0.45	0.71	0.07	1025±150	224± 94	159±25
Whitehorse	94	22-29	1.60	0.78	0.71	0.11	948±156	268±144	134±41
Stokes Super Snowball	94	20-28	1.77	0.68	0.98	0.11	954±192	132± 62	153±16
Snowball-Y	99	29-37	1.98	0.74	1.08	0.16	1172±176	217± 67	162±42
Snowball Imperial	100	29-42	1.86	0.72	1.01	0.13	1221±223	196±159	129±19
Snowmound	101	28-38	1.78	0.71	0.93	0.13	1087±247	164± 72	134±17
Clou	101	24-33	1.99	0.69	1.16	0.14	1089±109	292± 95	139±47
Imperial 10-6	101	33-46	2.09	0.84	1.11	0.14	1084±130	185± 85	115± 9
Igloo	116	24-40	2.80	0.85	1.72	0.23	1435±121	301± 75	139±17
Average	97		1.79	0.67	0.99	0.13	1028±160	210± 91	142±25

[1]Each value is the mean of 12 samples.

[2]Each value is the mean of 6 samples. Two plants were used for each sample. Each sample was analysed in triplicate, ± standard error.

The analysis of variance of SCN^- content in curd, showed significant differences ($p \leq 0.01$) between the cultivars (Appendix 4). There were also significant differences both for blocks ($p \leq 0.01$) and cultivars ($p \leq 0.05$) for the stem SCN^- content (Appendix 6). However there were no significant differences of SCN^- content of leaves for blocks or cultivars (Appendix 5).

Broccoli (Brassica oleracea var. botrytis)

As shown in Table 7, SCN^- content of broccoli heads from 6 cultivars averaged 830 µg/g dry weight. The analysis of variance showed no significant differences among the cultivars for head SCN^- content (Appendix 7). The leaf and stem tissue averaged 140 and 130 µg/g dry weight of SCN^- content respectively. There were significant differences ($p \leq 0.01$) in SCN^- content among cultivars both for leaf and stem tissue (Appendix 8 and 9). There was no relationship between plant size or days to maturity and the SCN^- content of the head.

Brussels sprouts (Brassica oleracea var. gemmifera)

In this experiment, it is quite noticeable that Brussels sprouts contained much higher SCN^- content than the other vegetables tested. As shown in Table 8, the

Table 7. Thiocyanate ion content in different tissues of six broccoli cultivars at the marketable stage in 1974

Cultivar	Days from seeding	Leaf stage	Weight (kg)[1] Top	Curd	Leaf	Stem	SCN^- (µg/g D.W.)[2] Curd	Leaf	Stem
Spartan Early	78	16-20	0.57	0.15	0.32	0.10	950±240	154±18	220±37
Italian Sprouting	85	16-23	0.90	0.15	0.57	0.18	868±295	150±37	146±30
Cleopatra	91	25-30	1.66	0.51	0.83	0.32	770±146	77± 9	86±12
Waltham 29	92	21-31	1.10	0.25	0.58	0.27	883±302	80±47	122±32
Green Mountain	101	19-38	1.59	0.35	0.87	0.36	625±134	150±22	98± 7
Early Purple Head	111	24-40	1.67	0.79	0.26	0.63	871±287	234±54	133±49
Average	93		1.25	0.37	0.57	0.31	828±234	141 31	134±28

[1]Each value is the mean of 12 samples.

[2]Each value is the mean of 6 samples obtained from 12 different plants. Each sample was analysed in triplicate, ± standard error.

Table 8. Thiocyanate ion content of marketable sprouts[1] of seven Brussels sprouts cultivars, 1974

Cultivar	Days from seeding	Sprouts	
		Weight(g)[2]	SCN^- (µg/g D.W.)[3]
Early Morn	129	16	1895 ± 201
Long Island Improved	130	13	2596 ± 92
Green Pearl	142	21	1536 ± 260
Topscore	142	13	2198 ± 132
Jade Cross	143	16	2471 ± 136
Indra	143	16	2291 ± 447
Peer Gynt	143	14	2120 ± 436
Average	139	16	2158 ± 243

[1]Samples were taken from basal portion of stalk.

[2]Average weight of 24 to 38 Brussels sprouts.

[3]Each value is the mean of 6 samples and 3 to 5 sprouts were used for each grinding. Each sample was analysed in triplicate, ± standard error.

cultivar Green Pearl was lowest (1,536 µg/g D.W.), but the other six cultivars had SCN^- contents ranging from 1,895 to 2,596 µg/g dry weight. However, analysis of variance showed no significant differences in SCN^- content among cultivars (Appendix 10).

The size of sprouts might be related to SCN^- content; for example the cultivar Long Island Improved had the smallest sprouts and the highest SCN^- content, whereas the cultivar Green Pearl had the largest sprouts and the lowest SCN^- content (Table 8).

A further sampling of sprouts was taken from different positions on the stalk from the cultivar Indra. The very small sprouts collected from the apical region of the stalk had more than twice the content of SCN^- of the largest sprouts at the base of the stem (Table 9).

According to Chong and Bible (1974), SCN^- precursors or possibly their enzyme(s) of hydrolysis are probably synthesized in young photosynthetic tissue. In addition to the above hypothesis, SCN^- precursors or their enzyme(s) of hydrolysis may be transferred to the young photosynthetic tissue from older photosynthetic tissue.

Several investigators (Clement and Wishart, 1956; Wills, 1966; Paxman and Hill, 1974) suggested that goitrogenic subs-

Table 9. Effect of stalk position on the SCN^- content of cv. Indra Brussels sprouts

Position of sprouts on stalk	Average weight of sprouts (g)[1]	Diameter of sprouts (cm)	SCN^- (µg/g D.W.)[2]
Apical	1.8	1.0 - 1.6	2945
Mid apical	3.8	1.6 - 2.2	2414
Mid basal	9.4	2.0 - 3.0	1651
Basal	16.7	3.0 - 4.3	1335

[1]Each value is the mean of 8 to 25 samples from 2 plants.

[2]Each value is the mean of 2 samples and each analysed in triplicate.

Table 10. Effect of boiling on SCN^- content of Brussels sprouts

Cultivar	Treatment	Average weight of sprouts[1](g)	SCN^- (µg/g D.W.)	Per cent decrease in SCN^- content relative to check
	Check	20	1966	0
Green Pearl	Boiled[2]	21	185	91
	Mixed[3]		1713	13
	Check	12	1910	0
Indra	Boiled[2]	13	209	89
	Mixed[3]		1654	13

[1]Samples of Brussels sprouts were taken mostly from the basal portion of the stalk.

[2]Samples were incubated in boiling water both for 10 minutes.

[3]Mixed sample was obtained by mixing 3 parts of boiled and 1 part of check sample respectively.

tances of cruciferous plants can be reduced by boiling the tissues before homogenizing. Since Brussels sprouts are usually boiled before being eaten, I tested the affect of boiling on the SCN^- content of Green Pearl and Indra sprouts by placing the sprouts in boiling water for 10 minutes prior to homogenizing. Boiling reduced the SCN^- yield of the sprouts by 90 per cent compared to the unboiled checks (Table 10). A mixed sample comprised of 3 parts boiled tissue and 1 part unboiled tissue yielded only 13 per cent less SCN^- content than the unboiled check. Presumably, when the Brussels sprouts were boiled before homogenization, myrosinase (enzyme) was destroyed. Apparently the precursor glucosinolate which gives rise to SCN^- content is not destroyed by boiling, since the mixed sample (which was 75 per cent boiled tissue) yielded almost as much SCN^- content as the unboiled checks.

Chinese cabbage (Brassica campestris [Pekinensis group])

Chinese cabbage can be eaten at any stage from transplanting to maturity. For this reason, both transplanting and mature stages were analysed for SCN^-. As shown in Table 11, SCN^- content of the transplanting stage of the three cultivars was fairly low compared with that of the mature stage, which had higher SCN^- levels than comparable western type cabbage (Brassica oleracea var. capitata).

Table 11. Thiocyanate ion content of three Chinese cabbage cultivars at two stages of development, 1974

Cultivar	Days from seeding	Leaf stage	Top weight (g/plant)	SCN^- content of top(μg/g D.W.)
		Transplanting stage[1]		
Chihli	44	6 - 9	15.0	159
Hybrid F-1 No.11	44	4 - 7	5.8	163
Michihli	31	7 - 8	21.4	389
Average	40		14.1	237
		Mature stage[2]		
Chihli	98		1060	894
Hybrid F-1 No.11	98		1755	1105
Michihli	98		2145	1124
Average	98		1653	1041

[1]Each value is the mean of 2 samples and 2 plants were used for each sample.

[2]Each value is the mean of 2 samples and each sample analysed in triplicate.

Experiment 2

Variation of SCN^- content during the life cycle of cauliflower and broccoli (field)

Although there are a few reports on the variation of SCN^- content in plant tissues during their life cycle, none are available for cauliflower and broccoli.

For this experiment plant tops included leaf and stem tissues and excluded curd, head and flower stalk tissues (Fig. 2, 3, 4 and 5). The mature stage of the cauliflower and broccoli was considered to be same as the marketable stage.

Cauliflower (cv. Jet Snow and Igloo)

The early cultivar Jet Snow matured 28 days earlier than Igloo. Top weight of Jet Snow increased up to the mature stage (820 g) and then decreased gradually with leaf stage until seeding (seed producing) stage (Fig. 2); whereas the top weight of Igloo increased continuously through to the final sampling (Fig. 3). However, this cultivar did not reach flowering because the curd rotted without flowering, thus there was no SCN^- analysis after the over-mature stage.

Curd. In the curd of both cauliflower cultivars, a rapid decreasing trend in SCN^- content was observed during the development of curd.

In Jet Snow, the SCN^- content of curd decreased rapidly from 960 µg/g D.W. when curds were small and pre-mature to 180 µg/g D.W. at full size or maturity.

In Igloo there was also a rapid decrease in SCN^- content from a maximum of 2,360 µg/g D.W. for pre-mature curds to 1,380 µg/g D.W. for over-mature curds. At the mature stage or marketable curd stage for Igloo, the SCN^- content was 1,970 µg/g D.W.

Foliage. In the foliage of both cauliflower cultivars, there was a decreasing trend in the SCN^- content during early vegetative growth prior to transplanting. Both Jet Snow and Igloo foliage showed only moderate fluctuation of SCN^- content after transplanting.

The SCN^- content of Jet Snow foliage decreased from a maximum of 690 µg/g D.W. for seedlings at the cotyledon stage to a minimum of 70 µg/g D.W. at senescence or the seeding stage, while that of Igloo decreased from a maximum of 1,570 µg/g D.W. for seedlings to 260 µg/g D.W. at the over-mature stage.

Stem. The stem tissue of both cauliflower cultivars showed a decreasing trend in SCN^- content during development.

In the stem of Jet Snow, the SCN^- content decreased rapidly from 910 µg/g D.W. when plants were at the cotyledon stage to 130 µg/g D.W. at maturity. Thereafter stem SCN^- content was relatively constant. The decreasing trend of stem SCN^- content was similar to that of leaf SCN^-, but the SCN^- content of stems was slightly higher than for leaves.

In Igloo, there was a similar rapid decrease in SCN^- content of stems from 1,460 µg/g D.W. at the cotyledon stage to 130 µg/g D.W. at the over-mature stage. For early growth of Igloo, the stems contained more SCN^- than the leaves while during the later stages of development the situation was reversed.

Seed. In the seed of both cauliflower cultivars, the SCN^- content was lower than the corresponding cotyledon stage. In the seed of Jet Snow the SCN^- content was 580 µg/g D.W. and in seeds of Igloo the SCN^- content was 860 µg/g D.W.

According to Neil (1971), the SCN^- content of radish root decreased as the plant matures and this agrees with my findings. Because, in this experiment, SCN^- content of young tissues was much higher than that of corresponding aging tissues. The curd tissues of the late cultivar Igloo showed a much higher SCN^- content than the corresponding

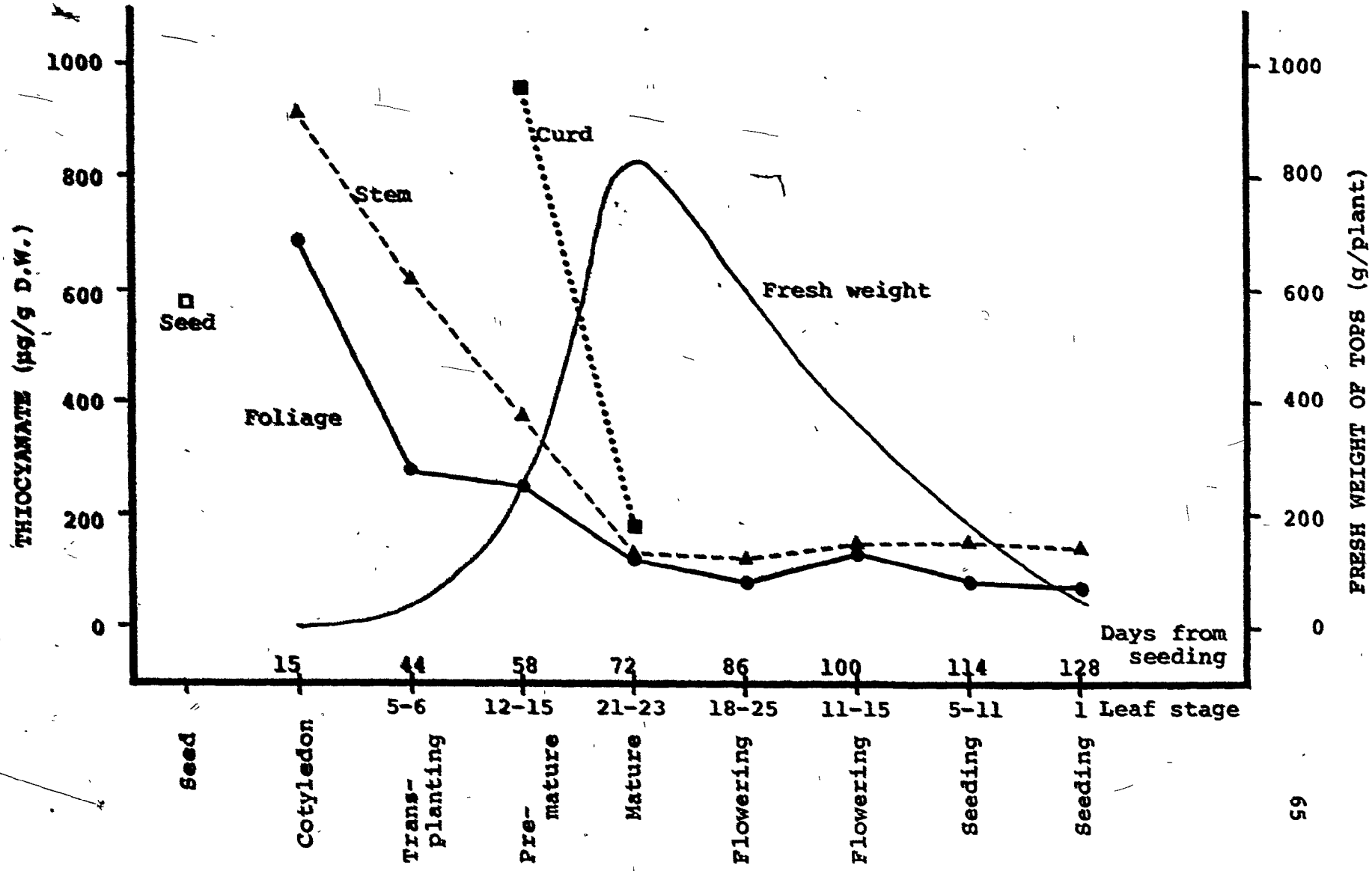


Figure 2. Variation of SCN^- content during the life cycle of cauliflower (cv. Jet Snow)

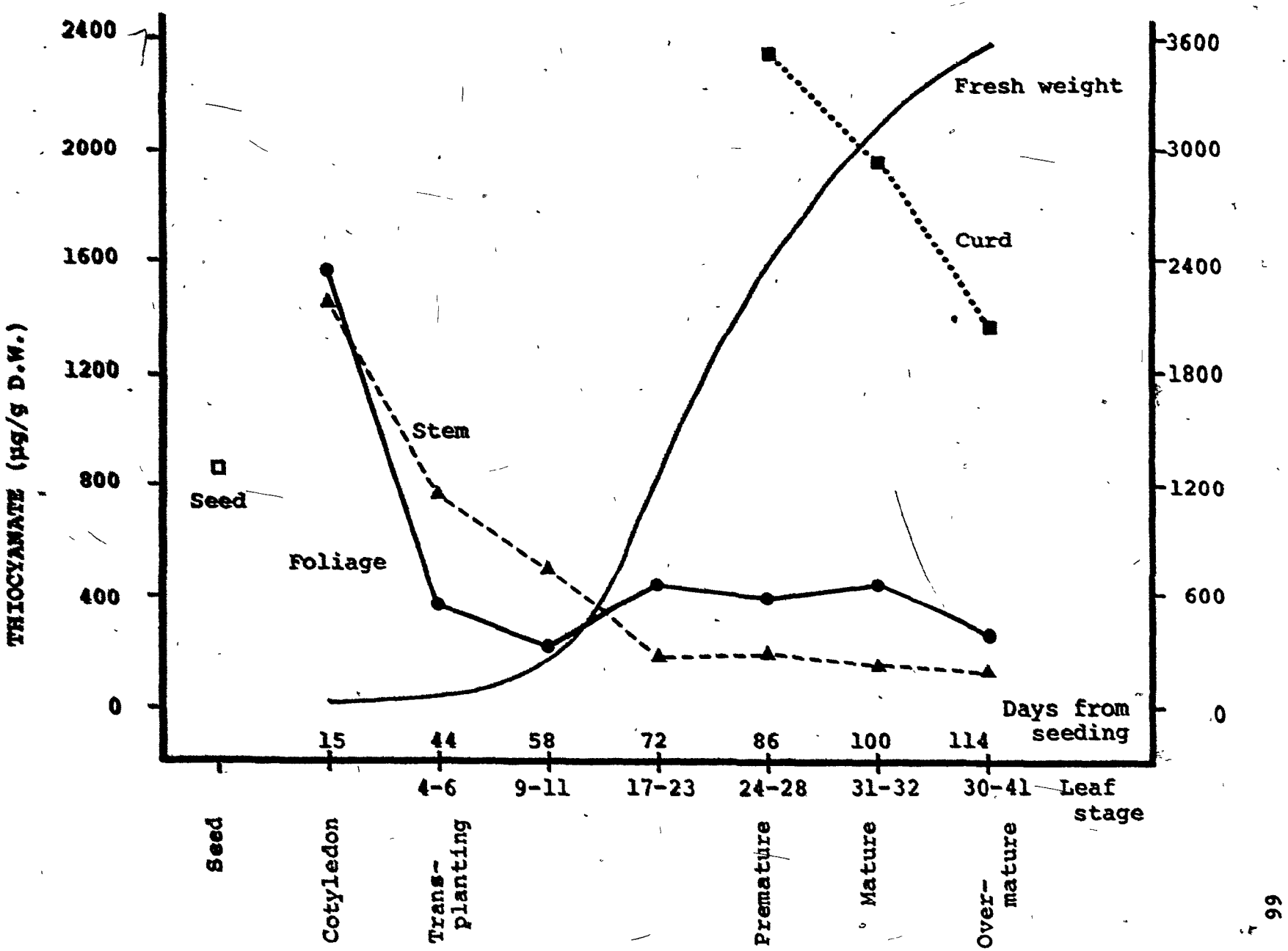


Figure 3. Variation of SCN^- content during the life cycle of cauliflower (cv. Igloo)

tissue of the early cultivar Jet Snow, while differences between the cultivars for stem and leaf SCN^- contents were much less.

Broccoli (cv. Spartan Early and Waltham 29)

The results for broccoli were very similar to those for cauliflower. The early cultivar Spartan Early matured 28 days earlier than the late cultivar Waltham 29. The top weight and leaf number of Spartan Early increased until the over-mature stage, then both decreased until the final sampling. For Waltham 29, top weight and leaf number showed about the same pattern as described for Spartan Early.

Head. For both broccoli cultivars, the head SCN^- content decreased with development. Only two samplings of head tissues were possible because of the rapid development of the heads. In Spartan Early, the SCN^- content decreased rapidly from 1,710 µg/g D.W. at maturity to 370 µg/g D.W. at over-maturity. However, in Waltham 29, the SCN^- content only decreased from 1,490 µg/g D.W. at the pre-mature stage to 1,000 µg/g D.W. at over-maturity.

Foliage. In Spartan Early, foliage SCN^- content decreased from 900 µg/g D.W. at the cotyledon stage to 180 µg/g D.W. at flowering. In Waltham 29 foliage SCN^- content decreased

from 610 µg/g D.W. at the cotyledon stage to 60 µg/g D.W. at flowering.

Stem. For both broccoli cultivars, stem SCN^- content was generally higher than the SCN^- content of leaf and it decreased with age.

In Spartan Early, stem SCN^- content decreased from 1,970 µg/g D.W. at the cotyledon stage to 180 µg/g D.W. at flowering and then increased slightly to 250 µg/g D.W. at the seeding stage. In Waltham 29, stem SCN^- content decreased from 1,250 µg/g D.W. at the cotyledon stage to 90 µg/g D.W. at the seeding stage.

Seed. For both cultivars, the seed SCN^- content was lower than leaves at the cotyledon stage. The seed of Spartan Early yielded 490 µg/g D.W. of SCN^-, and those of Waltham 29 440 µg/g D.W.

The SCN^- content of broccoli showed a trend of decreasing with age of plant tissue as shown for cauliflower. Trzebney (1962) reported that the content of isothiocyanate decreased as plants age. His results are based on analysis of root, stem and leaf tissue of two cultivars of kale and one cultivar of Indian mustard. However, no explanation has been put forward for this decrease in isothiocyanate and SCN^- content in aging plant tissue.

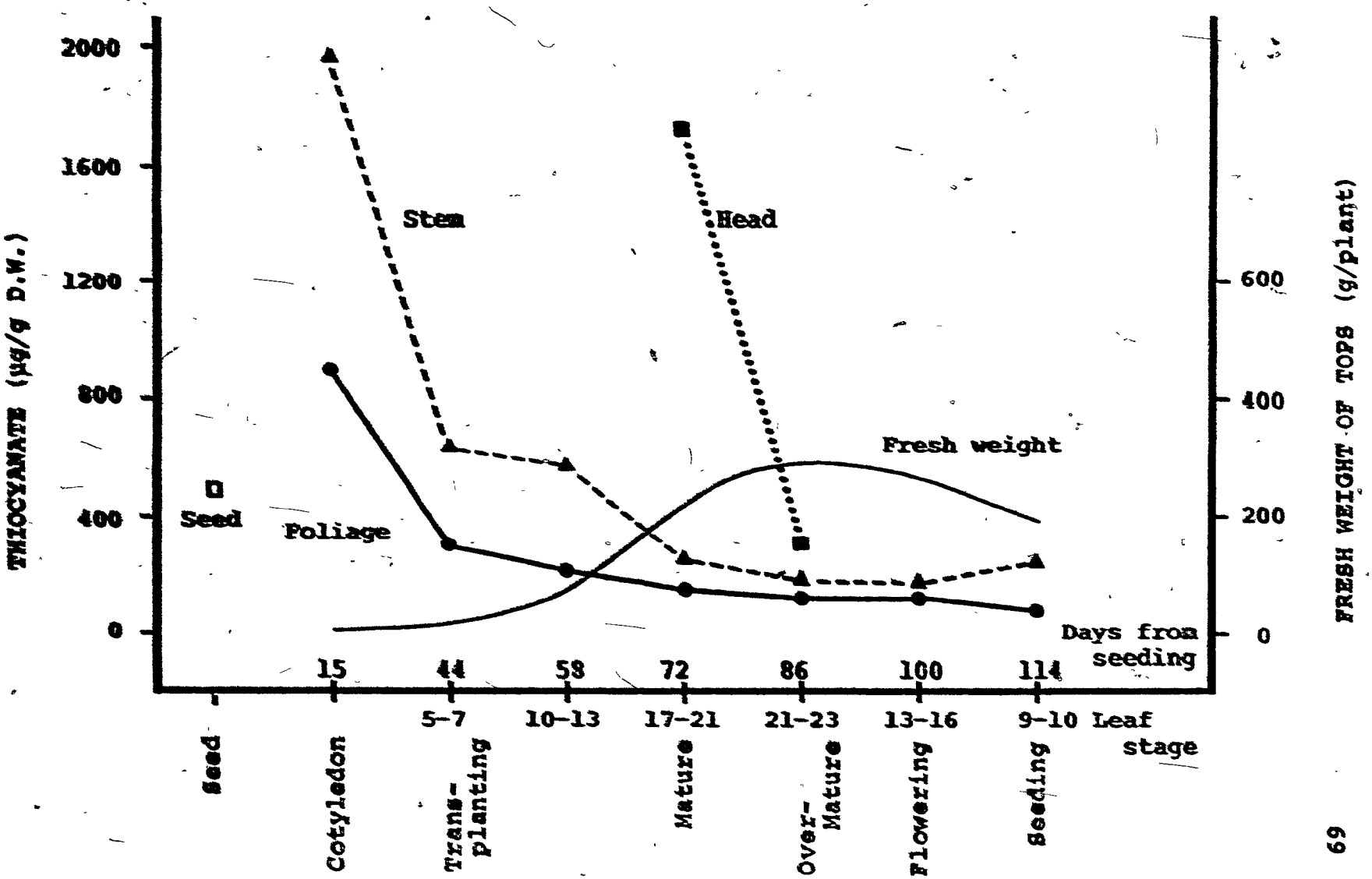


Figure 4. Variation of SCN^- content during the life cycle of broccoli (cv. Spartan Early)

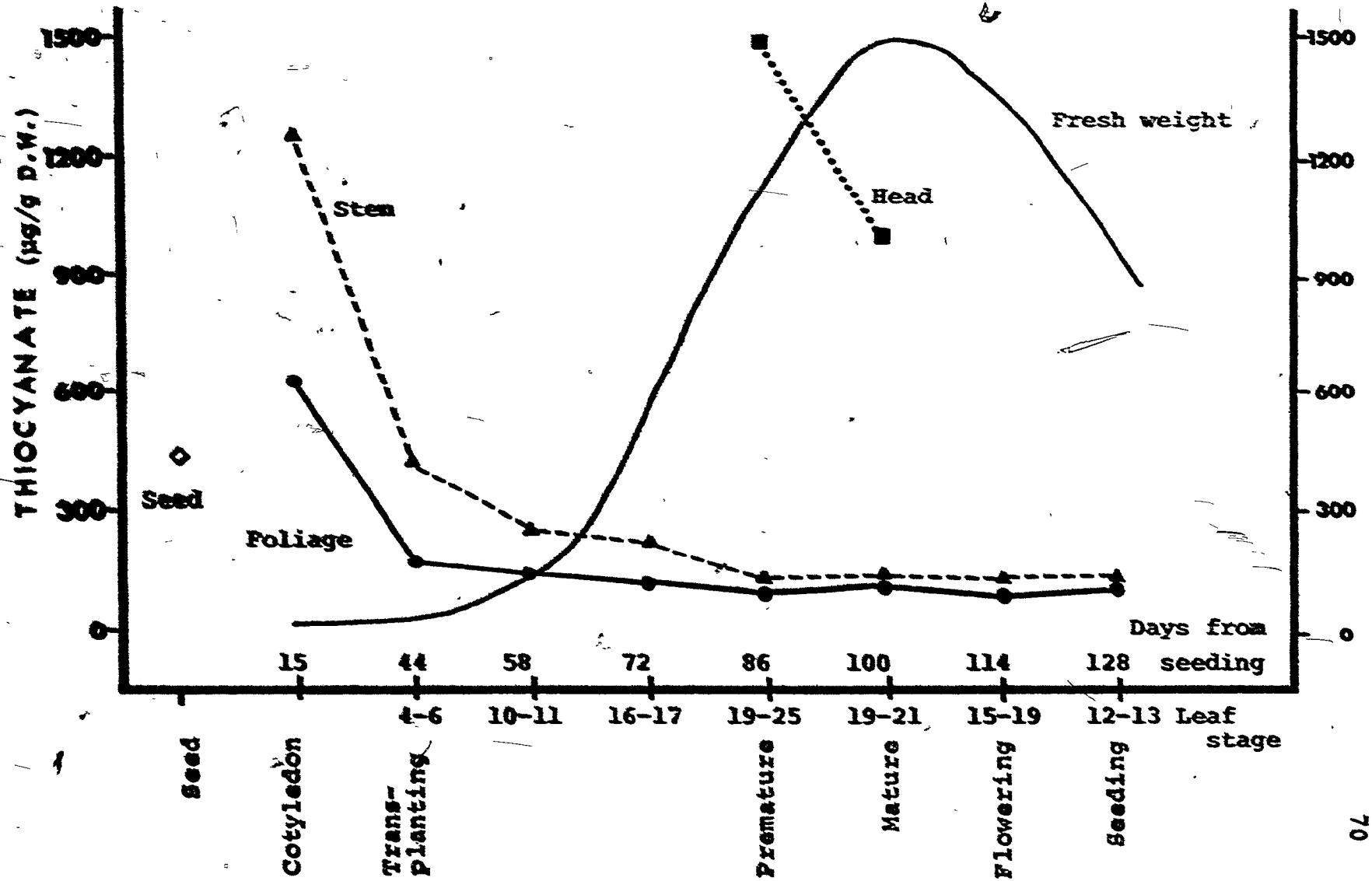


Figure 5. Variation of SCN^- content during the life cycle of broccoli (cv. Waltham 29)

Experiment 3

Relative variability of SCN^- content of kale in clonal plants versus plants grown from seed (cv. Dwarf Green Curled) (field)

The comparison of SCN^- contents of clonal plants versus plants from seed indicated that within the cultivar Dwarf Green Curled, genotypic differences accounted for 50 per cent and 67 per cent of the variation in SCN^- content of leaf blade and petiole tissues respectively (Table 12). The consistently higher stem content of SCN^- in the clone compared to plants from seeds of the same cultivar is surprising. Even more surprising is the larger variance for stem SCN^- content for clonal plants as compared to that of plants from seed. The reasons for these curious results are unknown.

The coefficients of variation for SCN^- contents of the various tissues for seeded plants were higher (about 30 per cent) than the corresponding values for clonal plants. As expected the clonal plants, identical genotypes, showed less variation for SCN^- content than did plants propagated from seed. Presumably even within a cultivar of

Table 12. Comparison of SCN^- content in different morphological parts of clonal plants versus plants from seed for kale (Dwarf Green Curled)

	Mean[1] SCN^- (μg/g D.W.)		Coefficient variation (%)		Variance (S^2)		Per cent variation due to genetics[3]
	clone	check[2]	clone	check	clone	check	
Leaf blade	169± 40	180± 69	24	38	2409.05	5424.39	56
Petiole	385± 74	409±130	19	32	5173.01	17350.80	70
Stem	496±168	250±105	34	42	22169.44	9944.96	-123
Average	350± 94	280±101	26	37	9917.17	10906.72	
Per cent change		-25 +7		+30		+10	

[1]Each datum is taken from the average of 9 plants.

[2]Checks refer to Dwarf Green Curled kale plants grown from seeds.

[3]The variance due to genetics is calculated by the formula:

$$\frac{(\text{check}-S^2)-(\text{clone}-S^2)}{(\text{check}-S^2)} \times 100$$

kale there is considerable genotypic variation for SCN^- content. However, the variation for SCN^- content even among clonal plants indicates strong environmental effects and possible variation arising from sampling and analytical techniques.

It is of interest that the coefficient of variation for top weight of plants is lower in clonal plants as compared to plants from seed (Appendix 11).

Various investigators (Josefsson, 1970; Josefsson and Appelqvist, 1968) have reported the influence of environmental factors on the variability of glucosinolate content in cruciferous seeds. No reports have been found in the literature on the relative difference in variation of SCN^- content between genotypically identical (clonal) plants and those with some genotypic variability (i.e. plants of one cultivar grown from seed).

Experiment 4

Effect of reciprocal grafts on SCN^- content of radish (growth chamber)

Although it has been suggested that SCN^- precursors or possibly their enzyme(s) of hydrolysis are probably synthesized in young photosynthetic tissue (Chong and Bible, 1974), there are no reports on their translocation within

the plant. This possibility was tested with reciprocal graft combinations of radish seedlings made between the cultivars Burpee White and White Icicle.

In this experiment, non-grafted plants were used as check plants for both cultivars. The edible portion of radish (hypocotyl-root tissue) consists of stem and root tissue. In this experiment the stem tissue was labelled (scion root) and the root tissue (stock root). It was easy to separate scion root from stock root tissues in grafted plants because the portion above the graft was scion root and that below stock root tissue (Fig. 7). Scion root tissue shows greening on exposure to light due to chlorophyll synthesis while stock tissues are not capable of chlorophyll synthesis. Thus the two types of tissues could be distinguished and separated for SCW determinations.

As shown in Table 13, the foliage weight of grafted White Icicle and Burpee White plants was reduced 25 per cent and 28 per cent respectively relative to the non-grafted check plants. Total root weight of Burpee White roots with White Icicle scions was reduced 26 per cent compared to roots of Burpee White check plants. But the total root weight of White Icicle roots with Burpee White scion was increased by 58 per cent compared to total root weight of White Icicle checks. This indicates that Burpee White foliage may sti-

Table 13. Effect of reciprocal grafts, with non-grafted plants as checks, on plant growth and SCN^- content of radish tissues

Scion cultivar	Rootstock cultivar	Fresh weight (g/plant)				SCN^- content (µg/g D.W.)			
		Foliage	Scion root	Stock root	Total root	Foliage	Scion root	Stock root	Total root
Grafted[1]									
White Icicle	Burpee White	21.4	12.9	29.8	42.7	144	138	235	206
Burpee White	White Icicle	22.4	9.5	67.2	76.7	271	103	409	371
Non-grafted checks[2]									
White Icicle		28.6	10.7	38.0	48.7	197	90	353	296
Burpee White		31.0	13.5	44.4	57.9	183	268	358	337

[1]Each value is the mean of 9 to 16 samples, each sample was analysed in triplicate.

[2]Each value is the mean of 3 to 4 samples, each sample was analysed in triplicate.

mulate the growth of White Icicle root.

The SCN^- content of White Icicle foliage grown on Burpee White rootstocks was decreased by 27 per cent compared to the foliage SCN^- content of White Icicle checks whereas the SCN^- content of Burpee White foliage grown on White Icicle rootstocks was increased by 48 per cent compared to the foliage SCN^- content of Burpee White checks.

White Icicle scions decreased the SCN^- content of Burpee White stock roots compared to Burpee White checks, whereas Burpee White scions increased the SCN^- content of White Icicle stock roots compared to White Icicle checks. This seems to indicate that Burpee White scions are better suppliers of SCN^- precursors to rootstocks than are White Icicle scions.

Burpee White rootstocks were associated with high SCN^- content of scion root tissue, while White Icicle rootstocks were associated with low SCN^- content of scion root tissue.

The same experiment was repeated with the exception that the check plants were also grafted. Generally the results for SCN^- contents were similar to the first trial, however the pattern of foliage and total root weights was different (Table 14).

These results indicate an interacting affect of foliage and roots on the SCN^- content of the tissues tested. However,

Table 14. Effect of reciprocal grafts, with grafted plants as checks, on plant growth and SCN^- content of radish tissues

Scion cultivar	Rootstock cultivar	Fresh weight (g/plant)[1]				SCN^- content (µg/g D.W.)[2]			
		Foliage	Scion root	Stock root	Total root	Foliage	Scion root	Stock root	Total root
White Icicle	White Icicle	15.8	7.7	27.1	34.8	229	79	441	361
	Burpee White	19.5	12.3	19.4	31.7	174	113	333	249
Burpee White	White Icicle	22.2	2.0	36.8	38.8	257	133	455	440
	Burpee White	25.0	9.6	19.7	29.3	163	230	374	327

[1,2]Each value is the mean of 6 to 15 samples, each sample analysed in triplicate.

the foliage may be the dominant partner in determining the over all amounts of SCN^- in radish plants.

Some difficulties arise in interpreting the results of these experiments because the SCN^- contents of the two cultivars, Burpee White and White Icicle were very similar. These cultivars were chosen because Burpee White was thought to have a much higher SCN^- content than White Icicle as reported by Chong and Bible (1974); however, this was not the case in these experiments.

Explanation of Figures

Figure 6. Reciprocal grafts of radishes (B.W. x B.W., W.I. x B.W., B.W. x W.I., W.I. x W.I.) at marketable stage. (Burpee White: B.W.; White Icicle: W.I.)

Figure 7. Scion roots are always formed from the stem tissue in the scion. A(foliage), B(scion root: stem tissue), C(stock root: root tissue), D(cotyledon trace of B.W. stock root).

Figure 8. The middle region is callus (b) formed between the scion root of Burpee White (a) and the stock root of White Icicle (c). Several xylems (d) appeared in the top, middle and in the bottom.

Figure 9. White Icicle (stock root) cells (B) showed much larger than callus tissue cells (A). Burpee White (scion) cells were not shown here.

Figure 6. (see Plate 1)
Reciprocal grafts between Burpee White and White Icicle radish cultivars

Figure 7. (see Plate 2)
Morphological aspects of reciprocal grafts of Burpee White and White Icicle radish cultivars

Plate 1

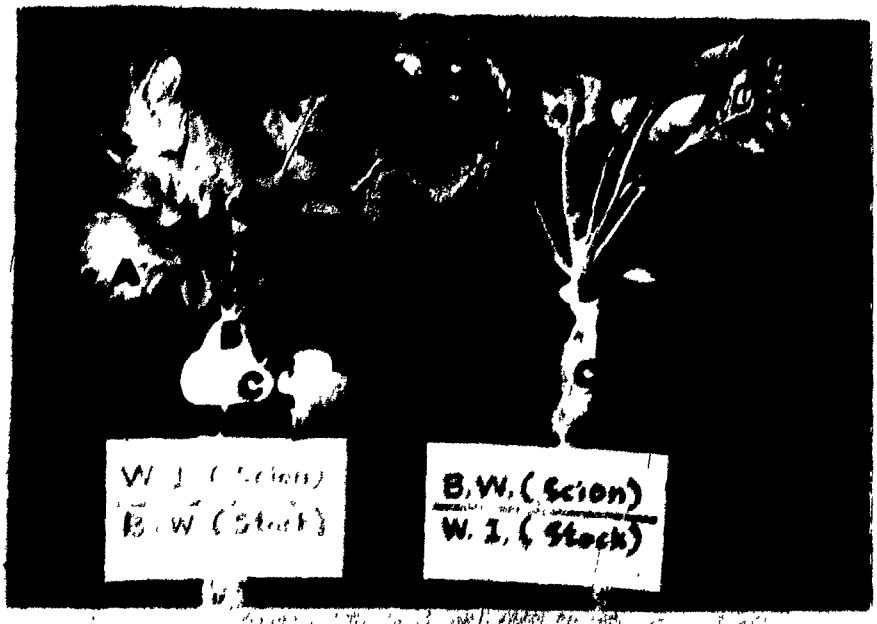


Plate 2

Figure 8. (see Plate 3)
Light micrograph (Ax100: REICHERT) of cross-section of grafted region (scion: Burpee White, rootstock: White Icicle)

Figure 9. (see Plate 4)
Light micrograph (Ax400: REICHERT) of cross-section of grafted region (scion: Burpee White, rootstock: White Icicle)

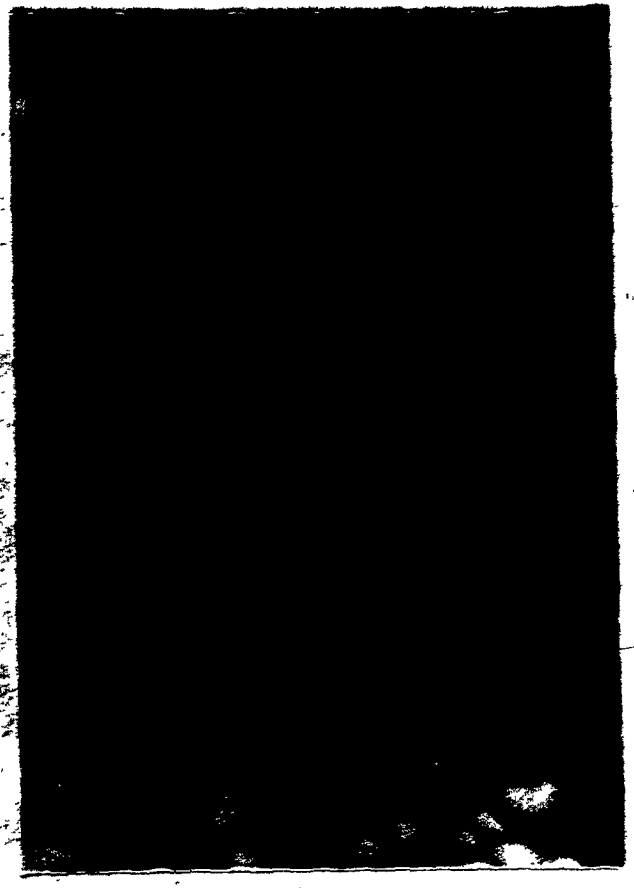


Plate 3

Plate 4

GENERAL DISCUSSION

It has long been considered possible that factors other than iodine deficiency might play a part in the etiology of endemic goitre, but not until the discovery of cabbage goitre (Chesney et al. 1928) was indisputable proof obtained of the existence of natural goitrogens. Since then, various goitrogens such as SCN^-, isothiocyanate and related compounds have been isolated mostly from common vegetables, fodder crops and weeds belonging to the Cruciferae family.

Even though there is a need for more knowledge on the content in foods of natural goitrogens such as SCN^- which pose a potential danger to human and animal health, only a few reports have appeared on SCN^- content of vegetables. The results of experiment 1 showed that SCN^- content of the edible parts of cruciferous vegetables varied among crops and within crops among cultivars (except Brussels sprouts and broccoli). The variation of SCN^- content of cabbage between 2 years was much higher than that reported previously by Josefsson (1970). Low rainfall, high temperature and bright sunlight may have caused the high yield of SCN^- for cabbage grown in 1975. However, little is known about the environmental factors that affect the biosynthesis of SCN^- precursors.

Late cabbage cultivars contained generally higher amounts of SCN^- than early cultivars. The results agree with previous work on radish and turnip (Chong and Bible, 1974). The precursors of SCN^- might have more time to accumulate given the longer period of growth for late cultivars, but no explanation has been reported.

Brussels sprouts contained much higher amounts of SCN^- than cabbage, cauliflower, broccoli and Chinese cabbage, but SCN^- in Brussels sprouts can be reduced by 90 per cent by boiling the tissue prior to homogenizing. Presumably, the enzymic system (myrosinase) which can hydrolysis glucosinolate was destroyed by this heat treatment.

Few reports have appeared on variation in SCN^- content of plant tissues during plant development. Thiocyanate ion content of both cauliflower and broccoli decreased as plants matured. The amount of SCN^- in curds and heads of cauliflower and broccoli was very high at the premature stage at which time the amounts of SCN^- in leaves and stems had already decreased rapidly. This may indicate that SCN^- precursors translocate to curds or heads from leaves and stem. The decreasing trend of SCN^- content in curds and heads from both late and early cultivars corresponded to results for other aging tissues. But the amount of SCN^- in curds and heads of late cultivars did not decrease as rapidly as

for early cultivars. The leaf condition may explain this, since leaves of late cultivars did not mature as rapidly as those of the early cultivars, they may have continued to supply SCN^- precursors to the developing curds and heads. Unfortunately the edible portions of cauliflower and broccoli have the highests SCN^- contents (i.e. curds and heads contain much more SCN^- than stems and leaves).

Previously Neil (1971) suggested that the variation among plants of a cultivar could be eliminated by using a clone. But the variability of SCN^- content in kale clonal plants was only 30 per cent lower than plants propagated from seeds. The individual variability of clone plants for SCN^- content was higher than expected. This suggests that environmental factors may mask genetic influence on SCN^- yield.

Studies on radish reciprocal grafts indicated that foliage (scion) was more important to SCN^- yield of root than rootstock was to SCN^- yield of foliage. Scion root SCN^- was affected mostly by the rootstock. Perhaps SCN^- precursors or their enzyme(s) of hydrolysis are synthesized independently in foliage and root tissues, but foliage somehow strongly influences the over all plant contents of SCN^-.

SUMMARY

Thiocyanate ion content of various cruciferous vegetables as influenced by stage of development, genotype and reciprocal grafting was studied.

The cultivar trials for SCN^- content of cabbage, cauliflower, broccoli, Brussels sprouts and Chinese cabbage showed that Brussels sprouts contained 2 times more SCN^- µg/g D.W. than cauliflower, broccoli and Chinese cabbage, and 4 to 7 times more than cabbage. A 2 year experiment showed that average amount of SCN^- in 16 cabbage cultivars varied by 92 per cent between the two seasons. The late maturing cabbage cultivars showed a tendency to yield higher SCN^- than early cultivars. This relationship was observed in some of the other crops tested. The amounts of SCN^- content in edible parts of vegetables was significantly different among cultivars in all crops except for broccoli and Brussels sprouts. The curds and heads of cauliflower and broccoli contained at least 5 times more SCN^- than their leaves and stems.

The SCN^- content of cauliflower and broccoli tissues decreased during growth of tissues. Generally, SCN^- content of leaf and stem was decreased rapidly until maturity thereafter remaining at a constant low level. It was quite

noticeable that SCN^- content of curds and heads in late cultivars of cauliflower and broccoli did not decrease to the same extent as it did in early cultivars.

From a study of SCN^- variation in clonal material versus plants propagated from seed, it was shown that difference in genotype within a cultivar can increase the variability of SCN^- readings. However, the SCN^- variability exhibited by clonal plants was larger than expected.

A study to determine the effect of reciprocal grafts on radish SCN^- content indicated that foliage influenced root SCN^- content. The results suggest that the SCN^- may translocate from foliage to root in radish, but this has not been proven.

SUGGESTION FOR FURTHER RESEARCH

1. Remarkable variation in SCN^- content of cabbage between 2 years was observed. Certain environmental factors such as rainfall, high temperature etc. may influence SCN^- content of cabbage. Further studies should be conducted with the same cabbage cultivars over a period of several years. Meterological conditions could be correlated with variation in SCN^- yield of the cabbage for the different years.

2. Studies on variation of SCN^- during development of cauliflower and broccoli could be improved by measuring SCN^- both in per gram dry weight and per total dry weight of plants. During early growth, high amounts of SCN^- were found in small curds and heads of cauliflower and broccoli while at the same time the amount of SCN^- in leaves and stems decreased rapidly. If leaves are the sites of glucosinolate synthesis, this can be tested by pinching off small curds and heads then follow the levels of SCN^- in the foliage.

3. Less variation of kale SCN^- content was observed in clones than in plants from seed but environmental factors in the field probably caused much variability. Perhaps clones should be tested under growth chamber conditions.

4. Foliage has been shown to influence SCN^- content in root tissue based on results of reciprocal grafts of radishes. Ettlinger and Tompson (1962) showed that turnip and radish have different isothiocyanates. Perhaps studies of reciprocal grafts between turnip and radish would yield more information on the relative role of foliage and roots in supplying isothiocyanates or their precursors.

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Appendix 1. Meteorological Conditions from April to September in 1974 and 1975[1]

	Mean temperature (°F)		Accumulated rainfall (inch)		Accumulated bright sunshine hrs		Average relative humidity (%)	
	1974	1975	1974	1975	1974	1975	1974	1975
April	42	38	3.09	1.45	155	132	69	65
May	51	64	3.61	2.66	161	157	71	69
June	66	68	3.16	2.57	248	279	71	68
July	70	73	2.96	4.36	250	299	70	68
August	69	71	2.49	4.02	250	241	71	68
September	59	56	2.08	6.82	148	-[2]	71	68

[1]The data were obtained from Seed Farm, Macdonald College.

[2]Data were not available.

Appendix 2. Analysis of variance of SCN^{-} content in cabbage cultivars in 1974

Source of variation	d.f.	S.S.	M.S.	F cal	F tab 0.01	F tab 0.05
Total	47	568,974.63				
Block	2	2,401.31	1,200.66	0.68	5.39	3.32
Cultivar	15	513,958.84	34,263.92	19.54 **	2.70	2.01
Exp. Error	30	52,614.48	1,753.82			

** significant at 0.01 level

Appendix 3. Analysis of variance of SCN^- content in cabbage cultivars in 1975

Source of variation	d.f.	S.S.	M.S.	F cal	F tab 0.05	F tab 0.01
Total	47	1,841,865.5931				
Block	2	12,424.7400	6,212.3700	1.2916 n.s.	3.32	5.39
Cultivar	15	1,685,142.7398	12,342.8494	2.5662 *	2.01	2.70
Exp. Error	30	144,298.1133	4,809.9372			

* significant at 0.05 level

Appendix 4. Analysis of variance of SCN^- content of curd in cauliflower cultivars

Source of variance	d.f.	S.S.	M.S.	F cal	F tab 0.05	F tab 0.01
Total	29	2,836,436.31				
Block	2	8,072.49	4,036.25	0.2035 n.s.	3.55	6.01
Cultivar	9	2,471,271.26	274,585.70	13,8411 **	2.46	3.60
Exp. Error	18	357,092.56	19,838.48			

** significant at 0.01 level

Appendix 5. Analysis of variance of SCN content of leaves in cauliflower cultivars

Source of variance	d.f.	S.S.	M.S.	F cal	F tab 0.05	F tab 0.01
Total	29	222,045.7696				
Block	2	3,949.3486	1,974.6743	0.3229 n.s.	3.55	6.01
Cultivar	9	107,992.8096	11,999.2011	1.9617 n.s.	2.46	3.60
Exp. Error	18	110,103.6114	6,116.8673			

Appendix 6. Analysis of variance of SCN^- content of stem in cauliflower cultivars

Source of variance	d.f.	S.S.	M.S.	F cal	F tab 0.05	F tab 0.01
Total	29	16,292.5670				
Block	2	4,578.5660	2,289.2830	7.8569 **	3.55	6.01
Cultivar	9	6,469.2804	718.8090	2.4670 *	2.46	3.60
Exp. Error	18	5,244.7206	291.3734			

** significant at 0.01 level

* significant at 0.05 level

Appendix 7. Analysis of variance of SCN^- content of head in broccoli cultivars.

Source of variation	d.f.	S.S.	M.S.	F cal	F tab 0.05	F tab 0.01
Total	17	713,832.6050				
Block	2	60,352.9434	30,176.4700	0.6623 n.s.	4.10	7.56
Cultivar	5	197,840.6317	39,568.1264	0.8684 n.s.	3.33	5.64
Exp. Error	10	455,639.0299	45,563.9030			

Appendix 8. Analysis of variance of SCN^- content of leaves in broccoli cultivars

Source of variation	d.f.	S.S.	M.S.	F cal		F tab 0.05	F tab 0.01
Total	17	57,776.3000					
Block	2	746.1434	373.0717	0.5847	n.s.	4.10	7.56
Cultivar	5	50,649.2200	10,129.8440	15.8752	**	3.33	5.64
Exp. Error	10	6,380.9366	638.0937				

** significant at 0.01 level

Appendix 9. Analysis of variance of SCN^- content of stem in broccoli cultivars

Source of variation	d.f.	S.S.	M.S.	F cal		F tab 0.05	F tab 0.01
Total	17	43,334.1000					
Block	2	4,735.5234	2,367.7617	4.9470	*	4.10	7.56
Cultivar	5	33,812.2600	6,762.4520	14.1288	**	3.33	5.64
Exp. Error	10	4,786.3166	478.6317				

* significant at 0.05 level

** significant at 0.01 level

Appendix 10. Analysis of variance of SCN^- content of Brussels sprouts

Source of variation	d.f.	S.S.	M.S.	F cal	F tab 0.05	F tab 0.01
Total	20	4,089,435.6900				
Block	2	140,743.7986	70,371.8993	0.51 n.s.	3.89	6.93
Cultivar	6	2,297,544.3300	382,924.0550	2.78 n.s.	3.00	4.82
Exp. Error	12	1,651,147.5614	137,595.6302			

Appendix 11. Comparison of top weight variability in clonal versus plants from seed of Dwarf Green Curled kale

	Clone	Check
Top weight (g/plant)[1]	143±37	175±57
Coefficient of variation(%)	26	33
Variance	1417.03	4329.62

[1]Each datum is taken from the average of 9 plants.