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Effect of Growth Stage and Pod Position on Soybean Seed Isoflavone Concentration

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Abstract

Field experiments were established during 2004 at two sites of the Emile A. Lods Agronomy Research Centre in Sainte-Anne-de-Bellevue, QC, Canada. Soil types were a fine sandy loam at one site (site A) and a Macdonald clay loam at the other (site B). During seed development and maturation, three replicates of 200 pods were collected from different parts of plants (from the top and base of plants) at three development stages; full-seed pod (R6), yellow pod (R7) and brown pod (R8), resulting in 6 treatments. Total and individual isoflavone concentrations were determined by high-performance liquid chromatography. Our results indicate that the individual and total isoflavone contents were significantly influenced by growth stage and pod position. The maximum individual and total isoflavone contents were recorded when the soybean seeds were collected from the base parts of plants at brown pod stage (R8). The maximum protein content of 468 g kg^{-1} was obtained by collecting soybean seeds at the earlier growth stage (R6). Also, the soybean seeds developing at the top parts of plants accumulate a greater amount of protein than those from the base region. On the other hand, soybean seeds developing at the base parts of plants (first 20 cm from soil surface) accumulate greater amount of oil than those from the top (last 20 cm from soil surface).

Keywords: soybean, isoflavone, daidzein, genistein, glycitein

Introduction

Soybean (*Glycine max* (L.) Merr.) seeds are rich in isoflavones including three aglycones (daidzein, genistein and glycitein), their glycosides and their corresponding acetyl and malonyl derivatives. Soybean isoflavones have a positive impact on human health including prevention of chronic diseases including cancer, heart disease, osteoporosis and menopausal symptoms (Messina, 1995). Ranich *et al*. (2001) demonstrated that soybean isoflavones have beneficial effect on diabetes and renal diseases. Setchell and Cole (2003) reported that these properties have also led to the amalgamation of soybean isoflavone extracts into a range of commercial functional foods. Several abiotic and biotic factors have been reported to affect soybean isoflavone concentration including: soil moisture levels (Chaves *et al*., 1997), pest pressure (Parr and Rhodes, 1996), temperature (Tsukamoto *et al*., 1995), mineral nutrition (Tiller and Parry, 1994) and light quality (Kubasek *et al*., 1992; Stapleton, 1992). Previous investigations have shown that isoflavone concentrations in soybean seeds (Hoeck *et al*., 2000; Wang and Murphy, 1994) are influenced significantly by location. For example, Carrao-Panizzi *et al*. (1999) reported that the highest isoflavone concentrations were observed in seeds of soybean plants grown in locations with high latitudes (cooler temperatures) when compared to locations with low latitudes (warmer temperatures).

The concentration and yield of isoflavones in plants vary among organs tissues. Alfalfa stores the largest amounts of isoflavones in the seed coat (Hartwig *et al*., 1990), whereas in soybean the amounts of isoflavones are much larger in the cotyledons than in the roots of hypocotyls (Tsukamoto *et al*., 1995). Chauser-Volfson and Gutterman (1997) found that all the lower and older *Aloe hereroensis* leaves contain significantly lower concentrations of the phenolic compounds than the terminal younger leaves.

In a study conducted by Bordignon *et al*. (2004) in the United States studied the effects of pod position on soybean seed isoflavone concentration. They reported that isoflavone concentration was lower in seeds collected from the top part of the plants and higher in seeds from the bottom parts. In Japan, Nakamura *et al*. (2001) reported that isoflavone concentrations appeared to be varied with the growth stages of soybean. They also reported that isoflavone concentrations were highest in mature bean seeds. Furthermore, Seguin *et al*. (2004 a) found that isoflavones concentration seemed to be varied with development stage in forage legumes.

Despite the fact that seeds are recognized as being a tissue with high isoflavone concentrations in soybean, there is still no information for this species on how it may vary with development stages and maturity under the Canadian conditions.

The objective of this study was to determine the effect of growth stage and pod position on soybean seed isoflavone concentration and other seed characteristics.

Materials and methods

Field experiments were established during 2004 at two sites of the Emile A. Lods Agronomy Research Centre in Sainte-Anne-de-Bellevue, QC, Canada. Plots (20x4.5 m) were seeded (cultivar 'OAC Chamption') on the 26th May 2004 at the rate of 500,000 plants/ha and were fertilized following recommendations of the Centre de référence en agriculture et agroalimentaire du Québec CRAAQ (2003). Soil types were a fine sandy loam at one site (site A) and a Macdonald clay loam at the other (site B). Seeds were inoculated at seeding with a commercial rhizobial inoculant (Liphatech, Milwaukee, WI, USA). Weeds were controlled by hand weeding later during the season. During seed development and maturation, three replicates of 200 pods were collected from different parts of plants (from the top and base of plants) at three development stages; full-seed pod (R6), yellow pod (R7) and brown pod (R8), resulting in 6 treatments (Tab.1). Harvested pods were placed in plastic bags and were brought to the lab within one hour of harvest. In the lab, three random samples of five pods from each treatment were taken to measure pod and seed characteristics, including pod length, pod width, seed length and seed width. The remaining pods were used for measuring 1000 seed weight and isoflavone concentrations.

Tab. 1. Description of the three development stages

Stages of maturity	Description
Full seed (R6)	Pods were green. Seeds filled the whole pod cavity. Seeds were dark green.
Yellow pod (R7)	Pods were yellow. Seeds filled the whole pod cavity.
Brown Pod (R8)	Pods were brown. Seeds began to dry down in the pod cavity. Seeds were light brown

Isoflavone extraction and HPLC analyses

Following harvest, seeds were stored at room temperature and within one month, they were extracted for determination of isoflavone. Extraction was done using a modified version of the protocol of Vyn *et al.* (2002), which relies on acid hydrolysis of the 12 endogenous isoflavones found in soybean seeds to their aglycone forms (i.e., daidzein, genistein and glycitein). In short, 0.25 g of fine ground seeds sample was hydrolyzed in a mixture of concentrated HCl (2 ml) and ethanol (10 ml) by boiling for 2 h (Pettersson and Kiessling, 1984; Choi *et al.*, 2000). Samples were then cooled and centrifuged at 10,000 rpm for 10 min.

Daidzein, genistein and glycitein were separated by HPLC using a Waters chromatograph system (Waters, Milford, MA), equipped with two model 510 pumps, a WISP 712 autosampler and a UV model 441 absorbance detector. Fifty µl of each extract were used for the analysis. The separation was carried out on C18 reversed-phase column (Bondapak, 10 µm, 3.9x300 mm, Millipore, Milford, MA, USA). Elution of isoflavones was performed using a linear gradient system from 20% methanol and 80% water to 80% methanol and 20% water over the course of 30 min, following an initial 5 minutes of steady elution with 20:80% methanol/water. All isoflavones were detected at 254 nm (Wang *et al.*, 2000). Purified isoflavones (daidzein, genistein, glycitein; (Sigma-Aldricht, Mississauga, ON, Canada) were used as standards to identify isoflavones on chromatograms and calculate their concentrations. All concentrations were expressed on a DM basis. Concentrations of aglycones were summed to obtain total isoflavone concentration.

Other measured variables

Seed samples from the harvested plots were also used to determine the crude protein (CP), oil and crude fiber (CF) concentrations. CP, oil and CF concentrations were determined on finely ground seeds using a FOSS NIR Systems Model 6500 (Silver Springs, MD, USA). All values are expressed on a dry matter (DM) basis (Al-Tawaha *et al.* 2005).

Statistical analysis

In both sites, the plots were arranged in a randomised complete block design with three replicates. All data were subjected to analysis of variance (ANOVA) using the general linear model (GLM) procedure in SAS (Statistical Analysis Software, 1989) to identify significant treatment effects and interactions. Thereafter, data were analysed using combined analysis that regrouped site, growth stage and pod position, (McIntosh, 1983). Comparisons between means were made using least significant differences (LSD) at a 0.05 probability. Histograms and standard error bars were produced according to Dytham (1999).

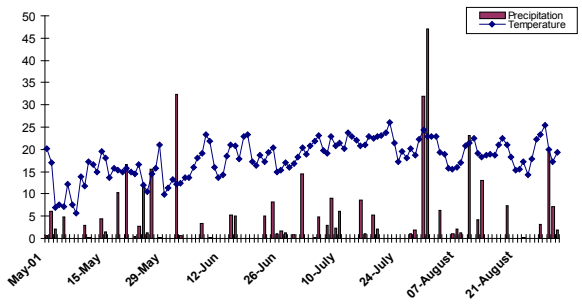

Fig. 1. Precipitation (mm) and average temperature (°C) in Montreal, QC from May to August 2004

Results and discussion

Average minimum and maximum air temperatures and rainfall for May, June, July, August and September for Montreal area are shown in Fig. 1. Growing conditions were positive (suitable at the experimental sites during the growing season).

Total isoflavone

Total isoflavone concentrations were influenced significantly (P<0.05), by growth stage (Tab. 2 and Tab. 3). However, total isoflavone concentration did not differ significantly due to site effects. This can be explained by the fact that soybean plants in both sites were exposed to the same environmental conditions (temperature and rainfall) during the pod development and seed filling stages. Carrao-Panizzi *et al*. (1999) reported that the accumulation of the isoflavones has been characterized during soybean seed development (between 35 and 60 days after flowering). They also reported that the highest isoflavone concentrations were observed in seeds of soybean plants grown in sites with cooler temperatures (high latitudes) when compared to sites with warmer temperatures (low latitudes). Furthermore, Tsukamoto *et al*. (1995) reported that high temperature during soybean seed-fill reduced total isoflavone concentrations by 5-50%. Overall, the maximum total isoflavone concentrations of 429 μg g^{-1} was obtained by collecting soybean seeds at the later growth stage (R8), which was superior to total isoflavone concentrations corresponding to the earlier date of seed collection; a total isoflavone reductions of 44 and 23% was recorded, with earlier dates of seeds collection on R6 (full-seed pod) and R7 (yellow pod), respectively. Nakamura *et al*. (2001) observed similar results and found that isoflavone concentrations were highest in mature soybean seeds. Site x growth stage (P<0.05) interactions were observed for total isoflavone concentrations (Tab. 2). In the clay loam soil site, the maximum total isoflavone concentrations of 360 μg g^{-1} were recorded when the soybean seeds were collected form developing pods at the R8 stage but it was not significantly different from R7, the value was 358μg g^{-1} (Fig. 2). However, in the sandy soil site, the maximum total isoflavone concentrations of 499 μg g^{-1} were recorded only when the soybean seeds were collected form developing pods at the R8 stage. Total isoflavone concentrations were influenced significantly (P<0.001) by pod position (Tab. 2 and Tab. 3). Across site and growth stage, the maximum total isoflavone concentrations of 414 μg g^{-1} were recorded when the samples were collected form base parts of the plants.

Site x pod position (P<0.05) interactions were observed for total isoflavone concentrations (Tab. 2). In the clay loam soil site, the maximum total isoflavone concentration was recorded when the samples were collected form the base parts of plants (Fig. 3). However, in the sandy soil site, total isoflavone concentrations did not differ significantly due to pod position effects. Growth stages x pod position (P<0.05) interactions were observed for total isoflavone concentrations (Tab. 2). The maximum total

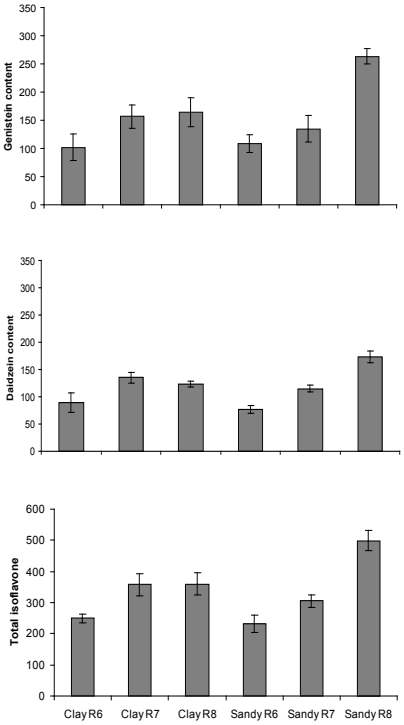

Fig. 2. Daidzein, genistein and total isoflavone content (μg g^{-1}) as influenced by site x growth stage interactions

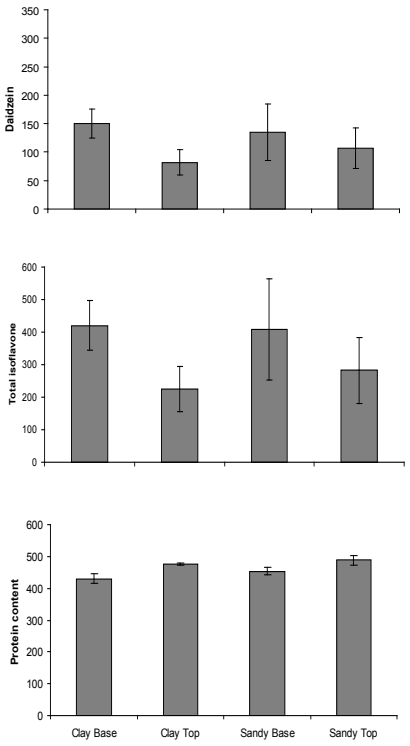

Fig. 3. Daidzein (μg g^{-1}), total isoflavone content (μg g^{-1}) and protein content (g kg^{-1}) as influenced by site x pod position interactions

Tab. 2. Analysis of variance of isoflavone concentration and other seed characteristics of field grown soybean as affected by site, development stage and pod position

Source of variation	Pod length cm	Pod width cm	1000-seed weight g	Seed length cm	Seed width cm	Crude protein g kg^{-1}	Oil g kg^{-1}	Crude fiber g kg^{-1}	Total daidzein μg g^{-1}	Total genistein μg g^{-1}	Total glycitein μg g^{-1}	Total isoflavone μg g^{-1}
S	**	**	ns	ns	ns	***	ns	ns	ns	ns	ns	ns
G	**	***	***	***	***	**	**	**	***	***	ns	***
S x G	**	ns	**	ns	ns	*	ns	ns	***	***	ns	***
P	***	**	***	***	ns	***	***	***	***	***	***	***
S x P	ns	ns	**	ns	ns	*	ns	ns	***	ns	ns	**
G x P	ns	ns	ns	ns	ns	ns	ns	ns	***	***	**	***
S x G x P	ns	ns	**	ns	ns	***	ns	ns	ns	ns	ns	ns
C.V	6.9	10.8	5.5	8.1	15.7	2.0	2.8	15.7	8.1	14.98	24.1	9.4

S site; G growth stage; P pod position; * P<0.05; ** P<0.01; *** P<0.001; NS non-significant

isoflavone contents were recorded when the samples were collected from base parts of plants at brown pod stage (R8) (Fig. 4).

Individual isoflavone concentrations

Across-site, growth stage and pod position, daidzein, genistein and glycitein represented respectively 118, 155 and 60 μg g^{-1}. The individual and total isoflavone contents of the investigated soybean seemed to be slightly lower than those reported by Eldridge and Kwolek (1983), Wang and Murphy (1994), Carrao-Panizzi *et al*. (1999), Choi *et al*. (2000), Hoeck *et al*. (2000), Wang *et al*. (2000). Total daidzein concentrations did not differ significantly due to site effects. However, total daidzein concentrations were influenced significantly (P<0.001) by growth stage (Tab. 2 and Tab. 3). Across-site and pod position the maximum total daidzein concentration of 149 μg g^{-1} was obtained by collecting soybean seeds at the later growth stage (R8), which was found superior to total daidzein content corresponding to the earlier date of collection: a total daidzein reduction of 44 and 16 % was recorded, with earlier dates of seeds collection at R6 (full-seed pod) and R7 (yellow pod), respectively. Site x growth stage (P<0.05) interactions were observed for total daidzein concentrations (Tab. 2). In the clay loam soil site, the maximum total daidzein concentrations of 135 μg g^{-1} were recorded when the soybean seeds were collected form developing pods at R7 stage but it was not significantly different from R8, the value was 124 μg g^{-1} (Fig. 2). However, in the sandy soil site, the maximum total daidzein concentrations of 173 μg g^{-1} were recorded only when the soybean seeds were collected form developing pods at R8 stage. Total daidzein concentrations of soybean were influenced significantly (P<0.001), by pod position (Tab. 2 and Tab. 3). Across site and growth stage, the maximum total daidzein concentrations of 143 μg g^{-1} were recorded when the samples

Tab. 3. Isoflavone concentration of field-grown soybean as affected by site, growth stage and pod position

	Site			Pod position			Growth stage		
Parameter	Sandy	Clay loam	LSD 0.05	Sandy	Clay loam	LSD 0.05	Sandy	Clay loam	LSD 0.05
Daidzein μg g^{-1}	122	116	ns	122	116	ns	122	116	ns
Genistein μg g^{-1}	169	142	ns	169	142	ns	169	142	ns
Glycitein μg g^{-1}	55	64	ns	55	64	ns	55	64	ns
Total Isoflavone μg g^{-1}	345	322	ns	345	322	ns	345	322	ns

NS non-significant

Tab. 4, Pod and seed characteristics of field-grown soybean as affected by site, growth stage and pod position

	Site			Pod position			Growth stage			
Parameter	Sandy	Clay loam	LSD 0.05	Base	Top	LSD 0.05	R6	R7	R8	LSD 0.05
Pod length (cm)	5.5	4.8	0.36	5.5	4.9	0.26	5.0	5.2	5.3	0.12
Pod width (mm)	10.1	8.6	0.79	10.0	8.7	0.74	9.2	10.3	8.7	0.62
Seed length (mm)	8.3	8.1	ns	9.2	7.7	0.50	6.9	8.0	10.5	0.85
Seed width (mm)	5.4	5.4	ns	5.4	5.4	ns	3.8	7.3	5.2	0.70
1000 seed weight (g)	169	171	ns	178	162	6.7	133	191	186	6.0

NS non-significant

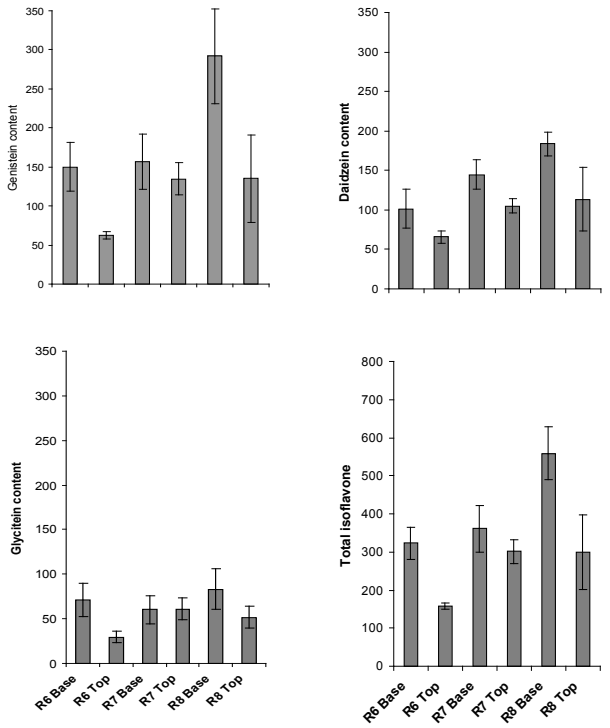

Fig. 4. Daidzein, genistein, glycitein and total isoflavone content (μg g^{-1}) as influenced by growth stage x pod position interactions

were collected form the base parts of plants. Site x pod position (P<0.01) interactions were observed for total daidzein concentrations (Tab. 2). In the clay loam soil site, the maximum total daidzein concentration was recorded when the samples were collected from the base parts of plants (Fig. 3). However, in the sandy soil site, total daidzein concentrations did not differ significantly due to pod position effects. Growth stage x pod position (P<0.05) interactions was observed for total daidzein concentrations (Tab. 2). The maximum total daidzein concentrations were recorded when the samples were collected from the base parts of plants at brown pod stage (R8) (Fig. 4). Glycitein did not differ significantly due to site and growth stage (main effects) and site x growth stage (interaction). It is tempting to speculate from these results that conditions affecting glycitein may be different from those affecting the other isoflavone contents. Total glycitein concentrations were influenced significantly (P<0.05) by pod position (Tab. 2 and Tab. 3). Across site and growth stage, the maximum total glycitein concentrations of 72 μg g^{-1} were recorded when the seed samples were collected form the base parts of plants. Total genistein concentrations were influenced significantly (P<0.05) by growth stage (Tab. 2 and Tab. 3). Across-site and pod position the maximum total genistein concentrations of 213 μg g^{-1}, was obtained by collecting soybean seeds at the later growth stage (R8), which was found superior to total genistein content corresponding to the earlier date of collection: a total genistein reduction of 50 and 31 % was recorded, with earlier dates of seeds collection on R6 (full-seed pod) and R7 (yellow pod), respectively. Total genistein concentrations were influenced significantly (P<0.001) by pod position (Tab. 2 and Tab. 3). Across site and growth stage, the maximum total genistein concentrations of 199 μg g^{-1} were recorded when the soybean seeds were collected form the base parts of plants (Tab. 3).

Growth stage x pod position (P<0.001) interaction was observed for total genistein concentrations (Tab. 2). The maximum total genistein concentrations were recorded when the soybean seeds were collected form the base parts of plants at brown pod stage (R8) (Fig. 4).

Other seed characteristics

The pod and seed measurements (pod length, pod width, seed length, seed width and 1000 seed weight) from different parts of plants (from the top and base of plants) at three development stages (full-seed pod (R6), yellow pod (R7), and brown pod (R8)) were similar at the two sites (Tab. 4). Overall, the pod width and seed width increased between R6 and R7. Our results are in general agreement with those of Samarah *et al*. (2003). Pod length, seed length increased to the maximum value at the R8 stage. These results are in agreement with those reported by Samarah *et al*. (2004). Seed lengths were influenced significantly (P<0.05) by pod position (Tab. 2 and Tab. 4). The maximum seed lengths were recorded when the samples were collected from the base parts of plants (Tab. 4).

1000 seeds weight was influenced significantly (P<0.05) by growth stage (Tab. 2 and Tab. 4). 1000 seed weight reached the maximum value at the R7 (191 g) but it was not significantly different from R8, the value was 186 g. The soybean seeds reached maximum dry weight (physiological maturity) at the R7 stage (data not shown). These results are in agreement with those reported by Samarah *et al*. (2003) who found that bitter vetch seeds reached maximum dry weight at the yellow pod stage. Site x growth stage (P<0.05) interaction was observed for 1000 seed weight (Tab. 2). The site x growth stage interactions

Tab. 5. CP, oil and CF concentrations (g kg^{-1}) of field-grown soybean as affected by site, growth stage and pod position

	Site			Pod position			Growth stage			
Parameter	Sandy	Clay loam	LSD 0.05	Base	Top	LSD 0.05	R6	R7	R8	LSD 0.05
CP g kg^{-1}	471	452	2.0	442	482	5.3	468	455	462	7.2
Oil g kg^{-1}	186	188	ns	195	179	3.9	178	189	192	6.3
CF g kg^{-1}	79	81	ns	68	92	9.1	95	82	63	14

CP crude protein; CF crude fiber; NS non-significant

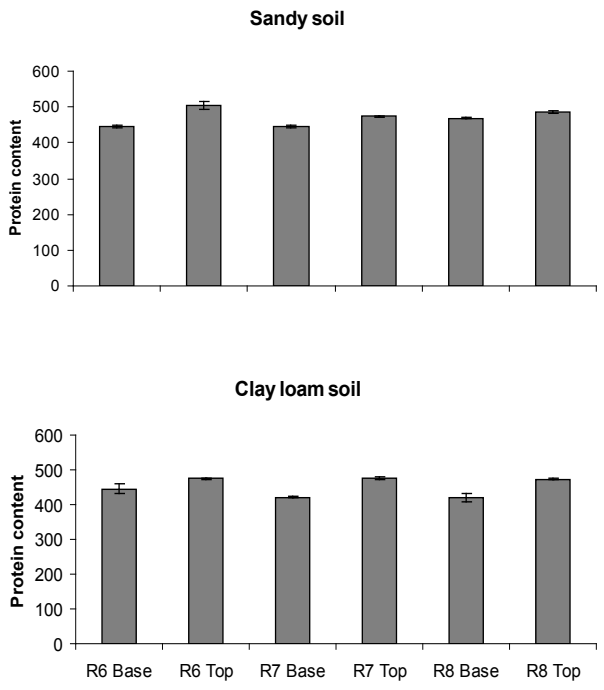

Fig. 5. Protein content (g kg^{-1}) as influenced by growth stage and pod position at two sites, clay loam and sandy soils

for 1000 seed weight were caused by magnitude 1000 seed weight differences between sites. On the other hand, 1000 seeds weight was influenced significantly (P<0.05) by pod position (Tab. 2 and Tab. 4). Samples collected from the base part of soybean plants had greater 1000 seed weight than those collected from the top part of plants. Soybean plants grown at sandy soil site had greater pod length and pod width (5.5 cm and 10.1 mm, respectively) than those grown at a clay loam soil site (4.8 cm and 8.6 mm, respectively). Samples collected from the base part of soybean plants had greater pod length and pod width than those collected from the top part of plants. Site x growth stage (P<0.05) interaction was observed for pod length (Tab. 2). The site x growth stage interaction for pod length was caused by magnitude pod length differences between sites.

Crude protein, crude fiber and oil contents

Across growth stage and pod position, soybean seeds collected from sandy soil site had greater crude protein content (471 g kg^{-1}) than those grown at clay loam soil site (452 g kg^{-1}). This contradicts previous results by Shaw and Laing (1966), Sionit and Kramer (1977), Krogman *et al.* (1980), Rose (1988) who concluded that environmental effects on harvested seed protein were generally relatively high. This also appeared to be true for our data. Crude protein content was influenced significantly (P<0.05), by growth stage (Tab. 2 and Tab. 5). Overall, the maximum protein content of 468 g kg^{-1} was obtained by collecting soybean seeds at the earlier growth stage (R6). Protein content was influenced significantly by pod position (Tab. 2 and Tab. 5). Across site and growth stage, the soybean seeds developing at the top parts of plants accumulate a greater amount of protein than those from base region. Seeds from the top nodes contained 482 g kg^{-1} proteins content while those from the base nodes were 442 g kg^{-1}. These values are in agreement with values reported by Wolf *et al.* (1982) who reported that the protein content of soybean seeds is usually high. Site x growth stage x pod position (P<0.05) interactions were observed for crude protein content (Tab. 2). In the clay loam soil site the maximum protein content was recorded when the samples were collected from the top parts of plants irrespective to the stage of growth (Fig. 5). On the other hand, in the sandy soil site, the maximum protein content was recorded when the samples were collected form the top parts of plants at full pod stage (R6). Crude fiber and oil content did not differ significantly due to site effect. On the other hand, oil content was influenced significantly by growth stage (Tab. 2 and Tab. 5). The maximum oil content of 192 g kg^{-1} was obtained by collecting soybean seeds at the later growth stage (R8), but it was not significantly different from soybean seeds, collected at the yellow pod

Tab. 6. Correlation coefficients for eleven characters of soybean plant

	Pod width (mm)	Seed length (mm)	Seed width (mm)	1000 seed weight (g)	CP g kg^{-1}	CF g kg^{-1}	Oil g kg^{-1}	Daidzein µg g^{-1}	Genistein µg g^{-1}	Glycitein µg g^{-1}	Total Isoflavone µg g^{-1}
Pod length (cm)	0.56***	0.45*	0.05	0.25	-0.26	-0.46**	0.46**	0.46**	0.49**	0.20	0.47**
Pod width (mm)		0.60***	0.32	0.19	-0.25	-0.07	0.26	0.28	0.26	0.23	0.28
Seed length (mm)			0.74***	0.64***	-0.42**	-0.28	0.47**	0.40**	0.29	0.22	0.34*
Seed width (mm)				0.66***	-0.16	-0.14	0.30	0.33*	0.11	0.06	0.19
1000 seed weight (g)					-0.47**	-0.61***	0.70***	0.60***	0.52**	0.41**	0.57***
CP g kg^{-1}						0.54***	-0.78***	-0.46**	-0.37*	-0.61***	-0.47**
CF g kg^{-1}							-0.90***	-0.69***	-0.62 ***	-0.38*	-0.66***
Oil gkg^{-1}								0.67***	0.54***	0.48**	0.62***
Daidzein µg g^{-1}									0.87***	0.58***	0.94***
Genistein µg g^{-1}										0.58***	0.97***
Glycitein µg g^{-1}											0.70***

* P<0.05; ** P<0.01; *** P<0.001

stage (R7) the value was 198 g kg^{-1}. Oil content and crude fiber were influenced significantly by pod position (Tab. 2 and Tab. 5). Soybean seeds developing at the base parts of plants accumulate lower amount of crude fiber (62 g kg^{-1}) than those from the top region (92 g kg^{-1}). However, the soybean seeds developing at the base parts of plants accumulate greater amount of oil than those from top region. Seeds from the base nodes contained of 195 g kg^{-1} oil while those from the top nodes had 179 g kg^{-1}. These results are in agreement with those reported by Wolf *et al*. (1982) who found nearly the same value for oil in soybean seed.

Correlations between isoflavone concentrations and other seed characteristics

Significant positive correlations were observed between pod length and daidzein (r=0.46; P<0.05), pod length and genistein (r=0.49; P<0.01), pod length and total isoflavone (r=0.47; P<0.01), seed width and daidzein (r=0.33; P<0.05), 1000 seed weight and daidzein (r=0.60; P<0.05), 1000 seed weight and genistein (r=0.52; P<0.01), 1000 seed weight and glycitein (r=0.41; P<0.01), 1000 seed weight and total isoflavone (r=0.57; P<0.05) (Tab. 6).

Significant negative correlations were observed between total isoflavone and CP (r=-0.47; P<0.05), total isoflavone and CF (r=-0.61; P<0.001), CP and genistein (r=-0.37; P<0.05), CP and daidzein (r=-0.46; P<0.01), and CP and glycitein (r=-0.66; P<0.05). This incongruity previous revealed by Allen *et al*. (2004) who found that the protein content did not correlate with the isoflavone concentration. Highly significant positive correlation (P<0.05) was observed between total isoflavone and oil (Tab. 6). Because oil content correlated strongly with isoflavone concentrations the use of oil content as an indicator for isoflavone concentration does appear to be warranted.

Highly significant positive correlations (P<0.05) were observed between individual and total isoflavone concentrations. These correlations are not surprising as individual isoflavones are synthesized via a common pathway (the phenylpropanoid pathway). These results are in agreement with those reported by several researchers (Seguin *et al*., 2004b; Al-Tawaha *et al*., 2005) who found that individual and total isoflavone were positively correlated.

Conclusions

This study demonstrates that the growth stage and pod position have a great impact on soybean seed isoflavone concentration. The maximum individual and total isoflavone contents were recorded when the soybean seeds were collected from the base parts of plants at brown pod stage (R8). Our results also indicated that maximum crude protein content was obtained by collecting soybean seeds at the earlier stage of growth (R6). Soybean seeds developing at the top parts of plants accumulate greater amount of protein and crude fiber than those from the base region. However, soybean seeds developing at the base parts of plants accumulate greater amount of oil than those from top region. Oil content correlated strongly with isoflavone concentrations and the use of oil content as an indicator for isoflavone concentration does appear to be warranted.

References

Allen, F. L., C. S. Charron, R. D. Johnson and C. E. Sams (2004). Associations between isoflavones and protein content in soybean (*Glycinemax*) seed. New directions for a diverse planet: Proceedings of the 4th International Crop Science Congress Brisbane, Australia.

Al-Tawaha, A. M., P. Seguin, D. L. Smith and C. Beaulieu (2005). Biotic elicitors as a means of increasing isoflavone concentration of soybean seeds. Annals of Applied Bio. 146:303-310.

Bordignon, J. R., S. P. Long and N. J. Engeseth (2004). Isoflavone content in soybean seeds from different parts of plants grown under elevated atmospheric CO_2 or O_3. Nutraceuticals and Functional Foods: Antioxidants and phytochemical analysis. International Food Safety and Quality Conference, Las Vegas, Nevada.

Carrao-Panizzi, M. C., A. D. Beleia, K. Kitamura and M. C. N. Oliveira (1999). Effects of genetics and environment on isoflavone content of soybean from different regions of Brazil. Pesq Agropec Bras. 34:1787-1795.

Chaves, N., J. C. Escudero and C. Gutierrez-Merino (1997). Role of ecological variables in the seasonal variation of flavonoid content of *Cistus ladanifer* exudate. J. Chem. Ecol. 23:579-603.

Chauser-Volfson, E. and Y. Gutterman (1997). Content and distribution of the secondary phenolic compound homonataloin in *Aloe hereroensis* leaves according to leaf part, position and monthly changes. J. Arid Environments. 37:115-122.

Choi, Y. S., B. H. Lee, J. H. Kim and N. S. Kim (2000). Concentrations of phytoestrogens in soybeans and soybean products in Korea. Journal of the Science of Food and Agriculture 80:1709-1712.

CRAAQ (Centre de référence en agriculture et agroalimentaire du Québec). (2003). Guide de Référence en Fertilisation. 1st edition; Centre de Référence en Agriculture et Agroalimentaire du Québec: Sainte-Foy, QC, Canada.

Dytham, C. (1999). Choosing and Using Statistics: Biologists Guide. Blackwell Science, Oxford.

Eldridge, A. and W. Kwolek (1983). Soybean isoflavones: effects of the environment and variety on composition. J. Agric. Food. Chem. 31:394-396.

Hartwig, U. A., C. A. Maxwell, C. M. Joseph and D. A. Phillips (1990). Chrysoeriol and luteolin released from alfalfa seeds induce nod genes in *Rhizobium meliloti*. Plant Physiol. 92:116-122.

Hoeck, J. A., W. R. Fehr, P. A. Murphy and G. A. Welke (2000).

Influence of genotype and environment on isoflavone contents of soybean. Crop Sci. 40:48-51.

Krogman, K. K., R. C. Mckenzie and E. H. Hobbs (1980). Response of faba bean yield, protein production and water use to irrigation. Can. J. Plant Sci. 60:91-96.

Kubasek, W. L., B. W. Shirley, A. McKillop, H. M. Goodman, W. Briggs and F. M. Ausubel (1992). Regulation of flavonoid biosynthesis genes in germinating *Arabidopsis* seedlings. Plant Cell 4:1229-1236.

McIntosh, M. S. (1983). Analysis of combined experiments. Agron. J. 75:153-155.

Messina, M. (1995). Modern applications for an ancient bean: soybeans and the prevention and treatment of chronic disease. J. Nutr. 125:567-569.

Nakamura, Y., A. Kaihara, K. Yoshii, Y. Tsumura, S. Ishimitsu and Y. Tonogai (2001). Content and composition of isoflavonids in mature or immature beans and bean sprouts consumed in Japan. Journal of Health Science 47:394-406.

Parr, A. J. and M. J. C. Rhodes (1996). Natural plant defence mechanisms. In: Protection Agents from Nature. LG Copping Royal Society of Chemistry Ed.

Pettersson, H. and K. H. Kiessling (1984). Liquid chromatographic determination of the plant estrogens coumestrol and isoflavones in animal feed. Journal of the Association of Official. Analytical Chemists 67:503-506.

Ranich, T., S. J. Bhathena and M. T. Velasquez (2001). Protective effects of dietary phytoestrogens in chronic renal disease. J. Ren. Nutr. 11:183-93.

Rose, I. A. (1988). Effects of moisture stress on the oil and protein components of soybean seeds. Aust. J. Agric. Res. 39:163-170.

Samarah, N., N. Allataifeh, M. A. Turk and A. M. Tawaha (2003). Effect of Maturity stage on germination and dormancy of fresh and air-dried seeds of bitter vetch (*Vicia ervilia* L.). NZ Journal of Agriculture Research 46:347-354.

Samarah, N., N. Allataifeh, M. A. Turk and A. M. Tawaha (2004). Seed germination and dormancy of fresh and air-dried seeds of common vetch (*Vicia sativa* L.) harvested at different stages of maturity. Seed Science and Technology 32:11-19.

SAS Institute Inc. (1989). SAS users guide, Version 6. Cary, USA.

Seguin, P., W. Zheng and A. Souleimanov (2004a). Alfalfa phytoestrogen content: Impact of plant maturity and herbage components. J. Agron. Crop Sci. 190:211-217.

Seguin, P., W. Zheng, D. L. Smith and W. Deng (2004b). Isoflavone content of soybean cultivars grown in eastern Canada. J. Sci. Food Agric. 84:1327-1332.

Setchell, K. D. R. and S. J. Cole (2003). Variations in Isoflavone Levels in Soy Foods and Soy Protein Isolates and Issues Related to Isoflavone Databases and Food Labeling. J. Agric. Food Chem. 51:4146-4155.

Shaw, R. H. and D. R. Laing (1966). Moisture stress and plant response, pp. 73-95. In: Plant Environment and Efficient Water use. W. H. Pierre *et al*. (Eds.). Am. Soc. of Agron. and Soil Sci. Soc. of America: Madison, Wisc.

Sionit, N. and P. J. Kramer (1977). Effect of water stress of growth of soybean. Agron. J. 69:274-278.

Stapleton, A. E. (1992). Ultraviolet radiation and plants: burning questions. Plant Cell 4:1353-1358.

Tiller, S. A. and A. D. Parry (1994). Isoflavonoid conjugates and their response to developmental change and abiotic stress in alfalfa *(Medicago sativa* L.) Acta Hort. 381:227-234.

Tsukamoto, C., S. Shimada, K. Igita, S. Kudou, M. Kokubun, K. Okubo and K. Kitamura (1995). Factors affecting isoflavones content in soybean seeds: changes in isoflavones, saponins, and composition of fatty acids at different temperatures during seed development. J. Agric. Food. Chem. 43:1184-1192.

Vyn, T. J., X. Yin, T. W. Bruulsema, C. C. Jackson, I. Rajcan, and S. M. Brouder (2002). Potassium fertilization effects on isoflavone concentrations in soybean (*Glycine max* (L.) merr.). J. Agric. Food. Chem. 50:3501-3506.

Wang, H. J. and P. A. Murphy (1994). Isoflavone composition of American and Japanese soybeans in Iowa: effects of variety, crop year, and location. J. Agric. Food. Chem. 42:1674-1677.

Wang, C., M. Sherrard, S. Pagadala, R Wixon and R. A. Scott (2000). Isoflavone content among maturity group 0 to II soybeans. Journal of the American Oil Chemistry Society 77:483-487.

Wolf, R. B., J. F. Cavins, R. Kleiman and L. T. Black (1982). Effect of temperature on soybean seed constituents: oil, protein, moisture, fatty acids, amino acids and sugars. J. Amer. Oil Chem. Soc. 59:230-232.