

## **Effect of sowing date on oil, protein and glucosinolate concentration of winter oilseed rape (*Brassica napus* L.)**

P. Lääniste, V. Ereemeev\*, E. Mäeorg and J. Jõudu

Estonian University of Life Sciences, Kreutzwaldi 1, EE 51014 Tartu, Estonia

\*Correspondence: vyacheslav.eremeev@emu.ee

**Abstract.** The effect of time of sowing on oil and meal quality of winter oilseed rape (*Brassica napus* L. cv. Express) was investigated at the Institute of Agricultural and Environmental Sciences of the Estonian University of Life Sciences in the period of 2001–2005. The rape seeds were sown at weekly intervals on four different dates: 8<sup>th</sup>, 15<sup>th</sup>, 22<sup>nd</sup> and 29<sup>th</sup> of August. The study shows that sowing date and environmental conditions affect the seed quality of winter oilseed rape. Early sown oilseed rape plants were more adapted to stressful conditions associated with high or low temperatures. The seeds of such plants had higher oil concentration (up to 50.2%) and a lower protein concentration (approximately 19%). Plants sown in late August were less tolerant to stressful conditions and their seed oil concentration was lower (47–48% DM). Oil and protein yield were higher in the early sown crops because the seed yield was higher. Also the glucosinolate (GSL) concentration of the seeds was affected by the time of sowing and weather conditions. Shortage of rainfall before harvest increased the GSL concentration in the seeds. Plants sown in late August did not tolerate the extreme environmental conditions and their seed glucosinolate concentration appeared to increase.

**Key words:** cv. Express; glucosinolate concentration; oil concentration; oil yield; protein concentration; protein yield.

### **INTRODUCTION**

Rapeseed cultivation has been strategically important agricultural sector for over 15 years in Estonia. It acts as profitable break crop in cereal crop rotations breaking the life-cycle of common cereal pathogens and pests and also improving the structural properties of the soil. The average yields (2.51 t ha<sup>-1</sup> in 2015; Statistics Estonia, 2015) have remained low compared to other European countries (EU average 3.12 t ha<sup>-1</sup> in 2013) (FAO, 2013), due to the large proportion (1/2) of spring rapeseed in the crop rotation. The average yield could be improved by using more winter rapeseed varieties that offer higher seed yield (3.1 t ha<sup>-1</sup> in 2015) (Statistics Estonia, 2015). Winter rapeseed that is usually sown at the end of summer or early autumn is characterized by more efficient use of resources (radiation, soil moisture etc) in autumn and at the start of the vegetation in spring, enabling approximately 50% yield increase compared to spring-sown rapeseed cultivars. Due to the short vegetation period in Estonia compared to Central European countries, the use of resources in early spring is of vital importance from the yield and environmental aspects (by decreasing erosion and nutrient leaching). The importance of efficient use of resources (for example, nutrients, water, temperature)

will gain more attention in the near future in the light of climate change, rising population and increasing input costs of crop production. The increased cultivation of winter rapeseed offers improved soil cover in the winter reducing the wind and water erosion and nutrient leaching in the soil (Sieling & Kage, 2010).

Rapeseed is grown worldwide in different climatic conditions. Even extreme conditions for crop growth are tolerated (Diepenbrock & Grosse, 1995). Sowing date of winter oilseed rape is an important determinant of length of growing season, insect infestation, seed and oil yields (Keshta & Leilah, 2003). Previous results Mus'nicki et al. (1999), Butkutė et al. (2006), Lääniste et al. (2007; 2008) indicate a strong relationship between growth and development of winter oilseed rape and environmental factors. Thus the sowing date has to be chosen such that yields are optimal taking negative environmental effects into account (Dejoux et al., 2003) and ensuring that leaf area and taproot reserves after the winter are sufficient to enable the crop to resume its growth quickly in spring (Mendham et al., 1981).

The major objectives of growing oilseed rape are its seed yield and seed oil concentration (Rathke & Schuster, 2001). Site-specific environmental conditions often account for large variations in seed yield and quality (oil, protein, glucosinolates) (Rathke et al., 2005). Glucosinolates (GSLs), which form a constituent of extracted meal, have antinutritive properties (Mika et al., 2003). On the other hand, some GSLs play an important role in the crop's resistance to pests and diseases (Zukalova & Vašak, 2002).

European growing technologies of winter oilseed rape vary little, with the exception of the time of the sowing, which ranges from 1<sup>st</sup> of August until 10<sup>th</sup> of September (Velička et al., 2000, Lääniste et al., 2007). According to various authors, very early sowing enhances crop growth and plants become more susceptible to frost, thus causing instability in seed yield (Graf & Heydrich, 2000, Lääniste et al., 2007, Lääniste et al., 2008). However, others have reported that plants grown from delayed sowing dates did not survive the winter (Lääniste et al., 2008) or that the seed yield was reduced (Graf & Heydrich, 2000). Compact plants with short internodes, that are in the six true-leaf stage usually show the highest survival rate (Behrens, 2002, Lääniste et al., 2007). However, under changing climatic conditions in Eastern Europe the rape sown during the second half of August (20.08) and at the end of August (30.08), whose autumnal growth and cold acclimation period was 64–76 days, was best prepared for wintering (Velička et al., 2010).

Oil and protein concentration of the seed are negatively correlated (Walton et al., 1999; Velička et al., 2011). Estonia is located on the eastern coast of the Baltic Sea. It represents a transition zone from the maritime climate type to the continental one. In spite of its comparatively small territory, climatic differences are significant, especially during the colder part of the year (Timothy & Granscog, 2001).

Field experiments were carried out to determine the effect of sowing date on seed oil, protein and glucosinolate concentration of the seeds and also oil and protein yield.

## MATERIALS AND METHODS

The crops were sown in the growing seasons of 2001–2002, 2003–2004 and 2004–2005 at the Eerika Experimental Station of Plant Biology (58°23'N, 26°44'E) near Tartu, Estonia. In each of the 3 seasons the rape seeds were sown weekly on four different dates: 8<sup>th</sup>, 15<sup>th</sup>, 22<sup>nd</sup> and 29<sup>th</sup> of August.

The soil of the experimental field is a Stagnic Luvisol (World Reference Base for Soil Resources; Deckers et al., 2002), with a texture of sandy loam and a humus layer of 20–30 cm (Reintam & Köster, 2006). Once a year before the sowing (early August), the soil samples were taken from depth of 0–25 cm. The results are presented as averages of three years. Soil analyses were carried out at the laboratories of the Department of Soil Science and Agrochemistry, EMU. Air-dried soil samples were passed through a 2 mm sieve. The following characteristics were determined: pH (in 1 M KCl and in 0.01 M CaCl<sub>2</sub> 1: 2.5 w: v); mobile P and mobile K were determined by AL-method (Egner et al., 1960); Ca was determined by Mehlich III method (Mehlich, 1984) and water-soluble S was determined by ISO 11048. The soil data of the topsoil of the experimental field were as follows: pH  $\approx$  6.2; humus, 2.4%; Ca, 5648 mg kg<sup>-1</sup>; P, 77.7 mg kg<sup>-1</sup> (Al-method; Egner et al., 1960); K, 169.8 mg kg<sup>-1</sup>; S 13.5 mg kg<sup>-1</sup>.

The experiments were carried out with *Brassica napus* cv. Express, bred in Germany. ‘Express’ is a productive cultivar with rapid autumn and spring development. The cultivar is resistant to winter conditions. Its plants have short stems, resulting in good lodging resistance.

The experiments were laid out in three replicates. Plot size was 1 m  $\times$  10 m. Seeds were sown at a rate of 150 seeds per m<sup>2</sup>, at a depth of 2–3 cm, previous crop being bare fallow. Prior to sowing, the herbicide Trifluralin (EK Trifluralin, AgroDan A/S) was applied at a rate of 0.72 kg a.i. in 400 l water per ha. The field was fertilised with complex granular combined fertiliser: Classic Brand 24-08-12, calculated at 120 kg of the active substance agent of nitrogen per hectare. The fertiliser was applied in April, when the plants had reached intensive growth phase of their vegetative mass. For insect pest control, the plants were sprayed with Fastac, with alpha-cypermethrine as the active substance agent. The rate of active substance agent was calculated at 0.15 l ha<sup>-1</sup>. The plants from test plots were harvested with a combine harvester and cleaned by a winnower. Thereafter the seeds were dried to a moisture concentration of 7% and then the yield was calculated.

The quality parameters of the seeds (oil concentration, protein concentration and glucosinolate concentration) were analyzed at Jögeva Plant Breeding Institute laboratory using NIR (near infrared spectroscopy) technology. The oil, protein and GLS concentration were presented as percentages of dry matter.

Weather data were obtained from the automatic weather station near the Institute of Agricultural and Environmental Sciences. The quality parameters of the seeds (oil concentration, protein concentration, glucosinolate concentration) were determined using near infrared spectroscopy. Below, the oil, protein and glucosinolate (GSL) concentrations are presented as percentages on a dry matter basis.

The Statistica version 11.0 (Statsoft Inc.) software package was used for all statistical analyses. Factorial analysis of variance (ANOVA) and one-way ANOVA were applied to test the results. The means are presented with their confidence limits. The level of statistical significance was set at  $P < 0.05$ , if not indicated otherwise. Pearson correlation analysis was used to study correlation between seed yield and oil seed rape seeds quality parameters (glucosinolate concentration, oil concentration, protein concentration). Linear correlation coefficients between variables were calculated, the significance of coefficients being  $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.05$ , ns: not significant ( $P > 0.05$ ).

## RESULTS AND DISCUSSION

The results from our experiments indicated that the quality of winter oilseed rape seed (oil, protein, and GSL concentration) directly depends on sowing date and weather conditions during the growth period.

The weather during the experiment period was monitored with Metos Compact (Pessl Instruments) electronic weather station, which automatically calculates the average daily temperatures and the sum of precipitation. To obtain the decade average of daily average temperatures at the weather station, the daily temperatures were averaged over each decade. The weather during the period April–May of the growing seasons of 2001–2002, 2003–2004 and 2004–2005 was typical for the Estonian climate (Table 1). The season of 2001–2002 was characterised by a long active growth period in autumn (until 19<sup>th</sup> of October) and by early spring growth (10<sup>th</sup> of April). The beginning of the active growth period in spring was warm and lacked rainfall (Table 1). The season of 2003–2004 was typical for the region. The period of active growth period ended on the 14<sup>th</sup> of October and started on the 5<sup>th</sup> of April. This period was sufficiently cold. Relatively more precipitation occurred in June and July and relatively less in April and May. The second part of the growing season was extremely rainy, especially June (Table 1). The growth period in autumn of 2004 was relatively short and ended on the 10<sup>th</sup> of October and the active growth period in spring started relatively late (26<sup>th</sup> of April). The beginning of the active growth period in spring of 2005 of oilseed rape was wet but June and July were sufficiently dry (Table 1).

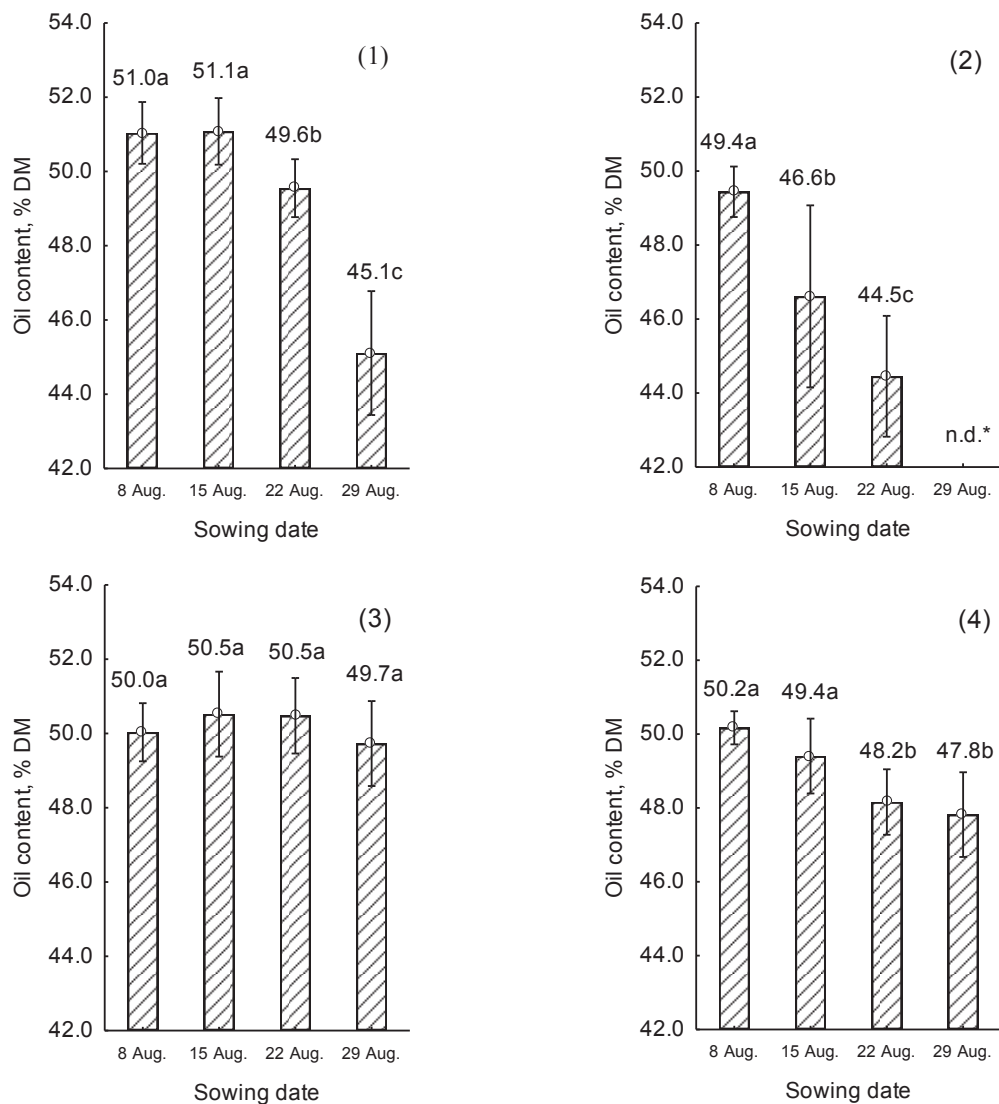
**Table 1.** Average monthly temperatures (°C) and precipitation (mm) during the growing period in the Eerika experimental field

Month	Temperature, °C				Precipitation, mm			
	2002	2004	2005	Average of 1966–1998	2002	2004	2005	Average of 1966–1998
April	6.4	5.7	5.0	4.4	20.1	12.8	21.8	32.4
May	19.3	12.1	10.9	11.0	15.4	34.4	114.0	55.0
June	16.5	13.4	14.5	15.1	50.1	210.6	54.2	66.0
July	20.1	16.4	19.2	16.7	44.7	113.2	21.8	72.0

### Sowing date and seed oil concentration and oil yield

Many authors have reported that sowing time affects the rapeseed yield and its major quality indicators. Higher seed yield results in higher oil yield and oil content (Kirkland & Johnson, 2000; Butkutė et al., 2006). However, some authors suggest that winter oilseed rape sowing time does not affect seed quality (Zhang & Zhang, 2012). Our research indicated that the oil concentration of the seeds was mainly affected by sowing date as well as by the weather during the growth period. Walton et al. (1999) found that temperature is an important environmental factor affecting the oil and protein concentration of winter oilseed rape. The weather data for the period April–July of the seasons 2001–2002 and 2004–2005 show that the temperatures were significantly higher and the rainfall less compared to the long-time averages (Table 1). In the season 2004–2005 the temperatures in June and July were markedly lower and precipitation was higher compared to the long-time averages (3 times more in July and 1.6 times more in June) (Table 1).

Walton (1999) found that high temperatures during ripening reduced oil concentration while increasing protein concentration in a Mediterranean-type environment. In our experiments it was observed that the higher temperatures, especially in June and July, affected the synthesis of oil positively and the oil concentration of the seeds increased significantly (more than 50% oil) (Fig. 1). The seeds produced by plants that had been grown in cool and rainy weather conditions had a lower oil concentration (less than 50%). The oil concentration of the different treatments in our experiment was very high (reaching above 50%).



**Figure 1.** Oil concentration (% in DM) in winter oilseed rape cv. 'Express' seeds: (1) 2001–2002, (2) 2003–2004, (3) 2004–2005 and (4) average of three growing seasons. Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences ( $p < 0.05$ ) between sowing dates. \* – no seeds were harvested.

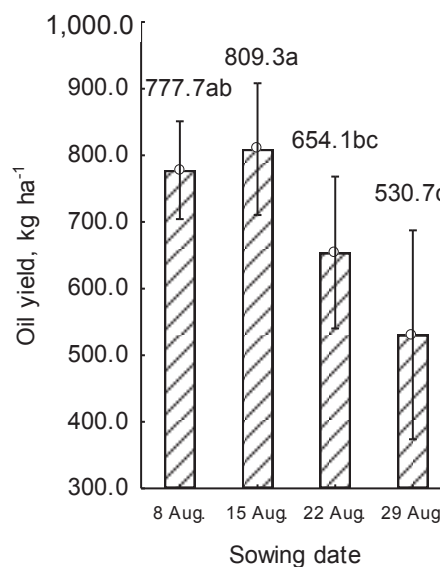
Also the oil concentration of the seeds is influenced by the time of sowing. Early sowing (8<sup>th</sup> and 15<sup>th</sup> of August) yielded seeds with an oil concentration of 50.2% and 49.4%, respectively. These data were significantly higher than the values for 22<sup>nd</sup> and 29<sup>th</sup> of August (48.2%,  $p < 0.05$  and 47.8%,  $p < 0.05$ ), respectively) (Fig. 1). The oil concentration was particularly negatively affected when the rape seeds was sown on the 29<sup>th</sup> of August and the plants were in the 3–4 leaves stage when winter started (7–8 foliage leaves is considered optimal) and also the root system was poorly developed (Lääniste et al., 2007). Such plants are very sensitive for the stress factors: extremely high/low temperatures, frost lift, excessive rainfall, and drought, and also the uptake of nutrients and water from the soil is hindered due to the poorly developed root systems. Under continuous stress conditions the synthesis of oil is inhibited.

Sowing date has an effect on the yield of rapeseed (Leach et al., 1999, Walton et al., 1999; Lääniste et al., 2008) as well as oil yield (kg ha<sup>-1</sup>). Leach et al. (1999) demonstrated that delayed sowing increased the seed yield and oil yield in Great Britain due to the maritime climate. In contrast, several authors reported that delayed sowing decreased grain yield in northern Germany (Schulz et al., 1994, Graf & Heydrich 2000, Sieling et al., 2005) and in Estonia (Lääniste et al., 2008).

The oil yield as the main important yield parameter highly correlated ( $P < 0.001$ ) with the seed yield. Kuht et al. (2013) observed, that the oil concentration of oilseed rape seeds was in strong negative correlation with seeds yield. A significantly higher oil yield was obtained from plants sown on the 15<sup>th</sup> of August (809 kg ha<sup>-1</sup>,  $p < 0.05$ ), a yield that exceeded the yield of plants sown on the 29<sup>th</sup> of August by 35%, and the yield of plants sown on 22 August by 20% (Fig. 2).

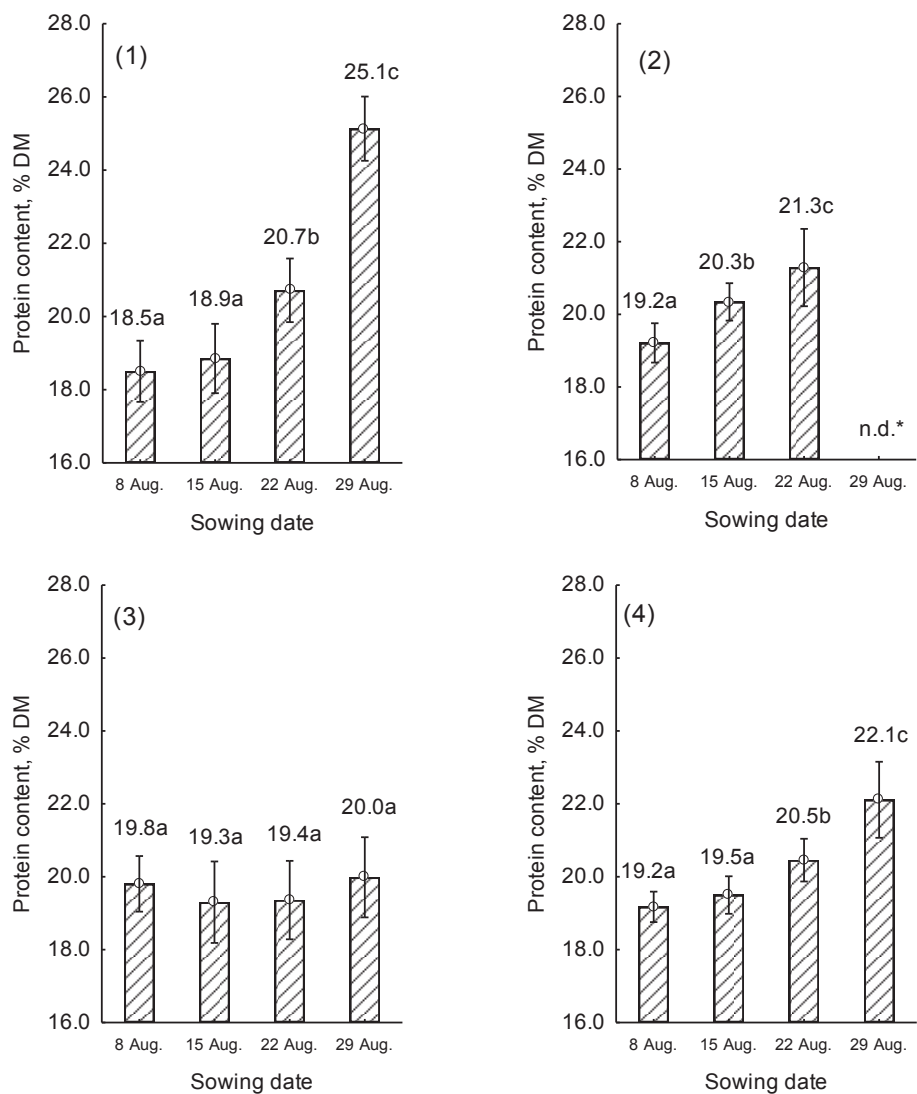
#### Sowing date and seed protein concentration and protein yield

There is a strong negative correlation between the protein concentration and the oil concentration of the seeds (Brennan et al., 2000; Kuht et al, 2015). The protein concentration of the seeds is strongly affected by the date of sowing. The protein concentration of the seeds from plants sown late in autumn (22<sup>nd</sup> and 29<sup>th</sup> of August) was significantly higher (20.5%,  $p < 0.05$  and 22.1%,  $p < 0.05$  protein, respectively), compared with the seeds from early sown crops (19.2% and 19.5% protein) (Fig. 3).

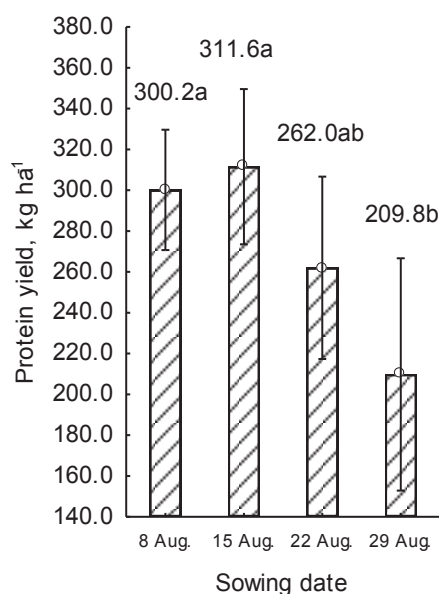


**Figure 2.** Oil yield (kg ha<sup>-1</sup>) average of three growing seasons in winter oilseed rape cv. 'Express'. Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences ( $p < 0.05$ ) between sowing dates.

Although the seed protein concentration of late sown crops was statistically higher  $p < 0.05$ , the seed protein yields remained lower (262.0 and 209.8 kg ha<sup>-1</sup>) than those of the early sown crops (300.2 and 311.6 kg ha<sup>-1</sup>) (Fig. 4). This effect can be explained by lower overall yields of late sown crops.



**Figure 3.** Protein concentration (% in DM) in winter oilseed rape cv. ‘Express’ seeds: (1) 2001–2002, (2) 2003–2004, (3) 2004–2005 and (4) average of three growing seasons. Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences ( $p < 0.05$ ) between sowing dates. \* – no seeds were harvested.



**Figure 4.** Protein yield (kg ha<sup>-1</sup>) average of three growing seasons in winter oilseed rape cv. 'Express' seeds. Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences ( $p < 0.05$ ) between sowing dates.

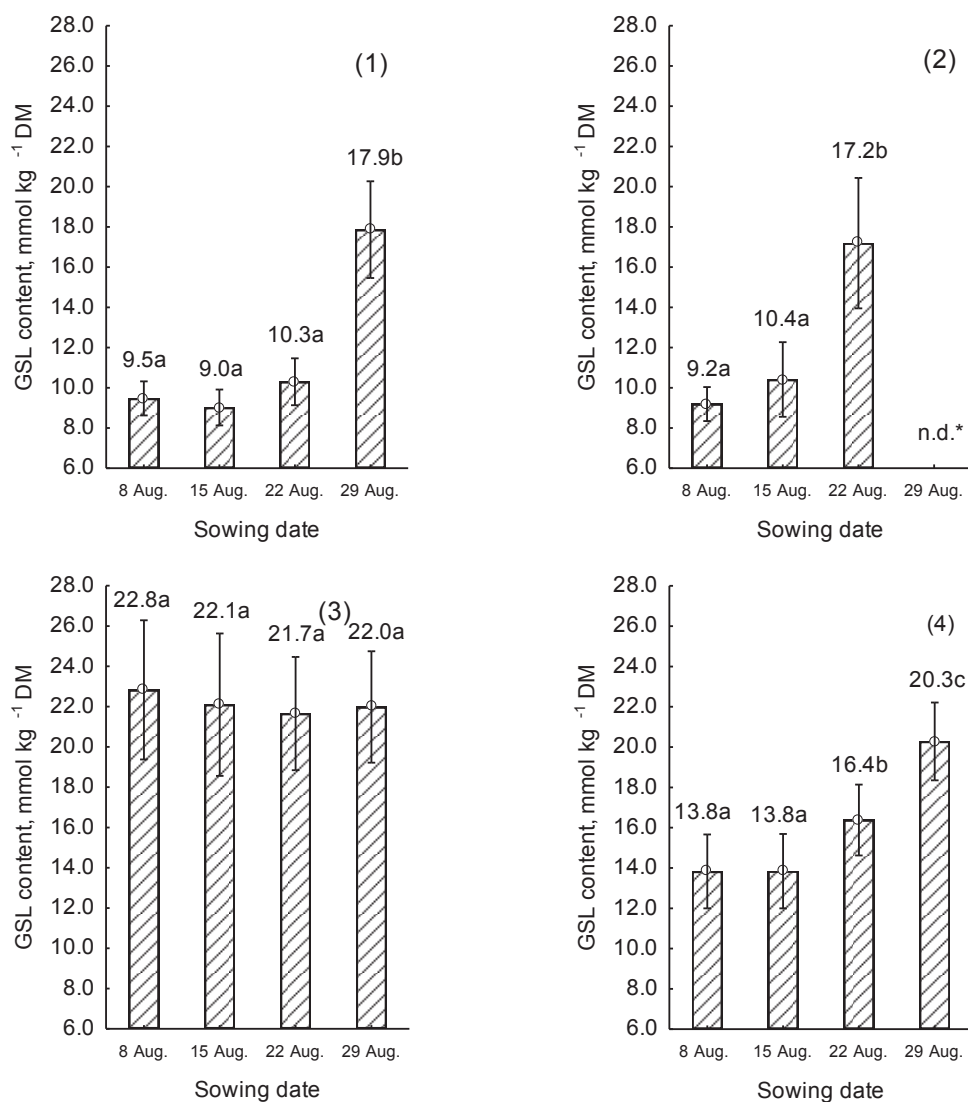
#### Sowing date and seed glucosinolate concentration

Glucosinolates are the main antinutritive components of oilseed rape seeds (Krzymanski, 1970). Breakdown products of glucosinolates, which occur during the crushing process, are partially volatile and are accumulated in circulating extraction solvents. They are chemically very active and can therefore reduce the quality and hence the value of the oil. The glucosinolate level of modern cultivars of oilseed rape is low enough to make rapeseed meal suitable for animal production (Krzymanski, 1993). The level of glucosinolate concentration depends mainly on the site, cultivar and sowing date. Generally, under optimum growth conditions nitrogen increases the glucosinolate concentration of the seeds (Bilsborrow et al., 1993).

In our experiments, the level of GSL remained low during the seasons 2001–2002 and 2004–2005 but exceeded 20 mmol per kg (Fig. 5) in 2004–2005. This suggests that drought (3.3 times less precipitation in July) before harvest tends to increase the GSL concentration of the seeds.

Date of sowing also affects the GSL concentration of the seeds. In the late sown crops (22<sup>nd</sup> and 29<sup>th</sup> of August) it increased significantly (16.4,  $p < 0.05$  and 20.3 mmol per kg,  $p < 0.05$  respectively) and in the early sown crops it was significantly reduced (13.8 mmol per kg) (Fig. 5). The crops sown later in autumn do not tolerate extreme environmental conditions (very cold temperatures, drought) and their glucosinolate concentration appears to increase.





**Figure 5.** Glucosinolate concentration ( $\text{mmol kg}^{-1}$  in DM) in winter oilseed rape cv. 'Express' seeds: (1) 2001–2002, (2) 2003–2004, (3) 2004–2005 and (4) average of three growing seasons. Vertical bars denote 0.95 confidence intervals. Different letters indicate significant differences ( $p < 0.05$ ) between sowing dates. \* – no seeds were harvested.

## CONCLUSIONS

This study shows that sowing date and environmental conditions affect the seed quality of winter oilseed rape. Early sowing (8<sup>th</sup> and 15<sup>th</sup> of August) yielded seeds with an oil concentration of 50.2% and 49.4%, respectively. These data were significantly higher than the values for 22<sup>nd</sup> and 29<sup>th</sup> of August (48.2% and 47.8%), respectively. The oil concentration was particularly negatively affected when the rape seeds were sown on the 29<sup>th</sup> of August and the plants were in the 3–4 leaf stage when winter started. The oil

and protein yield were higher in the early sown crops because the seed yield was higher. Also the GSL concentration of the seeds was affected by the time of sowing and weather conditions. Shortage of rainfall before harvest increased the GSL concentration in the seeds. Plants sown in late August did not tolerate the extreme environmental conditions and their seed glucosinolate concentration appeared to increase.

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