

Effect of Agronomical Factors and Harvest Year on Winter Rape Seed Yield, its Quality, Crude Fat and Protein Yield

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ABSTRACT

In this paper we present the results of studies on the impact of harvest year (1999-2002), sowing times, nitrogen rates in fungicide protected and unprotected plots on the seed yield, concentrations of crude protein (CP), glucosinolates (GSL), crude fat (CF), CF and CP yield of winter rape (*Brassica napus L.*) cv. 'Kasimir'. A split – split plot design was used in the field trial conducted on Endocalcari – Epihypogleyic Cambisol during 1998-2002. Due to different conditions of the year, mineral nitrogen fertiliser rates, sowing time, and fungicide application, the seed yield and chemical composition of oilseed rape varied within the range: yield 0.77-4.54 t ha⁻¹, glucosinolates 1.96-12.17 µmol g⁻¹, fat 39.2-50.1 % DM, protein 15.5-23.8 % DM, CF yield 337.7- 2034.2 kg ha⁻¹, CP yield 130.3- 987.3 kg ha⁻¹. The effect of the environmental conditions of harvest year was especially significant on the yield and on all seed chemical composition components tested, and the effect of agronomic factors was diverse in different years. Yield, CP, CF and GSL concentrations in seed were strongly affected by nitrogen rates each year. Sowing time had a significant effect on the seed yield in 2000-2002. Winter oilseed rape sown in the middle of August produced the highest seed, CP, CF yield. Sowing time significantly influenced (0.01 probability level) protein and fat accumulation in rape seed, but the trend of the effect depended on the growing conditions of the year. Fungicide application significantly increased winter rape seed yield in 2001 and 2002, but had the least effect of all the tested agronomical practices on rape seed yield quality formation, and the trend of the effect on the concentration of individual quality components depended on the year.

INTRODUCTION

Oilseed rape (*Brassica napus L.*) for both nutritional and industrial needs is required to produce seed containing an high oil content as high as possible with a fatty acid composition that is optimal for the intended end-use. Environmental and management factors can influence seed yield and quality (6-8, 11, 14, 21, 28). The most comprehensive studies in scientific publications have been done into the effect of nitrogen fertiliser (1, 2, 5, 8, 15). With the increase of nitrogen rates and a delay in nitrogen application the content of protein in double-low spring oilseed rape seed was increasing and the content of fat was decreasing (8, 24). The nitrogen rates, timing of application and plant supply with nitrogen, phosphorus and potassium during the growing season had some effect on the variation of GSL content (8.3 - 15.7 m mol/g) in the seed (9). GSL content varied more through the genotype effect and supply of plants with sulphur rather than other agronomic factors and the higher the GSL content is typical for the cultivar, the higher the response to sulphur fertilisation is (29, 30).

One of the major factors determining rape seed yield and its quality formation process is environmental conditions. Year and region could be far more important factors than cultivar on oil and seed protein concentration, but the opposite was true for GSL (13, 20). Weather conditions determine sowing time, soil moisture, temperature, during the crops' reproductive stage, and disease occurrence on crops. Quality is markedly affected by temperature fluctuations and moisture shortage at specific plant growth stages (3, 27, 28). This fact was corroborated by Mailer's results summarised over many years (13) : in 1994, a particularly dry year, the Australian crop averaged only 39% oil content on an as received moisture content and was as low as 34% at some sites. Radiation also had some effect on seed quality, it especially stimulated accumulation of fat (10). On average, oil content fell by 0.38% per 1.0°C increase in spring maximum temperatures (20).

There were significant differences in the effect of sowing time on yield and oil (21, 26, 28). Early sowing always gave high yield and oil, regardless of the location or variety. This effect was more dramatic in low than in high rainfall locations. Locations with long growing seasons, early sowing and flowering, all provide extended duration of plant development after flowering. The advance in flowering date allows seed development to occur in higher rainfall and cool temperatures, both factors correlated with yield and oil content (13, 21, 27).

Diseases and pests can significantly decline seed yield, and this decline is not compensated by nitrogen fertilisation (19). Crop morbidity affects yield and its quality. In winter rape experiments, disease appeared to affect seed oil content, but there were differences between double-low and high erucic acid rape cultivars (14). Seed from fungicide untreated plots of double-low cultivars usually had lower oil contents (up to a difference of about 5%) than corresponding fungicide treated plots. Glucosinolate content increases in the levels of specific glucosinolates

increased damage caused by aphid (25). However, enhanced levels of glucosinolates were not associated with correspondingly high levels of resistance to *Brassica* pathogens and may even have led to increased susceptibility (18).

Quite changeable climatic and soil conditions determine sowing and ripening time, therefore it is vital to estimate the factors that play a decisive role for the quality of seeds grown in Lithuania. The aim of the present study was to identify the significance of the effects of such agronomical factors as sowing time, nitrogen rates, fungicide use and harvesting year as well as environmental factor, on winter rape seed quality, CP, CF and seed yield.

MATERIALS AND METHODS

Material: rapeseed growing conditions. Winter oilseed rape was grown on an Endocalcari – Epihypogleyic Cambisol, neutral light loam in the Central lowland of Lithuania. The content of available phosphorus (P_2O_5) in the topsoil was 137-221, in the subsoil 80-217 $mg\ kg^{-1}$, available potassium (K_2O) 124-168 and 105-159 $mg\ kg^{-1}$, respectively, and the contents of P_2O_5 and K_2O were measured by Egner-Riem-Domingo method. The content of organic carbon (Tyurin method) in the plough layer ranged from 0.77 to 1.11 %, the plant available sulphur (turbidimetric method) amounted to 1.4 $mg\ kg^{-1}$. Pre-sowing the soil received granulated superphosphate and potassium chloride at a rate of $P_{90}K_{120}$. Hybrid cv. ‘Kasimir’ was sown at a rate of 1.5-2.0 million viable seed per ha at different dates: end of July – beginning of August, middle of August, and end of August. The plants were fertilised with ammonium nitrate at rates of $N_{0, 60, 120, 180, 240}$ in spring at the beginning of stem elongation stage. The effects of sowing time and nitrogen fertilisation on seed chemical composition were studied in the treatments with and without the fungicide Folicur (a. i. tebuconazole) application at a rate of 0.25 $kg\ a. i. ha^{-1}$ at the end of flowering stage. Weed and pest control was used according to the local recommendations.

The weather conditions during the resumption of vegetative growth of winter rape are provided in Fig. 1. The weather during the winter months was warmer and precipitation rate slightly higher than perennial average. Consequently, the plants exhibited a satisfactory over winter survival during all experimental period. The lowest temperatures during seed formation and maturation period were recorded in 2000. The year 2002 was exceptional with warm and dry weather from the beginning of flowering, which significantly advanced flowering and seed maturation. Part of the seed withered up in siliques.

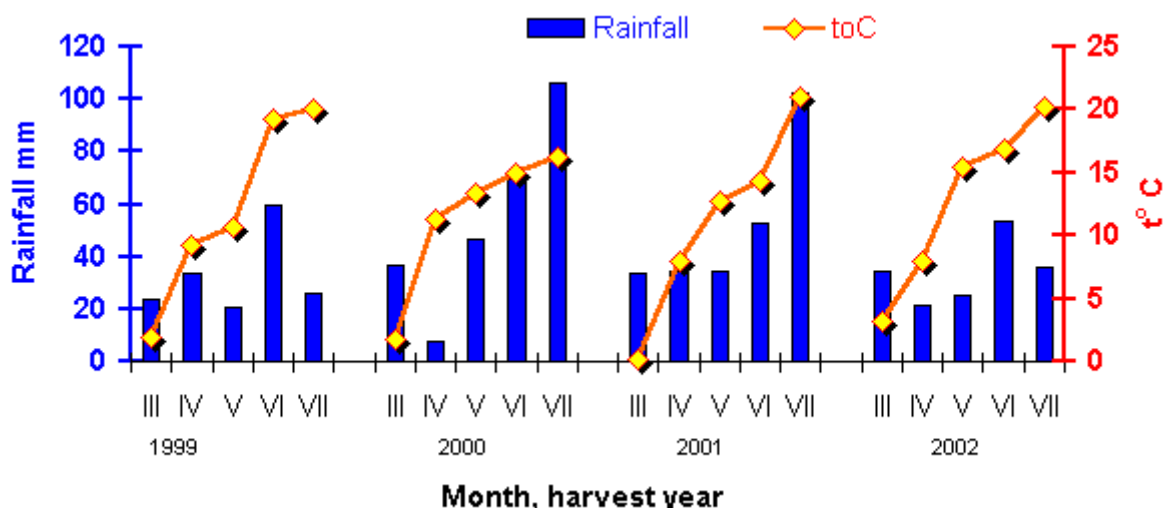


Figure 1. Monthly mean air temperature and rainfall rate (R) in mm during the resumption of vegetative growth of the experimental period

Methods of the quality analysis of rape seed. Intact seed samples were scanned on a monochromator NIR Systems model 6500 (Perstorp Analytical, USA). Quality components of seed were predicted by equations, developed at the Laboratory of Chemical Tests of LIA. The database of NIR spectra was composed of rape seed samples of different varieties grown in various areas of Lithuania from 1994 to 2001. The following statistics of equations are presented in Table 1: number of samples in calibration set (N), mean values by reference (*mean*), coefficients of determination in calibration and cross-validation (RSQ and 1-VR, respectively), standard errors in calibration and cross-validation (SEC and SECV, respectively). There are also presented the statistics of the accuracy in prediction of seed quality by the same equations in the set of 24 samples selected from the present experiment.

Table 1. Statistics of the accuracy of equations

<i>Rapeseed quality component</i>	<i>In calibration and cross-validation</i>						<i>In prediction</i>	
	<i>N</i>	<i>Mean</i>	<i>SEC</i>	<i>RSQ</i>	<i>SECV</i>	<i>1-VR</i>	<i>SEP</i>	<i>R²</i>
<i>Crude fat (CF)</i>	696	42.13	0.53	0.94	0.61	0.92	0.61	0.92
<i>Crude protein (CP)</i>	577	20.73	0.54	0.93	0.56	0.92	0.58	0.96
<i>Glucosinolates (GSL)</i>	311	12.34	1.84	0.96	2.16	0.95	1.20	0.94

Seed quality components contents for both calibration and prediction sets were determined by the following reference methods: crude protein (CP) - by Kjeldahl method; crude fat (CF) - by the method ISO 659 modification, when oil content in

a sample is calculated by the residue of defatted by hexane matter after extraction. Glucosinolate (GSL) content was analysed by different methods: for calibration set by gas chromatography of silyl derivatives at the Institute of Plant Breeding and Acclimatisation, Poland (17), and for prediction set by isocratic HPLC ISO/AWI 9167-3 in the Laboratory of Analyses of CETIOM.

All data were subjected to analysis of variance (ANOVA) to identify the significance of the tested factors on chemical composition of seed. Statistical tests, including variation of quality data, were computed using the software Statistica.

RESULTS AND DISCUSSION

Variability of rape seed quality, CP, CF and seed yield, data of analysis of variance. During the experimental period the seed yield varied with and within a year within the 0.77 - 4.54 t ha⁻¹ range (Table 2). The data due to the agronomic practices applied were especially varied in 1999, 2001 and 2002, when the yield in individual treatments differed more than 3 or even 4 times, and the variation coefficients were 29.09-29.82%. High seed yield matured under the conditions of the year 2000. This determined that crude fat and protein yield in 2000 was about 1.6-1.9 times higher, than in the other experimental years. Although the differences in seed yield and main quality components (CP and CF) between treatments were notably large that year, the statistical variation of data was the lowest.

Table 3. Variation of rape seed, crude fat, crude protein yield

<i>Variable</i>	<i>Harvest year</i>					<i>Coefficient of variation %</i>
		<i>Mean</i>	<i>Min.</i>	<i>Max.</i>	<i>SD</i>	
<i>Seed yield</i> <i>t ha⁻¹</i>	1999	1.89	0.81	2.74	0.56	29.82
	2000	3.57	2.37	4.54	0.50	13.88
	2001	2.03	0.77	3.14	0.59	29.09
	2002	2.16	0.79	3.05	0.64	29.52
<i>Crude fat yield</i> <i>kg ha⁻¹</i>	1999	859.4	386.1	1223.6	235.6	27.42
	2000	1583.0	476.4	2034.2	242.37	15.31
	2001	891.5	337.7	1392.0	268.15	30.08
	2002	936.2	348.2	1348.1	267.78	28.60
<i>Crude protein</i>	1999	381.0	130.3	616.2	134.41	35.28
	2000	745.4	240.8	987.3	139.46	18.71

<i>yield</i>	2001	405.3	150.5	657.8	119.87	29.58
<i>kg ha⁻¹</i>	2002	458.2	167.4	661.8	142.19	31.03

GSL content was found to be the most variable parameter among the tested parameters (content of crude protein, crude fat and GSL content). Variation coefficient (CV) of the total GSL content was as high as 21.98- 39.97 %. A more detailed discussion of the variability of rape seed yield and quality parameters will be provided in our forthcoming publication (6). The response of seed yield, its quality to the agricultural practices used was different annually.

To understand the reasons of such variability of the investigated parameters three-way analysis of variance (ANOVA) was applied for fungicide-treated and untreated treatments separately and was carried out according to the following scheme: A factor sowing date, B factor harvest year, C factor nitrogen rates were used to reveal the significance of factors for rape seed quality (Table 2, 3). All the tested factors were significant at $P < 0.01$ or $P < 0.05$ probability for rapeseed yield both in the crops protected from fungal diseases and in unprotected crops. The interactions of these factors significantly affected seed yield in fungicide –untreated plots.

Table 2. Analysis of variance (ANOVA) for significance of the effect of sowing time, year, nitrogen fertilisation rape on seed yield and quality. Without fungicide application.

<i>Variable</i>	<i>Yield</i>	<i>CF</i>	<i>CP</i>	<i>GSL</i>	<i>CF yield</i>	<i>CP yield</i>
<i>Sowing time (S)</i>	30.37**	9.55**	12.00**	1.93	32.81**	13.35**
<i>Harvest year (Y)</i>	830.8**	48.65**	34.93**	13.3**	678.46**	806.28**
<i>Nitrogen rate (N)</i>	330.1**	29.38**	51.46**	12.52**	216.97**	362.24**
<i>SxY</i>	15.22**	7.08**	14.03**	3.87**	14.14**	9.07**
<i>SxN</i>	2.37*	0.40	0.51	0.59	2	2.04*
<i>YxN</i>	4.53**	2.49**	6.36**	4**	4.41**	3.45**
<i>SxYxN</i>	2.03**	0.74	1.03	0.91	1.33	2.6**

Significant differences between treatments were measured after Fisher's PLSD at a significance level of * $P < 0.05$ and ** $P < 0.01$

In the protected plots the interaction between nitrogen fertilisation rates and sowing time and harvest year had a significant effect on seed yield (Table 3). The effect of the harvest year was significant at $P < 0.01$ probability for the tested seed chemical composition components both in the fungicide- treated and untreated plots. Sowing time had a significant effect on CP, CF concentration and their yield in rapeseed

harvested in the fungicide untreated plots, however the discussed factor was significant ($P < 0.01$) for GSL concentration in rape seed in fungicide treated plots. In the fungicide-treated plots oilseed rape sowing time did not have any significant impact on CP and CF concentration in seed, however nitrogen fertilisation was significant for the accumulation of all components of seed chemical composition, the same as on CP and CF yield in the fungicide-untreated plots.

Table 3. Analysis of variance (ANOVA) for significance of the effect of sowing time, year, nitrogen fertilisation on rape seed yield and quality. Fungicide applied at the end of oilseed rape flowering.

<i>Variable</i>	<i>Yield</i>	<i>CF</i>	<i>CP</i>	<i>GSL</i>	<i>CF yield</i>	<i>CP yield</i>
<i>Sowing time (S)</i>	4.79*	1.98	2.34	19.26**	5.40**	4.61*
<i>Harvest year (Y)</i>	248.0**	62.38**	41.37**	59.01**	235.65**	244.56**
<i>Nitrogen rate (N)</i>	93.43**	24.69**	35.11**	7.64**	74.98**	103.16**
<i>SxY</i>	1.63	7.53**	16.77**	3.22**	2.02	2.61*
<i>SxN</i>	2.86*	0.90	0.44	0.63	3.04**	2.37*
<i>YxN</i>	2.28*	3.90**	5.90**	1.21	2.39**	1.77
<i>SxYxN</i>	1.19	0.44	0.60	0.53	1.03	1.16

Significant differences between treatments were measured after Fisher's PLSD at a significance level of * $P < 0.05$ and ** $P < 0.01$

The interaction between sowing time and harvest year was significant at $P < 0.01$ probability for the concentration of all chemical composition components in seed irrespective of fungicide use, but less significant ($P < 0.05$) for CP yield and statistically not significant for CF yield in fungicide treated plots. No significance of effect of interaction nitrogen x sowing time was found on seed quality components concentration. The interaction of harvest year with nitrogen fertilisation was significant for the concentration of crude fat, protein and CF yield in rape seed irrespective of fungicide application, whereas GSL and CP yield significantly depended on the interaction between these factors only in the fungicide-untreated plots. Assessment of the significance of the tested factors for winter rape seed chemical composition according to F criterion revealed that environmental conditions during winter rape growth and seed maturation period are the main factor for the seed yield, and accumulation of each of the tested component. This observation does not contradict the inferences about the significance of the air temperature and amount of precipitation at different plant development periods for the seed yield and chemical composition of seed, made by other countries' researchers (10, 20, 28). To estimate which of the agronomic

practices applied exerted the most decisive effect on seed yield and quality in separate years, a three-way analysis of variance - nitrogen fertiliser rate, sowing time and fungicide use as factors, was applied to the data set separately for each experimental year.

Nitrogen rates. Nitrogen fertiliser rates had a significant effect on rape seed yield and quality composition formation (Fig.2).

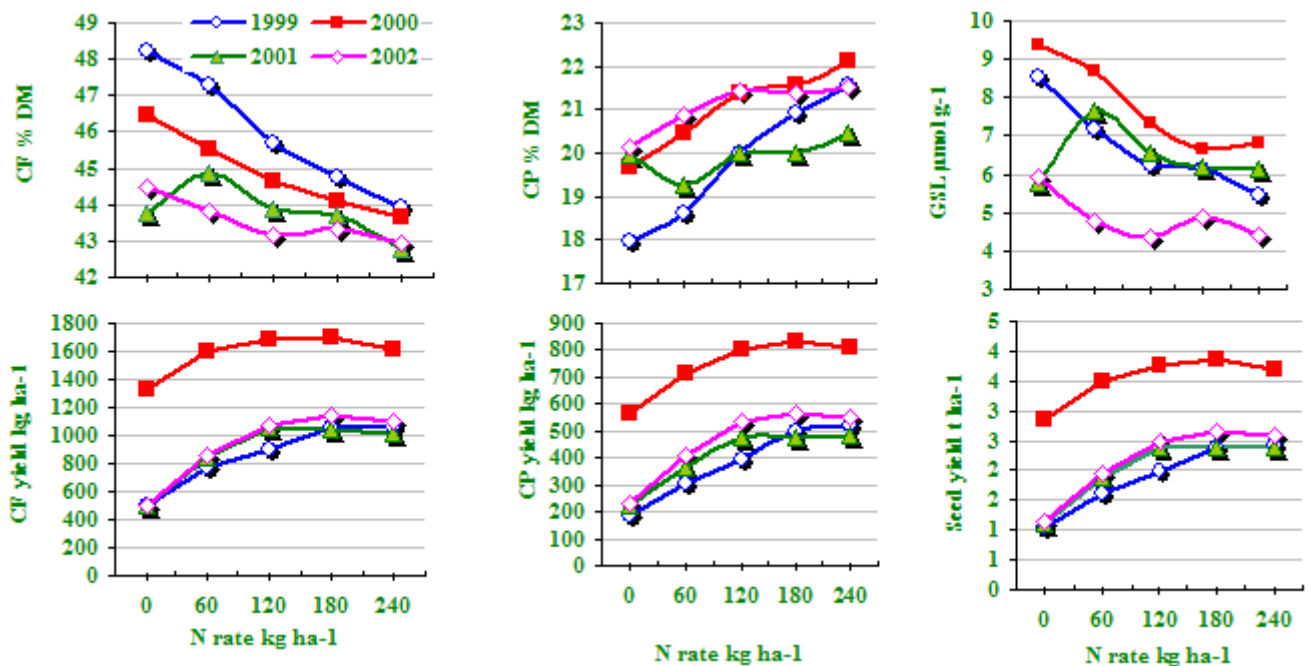


Figure 2. Mean of rape seed quality and yield for each nitrogen rate averaged over all other treatments, i.e., seeding date, fungicide treatment and replications.

It is noteworthy that statistical analysis of each year's data showed that the effect of this factor differed from year to year. Each year nitrogen fertilisation had a significant effect (at $P < 0.01$ probability level) on rape seed, CF and CP yield, however the relationship was different (Table 4). Only in 1999 the correlation between mean values of these parameters and nitrogen rates was linear with high coefficients: $R = 0.968^{**}$, 0.954^* and 0.983^{**} , respectively. In our experiments we observed, that in 2000-2002 only the initial rates of nitrogen effectively increased seed and its quality components yield: when nitrogen rates above 120 kg ha^{-1} had been applied, the yields either did not increase or increased inappreciably. Increasing nitrogen fertiliser rates reduced CF and GSL concentration and increased CP content in winter rape seed. These relationships were expressed by high linear or nonlinear regression correlation coefficients, except for the year 2001, when in nitrogen-untreated plots the seed contained more protein and less fat and GSL compared with the rape seed from nitrogen-treated plots (Fig. 2, Table 4). However, even in the year 2001 when nitrogen effect on seed chemical composition was not even, the inverse correlation between mean CP and CF values

was high. Many authors emphasise the significance of nitrogen fertilisation. Most of them point out that a better supply of rape with nitrogen stimulates the processes of protein biosynthesis in plants and an increase in crude protein (CP) content in rape seed, whereas it slows down fat synthesis (1, 4). Although the effects of nitrogen rates on CF concentration were negative, there was a trend towards increasing seed yield for nitrogen rate and the initial rates of nitrogen effectively increased the CF yield (24, 26). Our experiments showed a decrease in GSL content in seed of winter rape with increasing of nitrogen rate. That agrees with results of other researchers (22, 31).

Table 4. Correlations between nitrogen rates (N) and mean values of rape seed yield and quality components

Pair	1999	2000	2001	2002
NxCP	0.992**1	0.977** 1	NS	0.897* 1
NxCF	-0.993** 1	-0.986** 1	NS	-0.926* 1
NxGSL	-0.955* 1	-0.942* 1	NS	NS
NxSeed Yield	0.968** 1	0.998**q	0.991**e	0.999**q
NxCF Yield	0.954* 1	0.995**q	0.966**e	0.9997**q
NxCP Yield	0.983** 1	0.996**q	0.992*q	0.999**q
CPxCF	-0.999** 1	-0.995** 1	-0.988** 1	-0.988** 1
CPxGSL	-0.949* 1	-0.967** 1	NS	-0.940* 1
CFxGSL	0.960** 1	0.977** 1	NS	0.926* 1

Significant at *P<0.05 and **P<0.01 probability levels; NS not significant , NC- not computed, l-linear, q-quadratic, e- exponential relationship

When the plant supply with sulphur is insufficient the larger part of sulphur will be used in the first chain of metabolism - for the synthesis of sulphur-containing amino acids and proteins (2). Consequently biosynthesis of GSL might be suppressed and we found the negative correlation between CP and GSL content (Table 4). The other factor, having a negative effect on the accumulation of GSL, could be the "dilution" of concentration of GSL in the increased seed yield.

Sowing time. Sowing time significantly (0.01 probability level) affected protein and fat concentration in rape seed in each experimental year, however the trend of the effect depended on the growing conditions of the year (Fig. 3). Early sowing, i.e. end of July – beginning of August, was the most favourable sowing time in terms of higher concentration of fat in rape seed in 1999 and 2001 harvest year.

However, in 2000 and 2002 harvest years the seed harvested from the plots sown at a later date, i.e. middle of August had a higher fat content. With regard to GSL accumulation, the effect of sowing time was significant at 0.01 probability level for seed of the 1999-2001 harvest, and the highest content of these metabolites was accumulated in the seed of early sowing. This might be linked with the plant growth stage and soil moisture content in spring. In 2002, the year characterised by exceptional weather conditions the highest content of GSL was identified in the seed sown at the latest terms, however, GSL accumulation differences resulting from the sowing time were statistically not significant that year. The effect of the sowing time on seed yield was significant in all experimental years, except for the year 1999. On average, the highest seed yield 2.55 t ha⁻¹ was produced by rape crops sown in the middle of August. Oilseed rape crops sown at late dates were not able to properly develop in autumn and consequently prepare for wintering, which determined a much poorer development in spring. Rape crops sown too early, in very favourable conditions in the autumn tend to overgrow, the stem starts to grow, which makes the apical point sensitive to winter colds.

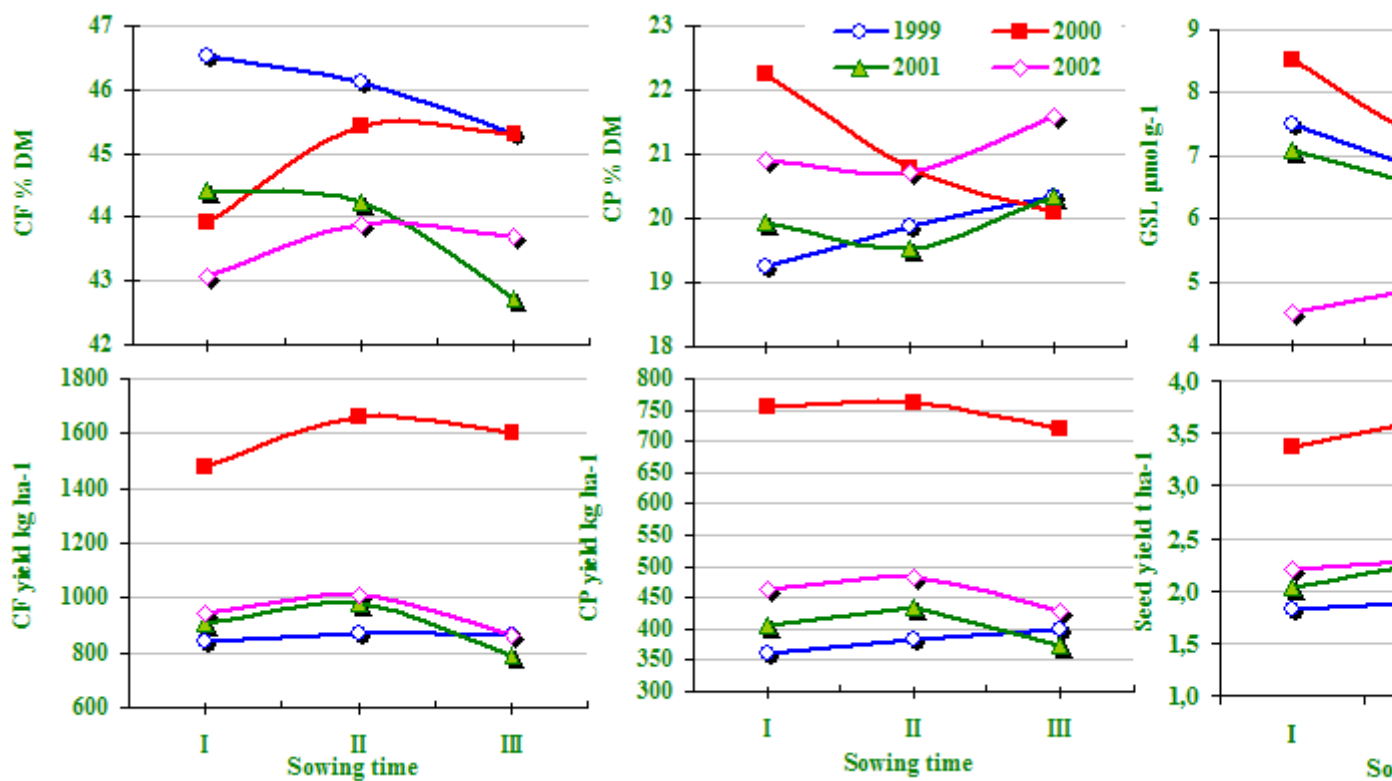


Figure 3. Mean of rape seed quality and yield for each sowing time averaged over all other treatments, i.e. nitrogen rate, fungicide treatment and replications. Sowing time: I- end of July/beginning of August; II- middle of August; III – end of August.

Early sowing, i.e. end of July – beginning of August, was the most favourable sowing time in terms of higher concentration of fat in rape seed in 1999 and 2001 harvest year. However, in 2000 and 2002 harvest years the seed harvested from the plots sown at later date, i.e. middle of August had a higher fat content. With regard to GSL accumulation, the effect of sowing time was significant at 0.01 probability level for seed of the 1999-2001 harvest, and the highest content of these metabolites was accumulated in the seed of early sowing. This might be linked with the plant growth stage and soil moisture content in spring. In 2002, the year characterised by exceptional weather conditions the highest content of GSL was identified in the seed sown at the latest terms, however, GSL accumulation differences resulting from the sowing time were statistically not significant that year. The effect of the sowing time on seed yield was significant in all experimental years, except for the year 1999. On average, the highest seed yield 2.55 t ha^{-1} was produced by rape crops sown in the middle of August. Oilseed rape crops sown at late dates were not able to properly develop in autumn and consequently prepare for wintering, which determined a much poorer development in spring. Rape crops sown too early, in very favourable conditions in the autumn tend to overgrow, the stem starts to grow, which makes the apical point sensitive to winter colds. Sowing time did not have any statistically significant effect on the CF yield only in one year -1999, for CP yield – 2000. Significant sowing time effect was found for seed yield, oil content, oil yield by researchers in Virginia, USA (26). The significant differences in the effect of sowing time on yield and oil regardless of the location in Australia or variety were found in the study of Walton and co-workers (28).

Fungicide use. Fungicide use was least effective of the three agronomical practices intended for yield quality formation. Averaged findings suggest that the use of fungicides had a significant effect on all the tested parameters of seed and oil only in 2002 (Fig. 4). The effect of plant protection measures against fungal diseases on CF concentration, seed, CP and CF yield was significant for rapeseed that matured

in the conditions of 2001 and 2002.

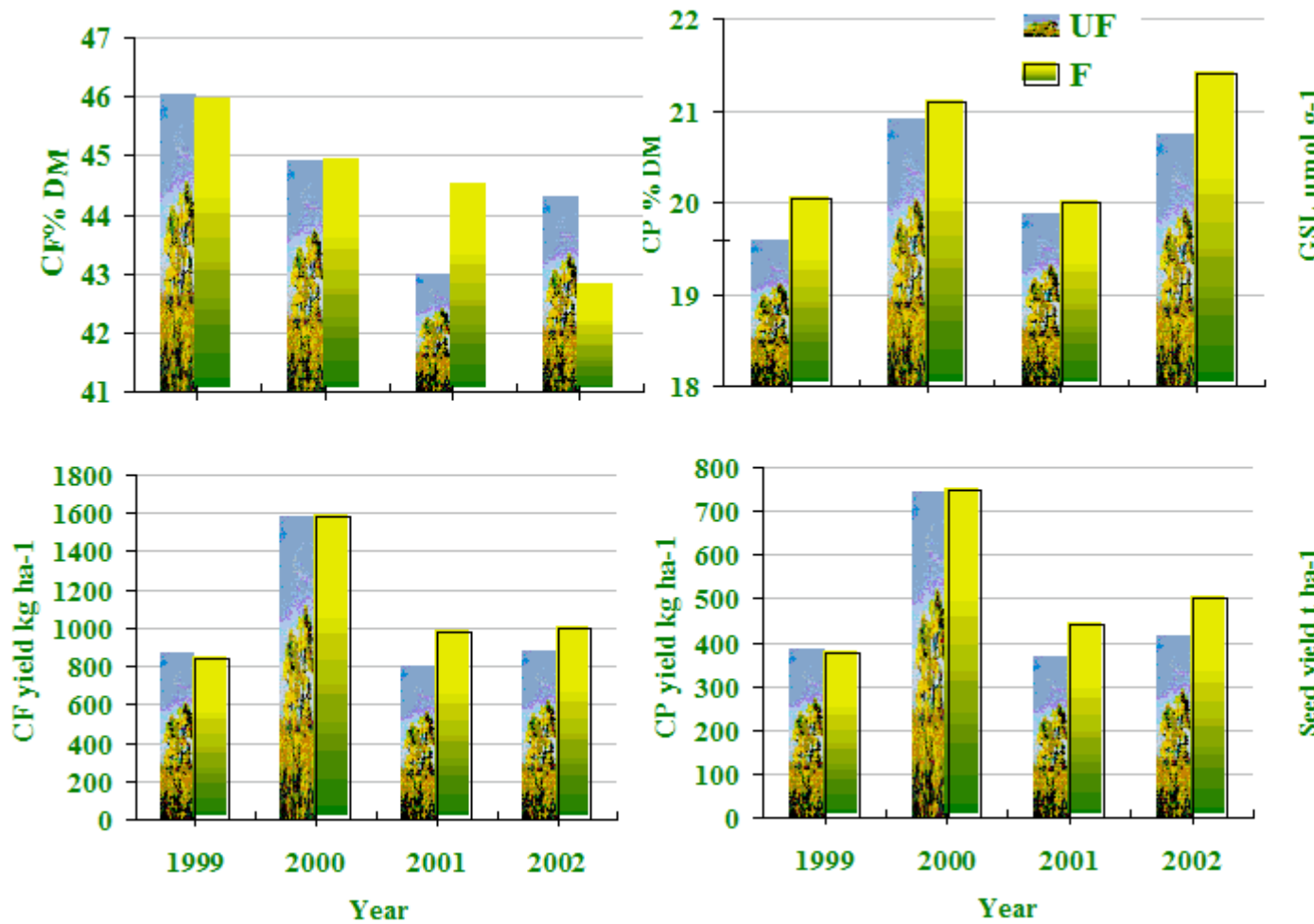


Figure 4. Mean of rape seed quality and yield for different fungicide use treatments, data averaged over all other treatments, i. e, nitrogen rate, sowing time and replications. Fungicide use: UF-fungicide unprotected, F- fungicide protected.

Significant effects of the fungicide on protein concentration were identified for the 1999 and 2002 harvest years. Significant effect (0.01 probability level) of the fungicide on GSL content was recorded only for the seed yield of 2002. The trend of fungicide use effect was different for the accumulation of each of the quality components and depended on the year. Only CP concentration in the seed yield from the fungicide protected plots was usually higher compared with unprotected plots. In general, the differences in most seed quality components, resulting from crop management factors, such as fungicide use, sowing time, were lower than those resulting from environmental factors and weather conditions in harvest year.

CONCLUSIONS

- Due to different conditions of the year, mineral nitrogen fertiliser rates, sowing time, and fungicide application, the seed yield and chemical

composition of winter oilseed rape cv. 'Kasimir' varied within the range: seed yield 0.77-4.54 t ha⁻¹, CF 39.2-50.1 % DM, CP 15.5-23.8 % DM, GSL 1.96-12.17 μmol g⁻¹, CF yield 337.7- 2034.2 kg ha⁻¹, CP yield 130.3- 987.3 kg ha⁻¹.

- The effect of the environmental conditions of harvest year was especially significant on the yield and all seed chemical composition components tested, the effect of agronomic factors was diverse in different years.
- Seed, CP, CF yields, and CP, CF, GSL concentrations in seed were strongly affected annually.
- Sowing time significantly influenced (0.01 probability level) protein and fat accumulation in rape seed, but the trend of the effect depended on the growing conditions of the year.
- Of the agronomical practices tested fungicide application was the least effective on rapeseed yield and quality formation in the conditions of the experimental years.

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