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Dissection of Year Related Climatic Variables and Their Effect on Winter Rapeseed (*Brassica Napus* L.) Development and Yield

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Abstract: Winter oilseed rape (WOSR) production is dependent on weather conditions, but is also characterized by low nitrogen (N) use efficiency. The objectives of this study were to: (i) evaluate sources of variability for the seed yield and oil content of four rapeseed cultivars under the influence of three sowing dates (SD trial) and five nitrogen dosages (N trial) during four growing seasons; (ii) understand year-related interactions and the effect of climatic variables in different growth stages; and (iii) assess the presence of interactions cultivar by year (C × Y) and treatment by year (T × Y). Six climatic factors were observed, during germination, overwintering, budding, flowering and ripening. The mixed effect split-plot analysis of variance was used, as well as factorial regression models. The C × Y interaction was the most important for the oil content in both trials. The precipitation at budding stage (75.8%), relative air humidity at overwintering (63.3%) and flowering stage (53.0%) accounted for the highest proportion of T × Y interaction for the seed yield, as well as precipitation at flowering (92.0%) and ripening (85.0%) for the oil content. Water availability was the main determinant of the seed yield and/or oil content accompanied with cooler temperatures during the seed development. The study successfully dissected the effect of year-related climatic variables on the agronomical traits in winter rapeseed. Based on this, appropriate agronomic practices can be applied at specific growing stages to ensure a high seed and oil yield.

Keywords: variability; sowing date; nitrogen dosage; climatic factors; phenology

1. Introduction

Rapeseed (*Brassica napus* L.) is the most produced oilseed crop in Europe, with a global production area exceeding 31 million hectares. The major producers are Canada, China, India, Australia, France and Germany. Rapeseed oil is the second most important vegetable oil in the world trade, with an annual production of over 21 million tons [1].

Considering the diversity of agro-ecological conditions in the areas where rapeseed is grown, a constant effort is made to define optimal agricultural practices to approach the genetic potential of cultivars' seed and oil yield. The seed composition of rapeseed varies widely, depending on both the genetic and environmental factors and their interaction [2]. The oil content ranges from 33% to 48%, while the oil-free meal contains 30% to 58% protein [3,4]. These variations are more affected by the environment and production system than the cultivar [5,6].

A number of authors have evaluated the performance of different cultivars in diverse locations and/or years to obtain information about their performance and stability [7–10]. The information on the (C × E) interaction provided valuable data to plant breeders and agronomists for the identification of superior cultivars in specific environments, and defined site-specific best management practices [11]. A further step in the C × E interaction analysis for rapeseed would be to investigate the effect of specific climatic variables throughout developmental stages. Such data could be used to dissect the year effect and determine which variables are the most significant for an optimal plant development at each growth stage.

Rapeseed growth is controlled from the seedling emergence to flowering by photo-thermal factors and from flowering to maturity by temperature [12]. In winter rapeseed, the juvenile growth with emergence, overwintering and stem elongation is the longest growth phase. If the field conditions are optimal, the temperature is the main factor determining seedling germination and emergence. Temperatures lower than 10 °C result in poor germination, while dry weather conditions and a high temperature can also negatively affect seedling emergence [13,14]. Because of the crop sensitivity in the early stages of development, the seed yield of canola is often limited by a poor plant establishment [15]. From the beginning of the leaf development to the end of stem elongation, the phenological development is controlled by the temperature, vernalisation and photoperiod [16]. If the optimal stand establishment is not achieved before the onset of winter, low temperatures and a low light intensity during winter can cause a dramatic loss of foliage and thus of stored N, as well as a reduced leaf area index [17,18]. Increased temperatures and photoperiod stop the vernalisation period as the day length increases [11].

When temperatures stay continuously above 0 °C, rapeseed grows rapidly and produces most of the aboveground biomass over a period of several weeks. Optimum temperatures for photosynthetic, vegetative, and reproductive processes vary from 21 to 25 °C [19]. Excessive precipitation during the pre-flowering phase can be detrimental for the yield, even though a large biomass is produced during flowering [5]. Flowering is the most critical stage of rapeseed development, because of the decrease in the total leaf area and reduced photosynthesis [20,21]. Frost at the beginning of the flowering increases branching, and the flowering lasts longer, resulting in an increased number of poorly filled pods, formed on the lower branches [22,23]. If the post-flowering period is water limited, the yield will be low, as canopy transpiration cannot be met [24]. Drought stress reduces the total dry matter production, but cultivars were found to possess varying levels of drought tolerance [25].

Rapeseed growth stages are used to define the main components in yield estimation models [24]. Each of them can be affected by yield-limiting factors, of which climatic conditions are the most important ones. Temperature, irradiation and precipitation directly and indirectly affect and determine the yield [5]. Unfavorable weather conditions may lead to a low sink-capacity, which is not necessarily yield-limiting because of the compensatory effect between the yield components [24]. In the regions with a warmer climate, each 1 °C increase in the ambient temperature results in shorter growth periods. However, the production of the highest seed and oil yields requires rather limited day/night average temperature ranges (20/15 °C and 15/13 °C) [26,27]. Late sowing dates can significantly decrease the number of primary branches and flowers per plant [23]. During the seed development, the temperature has a significant effect on the yield [23]. It affects the duration and rate of assimilation and therefore determines the assimilate availability for seed filling. The yield potential may be determined until the end of flowering, but whether this yield potential is realized or not mainly depends on the temperature and water availability in the subsequent growth phases [24].

Precipitation is the most important source of inter-annual variability that affects the genotype performance, varying significantly throughout the years and canola growing regions [5]. The difference is also caused by the amount of rainfall before sowing and the water holding capacity of the soil, because a greater water availability during flowering promotes a canola seed set [28]. Drought stress occurring before flowering reduces the total dry matter production, while during flowering it reduces the pod density. Both the seed weight and oil concentration decrease if a water shortage occurs between anthesis and maturity [24]. Oilseed rape is thus a water demanding crop, with studies showing that

> 300 mm of water must be available from flowering to maturity to support high yields (more than 4000 kg ha^{-1}) [29,30].

Winter oilseed rape (WOSR) production is dependent on weather conditions and characterized by a low nitrogen (N) use efficiency. The water supply plays a critical role when maintaining a high N use efficiency and reaching grain yields of 4000 kg ha^{-1}, simultaneously [11,31]. Defining site-specific fertilizer strategies based on field trials and crop modeling could help in the alleviation of the N limitation for this crop. The development of crop models has become widely popular for the improvement of site-specific management strategies [11,32]. Nonetheless, they have to be complemented by field trials by setting targets for the site-specific yield and improved N use efficiency and to obtain field data of the cultivar performance for the evaluated region.

The effect of several climatic variables on the winter rapeseed developmental stages and yield in Southeast Europe has not yet been analyzed simultaneously, although their interaction is important to breeders and growers. The objectives of the present study were to (1) evaluate the sources of variability for the seed yield and oil content of four rapeseed cultivars as affected by three sowing dates, five N dosages and four growing seasons, (2) understand the relations between the year-related interactions and the effect of climatic variables in different growth stages, and (3) assess the presence and nature of cultivar × year and treatment × year interactions.

2. Materials and Methods

2.1. Trial Design and Plant Material

The trials were arranged in a split-plot design with randomized complete block settings with four replications. The sowing date trial (SD) comprised three sowing dates (SD1, SD2, and SD3) in 10-day intervals in each year (Table 1). The basic plot in the sowing date trial consisted of 5 rows; it was 4 m long, with a 25 cm row spacing, making the total area of individual plots equal to 5 m^2 and the harvest area of the three central rows 2.93 m^2. In the N rate trials, N-min dosages were applied, as well as 0, 50, 100, and 150 kg ha^{-1}. N-min dosages were determined each year according to the N-min balance method [33,34], one week prior to N fertilization, at depths of 0–30, 30–60 and 60–90 cm. All N fertilizers were applied once in the early spring, on the same day. The values of N taken by the plants and N added to each N-min plot in the N trial are given in the Supplementary Table S1. The N trial was sown at approximately the same time as SD1 in the sowing date trial: 25 August 2001, 22 August 2002, 3 September 2004 and 10 September 2005. The basic plot in the N rate trial consisted of 7 rows; it was 4 m long, with a 25 cm row spacing, making the total area of the individual plots equal to 7 m^2 and the harvest area of the five central rows 4.88 m^2.

Four rapeseed cultivars were used: (1) 'Jet Neuf', a cultivar from France with 1.35% erucic acid, a medium-high shoot, small seeds and a 38% to 42% oil content; (2) 'Banaćanka', a 00 type cultivar from Serbia, with a medium-high shoot, large seeds and a 42% oil content; (3) 'Samourai', a 00 type cultivar from France, with a short shoot, medium-sized seeds and a 40% to 43% oil content; and (4) 'Falcon', a 00 type cultivar from Germany, with a high shoot, medium-sized seeds and a 37% to 43% oil content.

Both trials were performed on the experimental fields of the Institute of Field and Vegetable Crops, Novi Sad (IFVCNS), at the Rimski Šančevi trial site (45°19′51″ N; 19°50′59″ E; altitude 84 m), Serbia. The soil type was Mollisol, and it was fertilized with 250 kg ha^{-1} NPK (15:15:15). Herbicide Trifluralin (Chemical Agrosava, Belgrade, Serbia) (2 kg a.i. ha^{-1}) was applied. N fertilizer was applied in the N trial in the spring (March) at the HB3 (Harper Berkenkamp) growth stage, substage 3.1. [35]. The trials were sown every year on different fields, within a distance of a maximum of 1 km. Wheat was the preceding crop in all trials. The crop rotations were applied, and neither rapeseed nor sunflower or soybean was grown on the sites in the previous 5 years, to prevent carry-over diseases. Carry-over of in-soil nitrogen was controlled by analyses of the soil nitrogen content at each site before sowing. The trials were carried out under conventional tillage. The crops were kept free from weeds, insects and diseases according to the best local practices. Thinning of the crop stand was performed in the autumn at GS2 – the rosette stage [36] provided a plant spacing of 8–10 cm and plant density of 40–50 plants m^{-2}. The crops were harvested manually, when most plants reached the second technical level of maturity [35]. In the N trial, the harvest was done on 01 July 2002, 30 June 2003, 15 July 2005 and 11 July 2006. All plants from each separate plot were threshed together. The seed yield was measured per plot. After the moisture content was determined, the yield was adjusted to 10% moisture as a part of the internal procedure at IFVCNS, and expressed in t ha^{-1}. The oil content was determined using the NMR (Nuclear Magnetic Resonance) [37] and expressed as a percentage of seeds.

2.2. Calculation of Climatic Variables

The rapeseed growth stages were determined using the BBCH (Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie) standardized growth stage (GS) scale [36]: germination (GS0), GS1+GS2 seedling and rosette stage, including overwintering (no growth period), budding (GS3), flowering (GS4), and ripening (GS5). The overwintering period was not analyzed as a separate development stage due to the difficulty of determining the exact duration. The time span of overwintering is usually defined with temperatures below 5 °C that slow down the plant growth, but it also depends on other factors. Nonetheless, the period of time with temperatures below 5 °C is presented and discussed as a potential yield-determining phase (Table 1).

Six climatic factors were observed: the temperature (minimum—mn; minimum on 5 cm above ground—mn5cm; maximum—mx; and mean—mt), total precipitation (pr), and relative air humidity (rh). Each of the climatic factors was calculated for the duration of the individual growth stage based on daily values: germination (mn1, mn5cm1, mx1, mt1, pr1, rh1), overwintering (mn2, mn5cm2, mx2, mt2, pr2, rh2), budding (mn3, mn5cm3, mx3, mt3, pr3, rh3), flowering (mn4, mn5cm4, mx4, mt4, pr4, rh4) and ripening (mn5, mn5cm5, mx5, mt5, pr5, rh5) for each SD and N trial. The minimum values for mn and mn5cm, as well as the maximum values for mx, are not average values for individual growth stages, but absolute minimum and maximum temperatures. The average temperature is obtained by averaging all daily mean temperatures during a growth stage, while the total precipitation (pr) is a summed value for the precipitation during the individual growth stages. As a result, 30 climatic variables were obtained, out of which the mean temperature and precipitation are presented in Table 2 and Supplementary Table S2. Climatic data were obtained from the official meteorological station of the Hydrometeorological Service of the Republic of Serbia (http://www.hidmet.gov.rs/index_eng.php), located about 800 m away from the field trial.

Table 1. The onset and duration of five analyzed development stages (GS0–GS5) in the sowing date (SD) trial, starting with the sowing dates and ending with the harvest dates.

Vegetative and Reproductive Stages	2001/02			2002/03			2004/05			2005/06		
	SD1	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**
GS 0 Germination	25.8.01 *	5.9.01	14.9.01	21.8.02	30.8.02	9.9.02	1.9.04	10.9.04	20.9.04	5.9.05	15.9.05	26.9.05
GS1+2 Seedling and rosette	12.9.01	23.9.01	30.9.01	1.10.02	29.9.02	4.10.02	27.9.04	4.10.04	4.10.04	24.9.05	25.9.05	5.10.05
GS3 Budding	30.3.02	30.3.02	1.4.02	19.4.03	19.4.03	20.4.03	13.4.05	14.4.05	14.4.05	12.4.06	11.4.06	13.4.06
GS4 Flowering	8.4.02	8.4.02	10.4.02	25.4.03	25.4.03	26.4.03	23.4.05	23.4.05	23.4.05	21.4.06	20.4.06	22.4.06
GS5 Ripening	6.5.02	7.5.02	7.5.02	12.5.03	12.5.03	13.5.03	19.5.05	19.5.05	19.5.05	9.5.06	9.5.06	12.5.06
Harvest	26.6.02	28.6.02	30.6.02	20.6.03	23.6.03	24.6.03	2.7.05	4.7.05	4.7.05	1.7.06	1.7.06	2.7.06
Air temperature < 5 °C	5.11.01–14.2.02			7.11.02–12.3.03			15.11.04–18.3.05			5.11.05–24.3.06		

* The dates denote the sowing dates, which also determine the start of the GS 0 period lasting until the date in the following row (GS1+2 Seedling and rosette).

Table 2. The summed precipitation (pr) and mean temperature (mt) values calculated and averaged for the duration of the individual growth stages (GS) based on the daily values: GS0 germination (mt1, pr1), GS1+2 seedling and rosette (mt2, pr2), GS3 budding (mt3, pr3), GS4 flowering (mt4, pr4) and GS5 ripening (mt5, pr5).

Climatic Factor	Climatic Variable	2001/02			2002/03			2004/05			2005/06		
		SD1	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**	**SD1**	**SD2**	**SD3**
Mean temperature (°C)	mt1	16.9	14.3	15.2	17.6	16.8	14.5	16.2	14.7	13.7	17.4	15.3	14.6
	mt2	5.9	5.4	5.0	4.2	4.2	4.1	4.6	4.3	4.3	4.9	4.8	4.3
	mt3	**8.4**	**8.4**	**7.9**	12.7	12.7	13.4	11.6	11.6	11.6	12.4	12.2	13.0
	mt4	13.4	13.5	14.0	**21.2**	**21.2**	**21.8**	14.6	14.6	14.6	14.6	14.4	14.5
	mt5	20.6	20.7	20.7	22.0	22.1	22.2	19.6	19.6	19.6	18.8	18.8	19.2
Precipitation (mm)	pr1	**118**	**148**	**42**	55	46	52	48	43	38	56	47	15
	pr2	**217**	**163**	**160**	241	244	234	**387**	**381**	**381**	296	276	287
	pr3	**0**	**0**	**2**	2	2	3	15	11	11	**40**	**40**	**21**
	pr4	35	35	35	**3**	**3**	**3**	**52**	**52**	**52**	16	17	28
	pr5	101	97	107	**34**	**37**	**37**	**168**	**192**	**192**	163	163	157

The bold values represent extreme low and/or high values for each climatic variable. The detailed values for all analyzed climatic variables are given in the supplementary material.

2.3. Statistical Analysis

The analysis of the experimental data from both experiments was completed by asreml-R (version 3, Department of Primary Industries and Fisheries 2009, Brisbain, QLD, Australia) [38] and asremlPlus (version 4, University of south Australia 2016, Adelaide, SA, Australia) [39] packages. The experiments were set up as split-plot designs with four blocks (replications), where the treatments (N rate and sowing date) were considered the whole plot and cultivars the sub-plot across four years. The linear mixed model was formulated with the following fixed and random terms, where A stands for treatment and B for cultivar: fixed effects ~ Year + A + B + A.B + Year.A + Year.B + Year.A.B; random effects ~ Block.Year + A.Block.Year.

The significance of the fixed effects model terms was tested using F statistics [40], whereas the significance of the random terms was tested using a likelihood ratio (LR) test [41]. In order to properly account for different precision of the same trials in different years, the above-formulated model was fitted as the model with homogeneous residual error variances across the year. In addition, it was fitted as the model with heterogeneous residual error variances among the years, by imposing this relaxed assumption within the R structure of the linear mixed model. The choice among the models was made in accordance with the Akaike Information Criterion (AIC). The model with the lowest AIC value was selected for further discussion and presentation throughout the study. The presence of extreme and erroneous observations, as well as the overall quality of the models, was tested by an alternative outlier mixed model (AOMM) algorithm and diagnostic plots, as implemented in the asreml-R software [42]. The Best Linear Unbiased Estimates (BLUEs) of the fixed effect terms were estimated for all subsequent analyses. In addition, the fixed effects of both trials were separated out by the least significant differences (LSD) test at a given significance level alpha = 0.05.

A factorial regression (FR) model was used to determine the effect of a particular climatic variable on the year-related interactions (i.e., C × Y and T × Y) from the fixed effect part of the linear mixed models as described previously. In order to establish their importance, each environmental variable was included in a one-variable factorial regression model according to the catalogue of Van Eeuwijk [43]. An F test was carried out for each variable to estimate the significance in the effects of the treatments as described by [40], considering the unexplained interaction variation as the denominator [44]. Furthermore, the BLUEs of the fixed effects from the linear mixed model for the seed yield and oil content were organized into two-way tables for the graphical exploration of the interactions according to [45] using the R software (version 3.6.1, R Core Development Team 2012, R Foundation for Statistical Computing, Vienna, Austria).

3. Results

3.1. Weather Conditions

Season 2001/02 had an unusually high precipitation during germination for the first two sowing dates, but the total precipitation before the start of the budding phase (pr1+pr2) was similar to 2002/03 and 2005/06. The season 2004/05 had the highest precipitation values of the four trial years (670 mm) with maximum pr2, pr4 and pr5. In addition, this season was characterized by mild temperatures. In contrast, season 2002/03 was characterized by temperatures that were higher than average during the flowering stage, while precipitation during flowering and ripening was the lowest out of the four trial years (Table 2). A low precipitation also resulted in a relative air humidity of only 51% in the flowering stage, while in the other trial years it varied in the range of 68–71%. Relative humidity is expressed as the average of the values obtained at 7, 14 and 21 h each day, calculated for different growth stages separately. A low precipitation and high temperatures shortened the flowering periods to 17 days in 2002, and to 19 days in 2005. A higher precipitation (pr4) in 2001 and 2004 resulted in a flowering period of 28 and 26 days, respectively. Season 2002/03 was also specific for having the lowest precipitation during ripening (pr5), resulting in the shortest ripening period out of the four trial years.

Historically, according to the data since 1963, the lowest temperatures at the trial site usually occur in January, with an absolute recorded minimum temperature of −28 °C and an average of 24 frosty days. In the trial years, the absolute minimum temperatures during the winter were: −19.1 °C in 2001/02; −25.0 °C in 2002/03; −24.2 °C in 2004/05; and −13.8 °C in 2005/06. Each of those periods included snow covers of 15 cm or more (Table 3).

Table 3. The impact of the year, sowing date and cultivar on the rapeseed overwintering rate, expressed as a plant density (plants/m^2).

Plant Density	01/02	02/03	04/05	05/06	SD1	SD2	SD3	Jet Neuf	Banaćanka	Samourai	Falcon
(plants/m^2) autumn	44	35	90	57	48	61	60	57	67	47	55
(plants/m^2) spring	39	25	79	52	41	52	53	51	59	35	50
Overwintering (%)	89	71	88	91	85	85	88	89	88	74	91
Snow cover (cm)	17	18	27	15							

3.2. Variability of Seed Yield and Oil Content

A model comparison based on AIC favored models with heterogeneous variances for both trials, except for the oil content in the SD trial. A Wald F test showed a highly significant effect of cultivars (C) and years (Y) in both trials for the seed yield and oil content. Treatments (T) were not significant in the SD trial, while in the N rate trial it was highly significant for both the seed yield and oil content (Table 4).

Table 4. Wald F tests on the fixed effects from the mixed model analyses of the sowing date and nitrogen rate trials.

Source of Variation	SD Trial					N Rate Trial				
	df	Seed Yield		Oil Content		df	Seed Yield		Oil Content	
		F	*p*	*F*	*p*		*F*	*p*	*F*	*p*
Year (Y)	3	53.17	0.001 **	94.65	0.000 **	3	35.42	0.001 **	27.92	0.001 **
Treatment (T)	2	0.50	0.608 ns	0.98	0.379 ns	4	15.87	0.001 **	47.27	0.001 **
Cultivar (C)	3	6.19	0.001**	41.53	0.000 **	3	41.27	0.001 **	48.6	0.001 **
C × T	6	2.71	0.019 *	0.46	0.839 ns	12	1.62	0.088 ns	2.33	0.009 **
T × Y	6	0.86	0.535 ns	2.69	0.017 *	12	3.12	0.001 **	4.70	0.001 **
C × Y	9	2.34	0.022 *	7.60	0.000 **	9	4.38	0.001 **	52.42	0.001 **
C × T × Y	18	1.68	0.059 ns	0.81	0.691 ns	36	0.98	0.505 ns	1.49	0.052 ns

* significant at the 0.05 probability level, ** significant at the 0.01 probability level, ns: not significant.

The treatment × year (T × Y) interaction in the SD trial was only significant for the oil content, while the N rate was highly significant for both analyzed traits. The cultivar × year (C × Y) interaction was highly significant for both traits in both trials, except for the seed yield in the SD trial. The cultivar × treatment interaction was significant for the seed yield in the SD trial, while for the oil content it was significant in the N rate trial. A second order interaction was not significant in either trial (Table 4).

The BLUE values of the seed yield and oil content in the SD and N rate trials are summarized in Table 5. The seed yield varied from 1.11 to 2.79 t ha^{-1} in the SD trial, while the oil content exhibited BLUE values ranging from 43.4% to 49.8%. The lowest seed yield coincided with the lowest oil content in 2002. According to the LSD test, the seed yield was significantly higher in the N rate trial compared to the SD trial and varied from 1.82 to 3.75 t ha^{-1}, while the oil content varied from 43.9% to 46.6%. Similar relations were found for the seed yield, whose minimum was noted in 2002 and whose maximum was noted in 2005, while the oil content was the highest in 2004, just as it was in the SD trial (Table 5).

The SD treatments did not cause significant differences in the seed yield and oil content, while the N rates did. The N_0 treatment resulted in the lowest yield (2.36 t ha^{-1}), and the increase in the N rates consequently resulted in a seed yield increase (Table 5). However, the increase of the N rate from N_0 to N_{150} led to a decrease in the oil content from 45.8% to 43.9%.

Cultivars performed significantly differently in both trials. In the SD trial, the seed yield ranged from 1.88 ('Samourai') to 2.36 t ha^{-1} ('Jet Neuf'). A maximum oil content of 48% was found in 'Jet Neuf', while 'Banaćanka' had the minimum oil content of 44.5% (Table 5). In the N rate trial, the maximum seed yields were detected in 'Falcon' (3.08 t ha^{-1}) and 'Jet Neuf' (2.95 t ha^{-1}), which belonged to the same group. 'Banaćanka' and 'Samourai', with 2.67 and 2.26 t ha^{-1}, respectively, were significantly different from the first group and between themselves. The highest oil content was recorded in 'Jet Neuf' (45.7%), while 'Falcon' had the lowest oil content (43.9%), which was significantly different from the oil content of 'Banaćanka' and 'Jet Neuf' (Table 5).

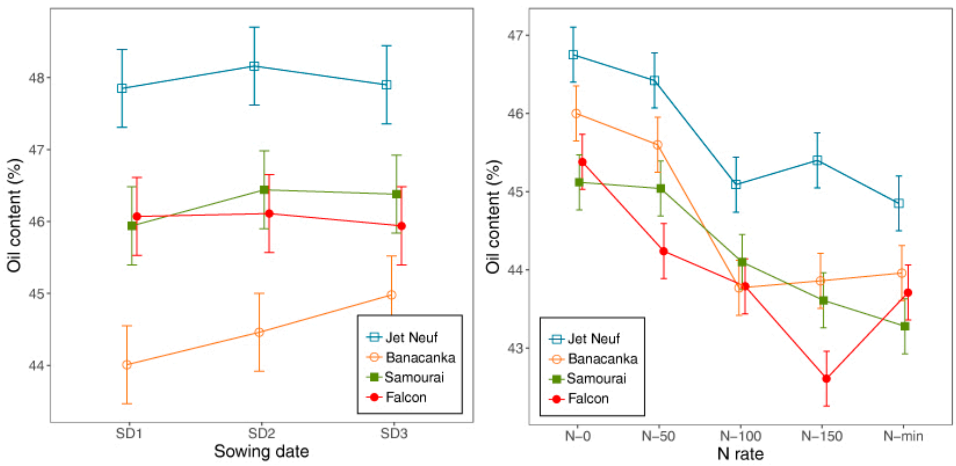
Table 5. The BLUE values for the seed yield and oil content in the sowing date and nitrogen rate trials for the fixed effects of the years, treatments and cultivars.

Factor	Sowing Date Trial		Factor	Nitrogen Rate Trial	
	Seed Yield (t·ha^{-1})	Oil Content (%)		Seed Yield (t·ha^{-1})	Oil Content (%)
Year (Y)			Year (Y)		
2001	2.721 ^{a}	44.91 ^{a}	2001	2.397 ^{ab}	43.96 ^{a}
2002	1.107 ^{b}	43.36 ^{b}	2002	1.815 ^{a}	44.00 ^{a}
2004	1.849 ^{c}	49.81 ^{c}	2004	2.983 ^{b}	46.60 ^{b}
2005	2.796 ^{a}	46.65 ^{d}	2005	3.753 ^{c}	43.94 ^{a}
Treatment (T)			Treatment (T)		
SD1	2.098 ^{a}	45.96 ^{a}	N_0	2.356 ^{a}	45.81 ^{a}
SD2	2.196 ^{a}	46.29 ^{a}	N_{50}	2.747 ^{b}	45.32 ^{a}
SD3	2.061 ^{a}	46.29 ^{a}	N_{100}	2.896 ^{bc}	44.19 ^{b}
			N_{150}	3.016 ^{c}	43.86 ^{b}
			$N_{min.}$	2.671 ^{b}	43.94 ^{b}
Cultivar (C)			Cultivar (C)		
'Banaćanka'	2.176 ^{ab}	44.48 ^{b}	'Banaćanka'	2.667 ^{b}	44.63 ^{b}
'Falcon'	2.060 ^{ab}	46.03 ^{c}	'Falcon'	3.075 ^{a}	43.94 ^{c}
'Jet Neuf'	2.359 ^{a}	47.97 ^{a}	'Jet Neuf'	2.950 ^{a}	45.70 ^{a}
'Samourai'	1.878 ^{b}	46.24 ^{c}	'Samourai'	2.257 ^{c}	44.22 ^{c}

$^{a, b, c, d}$ different at the 0.05 probability level.

3.3. *Cultivar Performance and the Effect of Year*

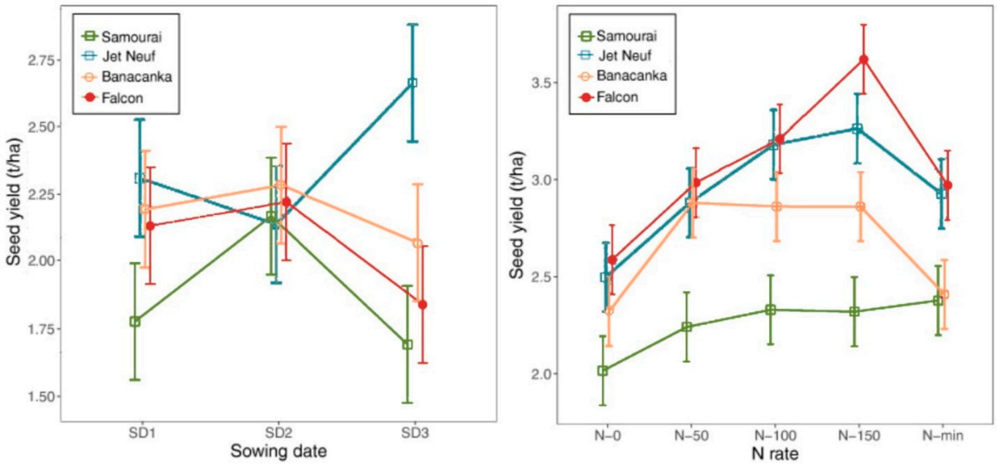
An evident variation between cultivars was present in both trials. The sowing dates only affected the oil content in the cultivar Banaćanka, increasing it by 1% between SD1 and SD3 (Figure 1a). The cultivar Samourai had the smallest reduction of oil content between the N_0 and N_{50} fertilization rates, while Jet Neuf had the highest overall oil content (Figure 1a,b). All cultivars showed an evident decrease in the oil content with an increase of the N rate.



(**a**) (**b**)

Figure 1. (**a**) The cultivar × Sowing date (SD) interaction plot for the oil content in the sowing date trial, and (**b**) the cultivar × nitrogen rate interaction plot for the oil content in the N rate trial.

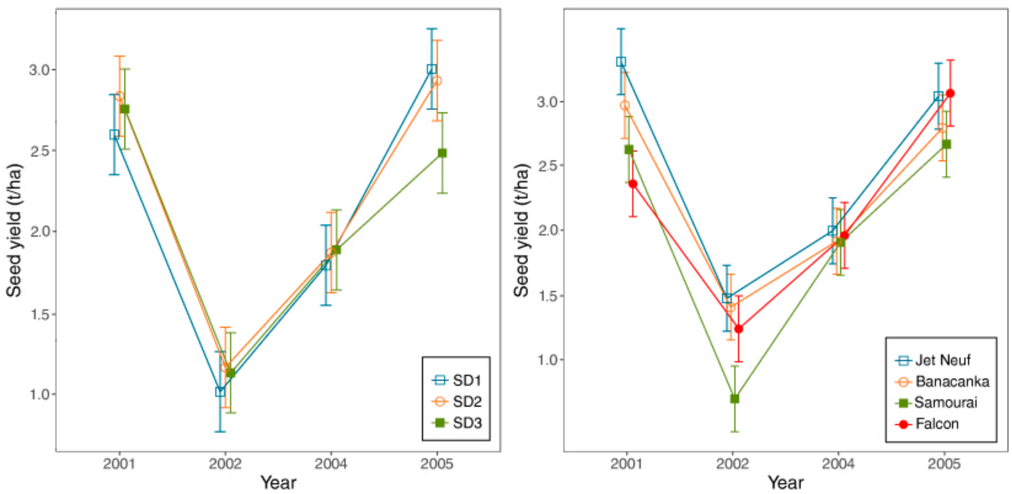
The sowing dates did affect the yield, so that SD2 was found to be optimal, especially for Samourai. Jet Neuf produced an almost 50% higher yield than the rest of the cultivars in SD3 (Figure 2a). The highest nitrogen use efficiency was found for Falcon and Jet Neuf, which were the only cultivars capable of using the additional nitrogen between the N_{100} and N_{150} fertilization rates (Figure 2b).



(**a**) (**b**)

Figure 2. (**a**) The cultivar × SD interaction plot for the seed yield in sowing date trial and (**b**) the cultivar × nitrogen rate interaction plot for the seed yield in the N rate trial.

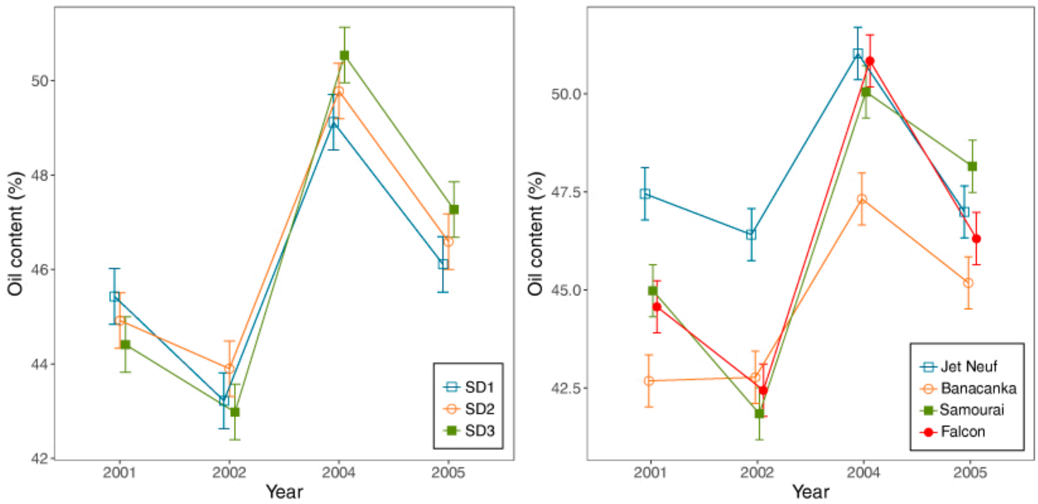
The lowest yield in the SD trial was observed for SD1 in 2002 (Figure 3a). The year affected the seed yield the most in 2005, when the largest variation was recorded between cultivars. In other years, the seed yield of the evaluated cultivars was similar, except for Samourai in 2002 with the lowest yield (Figure 3b).



(**a**) (**b**)

Figure 3. (**a**) The year × sowing date and (**b**) year × cultivar interaction plot for the seed yield in the sowing date trial.

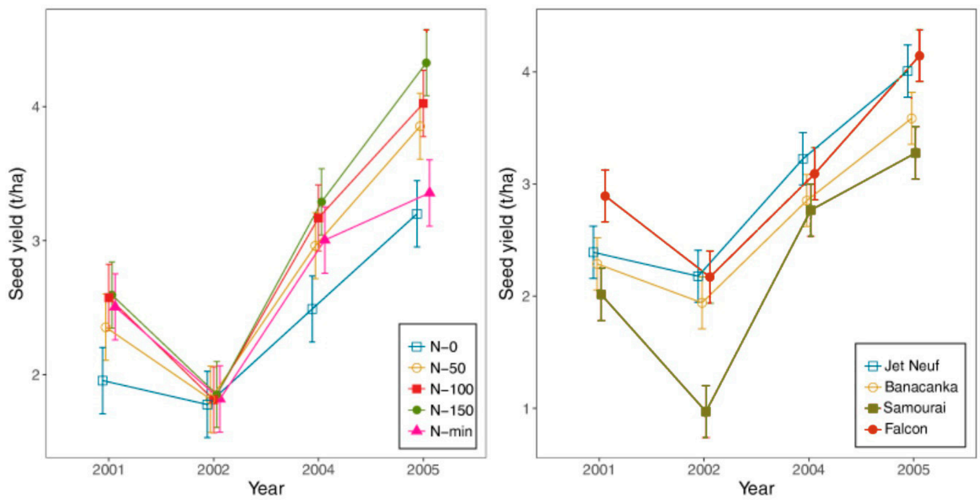
The recognizable effect of the sowing time on the oil content was only found in 2004 and 2005, while a later SD resulted in a higher oil content (Figure 4a). Jet Neuf was the most stable, with an oil content above 46% during all four years, while Banaćanka was the cultivar with the lowest oil content. Of the four trial years, 2002/03 had the most negative, while 2004/05 had the most positive effect on the oil content (Figure 4b). Compared to 2002/03, the 2004/05 season was characterized with more than double the precipitation and with milder temperatures for all growing stages, especially during flowering.



(**a**) (**b**)

Figure 4. (**a**) The year × sowing date and (**b**) year × cultivar interaction plot for the oil content in the sowing date trial.

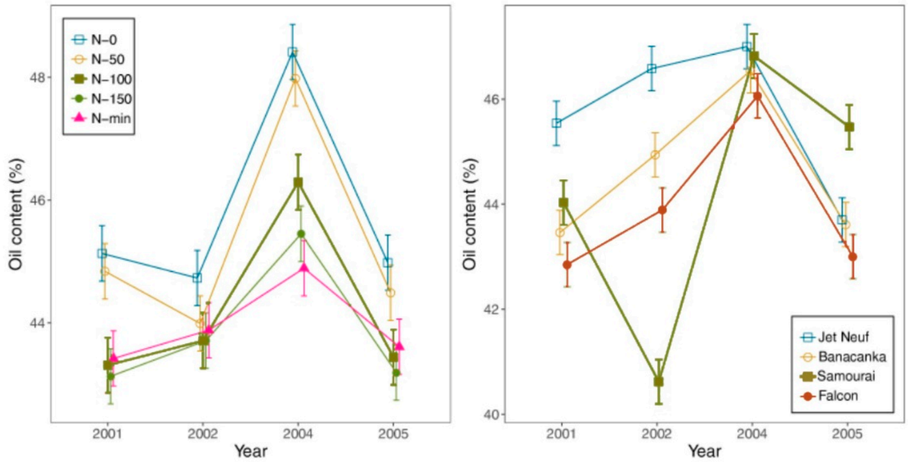
The effect of the year on the N rate treatment was visible only for the N_0 and N-min treatments in 2005. A higher influence of the year main effect in comparison to the treatment was visible in 2002, when the yields were almost the same for all N rates (Figure 5a). The year affected the cultivars the most in 2005, when the largest variation in yield was recorded, while Samourai had a more than 50% lower yield in 2002, compared to the other cultivars (Figure 5b).



(**a**) (**b**)

Figure 5. (**a**) The year × N rate and (**b**) year × cultivar interaction plot for the seed yield in the N rate trial.

Similar to the effect of the year on the N rate treatment for the seed yield, the smallest variation for the oil content was found in 2002. Nonetheless, a higher variation was found in other years, with 2004 being the most discriminative between treatments (Figure 6a). The highest effect of all second order interactions was found for the year × cultivar interaction in the N rate trial. Contrary to the year × N rate interaction, the largest differences were found in 2002, while in 2004 only Jet Neuf and Falcon had considerably different oil contents (Figure 6b).

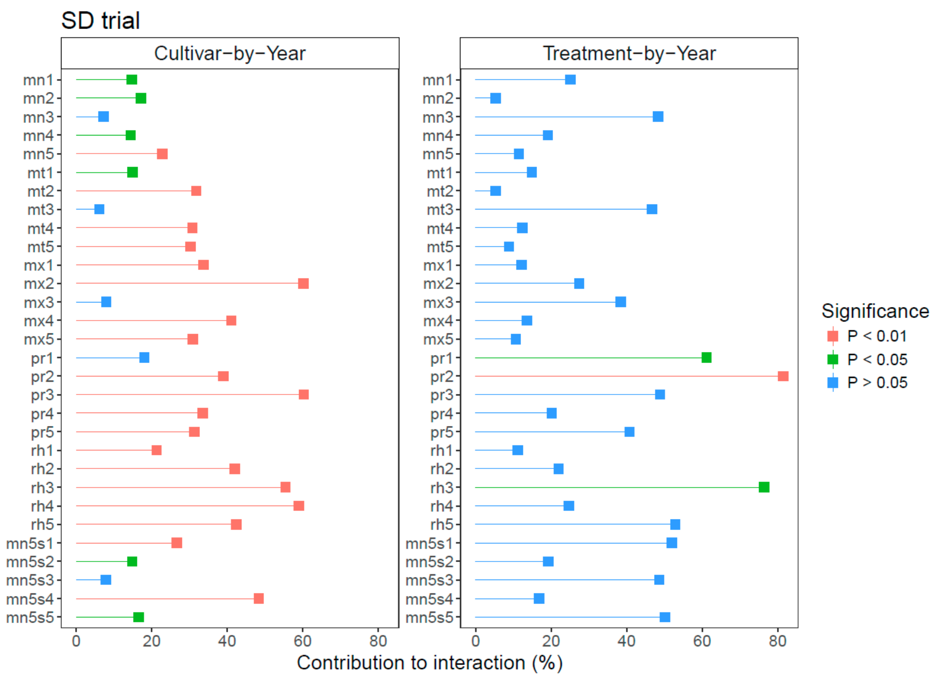


(**a**) (**b**)

Figure 6. (**a**) The year × N rate and (**b**) year × cultivar interaction plot for the oil content in the N rate trial.

3.4. The Effect of Climatic Variables on the Year-Related Interactions

A set of individual factorial regression models was developed in order to test the hypothesis about the effect of climatic variables on C × Y and T × Y interactions from ANOVA, as shown in Table 4. Out of thirty available climatic variables, nineteen had a highly significant effect on the C × Y interaction for the oil content in the SD trial. Six variables had a significant effect. The largest proportion of the explained interaction variance was obtained for precipitation at the budding stage (60.3%), the maximum temperature at overwintering (60.2%), and the relative air humidity at flowering (59.0%) (Figure 7a).



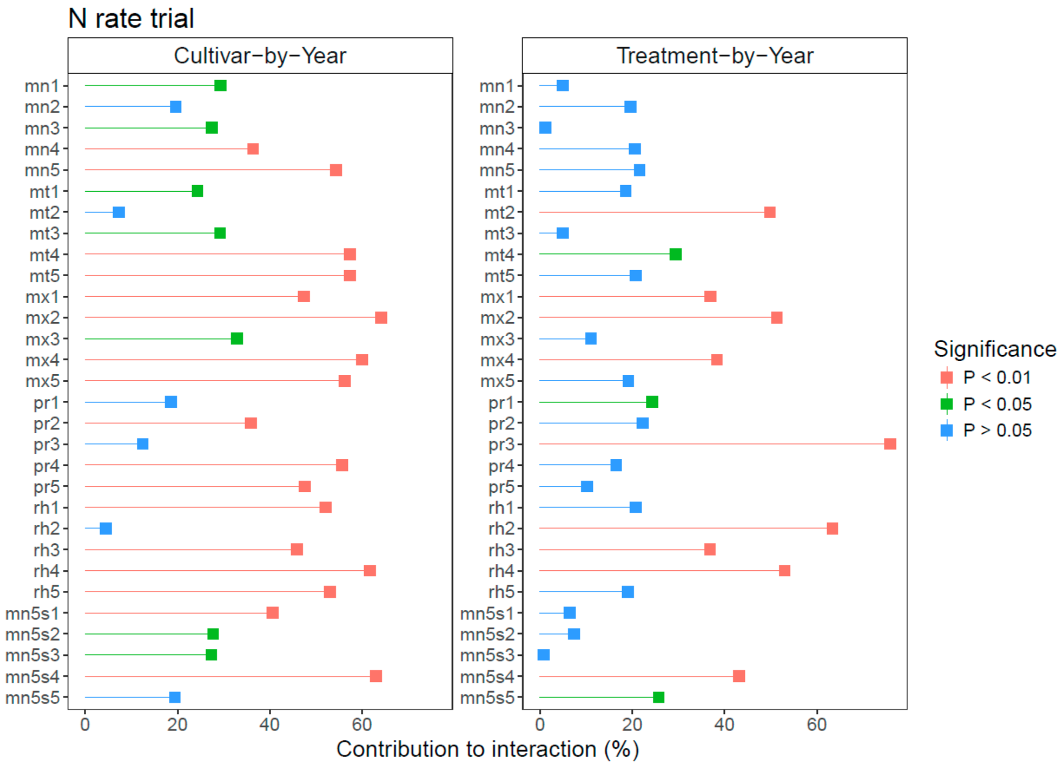
(**a**) (**b**)

Figure 7. Contribution of the environmental variables to (**a**) the C × Y interaction for the oil content in the sowing date trial and to (**b**) the T × Y interaction for the oil content in the sowing date trial. Minimum temperature—mn; minimum temperature on 5 cm above ground—mn5cm; maximum temperature—mx; mean temperature—mt, total precipitation (pr), relative air humidity (rh) during: germination (mn1, mn5cm1, mx1, mt1, pr1, rh1), overwintering (mn2, mn5cm2, mx2, mt2, pr2, rh2), budding (mn3, mn5cm3, mx3, mt3, pr3, rh3), flowering (mn4, mn5cm4, mx4, mt4, pr4, rh4) and ripening (mn5, mn5cm5, mx5, mt5, pr5, rh5).

As a consequence of the decreased level of significance of the T × Y interaction for the oil content in the SD trial, only three climatic variables were found to be important (Figure 7b). A highly significant effect was observed only for precipitation at overwintering (81.4%), whereas the effect of the relative air humidity at the budding stage (76.4%) and precipitation at the germination stage (61.1%) accounted for a significant proportion of the T × Y interaction (Figure 7b).

In the N rate trial, the C × Y interaction for the seed yield had a highly significant effect on seventeen climatic variables whose explained interaction proportion ranged from 35.9% (for precipitation at overwintering) to 64.1% (for the maximum temperature at overwintering). The range of the accounted proportion of the seven climatic variables with a significant effect was 24.3% (for the mean temperature at germination) to 32.9% (for the maximum temperature at budding). The largest proportion of the explained interaction variance was obtained for the maximum temperature at overwintering (64.1%), minimum temperature on 5 cm at flowering (63.0%), and relative air humidity at the flowering stage (61.7%) (Figure 8a).

Considering the T × Y interaction, nine highly significant climatic variables were identified (Figure 8b). Precipitation at the budding stage (75.8%) and the relative air humidity at overwintering (63.3%) and the flowering stage (53.0%) were identified as the most important. The mean temperature at flowering (29.4%), minimum temperature on 5 cm at ripening (25.7%) and precipitation at germination (24.3%) explained only a significant proportion of the T × Y interaction for the seed yield in the N rate trial (Figure 8b).



(**a**) (**b**)

Figure 8. Contribution of the environmental variables to (**a**) the C × Y interaction for the seed yield in the N rate trial and (**b**) the T × Y interaction for the seed yield in the N rate trial. Minimum temperature—mn; minimum temperature on 5 cm above ground—mn5cm; maximum temperature—mx; mean temperature—mt, total precipitation (pr), relative air humidity (rh) during: germination (mn1, mn5cm1, mx1, mt1, pr1, rh1), overwintering (mn2, mn5cm2, mx2, mt2, pr2, rh2), budding (mn3, mn5cm3, mx3, mt3, pr3, rh3), flowering (mn4, mn5cm4, mx4, mt4, pr4, rh4) and ripening (mn5, mn5cm5, mx5, mt5, pr5, rh5).

For the oil content in the N rate trial, the largest number of climatic variables had a highly significant effect on the C × Y interaction, except for the minimum temperature on 5 cm at the overwintering stage and the minimum temperature at the germination stage, which were not significant. The explained interaction variance ranged from 5.7% (for the minimum temperature on 5 cm at ripening) to 83.5% (for the relative air humidity at flowering). The most important climatic variables for the interaction variance explanation were the relative air humidity at the flowering stage (83.5%), the maximum temperature at overwintering (81.2%), and the minimum temperature on 5 cm at the flowering stage (72.2%) (Figure 9a).

Considering the T × Y interaction for the oil content in the N rate trial, twenty climatic variables were observed as highly significant. Only four climatic variables were found as significant, ranging from 14.1% (for the mean temperature at germination) to 18.7% (for the mean temperature at overwintering). The largest proportion of the explained interaction variance was obtained for the precipitation at the flowering (92.0%) and ripening (85.0%) stages, and the maximum temperature at ripening (84.9%) (Figure 9b).

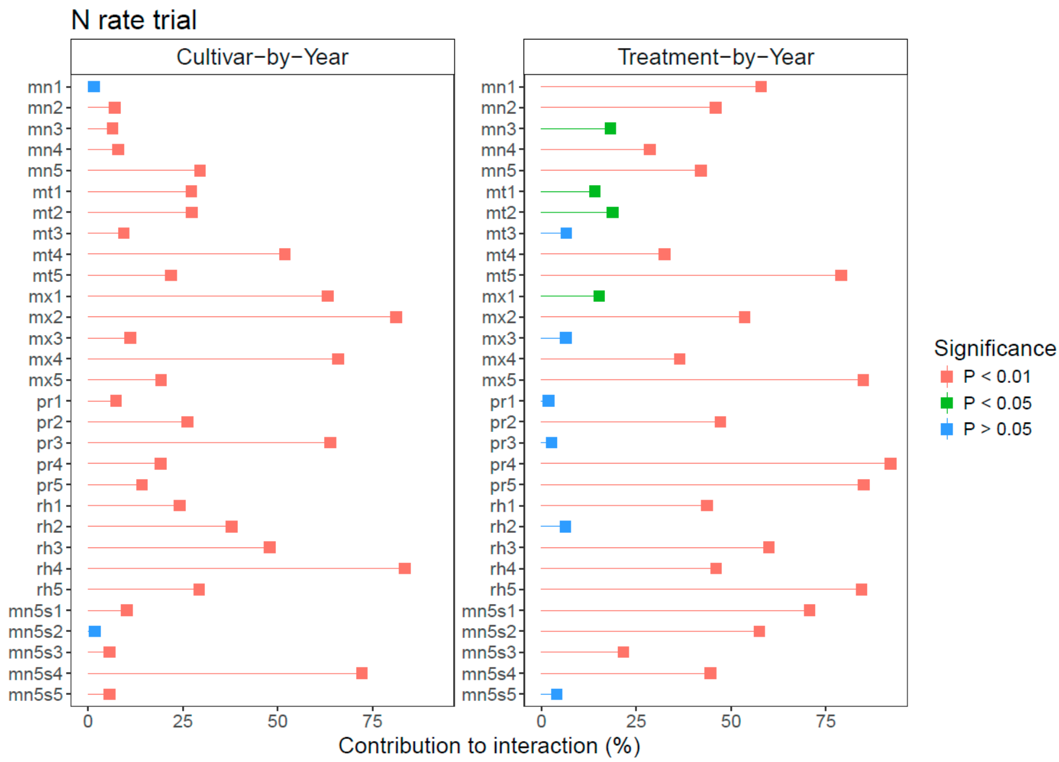


Figure 9. The contribution of the environmental variables to the C × Y interaction for (**a**) the oil content in the N rate trial and (**b**) the T × Y interaction for the oil content in the N rate trial. Minimum temperature—mn; minimum temperature on 5 cm above ground—mn5cm; maximum temperature—mx; mean temperature—mt, total precipitation (pr), relative air humidity (rh) during: germination (mn1, mn5cm1, mx1, mt1, pr1, rh1), overwintering (mn2, mn5cm2, mx2, mt2, pr2, rh2), budding (mn3, mn5cm3, mx3, mt3, pr3, rh3), flowering (mn4, mn5cm4, mx4, mt4, pr4, rh4) and ripening (mn5, mn5cm5, mx5, mt5, pr5, rh5).

4. Discussion

4.1. Sources of Variability for Seed Yield and Oil Content

For designing and selecting the most appropriate management practices for a specific combination of genotype and environment, the understanding and quantification of eco-physiological factors underlying crop growth and yield determination are critical. The basis for understanding the crop yield as determined by management practices, environmental conditions and genotype and the interactions among them was defined through the concepts of critical periods for the grain yield determination and vegetative and reproductive plasticity. That contributes to matching crop demands to the specific environment and an efficient use of the resources and inputs in a particular location [46].

4.2. Time of Sowing, N Rates and Growing Seasons

Identifying the optimal time for sowing rapeseed in a specific region is one of the most important steps for obtaining a high and stable production, as more significant changes in the photo thermal environment and water regime also lead to lower yields [47,48]. The selected SD range for winter rapeseed in this study in all trial years allowed for an adequate plants preparation for overwintering, but it was too narrow to have an adverse effect on the crop yield. The optimal period for rapeseed sowing in the Novi Sad region is from late August to late September, depending on the environmental conditions of the year confirmed in this study. A later sowing time, including October, can affect the seed yield and oil content as a result of inadequately prepared plants for overwintering [49,50].

Rapeseed genotypes included in this study responded differently to variable sowing dates. This was confirmed by the significant cultivar × sowing date interaction for the seed yield. Delayed sowing has also been found to affect the beginning of flowering and to force maturity, which adversely influenced the yield by the significant effect of the sowing time × genotype interaction [48,51]. Although the genotype × sowing time interaction was significant for the oil rate and other characteristics, its effect was not significant for the seed yield [52]. Our results indicate that the selection of suitable genotypes capable of a faster preparation for overwintering is important, as it can substantially influence the seed yield.

As opposed to the SD treatments, the N rate caused significant differences in the seed yield and oil content in rapeseed. The lowest yield was determined when no N fertilizer was applied (2.36 t ha^{-1}). The yield increased by increasing the N rates. The highest seed yield was achieved with the application of 150 kg ha^{-1}, while in the study of [53] the seed yield reached a plateau at 130 kg N ha^{-1}. Our findings were consistent with previous studies reporting a significant increase of the seed yield as the N application rate increased [54,55]. It was observed that the seed yield increased as the N application rate increased from 0 to 90 kg ha^{-1}, while at the highest N application rate (120 kg ha^{-1}), the rapeseed seed yield was significantly reduced [56].

The oil content decreased from 45.8% when the N_0 treatment was applied to a significantly lower oil content (43.9%) in the N_{150} treatment. This observation is in accordance with previous reports that recorded the highest oil content in unfertilized winter rapeseed, while the lowest one appeared at a high N supply [55–58]. The increase of the applied N dosage might increase the percentage of seed protein, as N is its major constituent [59]. Since the percentage of the oil content has an inverse relationship with the protein content [60–62], it can be a possible reason for the oil content decrease with the N increase.

The weather conditions were cold enough to influence the plant development, which was manifested by the lowest plant density in autumn and lowest survival rate in 2002/03. Used cultivars can tolerate temperatures between −14 °C and −17 °C without snow cover and between −20 °C and −24 °C with the snow cover when properly developed for overwintering. However, the cultivar Samourai still had a significantly lower survival rate than the other evaluated cultivars. The thickness of the snow cover is also important as it varies considerably in the Vojvodina region. During extremely low temperatures, not even the snow cover can protect the plants. The north wind is frequent during the winter, and it can partially or completely remove snow from the fields. It is not unusual that warm air currents speed up the reduction of the snow cover during the winter, especially in February. Such extreme changes of cold and warm periods negatively affect the rapeseed condition and survival rate, especially with frosts occurring in the first half of March. Temperature extremes are affecting the growth speed in the early spring, which is crucial for timely flowering, seed filling and ripening, as the temperatures in June during harvest are often above 35 °C.

The present results underscore the importance of multi-location and multi-year field testing, as overwintering was mostly affected by the year and cultivar in our trial. The results from such trials provide valuable information on the inter-annual variability of climatic factors and cultivar stability, which, combined, can support a more stable production [5,63].

Mild temperatures and a high precipitation in 2004/05 resulted in the maximum plant density and overwintering percentage out of the four trial years. In contrast, season 2002/03 was characterized by the lowest minimum temperature during winter and continuous drought conditions from the budding to ripening phase. The annual precipitation of 630–700 mm is optimal for rapeseed production in Vojvodina, but a significantly lower rainfall was registered in three of the four trial years. That suggests that breeding for drought tolerance is important for cultivars intended for this region. Season 2002/03 was specific because drought stress shortened the flowering period by 10 days in comparison to its length in 2001 and 2004, while the continued drought in ripening accelerated the development and lowered the yield. The cultivar Samourai had the lowest yield during 2002/03, both in terms of the N rate and SD trial. A low density in autumn and low survival rate, together with a lack of moisture in the spring, resulted in plants not being able to compensate the low density with other yield components. Falcon and Jet Neuf achieved significantly higher yields due to higher plant densities and a more efficient nitrogen use, especially in higher fertilization rates of N_{100} and N_{150}.

When searching for solutions, the selection for tolerance may provide better results than drought avoidance, because of the drought risk in the germination phase and early frost in October. Breeding for an earlier phenology is rather difficult and probably not beneficial to the seed yield, because too early flowering will result in a significant reduction of the total biomass production [64]. On the other hand, mutations in genes involved in the flowering time control can affect seed yield components [65]. The selection of the genes involved in the flowering time regulation may play a potential role in the adaptation of rapeseed to highly divergent environments [66]. Future cultivar selection should also consider the crop phenology and influence of the environment [67]. The modulation of circadian clock regulators in allopolyploids and hybrids enhances fitness and metabolism, and ultimately growth and development. That results in growth vigor and an increased biomass [68]. *Brassica* improvement by altering the flower time regulation can be done either through the development of new alleles via mutagenesis or through a targeted genetic transformation [69]. Such alterations could facilitate the introduction of genes into European rapeseed from different geographical origins, or affect the ability of the crop to avoid unfavorable environmental conditions in different growing regions.

4.3. The Effect of Climatic Variables on the Year Related Interactions

This study is among the first to dissect the effects of year-related climatic variables on the agronomical traits in winter rapeseed. Cultivars in different environments have previously been evaluated [47], but that did not provide information on the effect level of the individual climatic variables. However, because of the crops' economic importance and future concerns in agriculture under a possible climate change in the twenty-first century, the quantitative establishment of associations among crop yield values and climate variability is essential [70].

Thirty climatic variables were created and applied in order to test the effect of climatic variables on the cultivar × year and treatment × year interactions from ANOVA. In the SD trial, the C × Y and T × Y interactions were not significant for the seed yield, but only for the oil content. The precipitation at the budding stage (60.3%), maximum temperature at overwintering (60.2%), and relative air humidity at flowering (59.0%) explained the largest proportion of the C × Y interaction variance (Figure 7a). In the N trial, most of the climatic variables had a highly significant effect on the C × Y interaction for the oil content. The relative air humidity at the flowering stage (83.5%), maximum temperature at overwintering (81.2%), and minimum temperature on 5 cm in the flowering stage (72.2%) were the most important climatic variables for this interaction variance explanation (Figure 9a). The largest proportion of the explained C × Y interaction variance for the seed yield was obtained for the same climatic variables (Figure 8a).

Based on the F test, the C × Y interaction for the oil content in the N trial (F 52.4) is the only interaction more important than all of the main effects (year F 27.9; N treatment F 47.3 and cultivar F 48.6) in comparison with the other interactions in the SD and N rate trials. As the N treatment had a strong effect on the seed yield and even more so on the oil content, the cultivar (C) adaptability to increased N dosages had more impact than the year (Y). Climatic variables significantly contributed to the C × Y interaction for the seed yield only during flowering and ripening, while they were highly significant for almost all phases of plant development for the oil content (Figures 8a and 9a). For the seed yield, cultivars reacted positively to the N treatment, developed adequately and were less sensitive to the environment until flowering (climate contributing less to interaction). On the other hand, the oil content was under the negative influence of the N treatment, and it is a trait more dependent on the climate. That is why the C × Y interaction was more important, especially for the air temperature and humidity during the flowering period.

4.4. Cultivar Main Effect in Comparison to Interactions

Certain authors [64,71] emphasize the importance of cultivars' phenology to the specific rapeseed adaptation in different environments. The different treatments in both trials (sowing time and N rate) were mostly grouped according to the cultivation year (Figure 3a, Figure 4a, and Figure 5a). This confirms that matching the cultivar phenology to the growing season length and rainfall is essential for maximizing the rapeseed seed yield in the specific mega-environment [64]. The lower importance of all interactions, including T × Y, C × Y and C × T, compared to C main effect, in both trials for both traits, confirms the importance of cultivar adaptability to the environment. The cultivar main effect was only less important than the C × Y interaction for the oil content in the N rate trial due to the negative effect of higher N dosages on the oil content, and a higher sensitivity of cultivars to climatic conditions. On the other hand, increasing the N rate higher than N_{100} for the seed yield was only beneficial to Falcon and Jet Neuf, indicating that the optimal N dosage should be adapted to specific cultivars.

Variables that have the greatest influence on the seed yield and oil content during certain phenological phases are of the utmost importance. Such knowledge could support the adequate management of the cultivars to be planted in a specific environment and could help apply agronomical practices appropriately, in order to ascertain the highest yield and quality in rapeseed. In both trials of this study, the relative air humidity at the flowering stage and the maximum temperature at the overwintering stage were identified as the most important determinants of the seed yield. Plants from all three sowing dates flowered almost at the same time. The enhanced flower initiation was due to the prevalence of favorable environmental conditions, in particular a low temperature during the vegetative growth phase [72]. The longer the reproductive period in rapeseed, the higher the yield will be. That can be associated with a larger number of seeds per unit area [73]. The hypothesis behind this assumption is that extending the flowering period in rapeseed allows for the initiation of more floret primordia in the branches, and consequently more reproductive points, which would result in an increased number of seeds per plant. Their suggestion that the crop development phases need to be manipulated through environmental factors that regulate the development at one or more stages of the crop in order to test this hypothesis was confirmed through the results of our study. Mid and late flowering genotypes are better suited to the medium to high rainfall area, and the early flowering ones to low rainfall areas [73].

Considering the T × Y interaction in the SD trial, the largest proportion of the explained interaction variance was obtained for the precipitation at overwintering (81.4%) for the oil content (Figure 7b). In the N rate trial, the precipitation at the budding stage (75.8%), and the relative air humidity at the overwintering (63.3%) and flowering stages (53.0%) accounted for the highest proportion of T × Y interaction for the seed yield (Figure 8b), and the precipitation at the flowering (92.0%) and ripening (85.0%) stages accounted for the highest proportion of T × Y interaction for the oil content (Figure 9b). Based on the obtained results, it can be concluded that water availability at different growth stages was the main determinant of the seed yield and/or oil content in both trials. Similar results were reported in other studies. A higher rainfall and cooler temperatures during seed development resulted in a higher yield and oil content [74]. The relationships between the mean air temperature, radiation and rainfall during the flowering period were identified. It was concluded that these variables are generally applicable in the determination of the oil content in rapeseed [75]. Moreover, it was implied that the selection for specific adaptation to different environments should be the main mode of breeding. Considering that rainfall can be a main determinant for obtaining a high seed yield and oil content during different growth stages, growing and breeding varieties separately for low and high rainfall areas was suggested [62]. The selection of appropriate cultivars and adequate planting can increase the rapeseed yield potential, as an increased temperature during the grain filling stage results in an oil percentage reduction. Beside this, an adequate nitrogen addition is one of the key factors for increasing the yield due to the typically low nitrogen efficiency of rapeseed. Combined, the sowing date and nitrogen trials provided results that will help in improving the rapeseed production technology in the region, but may also be a starting point for a more complex analysis involving the crop model design.

In summary, this study is the first to successfully dissect the effect of year-related climatic variables on agronomical traits of winter rapeseed in Southeast Europe. The analysis of the effect of different climatic variables on the seed yield and oil content in rapeseed, over years, sowing dates, nitrogen dosages and genotypes, elicited the critical stages in rapeseed development, and the climatic factors that act as a main determinant for the target traits. Understanding the impact of different sowing dates and different N rates for specific genotypes, and determining the critical development stages, will help in crop management and yield and oil content modeling. Based on the obtained information, manipulations with one or a combination of these factors, the selection of the best performing cultivars for a particular region, and the application of appropriate agronomic practices at specific growing stages, will ensure the maximal usage of the available environmental resources during these intervals. This will inevitably secure a high seed and oil yield in rapeseed.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/9/517/s1, Table S1: Nitrogen taken by plants and N added to each N-min plot in the N trial (N kg/ha), Table S2: Climatic variables calculated based on daily values for the duration of each of the five studied development stages in the sowing date trial. Second stage represents joined GS1+GS2 stages of Seedling and Rosette.

Author Contributions: Conceptualization, A.M.-J., J.C. and M.Z.; methodology, A.M.-J., J.C. and M.Z.; software, M.Z. and M.J. (Mirjana Jankulovska); validation, M.Z.; formal analysis, M.Z.; investigation, A.M.-J., M.Z. and S.T.; resources, M.J. (Milan Jockovi) and N.H.; data curation, M.Z. and S.T.; writing—original draft preparation, S.T. and A.K.-S.; writing—review and editing, N.N., A.K.-S. and S.T.; visualization, M.Z.; supervision, A.M.-J.; project administration, A.M.-J.; funding acquisition, A.M.-J.

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Conflicts of Interest: The authors declare no conflict of interest.

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