




Sowing date and fungicide application in the agronomic performance of oleaginous brassica for the biodiesel production¹

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ABSTRACT

This work aims to evaluate the agronomic performance of crambe and juncea canola, sowed in the summer and winter season intervals with and without fungicide application. The cultivars used were FMS Brillhante and Terola 25A85 for crambe and juncea canola, respectively. The experimental design was randomized blocks in split plots, in which the plots were sowing dates 15th May (E1), 1st June (E2) and 15th June 2014 (E3), and the split plots with and without aboveground part with fungicide (trifloxystrobin 150 g L⁻¹ + prothioconazole 175 g L⁻¹). The evaluated variables were plant height, thousand seed weight, seed yield, oil content and oil yield. The sowing dates had had influence only on plant height for crambe, while juncea canola had a significant effect on plant height, seed yield and oil yield. The fungicide application gave higher seed yield for crambe and for juncea canola, higher thousand seed weight, seed yield and oil yield. With the production technologies adopted for the growth of these two brassicas, it is necessary to obtain higher yields of seeds and oil, to enable the commercial exploration in the interval between the summer and winter off-season in the Northern region of Paraná state, Brazil

Keywords: *Crambe abyssinica*; *Brassica juncea*; chemical control; winter oilseeds; off-season.

INTRODUCTION

The Brassicaceae oilseeds are an important source of biodiesel feedstock (Jham *et al.*, 2009) and special attention has been given to juncea canola (*Brassica juncea* L.) and crambe (*Crambe abyssinica* Hochst). Juncea canola has been developed in canola producing countries as an option to *B. napus* canola, because it is tolerant to drought, has high resistance to blackleg (*Leptosphaeria maculans*) and is more resistant to the opening of pods (Elliott, 2015). Meanwhile, crambe is considered a rustic plant, tolerant to droughts and frost (Reginato *et al.*, 2013) and short growth cycle (Zanetti *et al.*, 2016).

The characteristics of resistance to drought, rusticity and short cycle can allow the growth of these two

oilseeds in the summer and winter off-season, in places where the sowing of the second crop is anticipated to avoid possible damage caused by the frost. The sowing of these oilseeds in this crop window, besides providing feedstock for biodiesel production, is an important practice in soil conservation, as it contributes to the production of biomass and crop rotation for the no-tillage system and prevents the soil from being discovered.

The yield of the *Brassicaceae* oilseeds are generally higher with precipitation and with lower temperature during the grain filling (Hossain *et al.*, 2019). Specifically for the crambe crop, high temperatures at the end of the vegetative cycle can also cause reduced yield, because

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crambe is a winter crop (Zanetti *et al.*, 2016). The occurrence of adverse climatic conditions, cause reduction of oil content as reported by Costa *et al.* (2019).

With respect to diseases, have been reported in experiment with crambe in the western Paraná of the black rot, caused by the bacteria *Xanthomonas campestris* pv. *campestris*, alternaria leaf spot, caused by the fungus *Alternaria brassicae*, *Alternaria brassicola* and *Alternaria raphani*, besides white mold, caused by fungus *Sclerotinia* sp (Oliveira *et al.*, 2015). Wang *et al.* (2000) report the weak resistance of crambe to diseases and the lack of genetic diversity to perform genetic improvement through varietal resistance. Alternaria leaf spot and white mold control can be carried out using fungicides. Among the fungicides, triazoles represent the largest and most important group of systemic compounds used to control plant diseases, which influence the synthesis of sterol in fungi (Zamani-Noor & Knüfer, 2018). The prothioconazole (Proline® 480SC) is a triazole fungicide and is registered in Canada (Health Canada, 2019) for control of alternaria and white mold in crambe. This molecule also one of the best for the control of *Sclerotinia* in canola crop on Scotland, besides being recommended for the control of blackleg (Oxley & Evans 2009). In Brazil, there is availability of the mixture of active ingredient belonging to the strubirulin (trifloxystrobin) and triazole class (prothioconazole), registered for the soybean crop (Adapar, 2019).

The availability of information on the management of crambe and juncea canola in the North of the Paraná is limited and, in this way, the present work aims to evaluate the possibility of the insertion of these two oilseeds in the summer and winter cropping season.

MATERIAL AND METHODS

The experiment was conducted at the school farm of the State University of Londrina, Londrina - PR, at the geographic coordinates 23°20'30" S, 51°12'34" W and 551 m altitude, from May to September 2014. The soil of the experimental area was classified as Oxisol (Embrapa, 2013), with 70% clay, 15% silt and 15% sand. The chemical characteristics of the soil in the 0-0.10 and 0.10-0.20 m layers are shown in Table 1.

The climate of the region is Cfa type, according to classification of Köppen. Meteorological conditions were recorded daily at the Iapar weather station in Londrina, PR (Iapar, 2014) and precipitation, maximum and minimum temperatures are expressed every 10 days (Figure 1).

The experimental design was randomized complete block design, with subdivided plots and six replications, with three plots: 15th May (E1), 1st June (E2) and 15th June 2014 (E3). The sowing season E1 is the deadline established by the Fundação MS for the soying of the crambe in the north Paraná (Pitol *et al.*, 2010), while the dates E2 to E3 are the possible dates of sowing of the crambe after harvest of the winter maize. The subplots were constituted by the absence or application of fungicide trifloxystrobin 150 g L⁻¹ + prothioconazole 175 g L⁻¹. The fungicide doses were 0.4 L ha⁻¹ of the commercial product together with the adjuvant soy methyl ester at 0.25% concentration applied at 45 and 60 days after emergence. For the application of the fungicide, a costal spray was used, pressurized to CO₂ and equipped with a bar with 6 double-jet tips with air induction, AD-110.02, spaced apart at 0.50 m, with a pressure of 250 kPa and consumption of 180 L ha⁻¹ solution.

The subplots were constituted by 6 lines, with a spacing of 0.45 m between rows and 4 meters in length, making up an area of 10.8 m². The useful area was used the two central lines, being eliminated as a border, 0.50 m from each end.

The seeds of crambe cultivar, FMS Brilhante, the only cultivar registered in Brazil, were used, while for juncea canola it was cv. Terola 25A85. Both were previously treated with vitavax fungicides (150 g L⁻¹ active ingredient - i.a.) + thiram (350 g L⁻¹ i.a.) at the dose of 4 mL kg⁻¹ of seed. For the control of pre-emergence weeds paraquat (200 g of i.a.) + diurom (100 g of i.a.) were used at the dose of 2.0 L ha⁻¹, applied volume of 200 L ha⁻¹.

The sowing was done in no-tillage system, using to demarcate the 6-row planter furrow, model PA 6000 (Vence Tudo, Ibirubá, RS, Brazil). For the distribution of the seeds in the furrow, an adapted experimental planter was used with tank and seed distributor disk powered by driving wheel and pulled manually in the 0.45 m spacing between lines and distribution of crambe seed 60 and 30 canola seeds. The disks used for both crops possessed ø5mm

Table 1: Results of the chemical analysis of the soil in the layers of 0.00-0.10 and 0.10-0.20 m, before the implementation of the experiment in 2014, Londrina

Depth (m)	P ¹	C ²	pH ³	Al	H+Al	Ca ⁴	Mg ⁴	K ¹	BS	T	V
	(mg dm ⁻³)	(g dm ⁻³)									
0,00-0,10	38,9	22,24	6,0	0,00	3,42	10,20	2,59	1,15	13,94	17,36	80,29
0,10-0,20	16,6	16,75	5,6	0,00	4,96	8,10	2,30	0,71	11,11	16,07	69,13

⁽¹⁾ Extractor Mehlich I, ⁽²⁾ Method Walkley-Black, ⁽³⁾ CaCl₂ 0,01M, ⁽⁴⁾ KCL 1 M.

holes, however for canola were capped the first series of holes and half the second alternated, to reduce the number of seeds. After establishment of the plants, the excess plants were thinned to obtain 625,000 and 500,000 plants ha^{-1} , respectively.

Were applied manually, 300 kg ha^{-1} of 04-14-08 kg ha^{-1} (N-P-K) in sowing. The top dressing fertilization, applied manually also, consisted of 200 kg of ammonium sulfate applied 25 days after the emergence of the plants of each season of sowing.

In order to guarantee the uniform emergence of the plants, three irrigations were carried out in a sprinkler system with 20, 15 and 15 mm water, respectively, for the dates of 15th May, 19th May and 04th June.

During seed germination and seedling emergence, there was a dove attack, *Zenaida auriculata*, without significantly affecting the plant population, as there was a need to deplete the excess seedlings. Weed control in post emergence was performed manually. For the control

of *Diabrotica speciosa* and caterpillar complex the insecticide deltamethrin 25 g L^{-1} was used. The applied dose was 150 mL ha^{-1} in the total area of the experiment on 1st July and 30th July.

The plant height were determined in ten plants, at the time of harvest. The seed yield of each replicate obtained (g plot^{-1}) was transformed to yield (kg ha^{-1}). For the determination of the weight of one thousand seeds, 8 subsamples of 100 seeds were used, using the 2008 SANICK seed counter. The subsamples were weighed on a precision scale and the mean result was multiplied by 10, according to the Rules for Seed Analysis (Brasil, 2009). To determine the moisture of the harvested seeds, the Oven Method was used at 105 ± 3 °C for 24 hours, according to the Seed Analysis Rules (Brasil, 2009), with seed yield and weight of thousand seeds corrected for seed moisture by 13%.

The oil content in the seeds was determined in duplicate with the petroleum ether solvent in a Soxhlet

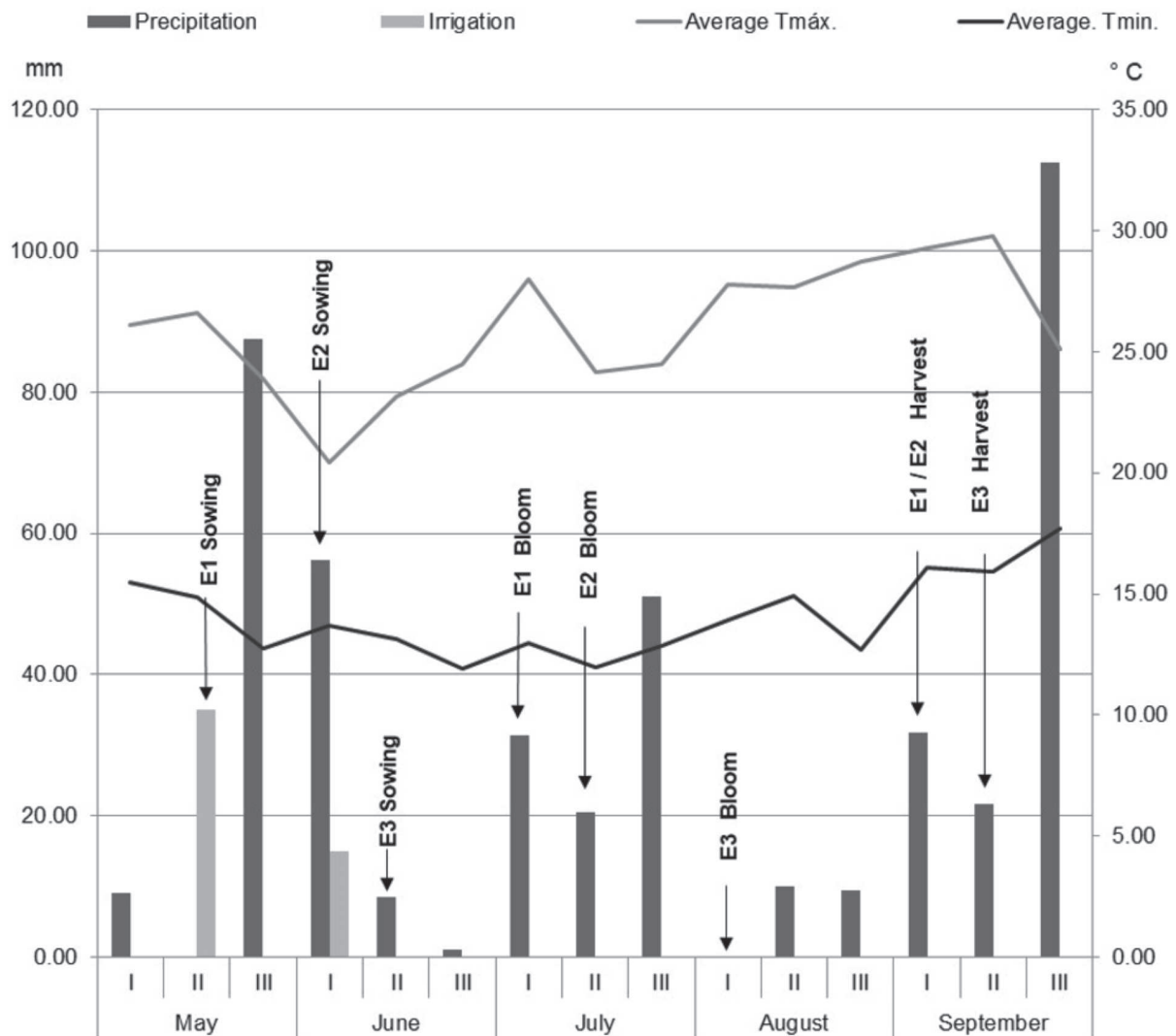


Figure 1: Main phenological phases of crambe and juncea canola and the meteorological conditions during the experiment

extractor, according to the method Aocs Ac 3-44 (AOCS, 2009) with extraction period of 6 hours. The oil yield were calculated using the harvested dry seed yield multiplied by oil content.

The data were submitted to analysis of variance, and the means were compared by the Tukey test, at 5% probability.

RESULTS AND DISCUSSION

Crambe

In the edaphoclimatic conditions of Northern Paraná, crambe cultivation showed a significant effect of sowing date on plant height and phytosanitary management, with fungicide application on seed yield (Table 2).

The lowest plant height was obtained at later sowing, possibly due to the lower availability of water for growth, since from sowing to flowering, the precipitations were 206, 133 and 112 mm, respectively for sowing dates 15th May (E1), 1st June (E2) and 15th June 2014 (E3). These values include the applied water to guarantee uniform emergence.

The recommended water requirement for crambe cultivation is 150-200 mm, concentrating mainly until full bloom (Pitol *et al.*, 2010). This condition was only reached in the first sowing season (E1 – 15th May).

The temperatures that occurred during the cultivation were high, reducing yield, since according to Pitol *et al.*, (2010), temperatures lower than 25 °C provide greater productive potential and greater tolerance to drought.

The higher plant height is associated with greater accumulation of biomass and, consequently, greater partition of the assimilates produced in photosynthesis for the seeds, providing seeds with greater mass, which promotes higher seed yield, greater oil accumulation and higher oil yield. Mastebroek *et al.*, (1994) verified a high correlation between the height of crambe plants and yield of seeds and oil.

In the present study, the influence of plant height on crambe yield was not observed, probably due to the incidence of *Xanthomonas campestris* pv. *campestris* (bacterial disease), causing little productive plants, which justifies the high coefficients of variation obtained (Table 2). The incidence of the disease directly impairs in photosynthesis, since it promotes necrosis and destruction of plant tissue, evolving to the complete decay of the affected plants (Maringoni, 2005).

For the thousand seed weight it was not possible to verify statistical difference. It is worth noting that the low values of the thousand seed weight and the seed yield obtained in the study were due to the incidence of bacterial disease and together with the low rainfall and high temperatures during the experimental period, as higher yields of the FMS Brilhante cultivar were recored in the literature. Pitol *et al.*, 2010 reported average yield of 1000 to 1500 kg ha⁻¹ in southern region of Mato Grosso do Sul and western Paraná, Viana *et al.*, 2015 obtained in Cascavel 2118 kg ha⁻¹ and Zoz *et al.*, 2018 reached 1246 kg ha⁻¹ in Marechal Cândido Rondon.

Considering the same production technology adopted in the experiment, the yield needed to achieve a balance between production costs and revenue obtained with crambe commercialization would be 3384 kg ha⁻¹. This yield for the current condition of the crop is very high, due to the relatively high cost of production and especially the crambe price (about R\$ 23.00 per bag of 60 kg on 20th October 2014), much lower than paid for soybean.

Regarding the oil content, there was no statistical difference between sowing times, possibly influenced by unfavorable soil and climatic conditions, as evidenced by the seeds used in the experiment that had a higher oil content (35.65% hulled seed) and the results obtained by Pitol *et al.* (2010), which obtained oil content for this same cultivar 37.0% hulled seed. The effect of the reduction of the oil content in the seeds of FMS Brilhante due to

Table 2: Yield variables in different sowing times and phytosanitary management of crambe⁽¹⁾

Sowing date	Plant height (m)	Thousand seed weight (g)	Seed yield (kg ha ⁻¹)	Oil content (%)	Oil yield (kg ha ⁻¹)	Cycle (days)
15 th May 2014	1.09 a	5.52	317.60	29.02	93.71	106
01 st June 2014	1.02 ab	6.06	461.10	30.70	127.11	101
15 th June 2014	0.83 b	5.47	524.89	27.93	41.56	96
Phytosanitary management						
With Fungicide	1.00	5.77	494.78 a	29.78	133.13	-
Without Fungicide	0.96	5.59	373.98 b	29.65	108.46	-
CV (%) Sowing Time	18.61	22.62	79.75	16.26	81.38	-
CV (%) Phytosanitary management	11.17	6.04	34.31	4.54	32.68	-

⁽¹⁾ Values within columns followed by the same letter are not significantly different at the P ≥ 0.05.

weather conditions are also reported by Costa *et al.*, 2019 in Portugal, where they obtained the oil content of 26% and other crambe cultivars by Zanetti *et al.* (2016), which showed a reduction in oil content in crambe sown in southern Italy (37% dehulled seed) compared to northern Italy (43% dehulled seed) due to the reduction of precipitation and increase in temperature.

The application of fungicide showed a significant effect on the agronomic performance of the crambe crop, in which the presence of *Alternaria sp* spores, mainly associated to the plants that were attacked by *Xanthomonas*, were observed. Bertelsen *et al.* (2001) report the increase in wheat yield with the application of azoxystrobin (a strubirulin) and epiconazole (a triazole) in fields with very low levels of visible diseases, since the fungicide controls the saprophytic fungi, and so the plants do not expend energy in defense against these fungi. Possibly the highest yields obtained with the application of fungicide in our experiment can be explained by control of *Alternaria sp* and this way, the plant has more photoassimilates to produce the largest number of seeds. The authors are not aware of researches reporting the efficacy of the fungicide trifloxystrobin + prothioconazole in crambe.

The oil yields were low at all sowing times, mainly due to the low seed yields and the lower oil content accumulated by the seeds. Reginato *et al.* (2013), studying the crambe crop in the region of Dourados - MS, verified that the highest yield of oil was reached in sowing on 9th April 2013, obtaining 303 kg ha⁻¹ of oil per hectare.

The cultivation of crambe during the interval between the summer and winter seasons was not feasible, regardless of the sowing season (E1: 15th May; E2: 1st June and E3: 15th June 2014), due to the climatic conditions of the year 2014 in the region of Londrina and the minimum yields necessary to at least balance the revenues with the very high cost of production.

Juncea canola

The juncea canola showed a significant effect of sowing date on plant height, seed yield and oil yield (Table 3).

The plant height of the first (15th May) and second sowing season (1st June) did not differ statistically among themselves, only the third season (15th June) showed lower plant height. Possibly, the lower availability of water for the growth occurred in the third season promoted the difference observed in the present study.

From sowing to harvest, accumulated rainfall was 340, 235 and 166 mm, for the first, second and third sowing seasons, respectively. According to Shekhawat *et al.* (2012), the need for water for rape and mustard crops is in the range of 240-400 mm.

They are reported by Enjalbert *et al.* (2013) mean height of plants higher (1.42 m) than those obtained in Londrina (ranging from 0.84 to 1.32), with genotypes of Russian and Chinese origin, in 2-year experiments conducted in the rainforest in Fort Collins, Colorado, United States.

Regarding the weight of one thousand seeds, there was no difference between the sowing times. The values obtained in the experiment are higher than those reported for rainfed cultivation, although lower than in the irrigated conditions by Enjalbert *et al.* (2013), which verified that the weight of a thousand seeds were of 2.08 and 2.85 g for the cultivation of rainfed and irrigated, respectively.

For seed yield, regardless of the sowing season, values were lower than those reported by Gesch *et al.*, 2015 (1462 - 2925 kg ha⁻¹) and Enjalbert *et al.*, 2013 (1462 - 2925 kg ha⁻¹). Later sowing dates provided lower yields due to lower water availability associated with high temperatures during flowering and grain filling, as reported by Kutcher *et al.* (2010), analyzing historical data on canola and canola yield in Saskatchewan, Canada.

The influence on water availability can be seen in semi-arid climates such as Fort Collins, Colorado, USA, where the average grain yield of 94 accessions of *B. juncea* in rainfed and irrigated crops in 2010 was 717 and 993 kg ha⁻¹, respectively (Enjalbert *et al.*, 2013).

With respect to temperature, Morrison & Stewart (2002) found that temperatures of 29.5 °C in bloom caused a reduction in grain yield of *B. juncea* due to the decrease in the number of flowers and the number and size of the seeds produced by each flower. Temperatures close to these during bloom were observed in the present experiment, especially in the first (15/05) and third sowing season (15/06) (Figure 1).

As canola and mustard are C3 metabolism plants, the best photosynthetic efficiency is achieved in the temperature range of 15 to 20 °C, where it reaches the maximum CO₂ exchange (Shekhawat *et al.*, 2012).

The seed yield for the breakeven between the production costs and the revenue obtained with the commercialization of the canola, considering the same production technology used in the experiment and with the quotation of R\$ 57.00 (60 kg bag) would be 1554 kg ha⁻¹. In this way, yields above those obtained at that time, must be reached to remunerate the producer. The higher value of canola versus crambe can be explained by the fact that it is a food oil, as it has no restriction on the use of meal in animal feed.

The results of experiments conducted in several locations in Canada over two agricultural years by Blackshaw *et al.* (2011) yielded grain yields ranging from 400 to 3580 kg ha⁻¹ for juncea canola and 610 to 3530 kg

Table 3: Yield variables in different sowing times and phytosanitary management of juncea canola⁽¹⁾

Sowing date	Plant height	Thousand seed weight	Seed yield	Oil content	Oil yield	Cycle
	(m)	(g)	(kg ha ⁻¹)	(%)	(kg ha ⁻¹)	(days)
15 th May 2014	1.32 a	2.38	482.67a	31.16	124.80a	106
01 st June 2014	1.21 a	2.52	394.49ab	30.22	101.93ab	101
15 th June 2014	0.84 b	2.73	237.81b	29.66	59.71b	96
Phytosanitary management						
With Fungicide	1.13	2.63 a	416.43 a	29.86	106.30 a	-
Without Fungicide	1.12	2.46 b	327.02 b	30.08	84.66 b	-
CV (%) Sowing Time	15.55	13.86	41.05	9.53	46.19	-
CV (%) Phytosanitary management	6.03	8.45	16.82	5.88	20.13	-

⁽¹⁾ Values within columns followed by the same letter are not significantly different at the $P \geq 0.05$.

ha⁻¹ for *B. juncea* and by Gan *et al.* (2016) which reached the yields with juncea canola open pollinated cultivars between 1410 kg ha⁻¹ and 1840 kg ha⁻¹, suggesting the possibility of achieving better levels of yield and thus better exploiting the potential of the crop in the region. In addition, these authors observed that great than the grain yield is related to the duration of flowering, so that the brassicas with less flowering period have a higher yield.

The oil contents obtained in the experiment were lower (29.66 to 31.16%) compared to the oil content of the seeds used in sowing (41%). Higher oil contents are cited in the literature, such as the experiments conducted at several sites in the semi-arid region of the Canadian prairies, where the oil content of juncea canola was 45% and *B. juncea* was 42% (Blackshaw *et al.*, 2011) and in experiments conducted in Minnesota, USA, by Gesch *et al.* (2015), which obtained 45.1% for juncea canola Oasis and 40.8% for *B. juncea* Pacific Gold.

The temperature stress during flowering probably anticipated the phenological cycle of the crop, thus occurring prematurely the end of flowering, which resulted in limited grain filling after accumulation of large amounts of dry matter (Johnston *et al.*, 2002). Schulte *et al.* (2013) observed the correlation between temperature during grain filling, defined during the 30 days prior to harvest, with the highest lipid content in canola.

For the oil yield, the statistical difference between sowing times was observed as a function of the significant effect of seed yield, with the first sowing season (15th May) being the most productive in vegetable oil (124.80 kg ha⁻¹).

The application of fungicide influenced the weight of one thousand seeds, seed yield and oil yield. The seeds of juncea canola showed a larger mass, which allowed greater yields of grain and oil. Similar results were obtained

by Blandino *et al.* (2012) in which they verified that the application of the fungicide based on strubirulin and triazole (azoystrobin + propiconazole) in maize, resulted in a greater weight of one thousand seeds and higher yield in comparison with the control, without application. These authors attribute this effect to increase photosynthetic efficiency.

In the present study, the incidence of white rust disease caused by the *Albugo candida* fungus was observed, which caused a 21.47% reduction in grain yield, since they promote negative effects on photosynthesis and on the dry matter partition for the (Dosio & Quiroz, 2010). Similar data are reported by Canola Council of Canada (2014) in Canada, where losses were greater than 20% when susceptible cultivars are severely attacked.

Similar to that reported for crambe, the authors are not aware of researchs evaluating the efficacy of the trifloxystrobin + prothioconazole in juncea canola.

CONCLUSIONS

With the use of the genotypes available on the market, FMS Brillhante for crambe and Terola 25A85 for canola juncea, at the sowing dates (E1: 15th May, E2: 1st June and E3: 15th June 2014), the agronomic performance and the oil content was severely limited by the occurrence of high temperatures during flowering, low precipitation and occurrence of diseases.

The phytosanitary management with fungicide application influenced the grain yields of crambe and juncea canola crops.

With the production technologies adopted for the cultivation of crambe and juncea canola, it is necessary to obtain higher yields of grain and oil, to enable commercial exploitation in the interval between the summer and winter offspring in the Northern region of Paraná.

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