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# Effect of Winter Canola Cultivar on Seed Yield, Oil, and Protein Content

Edmund T. Tetteh, Jason P. de Koff,\* Bharat Pokharel, Richard Link, and Chris Robbins

## ABSTRACT

Canola (*Brassica napus* L.) is an oilseed crop that can produce healthy cooking oil and animal feed byproducts. Although it is a relatively new crop, approved for human consumption less than 40 yr ago, advances in breeding have allowed for its production as a winter crop in the southeastern United States. There is little published research, however, related to its performance and quality in this region. Therefore, a study was conducted during the 2014–2015 (Year 1) and 2015–2016 (Year 2) seasons in Tennessee. Twenty-three varieties were planted in a randomized complete block design with four replications across both years to determine seed yield, seed oil, and seed protein content. Differences in fertilizer application rates, planting, and harvest management and differences in weather conditions probably led to significant interactions between years. Cultivar yields ranged from 1269 to 2647 and 1494 to 4199 kg ha<sup>-1</sup>, seed oil content ranged from 44 to 48% and from 43 to 46%, and seed protein content ranged from 20 to 24% and from 19 to 23% for Years 1 and 2, respectively. In each year, open-pollinated cultivars had significantly lower yield and oil content but significantly greater protein content than hybrid cultivars. There was also a strong negative correlation between seed oil and seed protein and the linear models were significant ( $r = 0.88$ ,  $p < 0.0001$  for Year 1;  $r = 0.85$ ,  $p < 0.0001$  for Year 2). Recommended winter canola cultivars include Exp1302 and Hekip.

## Core Ideas

- Little published research is available related to the performance of winter canola in the southeastern United States.
- Yield, oil, and protein content were identified across 23 potential winter canola cultivars over 2 yr.
- Significant linear relationships were observed between seed oil and protein content but not when compared with yield.

CANOLA IS an oilseed crop that is derived from rapeseed but has low levels of erucic acid and glucosinolates, which make it palatable to both humans and animals. In 2015, over 0.7 million ha of canola were harvested in the United States at an average yield of about 1900 kg ha<sup>-1</sup>, with North Dakota producing the majority of the canola (USDA-NASS, 2016). Canola is important for its oil as well as its meal. The oil is considered a healthy substitute to most other cooking oils because of its low saturated fat content and high level of omega-3 fatty acids (Gebauer et al., 2006; Lin et al., 2013). The oil can also be used as a feedstock in the production of biodiesel. In 2015, the United States produced only about one-third of the canola oil supply that it consumed (USDA-Economic Research Service, 2016). The canola meal remaining after the oil has been extracted is an important animal feed, particularly for dairy cattle (Paula et al., 2018). Depending on the cultivar, canola can be seeded either in the spring or fall. Spring canola generally does not require a period of vernalization like winter canola (Rahman and McClean, 2013) and is grown where environmental conditions during winter are not conducive to the survival of the canola plants. Winter canola has been observed as having a more vigorous root system than spring canola, which has been linked to higher seed yields (Rahman and McClean, 2013). As a result, winter canola, when winter survival occurs, generally produces higher yields than spring canola (Hunter et al., 2010; Boyles et al., 2012), and is usually grown in the High Plains, Great Plains, and Southeastern regions of the United States.

Winter canola can serve as a cover crop by restricting nutrient and soil losses during colder months (White et al., 2015), providing food for pollinators (Eberle et al., 2015; Thom et al., 2018) while also providing additional revenue to farmers once it is harvested in spring. As it is a winter crop, canola may compete less for land than corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] and can serve as an important rotational crop with winter wheat (*Triticum aestivum* L.). Bushong et al. (2012) observed that rotations with winter wheat could provide 10 to 22% higher wheat yields than continuous wheat production. Winter canola has also been identified for its potential dual-use as a forage crop for ruminants. Neely et al. (2015) observed that early planted winter canola could yield high-quality forage (5 Mg ha<sup>-1</sup>) and still produce modest seed yields (~2000 kg ha<sup>-1</sup>).

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**Abbreviations:** NWCVT, National Winter Canola Variety Trial.

Recent research involving participants in the National Winter Canola Variety Trials (2003–2012) across the United States identified that winter canola has the potential to yield seed up to 7 Mg ha<sup>-1</sup>, though most yields range from 0 to 4 Mg ha<sup>-1</sup> (Assefa et al., 2014). The few studies on winter canola available in the United States focused on the effects of planting date, tillage, and N and P fertilization on yield (Conley et al., 2004; Holman et al., 2011; Assefa et al., 2014).

As the approval of canola oil for human consumption occurred relatively recently (1985), breeding programs are comparatively new. Assefa et al. (2014) suggested the identification of genotypes as one factor that future canola research should focus on for increasing yields. As a large proportion (73%) of the variability in winter canola yields may be caused by the environment, with the remaining variability caused either by genetics or the interaction between genetics and the environment, it is particularly important to understand how cultivars interact in specific regions. Currently, there are few published scientific studies of winter canola in the United States, particularly for areas outside the Great Plains. Therefore, our objective was to evaluate the yield, oil, and protein content of 23 winter canola cultivars over a 2-yr winter canola harvest (summer fallow) period (2015 and 2016) in Tennessee.

## MATERIALS AND METHODS

### Field Methods

The study was conducted at the Tennessee State University Agricultural Research and Education Center in Ashland City, TN. The soils at the field site are Lindside–Nolin silt loam soil (fine-silty, mixed, mesic Fluvaquentic Eutrochrepts and fine-silty, mixed, mesic Dystric Fluventic Eutrochrepts) (Jenkins et al., 2002). Field plots were established on a site that had been previously planted to winter canola the year prior, followed by a summer fallow period. The plots measured 0.5 by 3.7 m and consisted of three 3.7-m rows of winter canola with about 0.2 m row spacing and 0.3 to 0.6 m in between plots in the same block. This was performed with a randomized complete block design with four replicates. In August 2014 (Year 1), glyphosate [2-(phosphonomethylamino)acetic acid] herbicide (3 kg a.i. ha<sup>-1</sup>) (Cornerstone, Winfield Solutions, St. Paul, MN) was applied to the field site. About 1 wk prior to planting, 19 kg N ha<sup>-1</sup>, 13 kg K ha<sup>-1</sup>, and 7 kg S ha<sup>-1</sup> fertilizers and trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl)aniline] herbicide (3 kg a.i. ha<sup>-1</sup>) (Treflan, Dow AgroSciences, Indianapolis, IN) was applied and tilled. Paraquat [1-methyl-4-(1-methylpyridin-1-ium-4-yl)pyridin-1-ium] (0.28 kg a.i. ha<sup>-1</sup>) (Gramoxone, Syngenta Crop Protection, Greensboro, NC) was applied 1 d before planting. Forty varieties of winter canola seed were received from the National Winter Canola Variety Trial (NWCVT), which had been treated with thiamethoxam [(*NZ*)-*N*-[3-[(2-chloro-1,3-thiazol-5-yl)methyl]-5-methyl-1,3,5-oxadiazinan-4-ylidene]nitramide] insecticide and fludioxonil [4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1*H*-pyrrole-3-carbonitrile], difenoconazole [1-[(2-(2-chloro-4-[4-chlorophenoxy]phenyl)-4-methyl-1,3-dioxolan-2-yl)methyl]-1,2,4-triazole], and (*R*)-[(2,6-dimethylphenyl)-methoxyacetylaminio]-propionic acid methyl ester fungicides. The insecticide and fungicides identified all make up the seed treatment called Helix (Syngenta). The canola varieties were planted on 10 September with a push

planter (EarthWay, Bristol, IN) with a seed spacing of about 14 cm within rows. In 2015 (Year 2), the herbicides paraquat (0.56 kg a.i. ha<sup>-1</sup>) and pendimethalin (3,4-dimethyl-2,6-dinitro-*N*-pentan-3-ylaniline) (1.6 kg a.i. ha<sup>-1</sup>) (Prowl, BASF Corp., Research Triangle Park, NC) and 93 kg N ha<sup>-1</sup>, 62 kg K ha<sup>-1</sup>, and 37 kg S ha<sup>-1</sup> fertilizers were applied about 2 wk prior to planting. No P was applied in either year, on the basis of the soil test recommendations. Thirty-five varieties of winter canola seed were again received through the NWCVT and had been treated with the same insecticide and fungicides as the year before. They were planted on 28 September with a push planter (EarthWay) with a seed spacing of about 1 cm within rows. Within each original block from Year 1, the open-pollinated cultivars in Year 2 were planted randomly in plots within one area of the original block and the hybrid cultivars were planted randomly in plots within a different area of the original block, with a buffer plot between the open-pollinated and hybrid groups of plots. Otherwise, the same design was used as in Year 1, with blocks of canola planted in the same locations as the blocks from the prior year. Areas between plots were maintained with a cultivator [Kentucky high wheel (Earthway) or STIHL Yard Boss MM 55 (STIHL Inc., Waiblingen, Germany)] and areas between blocks were tilled periodically with a three-point tiller pulled behind a tractor to reduce weeds. In March 2015 and 2016, 72 and 82 kg N ha<sup>-1</sup>, respectively, was broadcast applied as urea. Entire plots were harvested on 8 to 11 June 2015 and 7 to 8 June 2016. For the Year 1 harvest, a weed trimmer with saw blade attachments was used to direct-cut the winter canola plants, which were then fed through a belt thresher (BT14, ALMACO, Nevada, IA). In Year 2, a plot combine (HP5, ALMACO) was used for harvest. Following the Year 1 harvest, seeds were further sieved by hand to remove extraneous plant material, dried in an oven at 37 to 38°C, and further cleaned with an air blast seed cleaner (ABSC, ALMACO). Following the Year 2 harvest, seed was dried in an oven at 60°C and further cleaned with a tabletop seed cleaner (Clipper Office Tester, A.T. Ferrell Company Inc., Bluffton, IN). After final cleaning in both years, seeds were weighed and yields were directly derived from these weights.

Weather data were collected from the National Climatic Data Center for Charlotte, TN (approximately 30 km from our study site) (Fig. 1). Temperature data were calculated as the average between the minimum and maximum temperatures for each day and averaged across each month. Missing data occurred in Year 1 for February (1 d) and April (1 d) and in Year 2 for December (2 d) and January (6 d).

### Laboratory Methods

Protein and oil analyses of seed subsamples were performed by measuring four replications of the same sample with a near infrared analyzer (Da 7270, Perten Instruments, Stockholm, Sweden) and using the manufacturers' calibrations developed in 2011 for canola based on wet chemical methods. Values from the replications of each subsample were averaged for each plot. Both oil and seed protein values are reported on a moisture-free basis.

### Soil Analyses

Soil samples from 0- to 15-cm depth were collected prior to each planting season in 2014 and 2015. In 2015, these samples were collected from within blocks. Samples were dried and

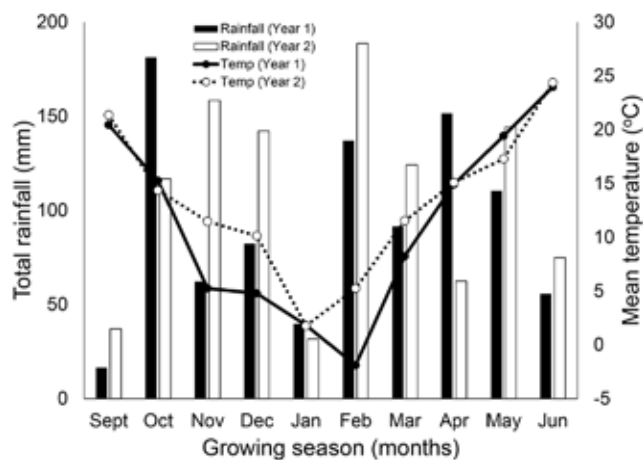


Fig. 1. Precipitation and air temperature data during the study period (September–July) in Year 1 (2014–2015) and Year 2 (2015–2016). Average air temperature values were calculated as averages between the minimum and maximum temperature for each month.

ground to <2 mm and analyzed for pH and P, K, Ca, Mg via Mehlich I extraction conducted by the University of Tennessee Soil, Plant and Pest Center (Nashville, TN). Nitrate and ammonium analyses were conducted according to the USEPA methods 353.2 and 350.1 (USEPA 1993a, 1993b), respectively, with a Lachat QuikChem (8000 series, Hach Co., Loveland, CO) by A&L Great Lakes Laboratories (Fort Wayne, IN). The results can be found in Table 1.

### Statistical Analyses

As some of the cultivars were only planted in one of the years because of seed availability through the NWCVT, only the 23 cultivars that were planted in both years were used for the analyses.

The seed yield ( $\text{kg}\cdot\text{ha}^{-1}$ ), seed protein content (%), and seed oil content (%) of 23 cultivars were evaluated in two planting seasons. For Year 1, data (seed yield, seed oil, and seed protein) from two plots (from different cultivars) were removed from the analysis because of very low yields ( $<15 \text{ kg}\cdot\text{ha}^{-1}$ ), probably caused by the low seeding rate or the belt thresher used. For Year 2, data from one whole block and either 10 (yield data) (cultivars DK Imistar, DK Sensei, DK Severnyi, Edimax CL, Einstein, Exp1302, Hekip, Mercedes, MH12AX37, and PX112) or three (oil and protein data) (cultivars PX112, MH12AX37, and Mercedes) additional plots were removed from the analysis because of mechanical error caused by the plot combine and/or human error. Each cultivar, however, had no less than two replicates. One-way ANOVA with Fisher's LSD post-hoc tests were performed in the R Statistical Computing environment (R Development Core Team, 2017). Probability values less than 0.05 were considered statistically significant. We evaluated whether seed yield, seed protein, and seed oil were significantly different by cultivar in each year. We then ranked cultivars in each year and assessed the monotonic relationship between years using Spearman's correlation test. As some cultivars were hybrids and others were open-pollinated, we also tested the effects of pollination on yield, protein, and oil content within each year. Other correlation analyses (between seed yield and seed oil, between seed yield and seed protein, and between seed

Table 1. Soil nutrient data prior to planting and fertilizer application in Year 1 and Year 2. No significant differences were observed between years for pH or nutrients at  $\alpha < 0.05$ .

	pH	P	K	Ca	Mg	$\text{NO}_3\text{-N}$	$\text{NH}_4^+\text{-N}$
	mg $\text{kg}^{-1}$						
2014	5.61	110	37	1152	84	2	10
2015	5.57	111	39	1152	82	1	9

oil and seed protein) were conducted with JMP version 9.0.0 (SAS Institute Inc., Cary, NC).

## RESULTS AND DISCUSSION

### Weather

Air temperature exhibited similar trends across both growing seasons; however, Year 1 had lower average temperatures, particularly in November, December, and February. Temperatures ranged from  $-2$  to  $24^\circ\text{C}$  in Year 1 and from  $2$  to  $24^\circ\text{C}$  in Year 2. The lowest temperatures recorded in Years 1 and 2 were  $-19$  and  $-13^\circ\text{C}$ , respectively, and the highest recorded temperatures in Years 1 and 2 were  $31$  and  $30^\circ\text{C}$ , respectively. Waalen et al. (2011) identified that *B. napus* species could tolerate short-term freeze periods (50% of plants were dead) down to between  $-17$  and  $-19^\circ\text{C}$ . They also identified long-term freeze periods (50% of plants were dead) at  $-8^\circ\text{C}$  between 9 and 21 d. In Year 1, there were five consecutive nights with temperatures ranging between  $-7$  and  $0^\circ\text{C}$  and six total nights with minimum temperatures in this range during the flowering period previously observed in this area (mid- to late March to early May). In Year 2, there were two consecutive nights with temperatures ranging between  $-1$  and  $0^\circ\text{C}$  and five total nights with minimum temperatures between  $-2$  and  $0^\circ\text{C}$  during the flowering period. These low temperatures probably led to some plant mortality and yield loss, though winter mortality was not measured.

Precipitation totals in Year 1 (929 mm) and Year 2 (1081 mm) were lower than the 30-yr (1980–2010) average of 1189 mm for Nashville, TN (35 km from the study site, 65 km from the weather station used) by about 22% in Year 1 and 9% in Year 2 (National Climate Data Center, 2014). Precipitation exhibited greater variability between years, with October having greater precipitation in Year 1, which was offset by greater precipitation in November and December in Year 2. In February and March, Year 2 had greater precipitation, which was again offset by greater precipitation in April for Year 1. In May and June, Year 2 had greater precipitation by 34 and 20 mm, respectively. According to Assefa et al. (2018), who aggregated canola yield data on the basis of water requirements, there is an average gain of  $7.2 \text{ kg}\cdot\text{ha}^{-1}$  for every mm of water above 125 mm and up to 600 mm. As both years in our study had total precipitation well above 600 mm, it is likely that this did not affect yields in either year. However, in looking at timing of rainfall for spring canola, Hergert et al. (2016) identified the need for  $1 \text{ mm}\cdot\text{d}^{-1}$  between emergence and the rosette stage,  $2$  to  $5 \text{ mm}\cdot\text{d}^{-1}$  between the late rosette and bud stages, and up to  $6 \text{ mm}\cdot\text{d}^{-1}$  at flowering and pod set (Diepenbrock, 2000; Assefa et al., 2018). According to this, September in Year 1 may have been low at  $0.6 \text{ mm}\cdot\text{d}^{-1}$  for emergence and rosette formation and both April ( $5.2 \text{ mm}\cdot\text{d}^{-1}$  in Year 1;  $2.1 \text{ mm}\cdot\text{d}^{-1}$  in Year 2) and May ( $3.6 \text{ mm}\cdot\text{d}^{-1}$  in Year 1;  $4.7 \text{ mm}\cdot\text{d}^{-1}$  in Year 2) in both years may have had low water availability when flowering and pod set usually occurs.

## Seed Yield

Table 2. LSD post-hoc tests among varieties for each year. Different letters denote significant differences at the 5% significance level within each year.

Cultivars and year	Seed yield† kg ha <sup>-1</sup>	Oil dry weight %	Protein dry weight
2015			
Claremore‡§	1611	45.1fghij	23.0ab
DK Imiron CL	2603	43.6jk	23.6a
DK Imistar CL	2272	45.6fg	22.8abc
DK Sensei	1731	44.0hijk	23.8a
DK Severnyi	1656	45.8ef	21.2e
Edimax CL§	2398	43.7ijk	22.6abcd
Einstein	2576	47.7abc	19.5f
Exp1302	2647	47.8ab	21.4cde
Hekip§	2346	46.3bcdef	21.3de
Hornet§	2008	46.2cdef	21.0e
Inspiration§	1404	46.1def	21.8bcde
Mercedes§	2321	47.4abcd	20.6ef
MHI1J41	2296	47.3abcd	20.9ef
MHI2AX37	1436	43.5k	23.4a
Popular§	2166	47.2abcde	21.5cde
PX112	1937	48.0a	21.0e
Riley‡§	1921	45.3fgh	22.9ab
Sumner‡§	1269	45.2fghi	23.4a
Torrington‡§	2013	44.3ghijk	23.5a
Virginia‡§	2054	43.6ijk	23.5a
VSX-3‡	1665	43.7ijk	23.8a
VSX-4‡	1321	44.0hijk	23.5a
Wichita‡§	1588	45.3fgh	23.0ab
2016			
Claremore‡§	3971	45.1bcd	22.6ab
DK Imiron CL	2023	44.8cde	20.8cde
DK Imistar CL	3349	43.9efgh	22.3ab
DK Sensei	3699	45.7abc	20.1efg
DK Severnyi	3097	44.9bcde	20.6cde
Edimax CL§	3252	45.7abc	19.0fg
Einstein	3356	46.4a	18.7g
Exp1302	4199	45.8abc	20.2def
Hekip§	3742	45.1bcd	19.9efg
Hornet§	3677	45.6abc	20.0efg
Inspiration§	2993	45.6abc	19.6efg
Mercedes§	1971	46.3ab	19.5efg
MHI1J41	2563	46.1ab	19.6efg
MHI2AX37	2848	43.8efgh	21.6bcd
Popular§	2592	46.0ab	19.7efg
PX112	1494	45.3abcd	20.2defg
Riley‡§	2179	44.5cdef	22.2ab
Sumner‡§	2887	44.6cdef	23.1a
Torrington‡§	2888	44.1defg	21.6bc
Virginia‡§	1785	42.8h	23.3a
VSX-3‡	3024	43.0gh	22.7ab
VSX-4‡	1609	43.4fgh	22.4ab
Wichita‡§	2453	44.2defg	22.9ab

† Seed yield values are presented as air dried weight and seed oil and protein contents are presented on a zero moisture basis. ANOVA for seed yield was not significant, therefore, no post-hoc test was conducted.

‡ Open-pollinated.

§ Commercially-available in the United States.

In Year 1, yield averages for cultivars ranged from 1269 to 2647 kg ha<sup>-1</sup>, with an overall average of 1967 kg ha<sup>-1</sup>; in Year 2, yield averages for cultivars ranged from 1494 to 4199 kg ha<sup>-1</sup> with an overall average of 2854 kg ha<sup>-1</sup> (Table 2). In 2015 and 2016, average yields (winter and spring canola) in the United States were 1880 and 2044 kg ha<sup>-1</sup>, respectively, which are somewhat similar to our yields (USDA-NASS, 2016; 2018). The difference between our average yield and the US average yield was greatest for Year 2. This may have been caused by the much greater application rates for N, K, and S as soil nutrients and fertilizer are one of the factors with the highest impacts on canola (Assefa et al., 2018). For example, a grain harvest yield of 2074 kg ha<sup>-1</sup> for winter canola grown in Kansas had nutrient uptake rates of 157 kg N ha<sup>-1</sup>, 132 kg K ha<sup>-1</sup>, and 23.5 kg S ha<sup>-1</sup> (Ciampitti et al., 2014). The difference may also reflect greater weather differences between Tennessee and other areas of the United States between the years as well as the differences in planting and harvest management identified previously.

Interactions by cultivar were not significant for yield within each year (Table 3). Averaged across all plots, Year 1 was significantly lower in yield than Year 2 (Fig. 2a). A Spearman's rank order correlation analysis found a very low correlation ( $r = 0.19$ ) between years, meaning that those cultivars performing well in 1 yr, probably did not perform well in the other year and vice versa (Fig. 3a). Though the environment produces the greatest variability in canola yields (Assefa et al., 2014), and weather is an annually variable component within the environment, the interactions by year may have also been caused by differences in fertilizer rates, planting rates, and harvest management between years.

In a comparison of open-pollinated (eight in total) and hybrids (15 in total) within each year, hybrid cultivars had a significantly greater yield (2120 vs. 1696 kg ha<sup>-1</sup> across plots in Year 1; 2959 vs. 2600 kg ha<sup>-1</sup> across plots in Year 2) (Fig. 4a). This is probably caused by the heterosis of the crop and breeding efforts to increase yield. Champagain and Good (2015) identified a 5% contribution of genetics to spring canola yield, whereas Assefa et al. (2014) found that around 27% of winter canola yield variability was caused by genetics or interactions between genetics and the environment. In Year 1, Exp1302 had the greatest yield and there were three other cultivars that had yields within 10% of Exp1302 (cultivars DK Imiron CL, Einstein, and Edimax CL) (Table 2). The five highest yielding cultivars in Year 1, which had a range of 2346 to 2647 kg ha<sup>-1</sup>, included Exp1302, DK Imiron CL, Einstein, Edimax CL, and Hekip. Of these cultivars, only Edimax CL and Hekip are currently commercially available in the United States and all are hybrids. In Year 2, Exp1302 had the greatest yield and cultivar Claremore had a yield that was within 10%. The five highest yielding cultivars in Year 2, which had a range of 3677 to 4199 kg ha<sup>-1</sup>, included the cultivars Exp1302, Claremore, Hekip, DK Sensei, and Hornet. Of these cultivars, Claremore, Hekip, and Hornet are currently commercially available in the United States and Claremore is open-pollinated.

In comparison with Year 1 data, a variety trial conducted during the same year in Griffin, GA, found that the cultivars MHI2AX37, DK Imiron CL, DK Imistar CL, DK Sensei, DK Severnyi, Edimax CL, Hornet, Inspiration, and VSX-3 were among a group of 13 higher-yielding cultivars out of 50 tested cultivars (Stamm and Dooley, 2016). As mentioned previously,

Table 3. ANOVA table partitioning the sources of variances for seed yield, seed oil, and seed protein content among 23 cultivars of winter canola for each year (Year 1 and Year 2). Seed yield values are the air-dried weight and seed oil and protein contents are on a zero moisture basis.

Sources	Seed yield			Seed oil			Seed protein		
	DF†	F-value	p-value	DF	F-value	p-value	DF	F-value	p-value
Cultivars (Year 1)	22	1.41	0.143 ns	22	8.56	<0.001***	22	6.38	<0.001***
Errors	67	–	–	67	–	–	67	–	–
Cultivars (Year 2)	22	1.73	0.071 ns	22	5.48	<0.001***	22	9.44	<0.001***
Errors	36	–	–	43	–	–	43	–	–

\*\*\* Significant at the 0.001 probability level.

† DF, degrees of freedom

DK Imiron CL and Edimax CL were also cultivars in our study that had numerically greater yields than other cultivars. Yields of the same cultivars ranged from 1270 to 3159 kg ha<sup>-1</sup> which was relatively similar to the range in our research (1269 to 2647 kg ha<sup>-1</sup>). In Orange, VA, for the same year, the cultivars DK Imistar CL, Einstein, Inspiration, Mercedes, and Popular were among a group of 11 higher-yielding cultivars out of 50 (Stamm and Dooley, 2016). In our study, Einstein was both within the five highest-yielding cultivars and within 10% of the highest-yielding variety (Table 2). Among those identified as having lower yields by Stamm and Dooley (2016) (cultivars Claremore, DK Imiron CL, DK Sensei, DK Severnyi, Edimax CL, Exp1302, Hekip, Hornet, MH11J41, MH12AX37, PX112, Riley, Sumner, Virginia, VSX-3, VSX-4, and Wichita), Exp1302, DK Imiron CL, Edimax CL, and Hekip were identified in our study as having some of the greatest yields, though they were not significant. Across the same cultivars between our study and the trial in Orange, VA in Stamm and Dooley (2016), yields ranged from 2338 to 3444 kg ha<sup>-1</sup>, whereas our yields were lower with a range from 1269 to 2647 kg ha<sup>-1</sup>. Fall N rates in the Orange, VA, study were slightly higher than in our study (34 vs. 19 kg ha<sup>-1</sup>) and spring N rates were relatively similar, which may indicate other environmental effects caused the lower yields in our study. The low seeding rates and harvest equipment in Year 1 may also have contributed to these lower yields. A variety trial conducted in Shorter, AL, (Stamm and Dooley, 2016) identified DK Sensei (2877 kg ha<sup>-1</sup>) and Hornet (2566 kg ha<sup>-1</sup>) as part of a higher yielding group of 4 out of 20 cultivars, which was different from our study, where DK Sensei had a yield of 1731 kg ha<sup>-1</sup> and Hornet had a yield of 2008 kg ha<sup>-1</sup>. DK Imiron CL, DK Imistar CL, DK Severnyi, Edimax CL, Hekip, MH11J41, and MH12AX37 all had comparatively lower yields than the other cultivars in that study, though DK Imiron CL, Edimax CL, and Hekip were among those with the greatest yields in our research. Overall, as with the previous example, the ranges in the yields of the same cultivars were lower in our study than the Shorter, AL trial (Stamm and Dooley, 2016) (1436–2603 kg ha<sup>-1</sup> vs. 1882–2877 kg ha<sup>-1</sup>). The difference was likely to be a combination of our lower N application rates, planting rates, and harvest management (which also led to lower yields than in Year 2), along with differences between environments.

In a variety trial in Springfield, TN, Edimax CL, Einstein, Hekip, Hornet, Inspiration, Mercedes, and Popular were all among the highest-yielding cultivars (a group of 11) out of 28 varieties harvested in 2016 and had yields ranging from 5575 to 6244 kg ha<sup>-1</sup> (Stamm et al., 2017). In comparison with our study, Hekip and Hornet in the Springfield, TN, trial were among the numerically highest-yielding cultivars (Stamm et al., 2017).

The range in the Springfield, TN, yields was much greater than those in the current study (3942–6244 kg ha<sup>-1</sup> vs. 1609–3742 kg ha<sup>-1</sup> for the same cultivars) and may have been caused by the difference in applied N. The Springfield, TN, trial applied 34 kg ha<sup>-1</sup> in fall and 134.5 kg ha<sup>-1</sup> in spring, whereas the current study applied 93 kg ha<sup>-1</sup> in fall and 82 kg ha<sup>-1</sup> in spring in the same year (Year 2). Even though Year 2 of the current study had more total N applied, the spring applied N was greater in the Springfield, TN, trial and may have had a stronger impact, as Ciampitti et al. (2014) observed a critical period for nutrient uptake about 2 to 3 wk before and after flowering in winter canola. Furthermore, researchers in Oregon found that spring N application could increase yields by 75% and identified a spring N rate of 112 kg ha<sup>-1</sup> as the optimal rate (Ferguson et al., 2016). It is also possible that environmental conditions may have led to these differences in yields, as both sites are <32 km apart and have similar soil but the Springfield, TN, site is 100 m higher in elevation (Stamm et al., 2017). In Year 2, a variety trial in Orange, VA, (Stamm et al., 2017) identified the cultivars Claremore (3078 kg ha<sup>-1</sup>), Torrington (2823 kg ha<sup>-1</sup>), and VSX-3 (2788 kg ha<sup>-1</sup>) as part of a higher-yielding group of 6 out of 24 cultivars studied, which is similar to our study. These three cultivars in our study all had greater yields than in Orange, VA for the same year though Torrington was similar. In the Orange, VA, trial, Sumner, Riley, Virginia, VSX-4, and Wichita all had lower yields with a range of 2429 to 2712 kg ha<sup>-1</sup> (Stamm et al., 2017). In our study, Sumner had greater yields (about 300 kg ha<sup>-1</sup> greater) and Riley, Virginia, VSX-4, and Wichita had lower yields (about 250–960 kg ha<sup>-1</sup>

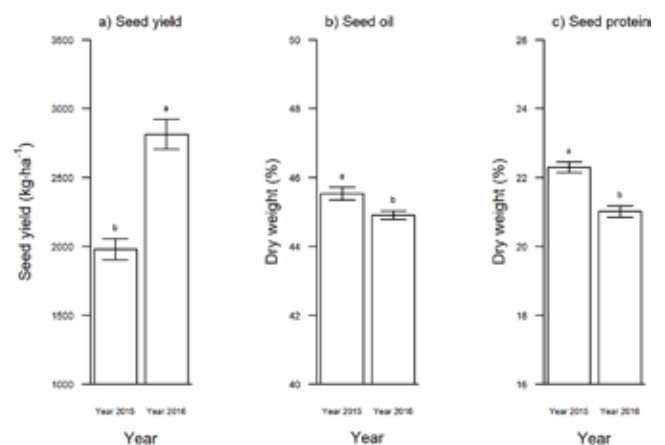


Fig. 2. Comparison of average (a) seed yield (kg ha<sup>-1</sup>), (b) seed oil content (%), and (c) seed protein content (%) by year. Seed yield values are presented as air-dried weight and seed oil and protein contents are presented on a zero moisture basis. Values followed by the same letter are not significantly different from each other ( $\alpha < 0.05$ ). Error bars represent one SE from the mean.

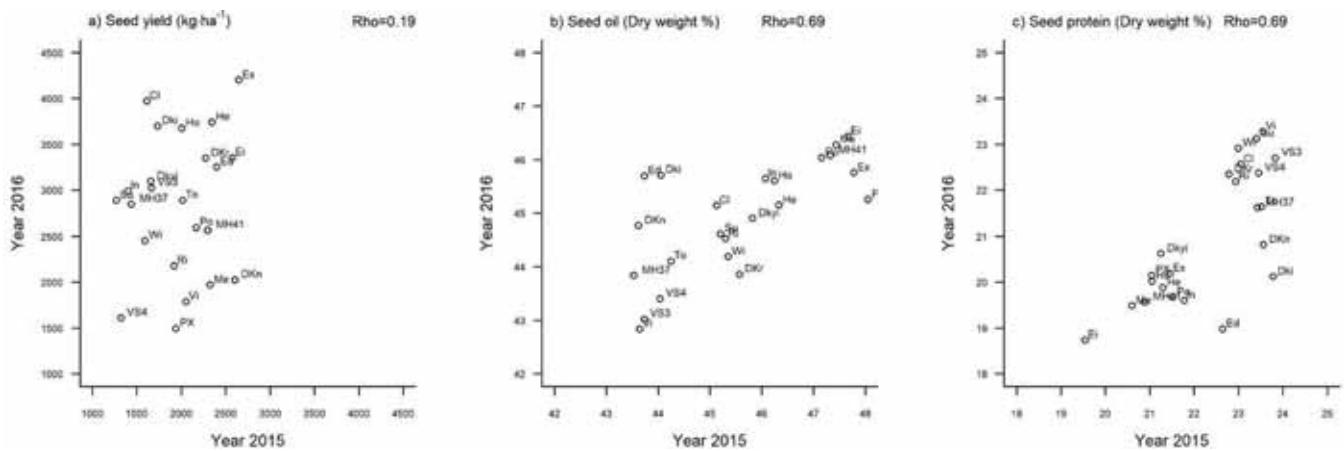


Fig. 3. Spearman's correlation test between Year 1 (2015) and Year 2 (2016) for (a) seed yield ( $\text{kg ha}^{-1}$ ), (b) seed oil (%), and (c) seed protein (%). Seed yield values are presented as air-dried weight and seed oil and protein contents are presented on a zero moisture basis. Labels represent cultivars as follows: Cl, Claremore; DKn, DK Imiron CL; DKr, DK Imistar CL; Dki, DK Sensei; Dkyi, DK Severnyi; Ed, Edimax CL; Ei, Einstein; Ex, Exp1302; He, Hekip; Ho, Hornet; In, Inspiration; Me, Mercedes; MH41, MH11J41; MH37, MH12AX37; Po, Popular; PX, PX112; Ri, Riley; Su, Sumner; To, Torrington; Vi, Virginia; VS3, VSX-3; VS4, VSX-4; Wi, Wichita.

lower) as compared to the Orange, VA trial. The Orange, VA, trial had a lower fall N application rate ( $34$  vs.  $93 \text{ kg N ha}^{-1}$ ) and slightly lower spring N application rate ( $67$  vs.  $82 \text{ kg N ha}^{-1}$ ) than our study; therefore, other factors, like weather and soil type, probably caused more of these differences. This was the second year where a number of cultivars in our study had lower yields than the Orange, VA, trial (Stamm et al., 2017), which further corroborates these likely causes. Another trial conducted in the same year in Shorter, AL, (Stamm et al., 2017) identified Einstein, DK Imistar CL, Inspiration, and Popular in a higher-yielding group of 6 out of 20 cultivars, which is similar to the results of our study for the same year. Other cultivars that had lower yields included DK Imiron CL, Edimax CL, Exp1302, Hekip, Hornet, Mercedes, MH11J41, MH12AX37, PX112, Virginia, VSX-3, and VSX-4. In our study, however, Exp1302, Hekip, and Hornet all had numerically greater yields than other cultivars. Though N fertilizer rates were greater ( $34 \text{ kg N ha}^{-1}$  in fall and  $135 \text{ kg N ha}^{-1}$ ) than in our study, the yields in the Shorter, AL trial were very low ( $490$ – $1657 \text{ kg ha}^{-1}$ ) and were likely to be caused by the late planting and significant disease pressure identified in Stamm et al. (2017).

### Seed Oil

In Year 1, cultivar averages for oil contents ranged from  $43.5$  to  $48.0\%$  with an overall average of  $45.5\%$ ; in Year 2, cultivar averages for oil contents were between  $42.8$  and  $46.4\%$ , with an overall average of  $44.9\%$  (Table 2). There was no significant linear relationship observed between oil and yield for either Year 1 ( $r = 0.18, p = 0.08$ ) or Year 2 ( $r = 0.19, p = 0.15$ ) (data not shown), which is consistent with other results (Gomez and Miralles, 2011). The range and average oil values were in the upper range of and higher than, respectively, oil contents observed during the National Winter Canola Variety Trials between 2003 and 2012, which ranged from  $30$  to  $47\%$  ( $32$ – $49\%$ , dry weight basis) with an average of  $39\%$  ( $41\%$ , dry weight basis) (Assefa et al., 2014).

Similar to the yield data, the main effects of cultivar and year and the cultivar  $\times$  year interaction were significant for oil content (Table 3). Year 2 oilseeds had a significantly lower oil content than Year 1 (Fig. 2b). A Spearman's rank order correlation analysis found a moderate correlation ( $r = 0.69$ ) between

years, meaning that those cultivars performing well in 1 yr may also have performed well in the other year and vice versa (Fig. 3b). Higher temperatures during canola seed development have been observed to result in lower concentrations of oil (Canvin, 1965); however, as average monthly temperatures (and average high temperatures, data not shown) were relatively similar during this period (May–June), this was not likely to be the cause. Walton et al. (1999) identified greater oil content with greater rainfall during seed development. This is different from our study, where Year 2 had greater rainfall during this period (May–June) than Year 1 but had lower oil concentrations. Nitrogen has been found to cause a decrease in oil content in *B. napus* seeds (Appelqvist, 1968; Harker et al., 2012) and as greater N rates were applied in both the fall and spring of Year 2 than in Year 1, this may have led to the lower oil content in Year 2. Alternatively, some studies have identified increases in oil content in winter and spring canola with lower seeding densities, though they have also identified a decrease or no change among different cultivars, locations, or years (Moore and Guy, 1997; Hanson et al., 2008). It is possible that a lower plant population density caused by potential winter mortality and a lower seeding rate (than in Year 2) could have supplied more nutrients overall to the plants and enhanced oil content in Year 1.

In a comparison of open-pollinated and hybrids for each year, the hybrid cultivars had significantly more oil content ( $46.0$  vs.  $44.5\%$  across plots in Year 1;  $45.4$  vs.  $44.0\%$  across plots in Year 2) (Fig. 4b). As with yield, breeding has been focused on increasing oil content and about  $6\%$  of the variance in oil content can be explained by genetic differences (Assefa et al., 2014).

In Year 1, PX112 had the greatest oil content ( $48.0\%$ ) and was statistically similar to five other cultivars (Einstein, Exp1302, Mercedes, MH11J41, and Popular) (Table 2). Of these cultivars, only Mercedes and Popular are currently commercially available in the United States and none is an open-pollinated variety. In Year 2, Einstein had the greatest oil content ( $46.4\%$ ) and was statistically similar to nine other cultivars (DK Sensei, Edimax CL, Exp1302, Hornet, Inspiration, Mercedes, MH11J41, Popular, and PX112) (Table 2). Of these cultivars, Edimax CL, Hornet, Inspiration, Mercedes, and Popular are currently commercially available in the United States and none are open-pollinated.

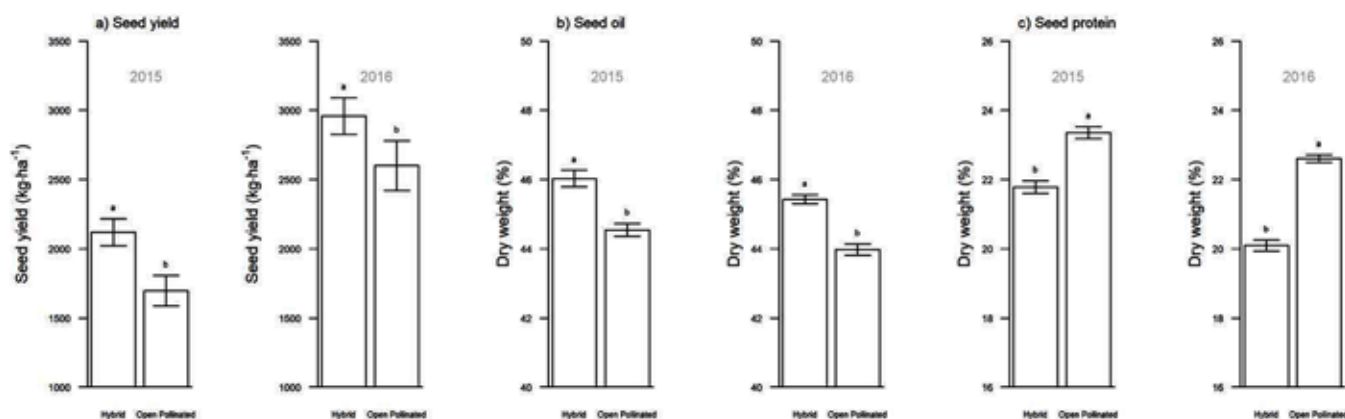


Fig. 4. Comparison of average (a) seed yield ( $\text{kg ha}^{-1}$ ), (b) seed oil content (%), and (c) seed protein content (%) between hybrid and open-pollinated cultivars for each year. Seed yield values are presented as air-dried weight and seed oil and protein contents are presented on a zero moisture basis. Values followed by the same letter are not significantly different from each other ( $\alpha < 0.05$ ). Error bars represent one SE from the mean.

In a variety trial harvested in 2015 in Griffin, GA, Einstein, Popular, Exp1302, PX112, Hekip, DK Imistar CL, Inspiration, Mercedes, and VSX-3 were identified within a group of 23 higher oil-producing cultivars out of 50 cultivars (Stamm and Dooley, 2016). MH11J41 was not among these high oil cultivars, although it was during the same year as our study. These cultivars had a range of 40.6 to 42.6% (42.7 to 44.8%, dry weight basis) at Griffin, GA, which was much lower than our high oil cultivars (47.2–48.0%) (Stamm and Dooley 2016). The overall range of oil content for the same cultivars was 37.6 to 42.6% (39.6–44.8%, dry weight basis) (Stamm and Dooley 2016), compared with 43.5 to 48.0% in our study. Unfortunately, there is no specific information related to the conditions of the trial, so the potential causes of this difference are difficult to estimate. A trial in Orange, VA, (Stamm and Dooley, 2016) containing 50 cultivars identified Inspiration as a high-oil cultivar, along with those identified in our study, but did not include Exp1302. The cultivars containing greater oil contents identified above (Einstein, Popular, PX112, MH11J41, Inspiration, and Mercedes) ranged from 41.5 to 43.6% (43.7–45.9%, dry weight basis), a range which again was lower than the range of high-oil cultivars in our study. Spring N application was lower in Orange, VA, than in our trial, which usually results in greater seed oil content. Therefore, the lower seeding density in our study (described above) and/or environmental factors probably caused this difference. In Shorter, AL, the same cultivars ranged from 40.4 to 41.4% (42.5–43.6%, dry weight basis) (Stamm and Dooley, 2016) compared with 43.5 to 47.3% in our study. The trial had a higher spring N application (135 vs. 72  $\text{kg ha}^{-1}$ ), which may have led to this difference; however, it is likely that the lower seeding density in our study and other environmental factors were also involved.

In a variety trial harvested in 2016 in Springfield, TN, Einstein, Hornet, Inspiration, Mercedes, MH11J41, and Popular were among a group of 13 higher oil-producing cultivars out of 28 cultivars (Stamm et al., 2017). Edimax CL was not identified within the higher oil yielding group, as in our Year 2 study, but Hekip and MH12AX37 were identified, which differed from our research. Those cultivars ranged in oil content from 44.6 to 45.7% (46.9–48.1%, dry weight basis) and were greater than the range of our high oil cultivars (45.3–46.4%). Though there was a greater spring N application in the Springfield, TN, trial (135

vs. 82  $\text{kg ha}^{-1}$ ), which would normally lead to lower oil contents, the environmental conditions may have led to these differences in oil contents, as both sites are <32 km apart and have similar soil, but the Springfield, TN, site is 100 m higher in elevation. In the Orange, VA, trial harvested in the same year (Stamm et al., 2017), none of the cultivars used in our study were identified within a higher oil yielding group. The same cultivars ranged in oil contents from 37.3 to 39.1% (39.3–41.2%, dry weight basis) in Orange, VA, which was lower than the 42.8 to 45.1% oil contents found in our research. The Orange, VA, trial had a lower spring N application rate (67–82  $\text{kg ha}^{-1}$ ), again indicating that environmental differences probably played a larger role.

### Seed Protein

In Year 1, the average cultivar seed protein contents ranged from 19.5 to 23.8%, with an overall average of 22.3%; in Year 2, the average cultivar seed protein contents ranged from 18.7 to 23.3%, with an overall average of 21.0%. These average values are comparable to observed protein contents for winter canola averaged across two sites and 4 yr in the state of Washington under similar N rates (N = 90  $\text{kg ha}^{-1}$ , 19.9%; N = 180  $\text{kg ha}^{-1}$ , 20.1%) (Hammac et al., 2017). Harker and Hartman (2016) identified a range for protein between 16.5 and 22.6%, with an average of 20.4% (at 8.5% moisture) for spring canola in Canada, which also relates well to our values. Ma et al. (2016) observed a range of 18.4 to 30.0% (dry weight basis) with an average of 24.2% for spring canola across sites in Canada.

Like the yield and oil content data, the main effects of cultivar and year and the cultivar  $\times$  year interaction were significant for protein content (Table 3). Year 1 had significantly more seed protein content than Year 2 (Fig. 2c). A Spearman's rank order correlation analysis found a moderate correlation ( $r = 0.69$ ) between years, meaning that those cultivars performing well in Year 1 may also have performed well in Year 2 and vice versa (Fig. 3c). High N application rates usually increase protein content (Seymour and Brennan, 2017; May et al., 2010; Lemke et al., 2009), though others have not found as consistent a relationship (Harker and Hartman, 2016; Hammac et al., 2017). In this study, the lower overall N rate in Year 1 (91  $\text{kg ha}^{-1}$ ) vs. Year 2 (175  $\text{kg ha}^{-1}$ ) led to greater seed protein contents, indicating environmental differences between years may have played a larger role. Though



temperature stress has been related to increased protein content, the temperatures in both years during the active growing season for winter canola in our study were relatively similar (Hammac et al., 2017). Ma et al. (2016) observed lower protein contents in seed planted earlier for spring canola grown in Canada. In our study, seed was planted earlier in Year 1 (10 September) than Year 2 (28 September) but had the greater overall protein content. As with oil content, the protein content may have been enhanced in Year 1 by the lower plant population density caused by potential winter mortality and the lower seeding rates, thereby allow individual plants access to more nutrients. As oil and protein content are usually inversely related (Hammac et al., 2017; Seymour and Brennan, 2017) and they both decreased from Year 1 to Year 2, this may be the most likely cause.

In a comparison of open-pollinated and hybrid cultivars across years, the open-pollinated cultivars had significantly more seed protein content (23.3 vs. 21.8% across plots in Year 1; 22.6 vs. 20.1% across plots in Year 2) (Fig. 4c). In Year 1, VSX-3 had the greatest seed protein content (23.8%) and was statistically similar to 12 other cultivars (Table 2). Of these cultivars, Claremore, Edimax CL, Riley, Sumner, Torrington, Virginia, and Wichita are currently commercially available in the United States and Claremore, Riley, Sumner, Torrington, Virginia, VSX-3, VSX-4, and Wichita are open-pollinated. In Year 2, Virginia had the greatest seed protein content (23.3%) and was statistically similar to seven other cultivars (Table 2). Of these cultivars, Claremore, Riley, Sumner, Virginia, and Wichita are currently commercially available in the United States and Claremore, Riley, Sumner, Virginia, VSX-3, VSX-4, and Wichita are open-pollinated.

Ferguson et al. (2016) planted the Virginia cultivar in Oregon over a 3-yr period and observed an average seed protein content across N rates of 17.5% (19.1%, dry weight basis). This is much lower than the same cultivar in our study, which had 23.5% in Year 1 and 23.3% in Year 2. The precipitation and temperature were similar between the two sites and the average total N application of 140 kg ha<sup>-1</sup> in Oregon was closest to our N application in Year 2. Their average spring application of 84 kg N ha<sup>-1</sup> was also similar to our spring rates in both years. The Oregon planting dates (11–19 September) were within the same range as ours; however, the harvest date of Ferguson et al. (2016) was 2 to 3 wk later than ours and the canola was harvested by swathing in late June, followed by threshing about 2 wk later. In a Springfield, TN trial, there were no significant differences identified between cultivars harvested in 2016 for seed protein content (Stamm et al., 2017). These values ranged from 20.7 to 24.8% (21.8–26.1%, dry weight basis) and were greater than values from the same cultivars in our trial (18.7–23.3%) for the same year (Year 2). The higher spring N application rate (135 vs. 82 kg ha<sup>-1</sup>) and/or environmental factors may have resulted in these differences. A trial performed in Orange, VA, also did not identify any statistical differences among cultivars harvested in 2016 for seed protein content (Stamm et al., 2017). The values ranged from 27.1 to 28.4% (28.5–29.9%, dry weight basis) and were considerably greater than the same cultivars in our study (21.6–23.3%) for the same year (Year 2). The spring N rate was lower in the Orange, VA, trial (67 vs. 82 kg ha<sup>-1</sup>), indicating that environmental conditions probably caused these differences.

Similar to oil, there was no significant relationship between yield and seed protein for either Year 1 ( $r = 0.20$ ,  $p = 0.06$ ) or

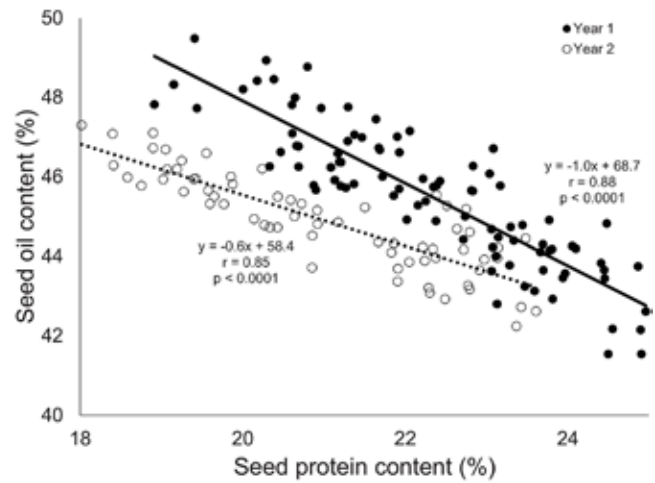


Fig. 5. Comparison of seed oil content (%) vs. seed protein content (%) for all plots in Year 1 and Year 2. Seed oil and protein contents are presented on a zero moisture basis.

Year 2 ( $r = 0.14$ ,  $p = 0.30$ ) (data not shown). There was, however, a significant negative relationship between oil and seed protein (Fig. 5) for both Year 1 ( $r = 0.88$ ,  $p < 0.0001$ ) and Year 2 ( $r = 0.85$ ,  $p < 0.0001$ ), which has been observed by others (Hammac et al., 2017; Seymour and Brennan, 2017). The relationship observed by Seymour and Brennan (2017) was Oil (%) = 64 – 0.95 × Protein (%), which was very close to our relationship for Year 1 (Fig. 5). Brennan and Boland (2009) identified the relationship as Oil (%) = 69 – 1.1 × Protein (%), which is also very similar to ours. In a comparison of seed protein to oil content (both as percentages), a slope of –1.0 was identified by Si et al. (2003), representing full substitution of protein for oil.

## CONCLUSIONS

Despite differences in fertilizer, planting, and harvest management, as well as weather between years, Exp1302 and Hekip had numerically greater yields than most of the other cultivars in each year. For oil, those exhibiting the greatest values in each year included Einstein, Exp1302, Mercedes, MH11J41, Popular, and PX112. The cultivars with the highest protein values in each year were Claremore, DK Imistar CL, Riley, Sumner, Virginia, VSX-3, VSX-4, and Wichita. Based on our data, if protein is of greater importance, such as for livestock feed, an open-pollinated variety is likely to provide more seed protein (seven out of the eight listed above are open-pollinated varieties) and therefore greater protein in the meal once the oil has been extracted. According to our results, Exp1302 is likely to perform best in Tennessee (and potentially across portions of the southeastern United States) under a variety of management practices and conditions, since it had the greatest numerical yield and had one of the highest oil contents in both years. This cultivar, however, is not currently commercially available in the United States. Although Mercedes, a cultivar that is commercially available in the United States, exhibited high oil contents, its yield in Year 2 was 53% lower than the highest-yielding cultivar. Popular, which is also available in the United States and also had high oil contents in both years, had yields that were 18 and 38% lower than the highest yielding cultivar in Years 1 and 2, respectively. Hekip had yields between 2346 and 3742 kg ha<sup>-1</sup> (11% lower than the cultivar with the greatest yields in each

year) and oil contents between 45.1 and 46.3%. This cultivar, therefore, which is commercially available in the United States, may be a reasonable alternative for farmers in this region.

### CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest.

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