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Kyle D. McGeary
Tennessee State University

Jason P. de Koff
Tennessee State University

Bharat Pokharel
Tennessee State University

Richard Link
Tennessee State University

Priya Saini
Tennessee State University

See next page for additional authors

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Authors
Kyle D. McGeary, Jason P. de Koff, Bharat Pokharel, Richard Link, Priya Saini, and Taqdeer Gill

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Effect of winter canola cultivar on seed yield, oil, and protein content

Kyle D McGeary | **Jason de Koff** | **Bharat Pokharel** | **Richard Link** | **Priya Saini** | **Taqdeer Gill**

Dep. of Agricultural and Environmental Sciences, Tennessee State Univ., 3500 John A. Merritt Blvd., Nashville, TN 37209, USA

Correspondence
Jason P. de Koff, Dep. of Agricultural and Environmental Sciences, Tennessee State Univ., 3500 John A. Merritt Blvd., Nashville, TN 37209, USA.
Email: jdekoff@tnstate.edu

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Abstract

Winter canola (*Brassica napus* L.) production has increased in the United States over the past several decades; however, there is little research in the southeastern United States on its agronomic production and growth characteristics under different nitrogen (N) fertilizer rates. The objectives of this study were to determine the effects of N rates on winter mortality, yield, oil and protein content, and seedpod shatter resistance across cultivars. Canola was grown in a randomized split complete block design with four replicates under five different N rates (0, 56, 112, 168, and 224 kg N ha^{-1}) with four different cultivars (Hekip, Inspiration, and Edimax CL or Phoenix CL). Seed yield was very low for the 2017–2018 season, likely due to extreme cold conditions in winter and/or frost at flowering. There was high winter mortality during this period, but Edimax CL had significantly lower mortality (79.7%) than Inspiration (85.7%) and Hekip (83.9%). In the following year, more mild temperatures led to low mortality and greater yields, where rates of 112, 168, and 224 kg N ha^{-1} had the greatest yields (1,612–1,857 kg ha^{-1}) with no significant differences according to cultivar. In 2018, shatter resistance was greatest for the rates of 112, 168, and 224 kg N ha^{-1} and was positively related to N rate. Across both years, Inspiration had greater shatter resistance. In 2019, the lowest N rate (0 kg N ha^{-1}) and Phoenix CL had significantly greater oil contents (48.4 and 47.3%, respectively), whereas the highest N rate (224 kg N ha^{-1}) with no particular cultivar had a significantly greater protein content (21.0%).

1 | INTRODUCTION

Winter canola (*Brassica napus* L.) is an oilseed crop that provides many benefits including high seed oil concentration, with subsequent use in cooking and as a feedstock for biofuel, effectiveness as a cover crop, benefit as a rotational crop, benefit as pollinator habitat, and use as an additive into animal feed for protein and carbohydrate value. Canola, or rapeseed, is the second largest oil crop produced and consumed in the world after soybean [*Glycine max* (L.) Merr.] (USDA-ERS, 2020). High winter canola yields are required to be a competitive alternative to corn (*Zea mays* L.) and soybean production (Wiedenhoeft et al., 2015). Canola yields from 0 to 4,000 kg ha^{-1} have been observed; however, winter canola has the potential to yield 7,000 kg ha^{-1} (Assefa et al., 2014).

Spring canola production is appropriate to areas with cold winters, like the northern Great Plains of North America, since winter hardiness is poor (Johnston et al., 2002). Winter canola cultivars are bred to exploit longer growing seasons in regions with mild winter climates, and the opportunity for vernalization such as experienced in the Pacific Northwest (Shafii et al., 1992) and the southeastern United States. Winter canola in the Pacific Northwest can yield more than twice that of spring canola in similar environments (Ehrensing, 2008). Winter canola is gaining acceptance as an alternative crop in the southern Great Plains, where production expanded to over 160,000 ha in 2014 (U.S. Canola Association, 2019).

Nitrogen (N) is the most limiting nutrient for canola production (CCC, 2021); however, canola typically requires less N than corn. Nitrogen applications are usually split with 33–50% applied at preplant and the remainder top-dressed in the spring (Lofton et al., 2017). Every 23 kg of spring canola requires about 1.2–1.5 kg N (2.7–3.3 lbs. N) (Jones & Olson-Rutz, 2016), and in North Dakota, which produces spring canola and the most U.S. canola, the average yield in 2020 was 2,197 kg ha^{-1} (USDA-NASS, 2021). Studies have shown that seed yield in canola is dependent upon available N (Jackson, 2000). Numerous studies conducted throughout the United States have shown that canola grown in various regions has distinct N requirements for successful seed yields. Canola N rates vary greatly depending on a multitude of complex interactions including region, geography, geology, soil texture, climate, spatial and temporal considerations, tillage and fertilizer application management, and cultivar. For example, a study conducted in Montana found that the total N requirement for spring canola was about 200 kg N ha^{-1} or about 7.5 kg N required for each 100 kg of seed yield (Jackson, 2000), and a study in eastern Oregon identified the total N requirement to be about 7 kg ha^{-1} per each 100 kg ha^{-1} of expected seed yield (Wysocki et al., 2007). Research at North Dakota State University indicated that the rate for optimum spring canola yield in cooler, moister regions should have an upper limit that includes the sum of residual soil N, contribution from previous crops (legumes), and fertilizer N rate of 168 kg ha^{-1} with 135 kg ha^{-1} for warmer, drier regions (Franzen, 2011). A study by Kazemeini et al. (2010) in southern Iran showed that N applied at the highest rate studied (150 kg ha^{-1}) resulted in the greatest seed yield. In a different study in India, significant increases in seed yields were observed at N rates of 100–150 kg ha^{-1} in the PGSH-51 cultivar (Buttar et al., 2006). Therefore, different cultivars of winter canola or environments may require different N rates to attain optimal seed yields. Porter et al. (2019) observed little effect of N on winter canola yields in Washington State, which were mainly affected by water availability and residual soil N concentrations. In Pakistan, Cheema et al. (2001) observed significantly enhanced seed yield with increasing N and phosphorus (P) rates up to 90 kg ha^{-1}. The seed yield increase was attributed to a larger number of pods per plant and seeds per pod. Similar results were recorded by Ahmadi and Bahrani (2009) in Iran where yield, branches per plant, pods per plant, and seeds per pod increased with increasing N rates and the highest value recorded was for 225 kg N ha^{-1}.

Core Ideas

- Cultivars like Edimax CL may help reduce winter mortality under extreme cold conditions.
- Higher nitrogen rates had significantly greater resistance to seedpod shatter.
- A nitrogen rate of 112 kg ha^{-1} and the Inspiration cultivar are recommended.

Nitrogen fertilization, however, can lower the oil concentration of canola seed, thus reducing its overall end value. According to Malhi and Gill (2004), increasing N application rates cause the oil concentration to decrease linearly. Oil concentration in spring canola at an N application rate of 120 kg ha^{-1} was 41.8% with sidebanded urea compared with 47.3% with no supplemental N applied. Cheema et al. (2001) and Ahmadi and Bahrani (2009) also observed a decrease in oil concentration with increasing N rates. Triboi-Blondel and Renard (1999) and Gunasekera et al. (2006) found a negative relationship between oil and protein concentrations. Most N rate research on winter canola has shown that higher protein results from higher N application rates (Asare & Scarisbrick, 1995; Kutcher et al., 2005).

To have a successful crop, stand establishment and winter survival are prerequisites for winter canola yields (Assefa et al., 2014; Holman et al., 2011). It has been noted that some *B. napus* cultivars can survive short-term temperatures as low as −19 ˚C, but only if adequate stand acclimation is achieved; however, cold hardiness varies among cultivars (Waalen et al., 2011). Secchi et al. (2021) determined that the number of days between −10 and −15 ˚C, the number of days with temperatures moving above and below freezing, and the wind chill temperature were important factors in determining winter mortality in winter canola. They also concluded that areas between 35 and 40˚ N latitude may have winter mortality issues. Canola requires a minimum of 43–54 plants m^{-2} to reach its yield potential (CCC, 2017) and must reach the fifth leaf stage (rosette stage) to have sufficient winter survival (Martinez-Feria et al., 2016). If canola grows poorly in the fall, it can fail to overwinter and regrow in the spring. Fall-applied N increases fall growth; however, it can result in decreased soil moisture, and the growing point going into winter may be raised above the soil surface leading to an increase in susceptibility to winter mortality (Christmas, 1996).

Some canola cultivars have a high potential for seedpod shatter during harvest, which results in yield loss. Also, a delayed harvest can result in an increase in pod shatter and reductions in seed yield (Oplinger et al., 1989). Therefore, shatter resistance is a key component that has been selected during crop improvement (Raman et al., 2014). Direct factors of pod shatter resistance are increased carbohydrates in pod walls including cellulose and lignin, which are important in pod cell walls. Hemicellulose content has also been identified as a defining factor in pod shatter resistance, with more hemicellulose leading to greater shatter resistance (Kuai et al., 2016). Kuai et al. (2016) observed that pod shatter resistance varied with cultivar and that pod shatter resistance was linearly related to pod wall weight and the water content in pod walls. According to Morgan et al. (1998), pod shatter resistance is positively correlated with pod wall thickness, but not with pod density, pod width, or seed number per pod. In other studies, pod shatter resistance was positively correlated with pod length (Summers et al., 2003), pod wall weight (Kuai et al., 2016), and vascular bundle size (Child et al., 2003).

Many plant properties of canola such as oil, protein, and seed yield are affected by N rates (Ahmadi & Bahrani, 2009), though these effects have not been thoroughly explored in canola grown in the southeastern United States. Therefore, the objectives of this research will focus on the effect of N rate on yield, oil and protein concentrations, pod shatter, and winter mortality for four commercially available winter canola cultivars in Tennessee.

2 | MATERIALS AND METHODS

2.1 | Field methods

Field research was conducted at the Tennessee State University Agricultural Research and Education Center in Ashland City, TN (36˚14′ N, 87˚2′ W) during the 2017–2018 and 2018–2019 growing seasons. The soil at the site is a Lindside–Nolin silt loam soil (fine-silty, mixed, active, mesic Fluvaquentic Eutrudepts and fine-silty, mixed, active, mesic Dystric Fluventic Eutrudepts) that is occasionally disturbed by inundation events due to flooding from the nearby Cumberland River. In the year prior to 2017–2018, the same study was performed using the same treatments, plot sizes, and locations. A randomized complete block split-plot design with four replications was established on a tilled field (33 × 36 m) with five N application rates (0, 56, 112, 168, and 224 kg N ha^{-1} as 46–0–0 urea [CO-OP]) per main effect experimental unit (5.9 × 3.7 m). These are referred to as 0N, 56N, 112N, 168N, and 224N throughout the manuscript. Main effect experimental units were separated by 3.7- or 4.3-m wide buffers, depending upon side. Each main effect experimental unit was split into three subeffect experimental units (1.7 × 3.7 m), with each of the three hybrids randomly selected for each subeffect experimental unit and separated by 0.5-m buffers for a total of 60 subeffect experimental units. Prior to fertilization in 2017, soil samples at 0- to 15-cm depth were collected from the 0N and 224N main effect experimental units and homogenized to give one sample from each of the four main effect experimental unit replicates. Samples were dried and ground to <2 mm prior to analyses.

In August, glyphosate [2-(phosphonomethylamino)acetic acid] herbicide (2.4 kg a.i. ha^{-1}; Cornerstone, Winfield Solutions) was applied to the field site. Preplant potassium (K) as commercial 0–0–60 muriate of potash was broadcast in September 2017 at 37.7 kg K ha^{-1}. In September 2018, K as 0–0–60 muriate of potash and sulfur (S) as 0–0–0–90 elemental S were broadcast prior to planting at 37.5 kg K ha^{-1} and 22 kg S ha^{-1}. The project area was treated with trifluralin (a.i. α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine; Trust, Winfield Solutions) at a rate of 850 g a.i. ha^{-1} using a Kawasaki Brute Force 650 4x4i ATV prior to planting to suppress labeled grassy and broadleaf weeds. A TeeJet spraying system affixed to the rear of the ATV was used for this application, delivering herbicide at 1.379×10^6 Pa pressure. After these fertilizer and herbicide applications, conventional tillage was implemented in an area under summer fallow in the fall with a tractor and pull-behind tilling system. Nitrogen was then applied via broadcast split (50:50) applications before planting in September and again before plants broke dormancy in March. The winter canola cultivars planted were Hekip, Edimax CL, and Inspiration, with Phoenix CL replacing Edimax CL in 2018–2019 because Edimax CL would no longer be commercially available. Each experiment was planted on 21 Sept. 2017 and 19 Sept. 2018 with 11 3.7-m rows (15-cm spacing between rows) using a push planter (EarthWay) equipped with a 1002-5 seed plate at a depth of about 0.6 cm. This provided a seeding rate of about 35 kg seed ha^{-1}. In 2017, Inspiration and Edimax CL seeds were treated with Helix Vibrance (Syngenta Crop Protection), which contains a 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-dimenthylphenyl)-methoxyacetylamino]-propionic acid methyl ester fungicides. Hekip seed was treated with Prosper EverGol (Bayer Crop Science), which contains a clothianidin {1-[(2-chloro-1,3-thiazol-5-yl)methyl]-3-methyl-2-nitroguanidine} insecticide and penflufen {*N*-[2-(1,3-dimethylbutyl)phenyl]-5-fluoro-1,3-dimethyl-1*H*-pyrazole-4-carboxamide}, trifloxystrobin {methyl (2*Z*)-2-methoxyimino-2-[2-({(*E*)-1-[3-(trifluoromethyl)phenyl] ethylideneamino}oxymethyl)phenyl]acetate}, and metalaxyl {methyl 2-[*N*-(2-methoxyacetyl)-2,6-dimethylanilino]propanoate}

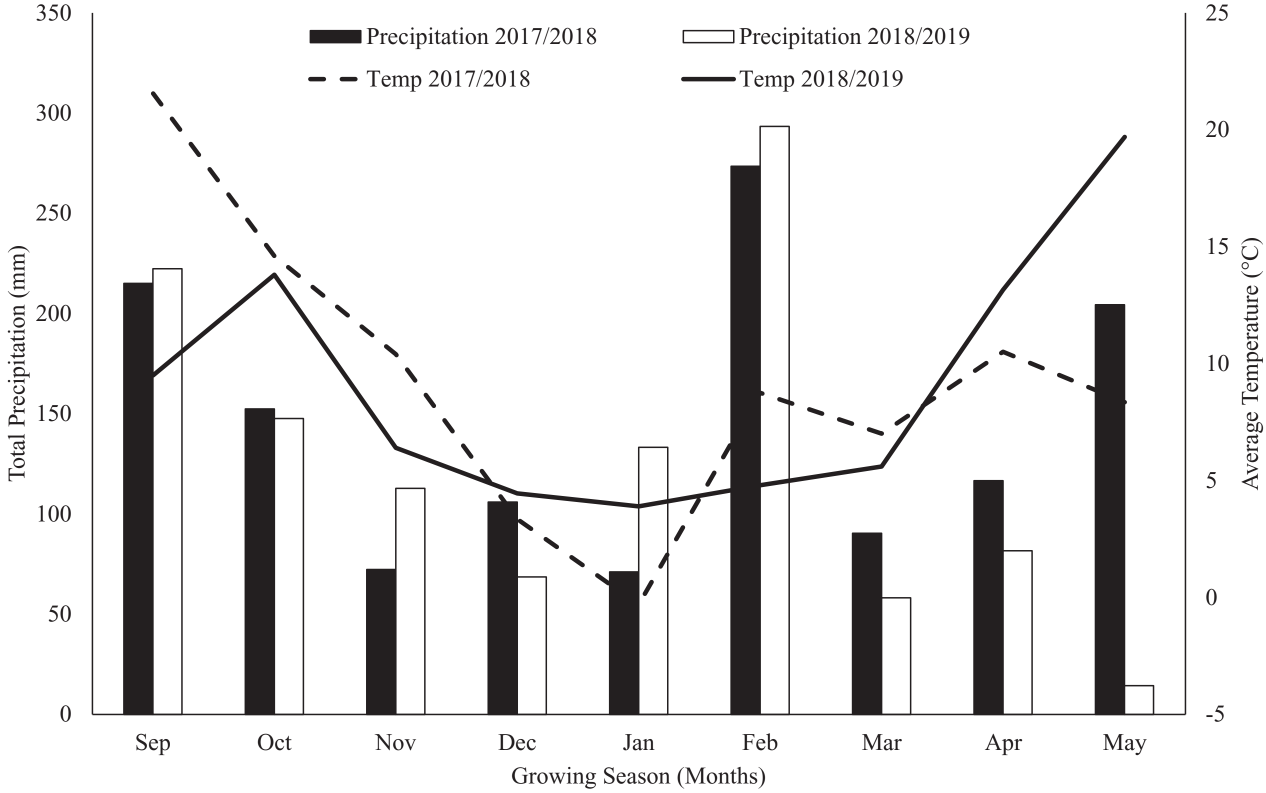

FIGURE 1 Average weather data for the 2017–2018 and 2018–2019 growing season months produced by a station in Ashland City, TN, approximately 7.24 km from the field site (U.S. Climate Data, 2019)

fungicides. In 2018, Inspiration, Phoenix CL, and Hekip seed were treated with Prosper EverGol insecticide and fungicides.

Stand counts were recorded from the second row on the left of each subeffect experimental unit. Stand counts were used to determine winter mortality beginning in October and resuming in March. Weekly weeding was conducted on all subeffect experimental units by manual hand removal and once using a push-behind Kentucky High Wheel for cultivating the 0.5-m buffers between each subeffect experimental unit. Ten seed pods from each subeffect experimental unit were collected randomly by cutting from the plant (one pod per plant) for shatter index measurements on 4 June in 2018 and on 3 June in 2019, just prior to the respective harvest dates.

Seed harvest was conducted on 5 June 2018 and 5 June 2019 using an Almaco HP 5 direct combine. In 2018, harvest data from two of the four replicates were lost due to issues with the combine. All seed samples were oven dried at 60 °C until a constant dry weight was achieved. After drying, seed was further cleaned using a Clipper office tester (A.T. Ferrell Company) and weighed to give seed dry weight yield.

Weather data for the field site was retrieved from a station in Ashland City, TN, located 7.24 km away (U.S. Climate Data, 2019) and is provided in Figure 1.

2.2 | Laboratory methods

Analysis was conducted by A & L Great Lakes Laboratories on soil samples for nitrate and ammonium. These analyses were performed using USEPA Methods 353.2 and 350.1, respectively, using a Lachat QuickChem FIA+ 8000 series on KCl soil extracts. The University of Tennessee Soil, Plant, and Pest Center (Nashville, TN) analyzed the soil samples for pH, available P, calcium (Ca), magnesium (Mg), and K. Soil Mehlich 1 extracts were analyzed for available P, Ca, Mg, and K using a Perkin Elmer 7300 inductively coupled plasma optical emission spectrometer (ICP–OES). Results of soil analyses can be found in Table 1. There were no significant differences between the 0N and 200N soils except for pH. Recommended pH for winter canola is 6.0–7.0 (Bushong et al., 2018), therefore, the pH differences between the soils likely had no effect.

Seed samples from each winter canola replicate and variety were introduced into a Da 7270 (Perten Instruments) near-infrared analyzer to determine seed oil and protein concentrations using the manufacturer's canola calibration from 2016. Both seed oil and protein concentration were adjusted for moisture based on near-infrared analysis data.

Seedpod shatter was determined by shaking pods in an orbital shaker with steel ball bearings. Initially in 2018, 10

TABLE 1 Soil nutrients prior to planting in 2017 with comparison between 0 kg N ha^{-1} (0N, $n = 4$) and 200 kg N ha^{-1} (200N, $n = 4$) treatment soils

N rate	pH	P	K	Ca	Mg	NH_4^+	NO_3^-
		mg kg^{-1}					
0N	6.6a	99.1a	44.6a	1,474a	149a	10.3a	1a
200N	6.3b	91.1a	40.5a	1,269a	129a	10.3a	1a

Note. Letters within each soil category that are the same are not significantly different at α < .05.

pods per subeffect experimental unit for 35 of the 60 subeffect experimental units were placed into compartments of a flat-bottomed Infinite Divider System box (Flambeau) with 33.34-cm × 22.54-cm × 4.92-cm dimensions. The box was separated into 12 equally spaced compartments (8.89 × 5.58 cm) using plastic dividers held in place by Flex Glue (Flex Seal Products). Five 9-mm steel ball bearings were placed inside each compartment. The box was placed in an orbital shaker at 300 rpm through five, 1-min intervals. These initial trials produced almost no shatter, nor when 10 steel ball bearings were used. Therefore, the harvested pods were weighed and dried in an oven at 60 ˚C. Eight to ten pods per replicate (7 of the 35 subeffect experimental units used in the initial shatter trial experienced some shatter and this led to fewer pods available from these samples for this analysis) were placed in the same box as described above. One sample in each year, from different subeffect experimental units, did not have enough seedpods for analysis. After drying, testing resumed with five 9-mm steel ball bearings inside each compartment in an orbital shaker at 300 rpm for five, 1-min intervals (5 min total shaking time). Pods were scored after each minute. All material was kept in the box throughout the entire experiment. Pods were scored every minute based on the number of pods remaining intact, including pods that were half open. Each full pod received a score of one and a half pod scored 0.5. For 10 initial seedpods, the maximum score was 10 intact for no shatter per 1-min interval and the lowest score was 0 indicating complete seedpod shatter. A maximum score of 50 after 5 min, for 10 initial seedpods, indicated that no pods shattered during the experiment, whereas a score of 0 indicated that all pods had shattered in the first minute. Shatter scores were added for the 5-min intervals and divided by 50 to give a number that would represent the shatter index. Therefore, the shatter index ranged between 0 (least shatter tolerant) and 1 (most shatter tolerant) for each replicate. Each replicate had a unique shatter index value, hence we treated it as a continuous variable for our statistical analysis. In cases where there were 9.5 initial seedpods, the scores were divided by 47.5; for 9 initial seedpods, it was divided by 45, and for 8 initial seedpods, it was divided by 40. For those subeffect experimental units (35 out of 60 total) that went through the initial shaking, prior to drying, about half had the lowest shatter index within the four replicates for a specific variety and N rate combination. Therefore, it was assumed that this initial shaking did not have any effect. In 2019, the same procedure was followed where seedpods were dried first and then shaken for shatter index measurements. In this year, some of the seedpods shattered upon drying, leading to similar calculations identified above.

2.3 | Statistical analysis

The main effects of N rate, and subeffects of cultivar and their interactions were analyzed using a split plot design ANOVA within the "agricolae" package in R statistical computing environment (version 3.6.2; de Mendiburu, 2019; R Development Core Team, 2020). When ANOVA was significant, LSD post-hoc tests were performed for multiple comparisons. *P* values greater than .05 were considered not significant and error bars represent one standard error (±1.0 SE) from the mean.

Regression analyses for oil vs. protein content and shatter vs. moisture content and soil nutrient comparisons were performed using JMP version 9.0.0 (SAS Institute).

Due to the combine issues during harvest in 2018, the yield and seed oil and protein concentrations for that year were not analyzed and are not provided, as the low number (two) of replicates might affect overall results and lead to incorrect conclusions.

3 | RESULTS AND DISCUSSION

3.1 | Winter mortality

Winter mortality values for each treatment are provided in Table 2. A difference was observed between cultivars in 2017–2018 where the average winter mortality was lower for Edimax CL (79.7%) than Hekip (83.9%) and Inspiration (85.7%), which were statistically similar (Table 3, Figure 2). Mean winter mortality was low for all cultivars in the 2018–2019 season with a range of 0–0.59% (data not shown). No differences were observed between N rates and winter mortality for either year. Differences between the cultivars were likely due to their genetic properties. Edimax CL may be more winter hardy in extreme temperatures as compared with Hekip and Inspiration. However, under mild winter temperatures all

TABLE 2 Average values for each cultivar and N rate treatment for winter mortality, shatter index, yield, oil content, and protein content for each growing season

Year	Cultivar	N rate	Winter mortality	Shatter index	Yield	Oil (dry wt.)	Protein (dry wt.)
		kg N ha^{-1}	%		kg ha^{-1}	——%——	
2017–2018	Edimax CL	0	78.0	0.69	NA[a]	NA	NA
		56	72.1	0.63	NA	NA	NA
		112	81.2	0.77	NA	NA	NA
		168	87.2	0.71	NA	NA	NA
		224	80.2	0.69	NA	NA	NA
	Hekip	0	80.2	0.31	NA	NA	NA
		56	85.5	0.41[b]	NA	NA	NA
		112	80.7	0.74	NA	NA	NA
		168	88.4	0.95	NA	NA	NA
		224	85.2	0.86	NA	NA	NA
	Inspiration	0	81.3	0.63	NA	NA	NA
		56	85.2	0.60	NA	NA	NA
		112	85.5	0.80	NA	NA	NA
		168	88.4	0.94	NA	NA	NA
		224	88.1	0.85	NA	NA	NA
2018–2019	Phoenix CL	0	0	0.74	688	49.0	17.3
		56	0	0.74	1190	48.7[b]	17.8[b]
		112	0	0.73	1637	46.3	20.1
		168	0	0.67	1395	47.0	19.3
		224	0	0.70	1667	45.3	21.3
	Hekip	0	1.0	0.23	326	48.4	17.2
		56	0	0.37	1019	47.4	17.9
		112	0	0.18[b]	1707	46.1	19.4
		168	0	0.34	1847	45.5	19.9
		224	0	0.27	1943	44.1	21.2
	Inspiration	0	2.2	0.62	315	47.9	17.3
		56	0.8	0.85	1267	47.5	18.2
		112	0	0.60	1493	46.6	19.5
		168	0	0.84	1865	45.5	20.3
		224	0	0.72	1961	45.4[b]	20.6[b]

[a]NA, not available.
[b]$n = 3$.

cultivars over wintered with little to no plant death. It has been noted that some *B. napus* cultivars can withstand short-term temperatures as low as −19 ˚C, but only if adequate stand acclimation is achieved (Waalen et al., 2011). However, during the overwintering period in 2017–2018, the canola experienced temperatures of about −14.3 ˚C as well as 5 d in April that reached below 0 ˚C with a minimum temperature of −1.7 ˚C during anthesis, which may have led to increased plant mortality. Therefore, greater cold temperature-tolerant cultivars like Edimax CL may help reduce winter stand losses in Tennessee.

3.2 | Shatter resistance

In 2018, the mean values for shatter index ranged from 0.31 to 0.95 with an average of 0.71. The effects of N treatment and the cultivar × treatment interaction were significant, but cultivar was not (Table 3). Nitrogen treatments of 112N, 168N, and 224N had greater shatter indices (i.e., greater resistance to shattering) than the 0N or 56N treatments (Figure 3a). Although there were no differences between cultivars when averaged across all N treatments, there were some differences between Hekip and the other cultivars for certain N treatments

TABLE 3 ANOVA table partitioning the sources of variances for shatter index in 2018 and 2019, winter mortality in 2018, and pod moisture content in 2018

Source	Shatter index 2019			Shatter index 2018			Mortality 2018			Pod moisture content 2018		
	df	*F* value	*p* value	df	*F* value	*p* value	df	*F* value	*p* value	df	*F* value	*p* value
Block	3	0.50	.6898	3	0.36	.7835	3	0.37	.7771	3	1.70	.2203
Treatment	4	2.01	.1564	4	11.86	.0004***	4	1.06	.4189	4	11.63	.0004***
Error (treatment)	12			12			12			12		
Cultivar	2	151.26	<.0001***	2	3.09	.0609	2	7.48	.0023**	2	2.09	.1419
Cultivar × treatment	8	2.17	.0604	8	3.94	.0030**	8	1.50	.1997	8	0.51	.8379
Errors	29			29			30			29		

**Significant at .01 probability level.
***Significant at .001 probability level.

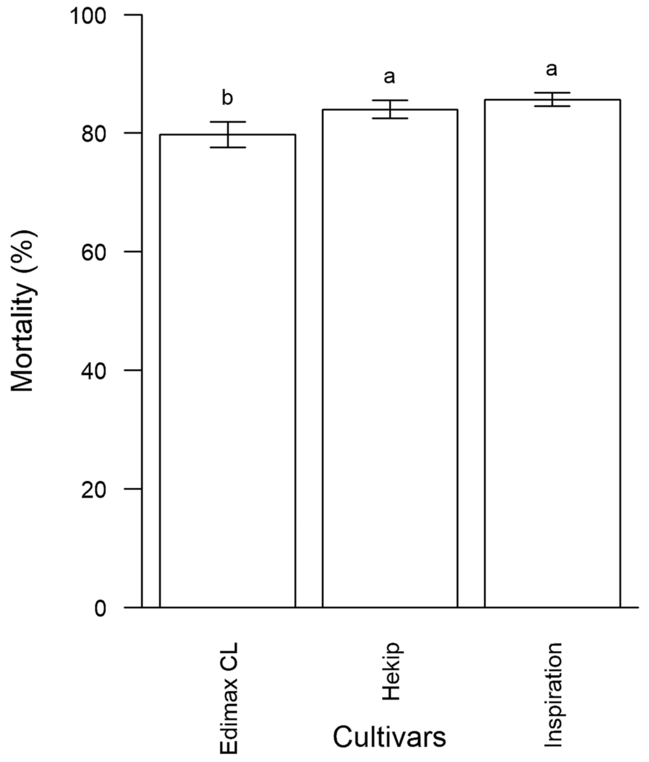

FIGURE 2 Differences in winter mortality (%) with cultivar in the 2017–2018 growing season. Values followed by the same letter are not significantly different from each other (α < .05). Error bars represent one standard error from the mean

(Figure 3b). Specifically, the shatter index values at 0N and 56N for Hekip were lower than the majority of other cultivar and rate combinations. Also, the shatter index values for Hekip and Inspiration at 168N were greater than the majority of other cultivar × N rate combinations.

In 2019, the average values for shatter index ranged from 0.18 to 0.85 with an overall average of 0.57. The effects of cultivar were significant, but the N treatment and N treatment × cultivar interaction were not (Table 3). For N rate, there was no increase in shatter index in 2019 with increasing N as in 2018. For cultivars, Hekip had a lower shatter index, which was 61% lower than the indices for Phoenix CL and Inspiration, which were statistically similar (Figure 4).

Canola has a high potential for seedpod shatter during harvest, which results in yield loss. The shattering analyses within these experiments, performed after oven-drying, may indicate the level of shatter that might be expected under a delayed harvest. If seeds are allowed to overripen, pods shatter more easily, particularly when exposed to inclement weather, such as rain, wind, or hail, resulting in yield loss (Salunkhe & Desai, 1986). In general, those pods that had a higher shatter index are more resistant to shatter and have higher cellulose, hemicellulose, and lignin levels (Kuai et al., 2016). Although Kuai et al. (2016) observed variation in pod shatter resistance with variety, where open-pollinated varieties were more resis-

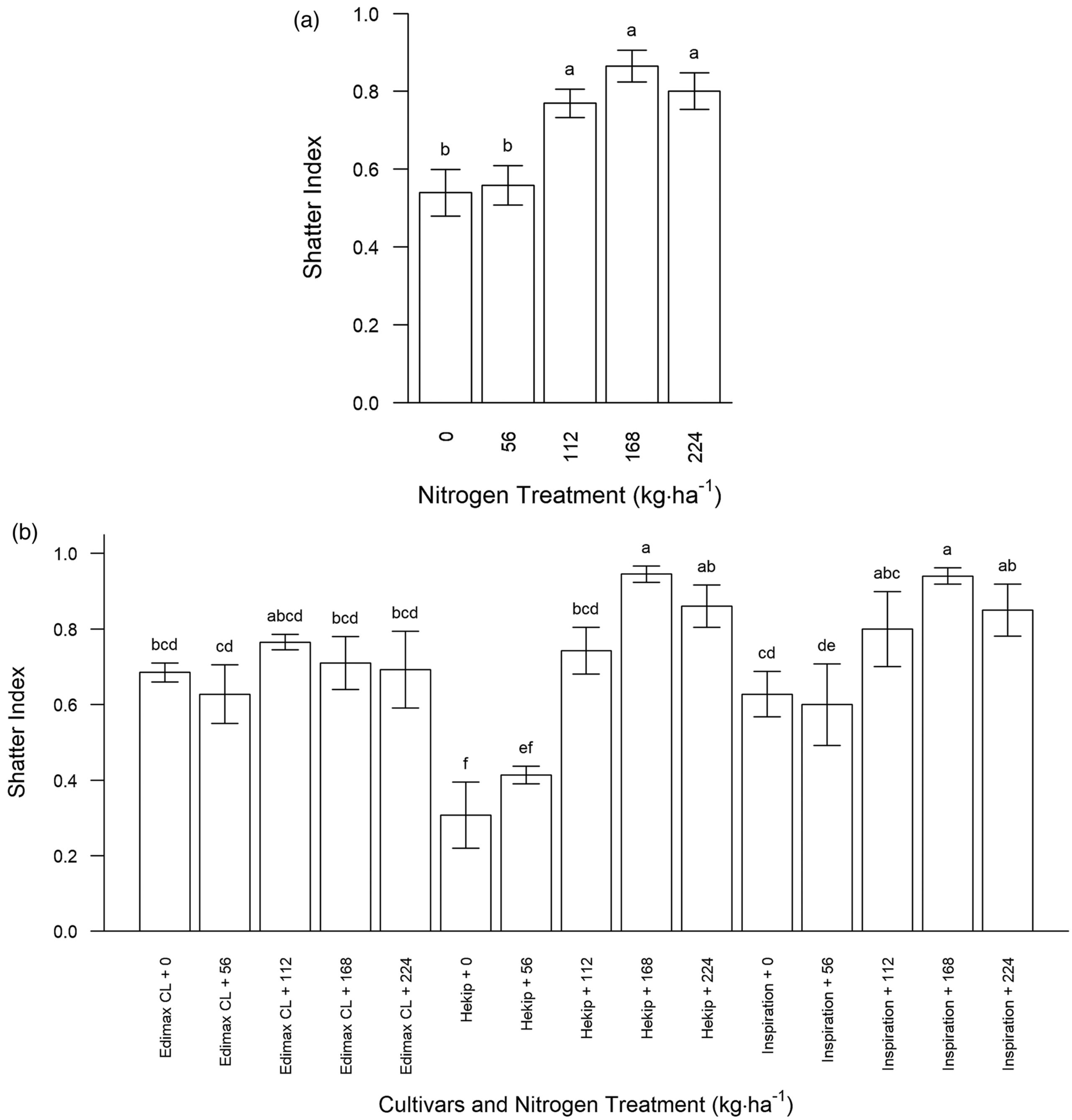

FIGURE 3 Differences in shatter index values in 2018 (a) with N rate and (b) with interactions between cultivar and N rate. Values followed by the same letter are not significantly different from each other ($\alpha < .05$). Error bars represent one standard error from the mean

tant than hybrid varieties, our study only included hybrid varieties. Genetic characteristics have been shown to be an important driver behind shatter resistance (Cavalieri et al., 2014). In the 2018 experiment, shatter resistance increased linearly with increasing moisture content of the entire intact seed pod ($r^2 = .23$, $p = .0001$). This was similar to Kuai et al. (2016), where shatter resistance was linearly related to the water content in pod walls where pod walls with greater water content led to a more shatter resistant pod. Also in the 2018 experiment, the pod moisture content increased with increasing N rate (Table 3, Figure 5). None of these relationships, however, were evident in 2019. The moisture content values in 2018 ranged from 17.0 to 54.5%, and in 2019, it ranged from 3.86 to 8.53%. Though the pods were collected around the same date in each year, those collected in 2018 may have occurred under cooler, more humid conditions as compared with those collected in 2019. Based on the shatter index data for both years, an N rate of 112, 168, or 224 kg ha^{-1} and the cultivar, Inspiration, provided the best treatments for reducing the potential for yield losses if harvest is delayed.

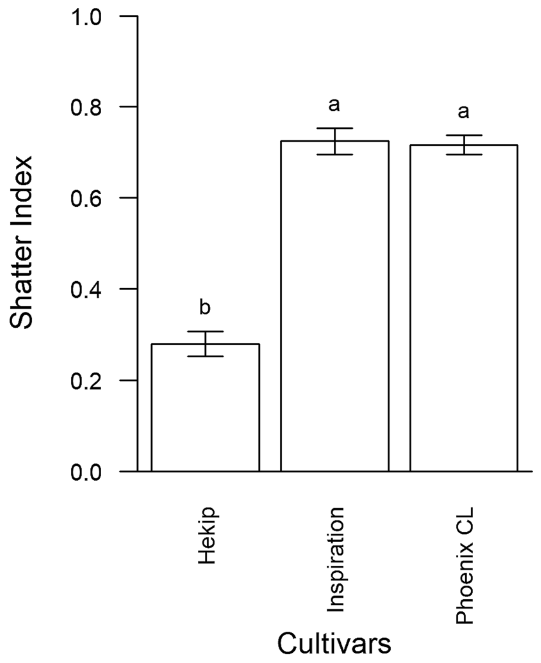

FIGURE 4 Differences in shatter index values in 2019 with cultivar. Values followed by the same letter are not significantly different from each other (α < .05). Error bars represent one standard error from the mean

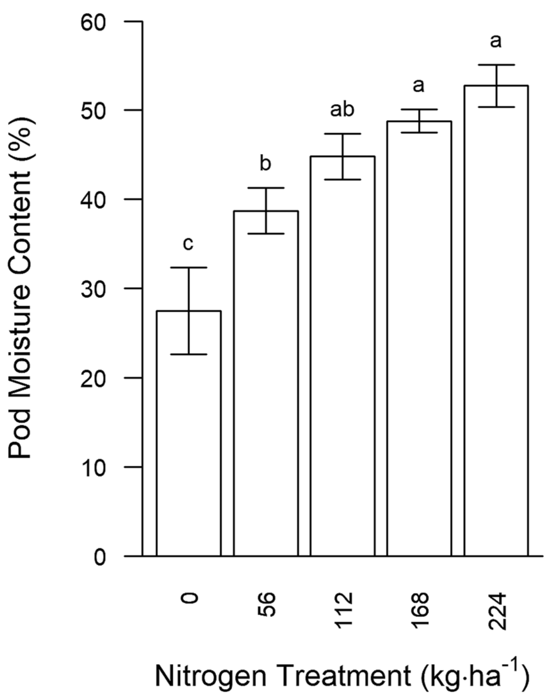

FIGURE 5 Differences in pod moisture content (%) in 2018 with N rate. Values followed by the same letter are not significantly different from each other (α < .05). Error bars represent one standard error from the mean

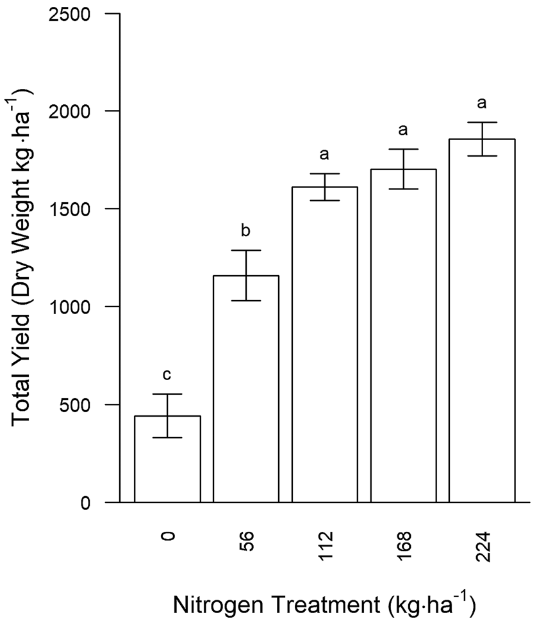

FIGURE 6 Differences in yield (kg ha^{-1}) in 2019 with N rate. Values followed by the same letter are not significantly different from each other (α < .05). Error bars represent one standard error from the mean

3.3 | Yield

Yield was very low (723 kg ha^{-1} for the highest yield across individual replicates) for the 2017–2018 season. The low yields are likely due to frost events that occurred during flowering in the early spring (April) as well as the low temperatures experienced during the overwintering period where temperatures reached lows of around −16.7 ˚C (U.S. Climate Data, 2017–2019) and had at least 72 d with average daily low temperatures below 0 ˚C between October and March. Frost at flowering delays maturity but in spring canola may only result in minor reductions in yield due to flower abortion. Only the flowers open during frost are affected (Kandel et al., 2019). Complete leaf loss over winter and high winter mortality was also observed in our experiment, which likely led to the lower seed yields observed.

The winter canola harvest in 2019 produced greater yields than 2018, which is likely due to the milder temperatures observed. There were no interactions for cultivar × N treatment for yield; however, yield increased with increasing N treatment (Table 4, Figure 6). The yields ranged from 443 kg ha^{-1} for the 0N treatment to 1,857 kg ha^{-1} for the 224N treatment, and the 224N treatment was not different from the 168N or 112N treatments. These results are similar to previous research in other environments. In southern Iran, Kazemeini et al. (2010) showed that N applied at 150 kg ha^{-1}, the highest rate studied, resulted in the greatest seed yield. In Pakistan, Cheema et al. (2001) observed greater seed yield with increasing N fertilizer rates, which was attributed to a

TABLE 4 ANOVA table partitioning the sources of variances for 2019 yield, protein, and oil

Sources	Yield 2019			Protein (%) 2019			Oil (%) 2019		
	df	*F* value	*p* value	df	*F* value	*p* value	df	*F* value	*p* value
Block	3	1.58	.2451	3	2.41	.1182	3	9.40	.0018**
Treatment	4	32.00	<.0001***	4	36.75	<.0001***	4	44.24	<.0001***
Error (treatment)	12			12			12		
Cultivar	2	0.21	.8100	2	0.07	.9298	2	11.55	.0002***
Cultivar × treatment	8	1.47	.2098	8	1.29	.2886	8	1.74	.1332
Errors	30			28			28		

**Significant at .01 probability level.
***Significant at .001 probability level.

larger number of pods per plant and seeds per pod. Similar results were recorded by Ahmadi and Bahrani (2009) in Iran where yield, branches per plant, pods per plant, and seeds per pod increased with increasing N fertilizer rates with the greatest yield achieved at a rate of 225 kg N ha^{-1}. In other research, significant increases in seed yields were observed with increasing N rates studied, 100–150 kg ha^{-1}, in India (Buttar et al., 2006).

The N rates or cultivars with lower shatter indices did not have an effect on yield. For example, Hekip had a very low shatter index in 2019 but was not accompanied by the lowest yield among cultivars. It may be because the winter canola was harvested early enough in the season where these effects were not as pronounced. If, however, harvest occurred later, there may have been more of these effects observed. Based on yield alone, an N application of 112 kg ha^{-1} was sufficient to produce the greatest yields in our experiment.

3.4 | Oil

In 2019, mean oil concentration ranged from 44.1 to 49.0% with an overall mean of 46.7%. The N rate and cultivar affected oil concentration; however, the interaction of cultivar × N rate did not (Table 4). Mean oil concentration of cultivars across all N treatments was 47.3% for Phoenix CL, which was greater than that of Inspiration (46.6%) and Hekip (46.3%) (Figure 7a). In an associated experiment by another group conducted in Springfield, TN, in 2019, Phoenix CL had an oil concentration of 41.8% (44.0%, dry weight basis) and Inspiration had an oil concentration of 40.8% (42.9%, dry weight basis) with a 235 kg N ha^{-1} application rate (Stamm & Dooley, 2020). At 224N, in our study, Phoenix CL and Inspiration had oil concentrations of 45.3 and 45.4%, respectively (Table 2). Mean oil concentration for N treatments ranged from 44.9 to 48.4% and decreased with increasing N rate (Figure 7b). When the oil concentration for each N rate was averaged across cultivar and year, there was an inverse relationship ($r^2 = .97$, $p < .003$) between oil concentration and N rate. The inverse relationship between N rate and oil concentration is common in canola (Ahmadi & Bahrani, 2009). One potential cause for the decrease in oil content with increasing N could be due to higher N levels delaying seed ripening causing immature seed with a lower oil content harvested (Scott et al., 1973), but it is more likely the result of excess N in the plants promoting C shunting towards protein production as opposed to oil storage. The most important economic parameter in the assessment of canola quality is oil content; therefore, Phoenix CL with lower rates of N application may have been the optimal choice for production in these experiments.

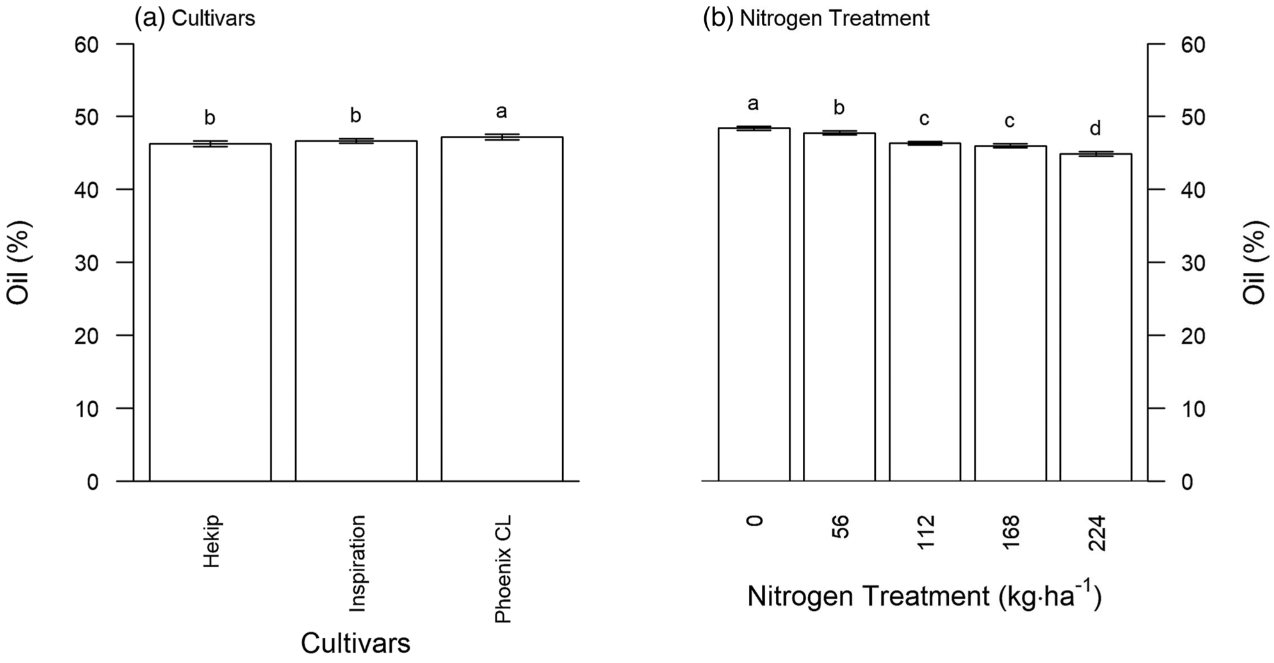

FIGURE 7 Differences in oil content (%) in 2019 with (a) cultivar and (b) N rate. Values followed by the same letter are not significantly different from each other (α < .05). Error bars represent one standard error from the mean

3.5 | Protein

In 2019, the mean protein concentration ranged from 17.2 to 21.3% with an overall mean of 19.2%. The effect of N rate on protein concentration was significant; however, the effects of cultivar and the cultivar × N rate interaction were not (Table 4). The mean protein concentration for N treatments ranged from 17.3 to 21.1% and increased with increasing N rate (Figure 8). In a related experiment conducted by another research group in Springfield, TN, the Inspiration and Phoenix CL cultivars had seed protein concentrations of 22.2% (23.4%, dry weight basis) and 22.8% (24.0%, dry weight basis), respectively, with a total N application of 235 kg ha^{-1} (Stamm & Dooley, 2020). In our study at 224N, Inspiration had 20.6% protein and Phoenix CL had 21.3% protein.

Most research on winter canola has shown that higher protein results from higher N application rates (Asare & Scarisbrick, 1995; Kutcher et al., 2005), which relates well with our study. A study performed in Pakistan recorded the highest seed protein content (23.5%) with an N rate of 80 kg ha^{-1} (Ahmad et al., 2007). Protein concentrations of 18.0 and 19.7% were recorded in the current study in 2019 at rates of 56 and 112 kg N ha^{-1}, respectively. The differences between studies were likely related to differences in soil availability and release due to environmental conditions or cultivar. For all data in both years, protein content was negatively correlated with oil ($r^2 = .77, p < .0001$). This relationship is similar to observations made in a cultivar trial at the same location in previous years (Tetteh et al., 2019) as well as other studies (Brennan & Boland, 2009; Seymour & Brennan, 2017). If the seed were being used as an animal feed, and protein was more important than oil, then higher rates of N application, using any of the three cultivars, may be the best option based

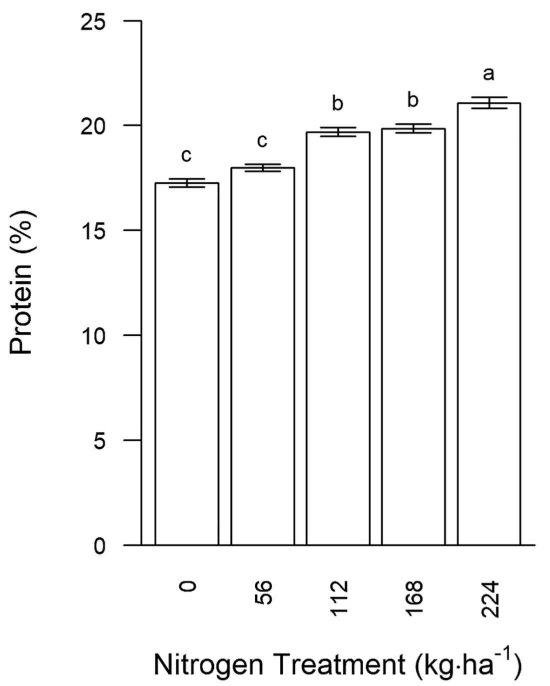

FIGURE 8 Differences in protein content (%) in 2019 with N rate. Values followed by the same letter are not significantly different from each other (α < .05). Error bars represent one standard error from the mean

on the data from 2019; however, most of the value of canola seed comes from oil in most regions where it is commonly grown.

4 | CONCLUSIONS

In 2018, yield was low, which was likely due to frost events that occurred during flowering in the early spring (April) as well as the low temperatures experienced during the overwintering period. This may affect winter canola's ability to be a viable cover crop in the winter months in Tennessee, particularly if these extreme weather events continue to occur or increase. Cold-tolerant cultivars with properties similar to or more enhanced than the Edimax CL cultivar used in this experiment may help decrease the susceptibility to winter mortality. Across both years, the Inspiration cultivar had greater shatter resistance. In 2018, the higher N rates of 112N, 168N, and 224N had the greatest resistance to shatter. These N rates also produced the greatest yields in 2019, with a rate of 112N preferred, as no further yield was achieved by N rates greater than this treatment. Therefore, an N rate of 112 kg N ha^{-1} and the Inspiration cultivar are recommended for winter canola production in Tennessee and other areas of the United States with similar environmental conditions. This new research provides important information for farmers, particularly those in the southeastern United States, as they consider the potential for growing winter canola in rotation with winter wheat or instead of winter fallow.

Further research should continue to focus on improved winter canola cultivars with respect to winter mortality resistance. More research on shatter resistance, in particular, is greatly needed, as this is little studied and can be of great importance where delayed harvests may occur due to increased rainfall events. Also, the potential relationship between N rate and shatter resistance is a viable research focus, as this could contribute to increased risk management options for farmers.

AUTHOR CONTRIBUTIONS

Kyle D. McGeary: Data curation; Formal analysis; Investigation; Writing – original draft; Writing – review & editing. Jason de Koff: Conceptualization; Methodology; Project administration; Supervision; Writing – review & editing. Bharat Pokharel: Formal analysis; Validation; Writing – review & editing. Richard Link: Investigation; Writing – review & editing. Priya Saini: Investigation; Writing – review & editing. Taqdeer Gill: Investigation; Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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