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Abstract

Reliance on summer annual crops in the Upper Midwest results in fallow land from late fall through early spring, providing opportunities to integrate winter crops, such as pennycress (*Thlapsi arvense* L.), onto the landscape. Pennycress agronomics have primarily been studied using unimproved wild-type lines prone to seed shatter, resulting in significant yield loss if not harvested early. However, high plant and seed moisture complicates harvest and seed storage. A new breeding line with a reduced-shatter mutation made it possible to use harvest aids to reduce plant moisture without the risk of seed loss. The objectives of this study were to quantify the reduction in pennycress seed and biomass moisture after applying a harvest aid and to assess the seed yield, oil content, and crude protein of the reduced-shatter line. This study was conducted over the 2018–2019 and 2019–2020 growing seasons with "'IO217" pennycress in Rosemount, MN. Seed moisture decreased to a similar level by harvest maturity regardless of treatment while swathing was the most effective method of reducing biomass moisture. Natural senescence decreased pennycress moisture content to a harvestable level at the same rate as treated plants, indicating that a harvest aid is not required at this time. Seed yield was two to six times higher than in studies using unimproved pennycress lines. Challenges associated with wild-type pennycress lines, such as uneven germination and late maturation, were prevalent in this study and further genetic improvement will be necessary to ensure successful pennycress production in the Upper Midwest.

Abbreviation: GDD, growing degree days.

1 | INTRODUCTION

Throughout the Upper Midwest, there is an opportunity to increase agricultural productivity and profit without displacing current production. These opportunities are the result of an almost exclusive reliance on summer annual crops in this region. Typically, crops are planted in April or May and harvested in October, which leaves land fallow in the early spring and late fall (Heggenstaller et al., 2008). Despite the norm, crop production can continue throughout this period through the use of temporal intensification (Heaton et al., 2013). Temporal intensification is a practice where a secondary crop is integrated onto the landscape during the fallow shoulder season using land that is already in production during the primary growing season. However, this practice is not widely adopted due to a lack of commercially viable winter annual species (Roesch-McNally et al., 2017; Sindelar et al., 2017). The most common suggestions for winter annual species in the Upper Midwest include winter rye (*Secale cereale* L.), triticale (x *Triticosecale* Wittmack), and barley (*Hordeum vulgare* L.; University of Minnesota Extension, 2021). While these species can be harvested for a commercially viable product, they are typically terminated during the vegetative growth phase, as they cannot be harvested for seed until August in Minnesota (University of Minnesota Extension, 2021; USDA National Agricultural Statistics Service, 2010). Based on this historical use, winter rye, triticale, and barley fill the role of a cover crop rather than a commercial crop meaning that they provide important environmental services to growers but do not yield a product that has a direct economic return. This has resulted in low adoption of winter crops across the Upper Midwest, where only 1%–10% of production land is annually cover cropped, depending on the state and county (Wallander et al., 2021).

Recent advances in the breeding and agronomic management of winter crops may present a solution to this problem. Pennycress is a winter annual oilseed Brassicaceae that can be grown during the typically fallow period between October and May and results in the harvest of a marketable oil product (Cubins et al., 2019; Moser, 2012). Pennycress is native to Eurasia, but has naturalized to many environments around the world including various growing regions in the United States (Holm et al., 1997; Warwick et al., 2002). Within the Upper Midwest, the pennycress growing season is relatively short and typically corresponds with a September planting date and mid-June harvest date (Cubins et al., 2022; Dose et al., 2017). While this time period somewhat overlaps with the summer annual growing season (i.e., May through September), pennycress production can make up for the reduction in summer annual crop yield that occurs as a result of this shift (Johnson et al., 2015, 2017; Ott et al., 2019). Following harvest, pennycress seed can be pressed for oil and usually contains between 260 and 360 g kg^{-1} of oil (Cubins et al., 2019). When compared with other common oilseed species grown in the region (e.g., soybean [*Glycine max* [L.] Merr] and canola [*Brassica napus* L.]), pennycress seed produces a comparable or greater amount of oil per seed (Balbino, 2017). In the context of the agricultural landscape in the Upper Midwest, pennycress may provide additional oil yield alongside a soybean double crop, providing growers with an additional source of revenue in a given growing season. Research on post harvest markets for pennycress oil has focused primarily on its use as a biofuel feedstock, but more recently, pennycress lines with low erucic acid content have shown promise for edible oil markets as well (Chopra et al., 2020; Moser, 2012; Moser et al., 2009).

Despite its promise, there are questions surrounding the basic agronomic management of pennycress that have yet to be answered. Prior research on pennycress in the Upper Midwest has focused on selected wild-type lines (e.g., "MN106" and "Beecher"), which retain weedy characteristics that are advantageous to weed seed production, but hinder production in an agricultural setting (Carlson, 2018; Chopra et al., 2020; Cubins et al., 2022; Isbell et al., 2015, 2017). One such characteristic is silicle shatter when plant moisture is low (Carlson, 2018; Chopra et al., 2020; Cubins et al., 2022). Seed shatter is a significant issue in Brassicaceae and it is estimated that pennycress yield losses due to silicle shatter can exceed 25% of the total seed yield (Carlson, 2018; Cubins et al., 2022; Sintim et al., 2016; Vera et al., 2007). To help combat this loss, chemical desiccation and swathing are common practices in Brassicaceae production to reduce plant moisture following physiological maturity (Kandel & Hanson, 2013; Sanderson, 1976; Sintim et al., 2016). By quickly drying and harvesting the crop using a harvest aid, it may be possible to minimize seed losses associated with decreased plant moisture content (Cangussú et al., 2018; Esfahani et al., 2012). However, the use of shatter-prone lines like "MN106" in agronomic research has made it difficult to understand the role of harvest aids in pennycress production (Cubins, 2019).

Core Ideas

- Pennycress treated with a harvest aid reached harvest maturity at the same time as naturally senescing pennycress.
- Seed moisture was not affected by harvest aid application.
- Swathing was the most effective method to reduce biomass moisture between physiological and harvest maturities.
- Non-shatter pennycress yielded two to six times higher than wild-type pennycress.

TABLE 1 Baseline soil samples for the Rosemount, MN experimental site over the 2018–2019 and 2019–2020 growing seasons.

Growing season	pH (H^+)	OM (g kg^{-1})	CEC (meq g^{-1})	Inorganic N (g kg^{-1})	P	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
2018–2019	5.9	47	0.227	0.218	31	158	1900	435
2019–2020	6.0	41	0.225	0.171	15	90	1954	460

Abbreviations: OM, organic matter; CEC, cation exchange capacity.

Recent advancements in pennycress breeding have led to the availability of reduced shatter lines that can be used to better assess the viability of harvest aids (Chopra et al., 2020). Thus, the objectives of this study were to (1) quantify the reduction in pennycress seed and biomass moisture between physiological and harvest maturities after the application of a harvest aid and (2) assess the seed yield, oil content, and crude protein of a reduced-shatter pennycress line.

2 | MATERIALS AND METHODS

2.1 | Cultural practices

Field experiments were conducted during the 2018–2019 and 2019–2020 growing seasons at the University of Minnesota Rosemount Research and Outreach Center in Rosemount, MN (44°42′35″ N 93°04′18″ W). The soil at the Rosemount site was classified as a Waukegan silt loam (fine-silty over sand-skeletal, mixed, superactive, and mesic Typic Hapludoll) over both growing seasons. Baseline soil samples were collected prior to pennycress planting on a site-wide basis and analyzed for active acidity, organic matter, total inorganic nitrogen, cation exchange capacity, phosphorous, potassium, calcium, and magnesium (Table 1). Monthly precipitation and mean air temperature were recorded by a weather station at the Rosemount site. The historical 30-year (1991–2020) air temperature and precipitation averages were available for the Rosemount Research and Outreach Center (National Centers for Environmental Information, 2021). The average monthly temperature and cumulative precipitation throughout the study period and the 30-year normal for both parameters are presented in Table 2.

In both years, spring wheat (*Triticum aestivum* L.) preceded pennycress and the field was prepared for seeding using a field cultivator. All plots were planted with "IO217" pennycress, which has a reduced-shatter mutation (*Ta-ind-2*) previously described by Chopra et al. (2020). The planting rate was 11.2 kg ha^{-1} and the plots were 3.0 m long × 3.0 m wide. In 2018, pennycress was planted on September 27 using a cone planter with 25 cm row spacing. In 2019, the pennycress was planted on September 15 using a grain drill with 19 cm row spacing. Fertilizer was broadcast by hand at a rate of 79–34–34 kg ha^{-1} of N–P–K on April 25, 2019 and May 4, 2020 for each respective growing season following spring thaw.

2.2 | Treatments and data collection

Plots were arranged in a randomized complete block design with four replications. The experimental treatments were harvest aids applied at or near physiological maturity (Table 3). Physiological maturity was characterized by growth stages 85–86 on the extended BBCH scale for camelina and corroborated by prior pennycress research (Cubins et al., 2022; Martinelli & Galasso, 2011). Chemical harvest aids included Defol 5 ($NaClO_3$), which was previously used as a chemical desiccant on pennycress, and Diquat ($C_{12}H_{12}N_2Br_2$), which was selected based on its broad use as a chemical desiccant for many agricultural crops including canola (Table 3; Cubins, 2019; Environmental Protection Agency, 2002). Swathing was also investigated as a potential pennycress harvest aid due to its historical use in canola production (Kandel & Hanson, 2013; Vera et al., 2007). Pennycress seed and biomass moisture were characterized at both physiological and harvest maturities to provide a baseline for comparison and to compare harvest aid effectiveness with a naturally senescing control, respectively. Physiological maturity is defined as the time when seed has achieved maximum dry weight while harvest maturity corresponds with the time when plant material has dried enough for mechanical harvest, typically when seed moisture is around 120 g kg^{-1} (Crookston & Hill, 1978; Martinelli & Galasso, 2011; Mousavi-Avval & Shah, 2020). In 2019, treatments were applied on July 2 when pennycress growth stage averaged 85.5 (Table 3). In 2020, treatments were applied on June 17 when pennycress was at an average growth stage of 86.2 (Table 3).

Pennycress reached harvest maturity on July 8 and June 24 in 2019 and 2020, respectively. All plots were hand harvested for seed yield by taking a 0.25 m^2 area from the center of the plot. Harvested plants were bagged and dried at 65°C for 48 h to a constant mass before threshing and screen cleaning seed. This seed was tested for moisture, which was used to adjust the seed yield to a moisture content of 80 g kg^{-1}. Seed and biomass moisture at harvest were calculated by taking an additional set of samples for each parameter within the plot outside the seed yield harvest area. Seed was hand threshed from the silicle and weighed immediately and then again after it was dried at 65°C to a constant mass to determine seed moisture at harvest. Biomass moisture was determined by weighing the

TABLE 2 Mean monthly air temperature and cumulative precipitation over the 2018–2019 and 2019–2020 growing seasons and the 30-year normal (1991–2020) for the Rosemount, MN experimental site.

	2018–2019				2019–2020			
	Temperature (˚C)		Precipitation (mm)		Temperature (˚C)		Precipitation (mm)	
Month	Mean	Normal[a]	Total	Normal	Mean	Normal	Total	Normal
Sept.	17.4	16.2	157	87	17.6	16.2	146	87
Oct.	6.2	8.7	91	73	6.8	8.7	109	73
Nov.	−3.3	0.3	38	43	−2.1	0.3	27	43
Dec.	−5.0	−7.1	47	31	−6.6	−7.1	35	31
Jan.	−11.1	−10.7	12	24	−7.7	−10.7	58	24
Feb.	−13.2	−8.3	73	23	−8.5	−8.3	15	23
Mar.	−4.3	−1.0	58	46	1.1	−1.0	35	46
Apr.	6.5	7.1	121	79	6.1	7.1	8	79
May	11.6	14.0	173	110	13.6	14.0	57	110
June	19.9	19.6	76	124	21.1	19.6	228	124
July	22.7	21.7	180	115	23.1	21.7	58	115

[a]The 30-year air temperature and cumulative precipitation averages from 1991 to 2020 were available for the Rosemount Research and Outreach Center.

TABLE 3 Harvest aid treatment active ingredients and rates and the intended pennycress growth stage at application versus actual growth stage at application during the 2019 and 2020 seasons.

			Growth stage at application[a]		
Treatment	Active ingredient	Rate (L ha^{-1})	Intended	2019	2020
Defol 5	Sodium chlorate	4.7	85–86	86.0	87.3
		9.4	85–86	85.2	85.5
Diquat	Diquat dibromide	2.3	85–86	85.3	86.5
		4.7	85–86	86.5	85.6
Swath	–	–	85–86	84.6	86.0

[a]Growth staging based on the extended BBCH scale for camelina (Martinelli & Galasso, 2011).

samples following harvest and then again after being dried at 65˚C to a constant mass.

2.3 | Post season analyses

Seed oil content and crude protein were measured using near-infrared spectroscopy (NIRS) with a DA 7250 At-line NIR Instrument with custom calibrations for pennycress (Perten Instruments). Spectra were collected at room temperature using 0.2–5.0 g of pennycress seed with the Micro Mirror Module from Perten Instruments with a 950–1650 nm wavelength range and a scan resolution of 5 nm. The methods to perform NIRS analysis were described in previous pennycress research (Chopra et al., 2019). Seed oil content and crude protein values were adjusted to a dry matter basis for analysis and presentation using the seed moisture content at the time of NIRS analysis.

Cumulative growing degree days (GDD) (˚C d) were retroactively calculated for pennycress using the daily maximum soil temperature (T_{max}), daily minimum soil temperature (T_{min}), and the established base temperature and ceiling temperature (T_b and T_c, respectively).

$$\text{Cumulative GDD} = \sum \left(\frac{T_{max} + T_{min}}{2} \right) - T_b$$

The T_b and T_c for pennycress are −2.5˚C and 25˚C, respectively (Royo-Esnal et al., 2015). In the instance that the daily minimum or maximum temperatures were lower than T_b, they were set equal to T_b; if the daily maximum temperature was higher than the T_c, the daily temperature was set equal to T_c (NDAWN Center, 2021). Total GDD were then calculated by summing the cumulative GDD from planting to the date when the harvest aids were applied (Table 3).

2.4 | Statistical analyses

All data were analyzed using linear mixed-effect models constructed using the lmer function of the *lme4* package (version 1.1.29) in R (Bates et al., 2015). Treatment was considered a fixed effect while year and replication were considered random effects. Biomass moisture at harvest maturity was heteroskedastic and violated the analysis of variance (ANOVA) assumption of equal variances and was log(x)-transformed for analysis and back-transformed for presentation. All other data met ANOVA assumptions and did not require transformation prior to analysis. Differences in treatment were determined using a threshold of $\alpha = 0.05$ with the ANOVA function of the *car* package (version 3.0.12; Fox & Weisberg, 2019). When significant differences were found ($p < 0.05$), mean separation was conducted using Tukey's honest significant difference (HSD) at $\alpha = 0.05$. The emmeans function from the *emmeans* package (version 1.7.4.1) was used for the mean separation, and the cld function from the *multcomp* package (version 1.4.19) was used to determine the compact letter display associated with the mean separation (Hothorn et al., 2008; Lenth et al., 2022).

3 | RESULTS AND DISCUSSION

3.1 | Environmental conditions

The primary pennycress growing season (i.e., September through November and April through June) over 2018–2019 was cooler and wetter compared with the 30-year normal (Table 2). Average conditions over the 2019–2020 primary growing season were similar to the 30-year normal. Despite these differences, conditions between both experimental years had similarities. The autumnal temperature and precipitation rates followed the same pattern across experimental years; however, the growing conditions differed in the spring (Table 2). There were contrasting levels of spring precipitation in 2019 and 2020. While April and May 2019 were consistently wet, the same period in 2020 was exceptionally dry. The opposite was true in June; in 2019, about half of the typical amount of rain fell, and in 2020, there was nearly twice as much precipitation as in an average year. Overall, the 2018–2019 primary growing season was 1.3°C cooler and accumulated 140 mm more precipitation than is typical while the 2019–2020 primary growing season was 0.4°C cooler and accumulated only 19 mm more precipitation compared with the 30-year normal (Table 2).

3.2 | Phenological development

Environmental conditions over the 2018–2019 and 2019–2020 growing seasons had a significant effect on pennycress development milestones. Pennycress that was harvested in 2019 did not reach physiological maturity until July 2, while the pennycress that was harvested in 2020 followed the expected growth and maturation timeline for Minnesota, reaching physiological maturity on June 17 (Cubins et al., 2022). These differences can likely be attributed to the early spring growing conditions during each experimental year. The monthly temperatures throughout spring 2020 were close to the 30-year normal, whereas cooler than average temperatures persisted throughout spring 2019 resulting in fewer available GDD (Tables 2 and 4). The pennycress grown during the 2018–2019 growing season accumulated 1586°C d GDD between planting and physiological maturity (Table 4). In comparison, it was determined that the wild-type "MN106" required 2230–2250°C d GDD to reach physiological maturity in Minnesota when grown under similar conditions (Cubins et al., 2022). This represents about 50% more GDD than was accumulated by the 2018–2019 pennycress in the present experiment. The low temperatures in early spring 2019 may have been the driving force behind these differences; however, this does not take into account the similarly low number of GDD accumulated in 2020 (Table 4). Only 1782°C d GDD were accumulated over the 2019–2020 growing season when pennycress matured at the expected time and temperatures were close the 30-year normal (Tables 2 and 4). Biologically speaking, winter pennycress is a long-day plant, meaning that it flowers when days are long after a period of vernalization (Best & McIntyre, 1972). Based on this, "IO217" was likely triggered to flower due to the change in day length alone despite a relative lack of GDD accumulation. The relative differences between the timing of pennycress maturity in 2019 and 2020 may have resulted from the annual temperature and precipitation shifts (Table 2). These results indicate that GDD accumulation may not be as useful a metric to predict pennycress physiological maturity as previously thought and other variables, such as photoperiod sensitivity, should be taken into account. Future experiments focusing on both flowering time and physiological maturity of "IO217" and other improved pennycress lines with stacked traits will be valuable for further development of pennycress best management practices.

Differences in time and GDD to physiological maturity compared with previous studies also underscored some of the difficulties of targeting management activities to specific growth stages. In this experiment, keeping track of phenological development was of critical importance since harvest aid application was targeted to a specific range of growth stages (Table 3). While this process was slow and drawn out for the majority of the 2019 growing season, pennycress maturation progressed quickly once it reached physiological maturity, which provided a temporal challenge. Rapid pennycress maturation was also observed in 2020 despite the timing of physiological maturity generally matching up with prior research. Pennycress had a slow start to phenological

TABLE 4 Pennycress cumulative growing degree days (GDD) at physiological maturity and mean values for pennycress harvest parameters at harvest maturity for the 2018–2019 and 2019–2020 growing seasons and the overall averages for both experimental years.

Growing season	Cumulative GDD (°C d)	Seed yield[a] (kg ha^{-1})	Seed moisture (g kg^{-1})	Oil content[b]	Crude protein[b]
2018–2019	1586	1962	57	309	153
2019–2020	1782	2580	78	337	224
Mean	1684	2271	67	323	188

[a]Adjusted to 80 g kg^{-1} moisture content.
[b]Presented on a dry matter basis.

development in 2020, but quickly made up for lost time in June, which was particularly hot and wet (Table 2). In the days prior to physiological maturity in 2020, visual indicators (i.e., seed texture and plant color) suggested that the pennycress stand was still at least a week from reaching physiological maturity (personal observation). However, maturation accelerated from that point as a result of consistent hot temperatures, which corresponded with rapid GDD accumulation (Table 2). Less than a week later the stand was near the end of physiological maturity and harvest aids were applied late relative to the targeted growth stages (Table 3). The speed at which pennycress matured after reaching physiological maturity in conjunction with the cost and labor required to apply a harvest aid to pennycress may make harvest aid application an unattractive option for growers considering pennycress for production on their farms.

These difficulties were also compounded by uneven stand maturation, a known issue with pennycress, which made it difficult to estimate pennycress growth stage on a plot-wide level (Sedbrook et al., 2014). It was typical to see plants that were visually mature directly adjacent to plants that were completely green throughout the second half of June 2019 (personal observation). This was likely a product of relatively late pennycress planting in 2018 and the resulting proportion of fall- versus spring-germinated seed. In prior research, early September planting dates led to better establishment than late fall or early spring planting dates (Dose et al., 2017). While germination in both the fall and spring were not specifically assessed, an earlier planting date in 2018 may have allowed for more consistent fall germination and establishment. In contrast, uneven stand maturation was not as much of an issue with the pennycress grown during the 2019–2020 season, which was planted about 2 weeks earlier than in the first experimental year. Overall, the "IO217" line in this study was predominantly used for its reduced-shatter mutation; however, it is clear that many of the difficulties associated with growing wild-type pennycress still persist and that future breeding targets should continue to focus on consistent germination at planting and uniform stand maturation.

3.3 | Seed and biomass moisture

There was a significant reduction in seed and biomass moisture between physiological maturity, when treatments were applied, and harvest maturity, when plants were harvested (data not shown; Figure 1). This was expected based on the biological parameters that define each stage of development (i.e., seed at maximum dry weight versus seed dry enough for mechanical harvest). While the main focus of this study was to examine the differences in biomass and seed moisture between harvest aid treatments at harvest maturity, these parameters were also measured at treatment application (i.e., at or around physiological maturity) as a reference parameter. Plant moisture at physiological maturity is typically too high for mechanical harvest and the seed is too wet for long-term storage. The pennycress in this experiment was no exception. Seed moisture at treatment application averaged 191 g kg^{-1} across experimental years while biomass moisture averaged 538 g kg^{-1} (data not shown; Figure 1). Biomass moisture at harvest aid application was similar in both years of the experiment, but it is interesting to note that seed moisture was quite different in each year. In 2019, when harvest aid application was more closely aligned with the targeted growth stages, seed moisture averaged 294 g kg^{-1} while seed moisture averaged 87 g kg^{-1} in 2020 when treatment application occurred late relative to the targeted growth stages (data not shown; Table 3). Based on the differences in growth stage at treatment application each year, 85.5 in 2019 and 86.2 in 2020, these results further demonstrate how rapidly pennycress matures once it reaches physiological maturity (Table 3). It is also important to recognize that pennycress seed in 2020 had nearly reached the recommended moisture content for post-harvest storage, 80 g kg^{-1}, prior to treatment application through natural senescence alone (Mousavi-Avval & Shah, 2020). The suggested pennycress seed moisture at harvest is 120 g kg^{-1}, so the 2020 pennycress may have been a candidate for direct harvest at the time of treatment application. However, taking the seed moisture at treatment application in 2019 into account, moisture reduction was necessary to facilitate combine harvest. It is interesting to note that, in both years, seed moisture at treatment application was below the estimated range for pennycress seed moisture at physiological maturity, 434–455 g kg^{-1}, established in a prior study (Cubins et al., 2022). This may mean that pennycress was past physiological maturity in both years of the study by the time treatment application occurred. In the future, it may be prudent to continuously sample for seed moisture once pennycress begins to mature to better estimate when physiological maturity is imminent. At harvest maturity, seed moisture

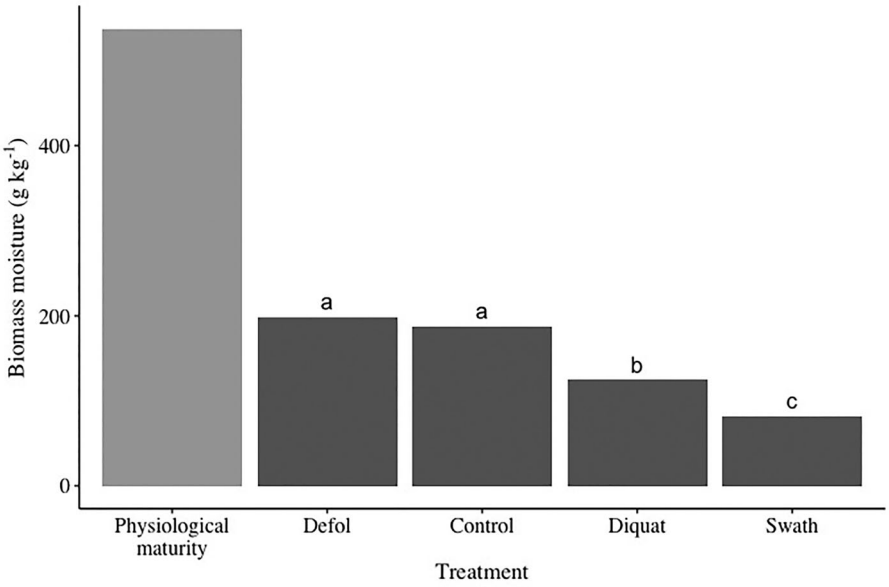

FIGURE 1 Biomass moisture of non-shatter pennycress at harvest maturity over the 2018–2019 and 2019–2020 growing seasons. Pennycress biomass moisture at physiological maturity (i.e., treatment application) is shown as a point of reference. Treatments that share a letter of significance are not statistically different from each other based on Tukey's HSD ($p < 0.05$).

was similar across treatments. The average seed moisture at harvest was 67 g kg^{-1}, meaning that pennycress seed was adequately dry for mechanical harvest as well as post-harvest storage (Table 4; Crookston & Hill, 1978; Mousavi-Avval & Shah, 2020). An important takeaway is that the seed moisture of the treated pennycress was no different than that of the control pennycress, indicating that harvest aid application was not necessary for pennycress seed moisture reduction.

Unlike seed moisture, there were significant differences in biomass moisture between treatments at harvest maturity (Figure 1). The swathing treatment produced the driest plants while the Defol treatment and control corresponded with the smallest reduction in biomass moisture (Figure 1). Plant moisture after swathing decreased to 82 g kg^{-1} by the time of harvest while the Defol and control treatments averaged 193 g kg^{-1}. There are few directives on optimal biomass moisture content for Brassicaceae at harvest. In canola, some green material is acceptable, but it is important to wait until the seed pods are dry enough to allow for seed to separate from the pod (Canola Council of Canada, 2018). In addition, a lower biomass moisture generally makes combine harvest easier with fewer chances of clogging machinery. Given this information, it is possible that pennycress in all treatments in this study were at an appropriate moisture level for combine harvest at harvest maturity despite the statistical differences (Figure 1). However, if a harvest aid was selected, these results demonstrate that Defol is not an appropriate choice for biomass moisture reduction in pennycress as it did not perform differently from the naturally senescing control. In contrast, Diquat or swathing the pennycress stand may be more effective moisture reduction tools (Figure 1). Swathing itself comes with challenges. Many farms that are targeted for pennycress production in the Upper Midwest (i.e., corn and soybean operations) have the infrastructure to harvest pennycress through direct combining but may not have the expertise or equipment readily available for swathing. In comparison with a chemical desiccant, the challenges of swathing may deter a grower from approaching swathing as a harvest method. Overall, it does not appear that a harvest aid is necessary for pennycress production using the current germplasm. As demonstrated, pennycress ripens between mid-June and early July when conditions allow for rapid moisture loss following physiological maturity. However, early-maturing pennycress lines are a primary breeding target (Chopra et al., 2020). When these lines are more readily available, it may be prudent to revisit the question of chemical desiccation or swathing prior to harvest as pennycress will hypothetically reach physiological maturity at a time when environmental conditions are cooler and therefore cannot facilitate rapid natural senescence following physiological maturity.

3.4 | Post-harvest measurements

There were no differences between treatments in terms of oil content, crude protein, or seed yield. This was an expected outcome given that pennycress treatments were deployed at or just after physiological maturity when both oil content and crude protein were fully developed (Table 3; Cubins et al., 2022). Oil content averaged 323 g kg^{-1} over both

experimental years (Table 4). In comparison, prior research has determined that pennycress oil content typically ranges between 260 and 360 g kg^{-1} (Cubins et al., 2019). Maximizing oil content is an important aspect of pennycress production as the primary post-harvest product is oil (Moser, 2012; Mousavi-Avval & Shah, 2020). However, oil yield is a combination of both oil content and seed yield. Pennycress yield in prior research has generally spanned 373–933 kg ha^{-1} though a few studies have reported maximum yield values up to 1387 kg ha^{-1} (Cubins et al., 2019). The seed yield in this study averaged 2271 kg ha^{-1}, much greater than the range typically reported (Table 4; Cubins et al., 2019). This is largely due to the reduced-shatter *Ta-ind-2* mutation that characterizes "IO217" (Chopra et al., 2020). Prior agronomic research has mainly dealt with shatter-prone wild-type lines such as "MN106" and "Beecher" meaning that yield loss up to 35% between physiological maturity and harvest maturity was commonplace (Carlson, 2018; Cubins et al., 2022; Dose et al., 2017; Johnson et al., 2017; Ott et al., 2019). While the oil content of seed in this experiment was comparable with prior research, the high seed yield of the "IO217" line greatly increases the pennycress oil yield potential.

Crude protein values were low relative to the values presented in prior pennycress research. Crude protein in the present study averaged 188 g kg^{-1} across experimental years while pennycress in prior studies had ranged between 230 and 270 g kg^{-1} (Table 4; Cubins et al., 2022; Dose et al., 2017). It is unclear why the crude protein content in this study was so low relative to prior research as similar rates of N were applied across experiments. There are trade-offs between oil content and crude protein, as oil is the primary form of carbohydrate storage in mature oilseeds (da Silva et al., 1997; Focks & Benning, 1998; Vigeolas et al., 2004). However, the oil content of this pennycress seed does not seem high enough to necessitate a lower crude protein content. While crude protein is not a primary concern in pennycress production right now, this may become more important in the future as there is an interest in pennycress meal for use as a nutritional supplement for livestock (Hojilla-Evangelista et al., 2013, 2015).

4 | CONCLUSIONS

Based on these results, harvest aids are not a necessary tool in pennycress production at this time as pennycress seed and biomass moisture was reduced at nearly the same rate for treated and untreated pennycress plants. Natural senescence is not only a physiologically efficient strategy for pennycress plant maturation, it also does not add to the material and labor costs of pennycress production like the addition of a harvest aid would. At present, the time between pennycress physiological and harvest maturity occurs when GDD are generally plentiful, meaning that the time between these milestone development stages is very short. However, this may change in the future. Early-maturing pennycress is a primary breeding target and when these pennycress lines are developed, they will reach physiological maturity earlier in the growing season when temperatures are cooler. This may necessitate another look at harvest aids once the window between physiological and harvest maturities lengthens.

While this experiment was not a germplasm study, the improvements and drawbacks of the "IO217" line were evident. Many of the challenges faced in this experiment, such as uneven stand germination and late maturation, stemmed from known issues with unimproved pennycress lines. It is clear that the development of further improved pennycress lines with stacked traits will be essential to successful production in the Upper Midwest. However, seed shatter has been a principal concern in pennycress research and production and the reduced-shatter mutation that is present in the "IO217" line resulted in a significant increase in harvestable seed. This mutation alone was able to increase potential pennycress oil production by two to seven times the amount predicted from the pennycress lines used in prior research regardless of the stand maturation issues. Oil is the primary product of pennycress production, so maximization of yield is essential to pennycress viability as an oilseed crop. Future improvements and trait stacking will continue to improve the feasibility of using pennycress as an agricultural crop in the Upper Midwest.

AUTHOR CONTRIBUTIONS

Julija A. Cubins: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; supervision; visualization; writing—original draft; writing–review & editing. **Samantha Wells**: Conceptualization; funding acquisition; investigation; methodology; resources; supervision; writing—review and editing. **Russ W. Gesch**: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing—review and editing. **Gregg A. Johnson**: Conceptualization; funding acquisition; supervision; writing–review & editing. **Maninder K. Walia**: Conceptualization; investigation; methodology. **Ratan Chopra**: Resources; writing–review & editing. **M. David Marks**: Funding acquisition; resources; writing–review & editing. **Rebecca D. Swenson**: Supervision; writing–review & editing. **Katherine Frels**: Conceptualization; resources; supervision; writing—review & editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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