frontiers | Frontiers in Agronomy

TYPE Original Research
PUBLISHED 12 October 2023
DOI 10.3389/fagro.2023.1205259

Seed treatment affected establishment and yield in two pennycress lines

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RECEIVED 13 April 2023
ACCEPTED 18 September 2023
PUBLISHED 12 October 2023

CITATION
Koirala N, Barker DJ, Gesch RW, Mohammed YA, Heller NJ, Hard AW, Wells SS, Phippen WB, Tas P and Lindsey AJ (2023) Seed treatment affected establishment and yield in two pennycress lines. *Front. Agron.* 5:1205259. doi: 10.3389/fagro.2023.1205259

Introduction: Oilseed pennycress (*Thlaspi arvense* L.) is an emerging biofuel crop for use in the aviation industry that has potential as a rotational crop in corn (*Zea mays* L.)–soybean [*Glycine max* (L.) Merr.] cropping sequences. Ensuring autumn emergence of pennycress after early broadcast seeding is key because this practice may result in uneven spatial distribution due to the small seed size and variable germination, soil contact, and moisture availability. The objective of this research was to evaluate the impact of five seed treatments and enhancements on autumn establishment in two pennycress lines (MN106NS and tt8-t/ARV1) in broadcast seeding compared with no treatment.

Methods: Tested treatments were (i) gibberellic acid (GA) soak, (ii) fludioxonil fungicide, (iii) pelleting with diatomaceous earth and a commercial binder, (iv) fungicide plus pelleting, or (v) fungicide plus pelleting with GA added to the binder. Seeds were planted at nine sites in four U.S. states to assess establishment (stand counts and percentage canopy cover) in the autumn and spring and seed yield after maturity.

Results: The MN106NS line had greater plants m^{-2} and percent green cover with treatments that included GA compared to the control. Line tt8-t/ARV1 had reduced stands when pelleted compared to the untreated, and establishment was unchanged when treated with GA compared to the untreated. Pelleting treatments without GA were not beneficial for stand establishment of either line. Seed yield for MN106NS was 20% greater than the untreated when treated with GA only or pelleted. Seed yield for tt8-t/ARV1 did not increase over the untreated with any treatment and was reduced by 20%–40% when pelleted.

Discussion: These results suggest minimal benefit of seed treatment and pelleting for the tested lines under broadcast seeding, though black-seeded lines may still benefit from GA treatment. Plant establishment and yield were negatively correlated with total precipitation post-planting, suggesting that excessive rainfall post-planting (>60 mm) may impede establishment when broadcast seeded.

KEYWORDS
pennycress, emergence, yield, oilseed, sustainable aviation fuel, crop rotation, seed pelleting, seed treatment

1 Introduction

Corn and soybean production dominate the U.S. Midwest agricultural landscape, which limits crop diversification. Crop diversification, while being challenged by climate and management practices (Mohammed et al., 2020), can increase agricultural productivity and sustainability (Brummer, 1998; Davis et al., 2012; Gaba et al., 2015). Increasing the bioeconomy of the agriculture sector through biofuel production can promote its sustainability (National bioeconomy blueprint, 2012). Oilseed pennycress grown as a winter annual crop for sustainable aviation fuel could fit into temperate climate regions such as the upper Midwest (McGinn et al., 2019). However, adoption of pennycress may be limited due to its poor germination and establishment (Hazebroek & Metzger, 1990; Sedbrook et al., 2014; Mohammed et al., 2020).

Recent studies that continue to demonstrate pennycress seed yields of 2,400 kg ha^{-1} (Cubins et al., 2019) and oil content of 270–390 g kg^{-1} (McGinn et al., 2019) highlight the suitability for biofuel generation (Moser et al., 2009b; Moser, 2012; Fan et al., 2013; Mousavi-Avval & Shah, 2020). Pennycress offers a suite of ecosystem services by providing food for pollinators (Eberle et al., 2015; Chopra et al., 2020), promoting the diversity of beneficial arthropods (Groeneveld & Klein, 2015; Groeneveld et al., 2015), suppressing weeds (Johnson et al., 2015), and scavenging residual N from the field (Weyers et al., 2019; Moore et al., 2020). Pennycress also has higher biofuel production potential and lower negative environmental impact compared to other existing oil-producing crops (Moser et al., 2009a; Mousavi-Avval & Shah, 2021).

Successful establishment and subsequent survivability are key requirements for crop production, and without achieving these, a crop is very unlikely to be adopted. Pennycress naturally has a higher degree of seed dormancy, though seed treatments with gas (especially GA4 + 7) have been found to be effective in breaking dormancy and increasing germination in seeds with a black seed coat (Metzger, 1983; Saini et al., 1987; Koirala et al., 2022). Koirala et al. (2022) also reported that seed pelleting increased germination in a black-seeded pennycress line under controlled conditions. Similarly, recent efforts in domestication have led to low-fiber golden pennycress lines that exhibit high germination potential in the absence of exogenous treatment (Ott et al., 2021; Koirala et al., 2023). Improved germination under ideal conditions does not always guarantee better establishment in field conditions (Finch-Savage & Bassel, 2016; Reed et al., 2022), but seed pelleting might be effective to improve field establishment of pennycress as has been demonstrated in other crops (Burns et al., 2002; Gesch et al., 2012; Javed & Afzal, 2020).

Improved establishment under broadcast seeding of pennycress in autumn is desired to facilitate easy intercropping and shortening the time between annual crop harvest and pennycress emergence. Being an emerging crop, pennycress lacks optimized crop management practices (Cubins et al., 2019; Zanetti et al., 2019; Verhoff et al., 2022). Previous studies on pennycress have reported using seeding rates ranging from 1.1 to 16.8 kg ha^{-1} (Cubins et al., 2019), which may have been an artifact of high seed dormancy and poor establishment. Planting depth and method are also reported to influence pennycress establishment. Phippen et al. (2010) reported drill seeding having a better crop stand than broadcast seeding while Moore and Mirsky (2020) did not observe a benefit of drill seeding over broadcast seeding. Similar studies comparing these two establishment methods did not find a significant difference in seed and biomass yield in pennycress (Phippen et al., 2010; Noland et al., 2018; Moore et al., 2020). CoverCress, Inc (2022) recommends farmers use a 5.6 kg ha^{-1} seeding rate with surface seeding or seeding <0.6 cm below soil surface during early September through mid-October for golden pennycress "CoverCress™" lines. Soil water availability is identified as the most limiting factor for field emergence (Hazebroek & Metzger, 1990), and precipitation of approximately 13 mm within 3 days of planting is stated as adequate to facilitate establishment (CoverCress, Inc, 2022). The integrated use of seed treatment such as fungicide, pelleting, and GA may increase pennycress establishment in autumn and may lead to increased seed yield. The objective of this study was to assess how different seed treatments (including seed pelleting) affect establishment and yield of different pennycress lines in a range of environmental conditions after broadcast seeding. The hypothesis was that treatments would vary in their efficacy of improving establishment, though gains in germination and establishment would be seen for all over the untreated.

2 Materials and methods

2.1 Study sites and experimental design

A field experiment was conducted in 2021–2022 at nine sites across the U.S. Midwest (Table 1, Figure 1). The design at each site was a randomized complete block with three replicates per site. The treatments included two varieties with six seed treatments: untreated control (Unt.); GA4 + 7 soak (Gibberellic acid A4+A7 90%, ThermoFisher Scientific Chemicals, Inc., Ward Hill, MA) at 0.01% w/w for 12 h (GA only); fludioxonil [4-(2,2-Difluoro-2H-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile] fungicide (Maxim 4FS, Syngenta Crop Protection Canada Inc., Guelph, ON) at 50 µg ai per g of seed (Fung only); pelleting with diatomaceous earth (Spectrum Chemical Mfg. Corp., Gardena, CA) and a commercial binder (Seedworx Lite, AgInnovation, Walnut Grove, CA) (Pellet); and two pelleting treatments with the addition of fungicide alone (Pellet + Fung) or fungicide and GA at 0.01% w/v added to the pelleting binder solution (Pellet + GA + Fung). Fludioxonil as the fungicide was chosen due to its availability in other agronomic crops, and to combat potential fungal infection that could influence seedling survival and crown integrity. The first line was an improved, black-seeded pennycress from a public source ("MN106NS"; USDA-ARS, St. Paul, MN), and the second was a golden-seeded pennycress from a private source ("tt8-t/ARV1"; CoverCress, Inc., St. Louis, MO). Both lines are improved beyond

TABLE 1 State, city, site abbreviation (ID), GPS coordinates, and predominant soil series and texture for each site as identified from USDA-NRCS Web Soil Survey.

State	City	Site ID	GPS coordinates	Predominant soil series and texture
Ohio	Piketon	OSU_South	39.0472, −82.9938	Omulga Silt Loam
Ohio	Custar	OSU_NWB	41.2142, −83.7648	Hoytville Silty Clay Loam
Ohio	Columbus	OSU_Wat	40.0085, −83.0393	Kokomo Silty Clay Loam
Minnesota	Rosemount	UMn_2	44.7149, −93.0652	Waukegan Silt Loam
Minnesota	Morris	USDA_Mn	45.6843, −95.7991	Aazdahl-Formdale-Balaton clay loams
Illinois	Normal	ISU	40.5271, −89.0070	Catlin Silt Loam
Illinois	Macomb	WIU	40.4918, −90.6876	Sable Silty Clay Loam
Wisconsin	Madison	UW_1	43.0604, −89.5315	Kegonsa Silt Loam
Wisconsin	Lancaster	UW_2	42.8351, −90.7883	Fayette Silt Loam

the wild type, though the golden-seeded line is a transparent testa type that has a modified seed coat and may be less responsive to seed treatments to stimulate germination (Ott et al., 2021; Koirala et al., 2023). The seed of both tested lines was harvested in June 2021. The seed treatments were applied to a 40-g sample of both lines using the same materials and methods described in Koirala et al. (2022). Each batch of seed was subdivided into envelopes containing 1,000 seeds counted using a seed counter (Seedburo 801, Seedburo Equipment Company, Des Plains, IL) and were distributed in replicate for planting at each field location.

2.2 Cultivation practices

The plots were 1.2 × 1.2 m with a 0.6-m border between them. Planting dates ranged from 13 September to 12 October 2021

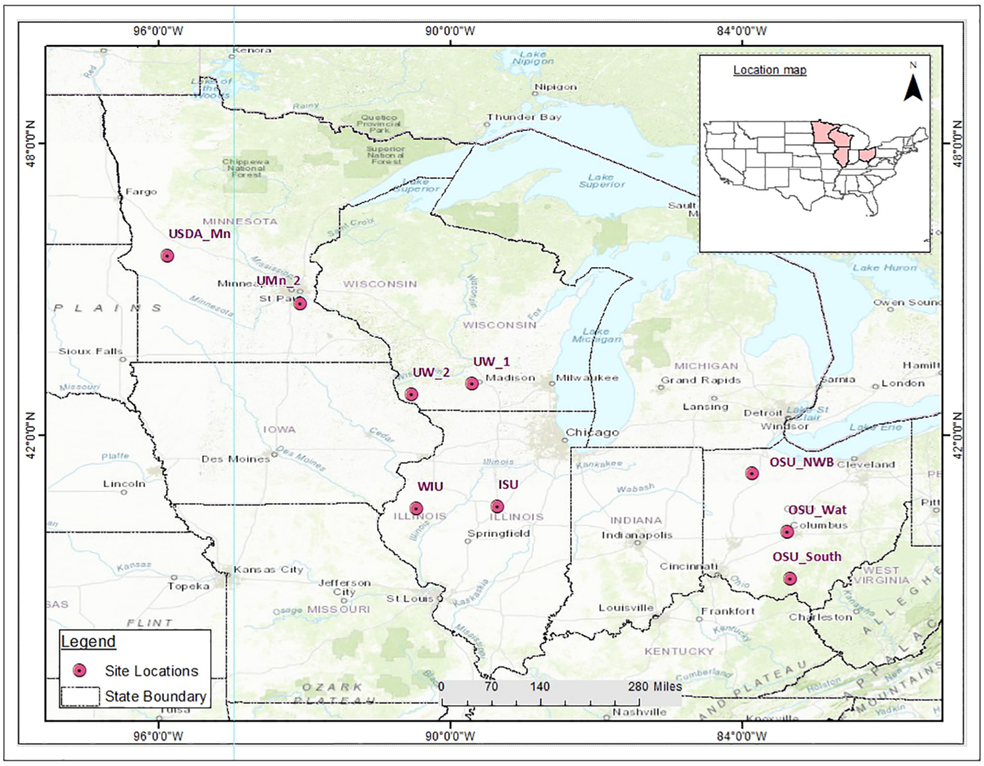

FIGURE 1
Map illustrating state and location for study sites. Abbreviations for sites are described in Table 1.

TABLE 2 Planting date, previous crop, tillage before planting, percentage residue cover, and surface soil moisture rating on a scale of 1–5 (1 = dry surface, 2 = 25% wet, 3 = 50% wet, 4 = 75% wet, and 5 = 100% wet) for each site (site abbreviations in Table 1).

Site	Planting date	Previous crop	Tillage before planting	Residue level (%)	Surface soil moisture rating (1–5)
OSU_South	14 September	Fallow	Autumn disc	28	1.18
OSU_NWB	16 September	Wheat (*Triticum aestivum* L.)	Autumn disc	40	1
OSU_Wat	12 October	Sweetcorn (*Zea mays* subsp. *mays* L.)	Autumn disc and cultipack	30	1.25
UMn_2	13 September	Spring wheat	Autumn disc and cultipack	7	1
USDA_Mn	24 September	Soybean	No-till	80	4
ISU	1 October	Soybean	No-till	50	1
WIU	27 September	Corn silage	Field cultivated and cultipack	10	1
UW_1	25 September	Corn	Autumn disc	20	1
UW_2	26 September	Alfalfa (*Medicago sativa* L.)	Autumn disc	30	1

(Table 2). The previous crop and tillage practice used before sowing for each site are also listed in Table 2. The percentage residue cover from the previous crop at planting and soil surface moisture condition (rated on a scale of 1–5: 1 = dry surface, 2 = 25% wet, 3 = 50% wet, 4 = 75% wet, and 5 = 100% wet) for each site was noted using visual assessment (Table 2).

Plots were seeded with 1,000 seeds each as a consistent seed number was desired for treatment evaluation. Seeding rate was not calculated on weight basis because of the difference in weight between pelleted and non-pelleted seeds, and MN106NS naturally had a greater 1,000 seed weight than tt8-t/ARV1. Assuming the non-pelleted seeds had an approximate weight of 0.9 mg (Koirala et al., unpublished data), the seeding rate would equate to 6 kg ha^{-1}, which is similar to the rates used in previous studies on pennycress (Phippen et al., 2010; Johnson et al., 2015; Dose et al., 2017; Cubins et al., 2019; Zanetti et al., 2019). Owing to the small area of each plot and low volume of seed, pennycress seeds were blended with 5 g of inert teff [*Eragrostis tef* (Zucc.) Trotter] seed prior to hand-broadcasting to increase uniformity of seed distribution.

2.3 Measurements collected

A composite soil sample to 20-cm depth was collected from each site for baseline analysis of cation exchange capacity (CEC), organic matter (OM) (measured as loss on ignition), pH (in water), P (Bray P1), and Mehlich-3 extraction of K, Ca, and Mg (Table 3). Daily total precipitation and mean air temperature data were recorded from 7 days prior to seeding up to 14 days after seeding. The rainfall data were extracted from the respective research station's weather records from each site. Temperature data were obtained from the records available at the Parameter-elevation Regressions on Independent Slopes Model (PRISM) Climate Group's website maintained by Northwest Alliance for Computational Science and Engineering

TABLE 3 Soil test results including organic matter (OM) measured as loss on ignition, cation exchange capacity (CEC), pH (in water), P (Bray P1), and Mehlich-3 extraction of K, Ca, and Mg for each site (site description in Tables 1 and 2).

Site	OM	CEC	pH	P	K	Ca	Mg
	g kg^{-1}	cmol(+) kg^{-1}		––––– mg kg^{-1} –––––			
OSU_South	32.0	7.4	6.8	15	73	1000	235
OSU_NWB	32.0	14.1	6.8	17	173	2100	330
OSU_Wat	34.0	16.6	7.0	164	590	2250	455
UMn_2	33.0	13.9	5.0	36	99	1100	260
USDA_Mn	37.0	17.4	6.9	18	125	2400	575
ISU	42.0	19.5	5.9	85	155	2300	340
WIU	34.0	22.0	5.8	25	165	2500	515
UW_1	32.0	14.8	7.2	35	163	1800	645
UW_2	40.0	12.1	7.1	26	153	1600	440

(NACSE), based at Oregon State University using the coordinates for each site (NACSE, 2022). Stand count was assessed early to mid-November (after the first frost) using a 0.25-m^2 area quadrat in each plot. One image per plot was taken from a consistent height and was processed using the “Canopeo” mobile application (Oklahoma State University, Stillwater, OK) to obtain green cover area percentage for each quadrat. Spring stand counts and canopy coverage using the same procedures were conducted in late March to mid-April at each site. Following spring stand assessment, plots were top-dressed with 45 kg N ha^{-1} of urea (46-0-0).

Because of excessive spring weed competition after spring stand assessment, OSU_South and UW_2 were unable to be harvested. Prior to harvest, each plot was visually assessed for stem lodging (0–100%) where higher percentages indicated greater lodging in each plot. Each plot was harvested between 9 and 30 June 2022. Seed yields were determined following hand-harvest and threshing using a small batch thresher (i.e., SBT Thresher, Almaco, Nevada, IA) or by mechanically harvesting using a Wintersteiger plot combine (Quantum model, Salt Lake City, Utah). The weight of 1,000 dried seeds was also measured from each plot.

2.4 Statistical analysis

Data analysis was conducted using a general linear mixed model (GLIMMIX procedure) in SAS 9.4 (SAS Institute, Cary, NC, USA). To address issues with assumptions of normality, the data for autumn stand count and canopy coverage were processed using a negative binomial distribution and a log link function. Spring stand count data were processed using a Poisson distribution with a log link function, and spring canopy coverage and lodging at harvest were analyzed using a beta distribution and logit link function.

Seed treatment and pennycress line and their interaction were considered as fixed factors, with site and replication nested within site being set as random factors. Site was treated initially as a fixed factor, but majority of interactions with site and other fixed effects were of magnitude (rather than direction). Site was treated as a random effect to assess the overarching ability of seed treatment influence on germination of the two lines when broadcast seeded. When the Global F-test was found to be significant (α = 0.05), means separation was conducted using LSMEANS statement in SAS after applying the ilink adjustment. Correlation analysis (CORR procedure) was conducted to determine potential relationships between percentage green area cover and number of plants m^{-2} and other measured variables (surface moisture level, residue cover percentage, precipitation after planting, precipitation 7 days before planting to 14 days after planting, day of year, and latitude).

3 Results

3.1 Weather at different sites

During the daily total precipitation measurement period (7 days prior to planting to 14 days after planting), there was one site with a single-day precipitation value of 40 mm (OSU_NWB), three sites with a single-day total between 20 and 40 mm (UMn_2, UW_1, and UW_2), and five sites with a single-day precipitation value below 20 mm (OSU_South, OSU_Wat, USDA_Mn, ISU, and WIU) (Table 4). Of the 84 mm precipitation received at OSU_NWB after planting, 71 mm of that total occurred in a single day. All other sites received some precipitation in the first 2 weeks after planting though distribution of precipitation in each site varied. Average daily temperature at each site ranged between 15 and 20°C, suggesting temperatures were adequate for pennycress germination.

3.2 Autumn and spring establishment

Data from OSU_NWB are not included in the analyzed results as autumn establishment was 0 plants m^{-2} for all treatments likely due to the excessive rainfall event (71 mm) that occurred after planting. Across all other sites, a significant treatment by line interaction was evident for autumn and spring stands (Table 5). The MN106NS line was more responsive to GA treatment, exhibiting the greatest number of plants and greatest canopy cover of all treatments when soaked or pelleted with GA included in the binder solution (55%–70% more plants m^{-2} and 16%–37% greater canopy coverage compared to untreated MN106NS). Pelleting MN106NS without GA application and treatment with fungicide alone improved establishment compared to the untreated check, though they were statistically similar in most cases (12%–40% more plants m^{-2}, 0%–25% greater canopy coverage). In contrast, tt8-t/ARV1 establishment was negatively affected by all pelleting treatments (Table 5) reducing plant numbers by 60%–75% and canopy coverage by 31%–61% compared to untreated tt8-t/ARV1. Treatment of tt8-t/ARV1 with fungicide or GA alone had marginal effects on establishment compared to the untreated (−15% to 20% compared to untreated). These results indicate that seed treatment improved establishment for MN106NS, whereas establishment of tt8-t/ARV1 was impeded by pelleting.

TABLE 4 Precipitation total 7 days prior to planting and 14 days post-planting, as well as average daily temperature for the first 14 days post-planting.

Site	Total precipitation 7 days before planting	Total precipitation 14 days after planting	Average daily temperature for 14 days after planting
	mm		°C
OSU_South	3	41	18.9
OSU_NWB	2	84	18.3
OSU_Wat	2	29	15.1
UMn_2	0	59	15.9
USDA_Mn	19	7	17.6
ISU	3	79	19.2
WIU	0	46	20.5
UW_1	24	44	18.8
UW_2	27	33	19.2

TABLE 5 Effects of seed treatment (Trt) and pennycress line on autumn and spring stand count, percentage canopy coverage, percentage stem lodging at harvest, seed yield, and 1,000 seed weight after harvest.

Trt	Line	Autumn Stand Count[a]	Autumn Canopy Cover[a]	Spring Stand Count[b]	Spring Canopy Cover[a]	Stem Lodging at Harvest[c]	Seed Yield[c]	1,000 Seed Weight[c]
		Plants m^{-2}	%	Plants m^{-2}	%	%	kg ha^{-1}	g
Untreated	tt8-t/ARV1	75b	2.9d	113e	14.1ab	34.2	947ab	0.920
	MN106NS	69b	3.8bcd	96g	13.6b	34.3	865bc	0.974
GA only	tt8-t/ARV1	75b	3.4bcd	103f	16.3ab	43.3	936ab	0.925
	MN106NS	110a	6.0a	164a	18.7a	23.5	1,034a	0.965
Fung only	tt8-t/ARV1	78b	3.2cd	96g	14.1ab	34.7	1,079a	0.930
	MN106NS	107a	4.7ab	137c	17.2ab	26.0	972ab	0.987
Pellet	tt8-t/ARV1	27c	1.1e	32i	8.4cd	49.5	738c	0.940
	MN106NS	81ab	4.5abc	138c	17.0ab	37.9	1,039a	0.967
Pellet + Fung	tt8-t/ARV1	31c	1.6e	44h	9.7c	63.1	759c	0.941
	MN106NS	77b	3.8bcd	124d	15.5ab	18.7	947ab	0.966
Pellet + GA + Fung	tt8-t/ARV1	19d	1.5e	28j	6.1d	56.6	565d	0.919
	MN106NS	107a	4.5abc	154b	17.4ab	39.6	1,051a	0.982
p-values								
Trt		<0.001	<0.001	<0.001	<0.001	0.241	0.004	0.905
Line		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Trt × Line		<0.001	0.002	<0.001	<0.001	0.118	<0.001	0.537

Treatment abbreviations are as follows: GA only = gibberellic acid (GA) soak at 0.01% w/w for 12 h, Fung only = fludioxonil at 50 µg ai per g of seed; Pellet = pelleting with diatomaceous earth and a commercial binder, Pellet + Fung = Pelleting with the addition of fungicide, and Pellet + GA + Fung = Pelleting plus GA and fungicide. Different letters within a column denote mean separation for the interaction of Trt × Line.
[a]Included data for all sites except OSU_NWB due to lack of autumn emergence.
[b]Includes same sites as for autumn data minus WIU where spring counts were not collected.
[c]Includes all sites except OSU_South and UW_2 where substantial summer annual weed occurred to impede accurate yield data collection.

3.3 Stem lodging, yield, and 1,000 seed weight

Visual rating of stem lodging at harvest was unaffected by seed treatment, but was greater for tt8-t/ARV1 (46.8%) than for MN106NS (29.4%) across seed treatments. Seed treatment effects on seed yield for MN106NS mirrored the effects observed for autumn and spring establishment values (Table 5) in that all treatments were similar or greater yielding than the untreated by 9% to 21%. Conversely, tt8-t/ARV1 treated with fungicide produced the greatest yield but was statistically similar to untreated tt8-t/ARV1. All treatments where pelleting was used reduced yield by 20%–40% compared to untreated tt8-t/ARV1. Seed treatment did not affect 1,000 seed weight after harvest, though MN106NS seeds were heavier than tt8-t/ARV1 seeds (0.974 vs. 0.929 g, respectively).

3.4 Correlation results

Different correlations were observed between the dependent variables and other variables independent to our treatments (Table 6). Plant number (at both autumn and spring) was positively correlated to precipitation before planting and negatively correlated to precipitation after planting, possibly due to heavy precipitation post-planting reducing establishment (Figure 2). Percentage canopy coverage correlations were less impacted by precipitation patterns, though they were correlated to their respective plant numbers within each measurement period. Canopy coverage was negatively correlated to residue percentage, suggesting that emerged plants may have been less visible in sites with heavier surface residue. Spring plant number exhibited the strongest correlation to yield. Drier soil at planting and greater

TABLE 6 Pearson correlation coefficient (*r*) for precipitation from 7 days before planting (rain pre-planting), surface residue coverage at planting, soil surface moisture rating at planting, precipitation up to 14 days after planting (rain post-planting), autumn and spring stand counts, percentage green area cover during autumn and spring, and other parameters: percentage stem lodging, seed yield, and 1,000 seed weight across locations, lines, and seed treatments.

	Autumn Stand Count	Autumn Canopy Cover	Spring Stand Count	Spring Canopy Cover	Stem Lodging	Seed Yield	1,000 Seed Weight
Rain Pre-Planting	0.465***	−0.145*	0.300***	0.0653	0.138*	−0.189***	0.068
Residue at Planting	−0.041	−0.453***	0.083	−0.388***	0.032	0.203**	−0.319***
Soil Surface Moisture Rating at Planting	0.101	−0.255***	0.257***	−0.283***	−0.320***	0.079	−0.619***
Rain Post-Planting	−0.260***	0.091	−0.536***	−0.071	0.260***	−0.354***	0.587***
Autumn Stand Count		0.210***	0.645***	0.122	−0.140*	−0.100	0.184**
Autumn Canopy Cover			0.213**	0.511***	−0.202**	−0.189**	−0.0570
Spring Stand Count				0.624***	−0.132	0.522***	−0.104
Spring Canopy Cover					0.118	0.308***	0.016
Stem Lodging						0.177**	0.464***
Yield							−0.080

*, **, and *** show significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

precipitation post-planting were correlated to greater 1,000 seed weights, though further investigation into these relationships may be warranted to draw further conclusions. Stems with fewer or smaller seeds in their silicles may have been less prone to lodging at the time of harvest, which may explain the significant correlation between lodging and 1,000 seed weight.

4 Discussion

Similarities in pennycress seedling establishment occurred across all sites, suggesting that its potential for production exists over a wide area of the upper U.S. Midwest. Establishment was demonstrated over a wide range of soil pH from 5.0 to 7.2 (Table 3) under broadcast seeding conditions, though autumn precipitation played an important role in establishment. The OSU_NWB site experienced heavy rainfall (71 mm) on the 6th day after sowing, which likely washed out or killed any emerged seedlings. With the failure of plant establishment in all seed treatments of both lines at OSU_NWB, forecasted rainfall may be key in ensuring autumn establishment. Industry recommendations are for 13-mm precipitation within 3 days of planting to facilitate establishment (CoverCress, Inc, 2022); the current study suggests between 10 and 60 mm in the 14 days following seeding may be sufficient for the establishment of surface broadcast seeds. Both OSU_South and UW_2 were not harvested due to excessive weed competition, and both sites were planted after a non-grain crop (Table 2). This indicates that annual crop herbicide programs or past management practices may be more conducive to pennycress establishment compared to other cropping systems. The inability to harvest in these two sites due to high competition supported the idea of low water use efficiency and competing ability of pennycress as discussed by Johnson et al. (2015).

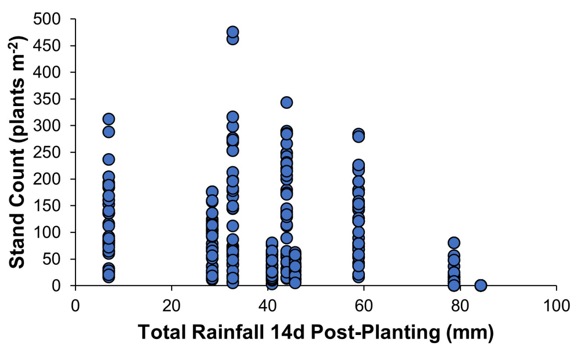

FIGURE 2
Autumn stand count (*y*-axis) as affected by total precipitation during the first 14 days post-planting (*x*-axis).

There was an overall increase in plant stand count (averaged across all sites counted) in spring compared to autumn stand count across all seed treatments and both varieties (Table 5). This indicated the presence of spring emergence even with the GA seed treatments that were applied to break dormancy and accelerate germination shortly after planting. A similar increase was reported by Wohrley (2022), though Mohammed et al. (2020) reported a reduction in stand count in spring compared to autumn. All pelleted treatments with or without GA improved number of established plants in spring for black-seeded MN106NS. This increase supported the idea presented by Koirala et al. (2023) of improved seed vigor with these treatments in MN106NS. Conversely, pelleted seed treatments on golden-seeded tt8-t/ARV1 had a negative impact in plant stand count. These results were supported by Koirala et al. (2023) that showed that seed pelleting of golden-seeded decreased seed vigor.

The positive correlation observed between number of plants and green canopy cover (Table 6) was similar to a study reported by Wohrley (2022). However, a negative relationship between these two variables was observed and discussed in Koirala (2022) and was reported in another study of Wohrley (2022) possibly due to plant thinning along with growth and rosette development. Canopy cover of at least 30% of the soil surface is expected in a cover crop to protect soil from erosion (Allmaras & Dowdy, 1985); the maximum observed spring canopy cover averaged across sites (18.7%) was lower than the desired cover of more than 30% to fully realize erosion prevention benefits. This might be due to the use of broadcast seeding in this study, which was reported to have a poor establishment than drill seeding (Phippen et al., 2010). However, the number of plants m^{-2} even with drill seeding in the study by Phippen et al. (2010) was less than what was observed for most treatments in the current study.

Despite the positive correlation observed between spring stand count and canopy cover with seed yield, non-pelleted tt8-t/ARV1 had a similar yield to that of MN106NS. The observed yields were similar and within the range of the studies reported by Cubins et al. (2019) in their review of pennycress agronomics. The same article mentioned that a pennycress seed yield of 1,684 kg ha^{-1} is required to be an economically viable crop, while Trejo-Pech et al. (2019) estimated that a seed yield of 1,335 kg ha^{-1} was needed to cover production costs. The seed yields observed in the current study across sites were 25% or more below these desired estimates, indicating that the efforts to improve establishment and yield through seed treatments under broadcast seeding were inadequate. Further advances in both genetics and other management practices (i.e., seeding method and seeding rate) will be needed to ensure critical yield levels are achieved.

The golden-seeded line tt8-t/ARV1 experienced a reduction in establishment and yield following most treatments in this study, suggesting that treatment using these methods was not necessary or was detrimental (e.g., pelleting). Even though seed pelleting with GA improved establishment and yield for MN106NS, there was no clear advantage of pelleting over the GA-only treatment as suggested in literature (Scott, 1989; Gesch et al., 2012; Vinod Kumar et al., 2015; Su et al., 2017; Javed & Afzal, 2020). Pelleting could result in improved flow rate in mechanical planters (Burns et al., 2002), though mechanized planting was not assessed in the current study. The treatments from the current study may be used for MN106NS depending on the availability of seed treatment facility, cost of treatment, and goals for treating the seed.

In conclusion, the seed treatments applied in the current study were not enough to fully address the challenge of establishing pennycress as a viable winter annual cash cover crop in the Midwestern United States with broadcast seeding. Rainfall accumulation of more than 60 mm within 14 days of planting can reduce plant establishment; thus, a weather forecast should be considered when deciding a planting period to avoid heavy rain events. Similarly, as rainfall within 7 days prior to planting improved plant stand, it is recommended to broadcast pennycress after a rainfall during autumn for better establishment. It is not recommended to treat the golden-seeded line with the treatments used in the present study, while the black-seeded line had better establishment and yield from seed treatment with GA and the novel seed treatment—seed pelleting with added GA. Pennycress had lower competing ability with other crops and weeds; therefore, a herbicide program needs to be developed for pennycress especially if it is broadcasted following non-grain crops or fallow conditions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

NK: study design, treatment implementation, data collection, and drafted original manuscript; DB: study design and edited manuscript draft; RG: data collection and edited manuscript draft; YM: data collection and edited manuscript draft; NH: data collection and edited manuscript draft; AH: data collection and edited manuscript draft; SW: data collection and edited manuscript draft; WP: data collection and edited manuscript draft; PT: data collection and edited manuscript draft; AL: study design, treatment implementation, data collection, drafted original manuscript, and incorporated revisions/corresponding author. All authors contributed to the article and approved the submitted version.

Funding

Funding support was provided through the Sustainable Agricultural Systems grant program from USDA-NIFA, award number 2019-69012-29851. The authors acknowledge support of these projects through the Integrated Pennycress Research Enabling Farm and Energy Resilience (IPREFER) group. Salary and research support provided in part by state and federal funds appropriated by the Ohio Agricultural Research and Development Center (OARDC) and The Ohio State University, manuscript number HCS23-12.

Acknowledgments

Some of the data included in this publication are also a component of NK's MSc thesis entitled *Effects of Seed Treatment on Germination and Establishment of Oilseed Pennycress* (*Thlaspi arvense* L.), The Ohio State University, Columbus, OH. We thank Chris Aulbach for his suggestions of seed treatment types to consider. We are also grateful for the field and technical support provided by Allison Pohlmann, Tad Wesley, Kevin Schrock, Glenn Mills, Wayne Lewis, and Matt Davis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Allmaras, R. R., and Dowdy, R. H. (1985). Conservation tillage systems and their adoption in the United States. *Soil Tillage Res.* 5, 197–222. doi: 10.1016/0167-1987(85)90030-3

Brummer, E. C. (1998). Diversity, stability, and sustainable American agriculture. *Agron. J.* 90 (1), 1–2. doi: 10.2134/agronj1998.00021962009000010001x

Burns, J., Bennet, B., Rooney, K., Walsh, J., and Hensley, J. (2002). "Coatings for legume and grass seed," in *57th Southern Pasture and Forage Crop Improvement Conference*, Athens, GA, USA. Southern Pasture & Forage Crop Improvement Conference: Texas A&M AgriLife Extension, College Station, TX

Chopra, R., Johnson, E. B., Emenecker, R., Cahoon, E. B., Lyons, J., Kliebenstein, D. J., et al. (2020). Identification and stacking of crucial traits required for the domestication of pennycress. *Nat. Food* 1 (1), 84–91. doi: 10.1038/s43016-019-0007-z

CoverCress, Inc (2022) *Agronomic recommendations*. Available at: https://www.covercress.com/agronomic-recommendations.cfm.

Cubins, J. A., Wells, M. S., Frels, K., Ott, M. A., Forcella, F., Johnson, G. A., et al. (2019). Management of pennycress as a winter annual cash cover crop. A review. *Agron. Sustain. Dev.* 39 (5), 46–57. doi: 10.1007/s13593-019-0592-0

Davis, A. S., Hill, J. D., Chase, C. A., Johanns, A. M., and Liebman, M. (2012). Increasing cropping system diversity balances productivity, profitability and environmental health. *PloS One* 7 (10):e47149. doi: 10.1371/journal.pone.0047149

Dose, H. L., Eberle, C. A., Forcella, F., and Gesch, R. W. (2017). Early planting dates maximize winter annual field pennycress (Thlaspi arvense L.) yield and oil content. *Ind. Crops Prod* 97, 477–483. doi: 10.1016/j.indcrop.2016.12.039

Eberle, C. A., Thom, M. D., Nemec, K. T., Forcella, F., Lundgren, J. G., Gesch, R. W., et al. (2015). Using pennycress, camelina, and canola cash cover crops to provision pollinators. *Ind. Crops Prod* 75, 20–25. doi: 10.1016/j.indcrop.2015.06.026

Fan, J., Shonnard, D. R., Kalnes, T. N., Johnsen, P. B., and Rao, S. (2013). A life cycle assessment of pennycress (*Thlaspi arvense* L.) -derived jet fuel and diesel. *Biomass Bioenergy* 55, 87–100. doi: 10.1016/j.biombioe.2012.12.040

Finch-Savage, W. E., and Bassel, G. W. (2016). Seed vigour and crop establishment: Extending performance beyond adaptation. *J. Exp. Bot.* 67 (3), 567–591. doi: 10.1093/jxb/erv490

Gaba, S., Lescourret, F., Boudsocq, S., Enjalbert, J., Hinsinger, P., Journet, E. P., et al. (2015). Multiple cropping systems as drivers for providing multiple ecosystem services: from concepts to design. *Agron. Sustain. Dev.* 35 (2), 607–623. doi: 10.1007/s13593-014-0272-z

Gesch, R. W., Archer, D. W., and Spokas, K. (2012). Can using polymer-coated seed reduce the risk of poor soybean emergence in no-tillage soil? *Field Crops Res.* 125, 109–116. doi: 10.1016/j.fcr.2011.09.005

Groeneveld, J. H., and Klein, A. M. (2015). Pennycress-corn double-cropping increases ground beetle diversity. *Biomass Bioenergy* 77, 16–25. doi: 10.1016/j.biombioe.2015.03.018

Groeneveld, J. H., Lührs, H. P., and Klein, A. M. (2015). Pennycress double-cropping does not negatively impact spider diversity. *Agric. For. Entomol* 17 (3), 247–257. doi: 10.1111/afe.12100

Hazebroek, J. P., and Metzger, J. D. (1990). Environmental control of seed germination in Thlaspi arvense (Cruciferae). *Am. J. Bot.* 77 (7), 945–953. doi: 10.1002/j.1537-2197.1990.tb15189.x

Javed, T., and Afzal, I. (2020). Impact of seed pelleting on germination potential, seedling growth and storage of tomato seed. *Acta Hortic.* 1273, 417–423. doi: 10.17660/ActaHortic.2020.1273.54

Johnson, G. A., Kantar, M. B., Betts, K. J., and Wyse, D. L. (2015). Field pennycress production and weed control in a double crop system with soybean in minnesota. *Agron. J.* 107 (2), 532–540. doi: 10.2134/agronj14.0292

Koirala, N. (2022). Effects of Seed Treatment on Germination and Establishment of Oilseed Pennycress (*Thlaspi arvense L.*). Masters of Science Thesis. (Columbus, OH, United States: Ohio State University). Available at: http://rave.ohiolink.edu/etdc/view?acc_num=osu1658311918285856.

Koirala, N., Barker, D., Gesch, R. W., Heller, N. J., Hard, A. W., Wells, S. S., et al. (2023). Seed pelleting and storage effects on germination of pennycress (*Thlaspi arvense* L.). *Crop Sci* 63(5):3025–3036. doi: 10.1002/csc2.21077

Koirala, N.. (2022). Effects of Seed Treatment on Germination and Establishment of Oilseed Pennycress (Thlaspi arvense L.). Masters of Science Thesis. Ohio State University. Available at: http://rave.ohiolink.edu/etdc/view?acc_num=osu1658311918285856

McGinn, M., Phippen, W. B., Chopra, R., Bansal, S., Jarvis, B. A., Phippen, M. E., et al. (2019). Molecular tools enabling pennycress (*Thlaspi arvense*) as a model plant and oilseed cash cover crop. *Plant Biotechnol. J.* 17 (4), 776–788. doi: 10.1111/pbi.13014

Metzger, J. D. (1983). Promotion of germination of dormant weed seeds by substituted phthalimides and gibberellic acid. *Weed Sci.* 31 (3), 285–289. doi: 10.1017/S0043174500069010

Mohammed, Y. A., Matthees, H. L., Gesch, R. W., Patel, S., Forcella, F., Aasand, K., et al. (2020). Establishing winter annual cover crops by interseeding into maize and soybean. *Agron. J.* 112 (2), 719–732. doi: 10.1002/agj2.20062

Moore, V. M., and Mirsky, S. B. (2020). Cover crop biomass production across establishment methods in mid-Atlantic corn. *Agron. J.* 112 (6), 4765–4774. doi: 10.1002/agj2.20414

Moore, S. A., Scott Wells, M., Gesch, R. W., Becker, R. L., Rosen, C. J., and Wilson, M. L. (2020). Pennycress as a cash cover-crop: Improving the sustainability of sweet corn production systems. *Agronomy* 10 (5). doi: 10.3390/agronomy10050614

Moser, B. R. (2012). Biodiesel from alternative oilseed feedstocks: Camelina and field pennycress. *Biofuels* 3 (2), 193–209. doi: 10.4155/BFS.12.6

Moser, B. R., Knothe, G., Vaughn, S. F., and Isbell, T. A. (2009a). Production and evaluation of biodiesel from field pennycress (*Thlaspi arvense* L.) oil. *Energy Fuels* 23 (8), 4149–4155. doi: 10.1021/ef900337g

Moser, B. R., Shah, S. N., Winkler-Moser, J. K., Vaughn, S. F., and Evangelista, R. L. (2009b). Composition and physical properties of cress *(Lepidium sativum* L.) and field pennycress (*Thlaspi arvense* L.) oils. *Ind. Crops Prod* 30 (2), 199–205. doi: 10.1016/j.indcrop.2009.03.007

Mousavi-Avval, S. H., and Shah, A. (2020). Techno-economic analysis of pennycress production, harvest and post-harvest logistics for renewable jet fuel. *Renewable Sustain. Energy Rev.* 123, 109764. doi: 10.1016/j.rser.2020.109764

Mousavi-Avval, S. H., and Shah, A. (2021). Life cycle energy and environmental impacts of hydroprocessed renewable jet fuel production from pennycress. *Appl. Energy* 297, 117098. doi: 10.1016/j.apenergy.2021.117098

NACSE, O.S.U (2022) *PRISM Climate Group*. Available at: https://www.prism.oregonstate.edu/explorer/.

National bioeconomy blueprint (2012). Available at: https://obamawhitehouse.archives.gov/sites/default/files/microsites/ostp/national_bioeconomy_blueprint_april_2012.pdf.

Noland, R. L., Wells, M. S., Sheaffer, C. C., Baker, J. M., Martinson, K. L., and Coulter, J. A. (2018). Establishment and function of cover crops interseeded into corn. *Crop Sci.* 58 (2), 863–873. doi: 10.2135/cropsci2017.06.0375

Ott, M. A., Gardner, G., Rai, K. M., Wyse, D. L., Marks, M. D., and Chopra, R. (2021). TRANSPARENT TESTA 2 allele confers major reduction in pennycress (Thlaspi arvense L:) seed dormancy. *Ind. Crops Prod* 174, 114216. doi: 10.1016/j.indcrop.2021.114216

Phippen, W., Gallant, J., and Phippen, M. (2010). *Evaluation of Planting Method and Seeding Rates with Field Pennycress (Thlaspi arvense L.)* (Western Illinois University, Macomb IL: Western Illinois University). Available at: http://www.wiu.edu/pennycress/current-experiments/PlantDensity2010.pdf.

Reed, R. C., Bradford, K. J., and Khanday, I. (2022). Seed germination and vigor: ensuring crop sustainability in a changing climate. *Heredity* 128 (6), 450–459. doi: 10.1038/s41437-022-00497-2

Saini, H. S., Bassi, P. K., Goudey, J. S., and Spencer, M. S. (1987). Breakage of seed dormancy of field pennycress (Thlaspi arvense) by growth regulators , nitrate , and environmental factors. *Weed Sci.* 35 (6), 802–806. doi: 10.1017/S0043174500079376

Scott, J. M. (1989). Seed coatings and treatments and their effects on plant establishment. *Adv. Agron.* 42, 43–83. doi: 10.1016/S0065-2113(08)60523-4

Sedbrook, J. C., Phippen, W. B., and Marks, M. D. (2014). New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: Example pennycress (Thlaspi arvense L.). *Plant Sci.* 227, 122–132. doi: 10.1016/j.plantsci.2014.07.008

Su, L. Q., Li, J. G., Xue, H., and Wang, X. F. (2017). Super absorbent polymer seed coatings promote seed germination and seedling growth of Caragana korshinskii in drought. *J. Zhejiang University: Sci. B* 18 (8), 696–706. doi: 10.1631/jzus.B1600350

Trejo-Pech, C. O., Larson, J. A., English, B. C., and Edward Yu, T. (2019). Cost and profitability analysis of a prospective pennycress to sustainable aviation fuel supply chain in southern USA. *Energies* 12 (16). doi: 10.3390/en12163055

Verhoff, K., Phippen, W., Heller, N., and Lindsey, A. (2022). Winter-type oilseed pennycress crop staging guide. *Crop Forage Turfgrass Manage.* 8 (1), 1–9. doi: 10.1002/cft2.20165

Vinod Kumar, S., Vyakaranahal, B., Deshpande, V., Raikar, S., Nadaf, H., and Aravinda Kumar, B. (2015). Effect of seed polymer coating on growth and yield of pigeonpea. *J. Farm Sci.* 27 (4), 19–22.

Weyers, S., Thom, M., Forcella, F., Eberle, C., Matthees, H., Gesch, R., et al. (2019). Reduced potential for nitrogen loss in cover crop-soybean relay systems in a cold climate. *J. Enviornmental Qual.* 48 (3), 660–669. doi: 10.2134/jeq2018.09.0350

Wohrley, B. (2022). Pennycress establishment in response to management of corn residue and harvest date. Masters of Science Thesis. (Normal, IL, United States: Illinois State University), 1583. Available at: https://ir.library.illinoisstate.edu/etd/1583.

Zanetti, F., Isbell, T. A., Gesch, R. W., Evangelista, R. L., Alexopoulou, E., Moser, B., et al. (2019). Turning a burden into an opportunity: Pennycress (Thlaspi arvense L.) a new oilseed crop for biofuel production. *Biomass Bioenergy* 130, 105354. doi: 10.1016/j.biombioe.2019.105354