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# Short title: Barley outperforms mustard

Barley cover crops outperform brown mustard for early-season weed control in New Mexico chile pepper

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## Abstract

Barley (Hordeum vulgare L.) and brown mustard (Brassica juncea L.) are winter cover crops known to produce allelochemicals that suppress plant growth. Incorporating barley or brown mustard residues into the soil prior to planting a spring-seeded cash crop may suppress early season weeds in the cash crop; however, the comparative levels of weed suppression offered by barley and brown mustard cover crops incorporated into soil have not been determined. This study analyzed the relative capacities of barley and brown mustard cover crops to suppress early season weeds of spring-seeded chile pepper (Capsicum annuum L.). Reductions in weed density or hand hoeing time as a result of barley and/or brown mustard cover crop treatment were determined in two chile pepper fields in New Mexico over two growing seasons. For cover crop species that suppressed weeds in multiple site-years, a controlled environment study clarified possible growth stages adversely affected by determining the effects of cover crop-amended soil on the germination and seedling development of Palmer amaranth (Amaranthus palmeri S. Watson). Field study results indicated barley reduced early-season weed densities of chile pepper by up to 80% compared to the noncover control. Barley also reduced hoeing time in three of four site-years without affecting chile pepper fruit yield. Mustard cover crops reduced weed density in only one site-year (56% reduction relative to noncover control) and did not decrease hoeing time. The controlled environment study indicated that soil amended with barley slowed germination of A. palmeri without inhibiting seedling development. The results of this study indicate that a barley cover crop is more effective than brown mustard for early-season weed control of chile pepper in the southwestern United States.

Keywords: allelopathy; chili; green manure; integrated weed management

# Introduction

In 2022, New Mexico produced 61% of all chile peppers (*Capsicum annuum* L.) harvested in the United States, generating over 47 million USD in revenue (USDA-NASS 2024). In the same year, New Mexico chile pepper acreage was 72% of the U.S. total (USDA-NASS 2024). Ninety one percent of the chile pepper produced in New Mexico in 2022 were destined for processing. Fifty six percent of the chile pepper acreage in New Mexico occurred in three contiguous counties in the southern part of the state in 2021, which is the most recent year in which county-level acreage data are available (USDA-NASS 2024).

Southern New Mexico farmers who grow chile pepper for processing typically do not use plastic mulch and often directly sow chile pepper seed into raised beds (Bosland and Walker 2014). Because direct seeded chile pepper is slow to establish and therefore a weak competitor with weeds (Schroeder 1993), early season weed control is important for maximizing fruit yield (Amador-Ramírez 2002; Tursun et al. 2012). Early-season weeds can be managed with soil-residual herbicides and cultivation (Bajagain et al. 2023; Schutte et al. 2021). Weeds that establish despite these methods are typically controlled by hand hoeing. Minimizing the time required for hand hoeing is necessary for reducing labor costs, which are often prohibitively expensive for farm operations in southern New Mexico (Hawkes et al. 2008). Therefore, to sustain profitable chile pepper production in New Mexico, novel methods of controlling early-season weeds should be explored.

Residues of mustard (Brassicacea) cover crops tilled into the soil have a documented history of suppressing early-season weeds in potato (*Solanum tuberosum* L.) (Boydston and Hang 1995), pea (*Pisum sativum* L. 'Bolero') (Al-Khatib et al. 1997), tomato (*Solanum lycopersicum* L.) (Bangarwa and Northworthy 2014), soybean [*Glycine max* (L.) Merr.] (Krishnan et al. 1998), and bell pepper (*Capsicum annuum* L.) (Norsworthy et al. 2007). Such suppression is caused by the release of specific secondary metabolites from mustard residue in soil. When mustard tissue is macerated, glucosinolates — a type of secondary metabolite — are exposed to the enzyme myrosinase and converted to volatile isothiocyanates (ITCs) (Borek et al. 1994, Brown and Morra 1995, Vaughn and Boydson 1997). ITCs suppress weeds by inhibiting radicle protrusion at low concentrations and suppressing seedling growth at higher concentrations (Angelini et al. 1998). ITCs typically reach maximum concentrations between 5 and 21 h after hydrolysis, creating a very narrow period in which these volatiles may exhibit

herbicidal activities (Brown and Morra 1995, 1996). Although brief, weed suppression by a mustard cover crop incorporated into the soil is an example of allelopathy, which is the phenomenon by which secondary metabolites released into the environment from a plant, including plant residues, inhibit growth of other organisms (Rice 1984).

Previous studies determined that brown mustard (*Brassica juncea* L.) cover crops mowed and incorporated into soil in spring suppressed early-season weeds in spring-seeded chile pepper (Bajagain et al. 2024) and reduced seed survival and germination of Palmer amaranth (*Amaranthus palmeri* S. Watson; Nagila et al. 2022), which is a weed commonly found in New Mexico chile pepper. Although these results suggest brown mustard is a promising method for managing weeds in chile pepper, it may not be suitable for widespread use throughout New Mexico due to its susceptibility to cold injury shortly after seeding (Nagila et al. 2022), its potential to host beet leafhoppers (*Circulifer tenellus*) that vector curly top disease to chile pepper (Golino et al. 1989), and its potential to increase population densities of southern rootknot nematode (*Meloidogyne incognita*; Rudolph et al. 2015), which is a pest that can damage chile pepper. The potential for failed establishment due to frost damage, combined with the possibilities for harboring crop pests, compels a need for an alternative allelopathic cover crop for suppressing weeds in chile pepper.

In contrast with brown mustard, barley (*Hordeum vulgare* L.) is not considered a host plant of *C. tenellus* (Cook 1967). Barley also appears to be a cover crop well-suited for New Mexico because it can produce relatively large amounts of biomass in water-limited (Pratt et al. 2022) or salt-affected soil (Ayers et al. 1952) — conditions common in southwestern U.S. When grown as a fall-sown cover crop in New Mexico, barley suppresses cool-season weeds more than cereal rye (*Secale cereale* L.) (Pratt et al. 2022), suggesting its utility as a weed suppressive grass cover crop in the southwestern U.S. In germination assays, aqueous leachates of barley reduced germination of yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult. ssp. *pumila*] by 86% (Creamer et al. 1996), rigid ryegrass (*Lolium rigidum* Gaudin) by up to 81.5% (Kotzamani et al. 2021), and great brome (*Bromus diandrus* Roth.) by up to 74% (Bouhaouel et al. 2019).

Little is known about either the comparative abilities of barley and brown mustard cover crops to suppress weeds in chile pepper or the effects of barley-amended soil on a weed species common in chile pepper. Cover-crop induced suppression of weeds in subsequent cash crops, as well as other ecosystem services derived from cover crops, is related to cover crop biomass production (Ryan et al. 2011, Teasdale et al. 2007). Thus, the first objective of this study was to quantify amounts of aboveground biomass produced by barley and brown mustard cover crops grown in southern and central New Mexico. The second objective of this study was to evaluate mowed and incorporated barley and brown mustard monocultures, and barley and brown mustard combinations, for their effects on weed density, hand hoeing time, and fruit yield in chile pepper. The third objective was to determine barley-amended soil effects on germination and seedling development of *A. palmeri*.

#### **Materials and Methods**

#### Field descriptions

A field study was conducted at two sites. One study site was at the New Mexico State University (NMSU) Leyendecker Plant Science Research Center (hereafter "Leyendecker") located near Las Cruces, NM (32.20 N 106.74 W). Leyendecker has a cold arid desert climate (Köppen *BWk*). The second study site was at the NMSU Los Lunas Agricultural Science Center (34.77 N 106.76 W; hereafter "Los Lunas"), approximately 338 km north of Leyendecker. Los Lunas has a cold semi-arid climate (Köppen *BSk*).

The study was conducted from fall 2021 to fall 2022 and repeated fall 2022 to fall 2023. For each site-year, cover crops were seeded in fall and terminated in spring, when chile pepper was subsequently seeded (Table 1). Hereafter, site-years are referred to by the combination of site and year of chile pepper seeding. For Leyendecker 2022 and 2023, the study site was fallow and planted to chile pepper the summer prior to cover crop seeding, respectively. For Los Lunas 2022 and 2023, study sites were planted to guar [*Cyamopsis tetragonoloba* (L.)] and oat (*Avena sativa* L.), respectively, the summers prior to cover crop seeding. For both Leyendecker site-years, soil was a Belen series (clayey over loamy, smectic over mixed, superactive, calcareous, thermic Vertic Torrifluvents) (USDA-NRCS 2024). Soil samples taken from Leyendecker both years revealed an average textural composition of 43.5% clay, 33.5% silt, 23% sand, and 2.1% organic matter (Ward Laboratories, Kearney, NE, USA). In Los Lunas 2022, soil was a Vinton series (sandy, mixed, thermic Typic Torrifluvents) (USDA-NRCS 2024a), with 14% clay, 5% silt, 81% sand, and 0.9% organic matter (Ward Laboratories, Kearney, NE, USA). Soil for Los Lunas 2023 was a Bluepoint series (mixed, thermic Typic Torripsamments) (USDA-NRCS

2024a), with 10% clay, 7% silt, 83% sand, and 1.1% organic matter (Ward Laboratories, Kearney, NE, USA).

### Field study

Treatments were arranged in a randomized complete block design with four replications. Experimental units were plots measuring 19.81 m by 4.05 m at Leyendecker and 19.81 m by 3.05 m at Los Lunas. Differences in plot sizes between Leyendecker and Los Lunas reflected regional differences in row spacing for chile pepper. At Leyendecker, plots contained 22 rows of cover crops at 18-cm row spacing. Following cover crop termination, plots contained four rows of chile pepper at 1-m row spacing. At Los Lunas, plots contained 16 rows of cover crop at 18-cm row spacing, and 4 rows of chile pepper at 0.7-m row spacing. Cover crop treatments consisted of barley monocultures, mustard monocultures, a barley-mustard combination, and a no cover crop control.

The barley cultivar 'Stockford' (Helena Agri-Enterprises LLC, Mesquite, NM, USA) was used in the following site-years: Leyendecker 2022, Leyendecker 2023, and Los Lunas 2022. This cultivar was previously shown to be suitable as a winter cover crop in New Mexico (Agarwal et al. 2022b). However, relatively low amounts of barley biomass for Los Lunas 2022 compelled a change in cultivar for the Los Lunas study site. The barley cultivar 'Valor' (TriCal Superior Forage, Butte, MT, USA) was selected as a replacement of Stockford for Los Lunas 2023. For all site-years, the brown mustard cultivar 'Caliente Rojo' (Caliente Brand; Stokes Seeds Inc, Holland, MI, USA) was selected because it is marketed to contain high levels of glucosinolates.

Prior to cover crop sowing, fields were disked and then levelled with a laser-guided land levelling system (Laser Alignment Inc., Grand Rapids, MI, USA). Following seeding rate recommendations in Idowu and Grover (2014), plots featuring a mustard monoculture were seeded at 8.96 kg ha<sup>-1</sup>, and barley monoculture treatments were seeded at 112 kg ha<sup>-1</sup>. Plots featuring a combination treatment were seeded at a rate of 4.5 kg ha<sup>-1</sup> for mustard and 56 kg ha<sup>-1</sup> for barley. At Leyendecker, barley was sown with a mechanical grain drill (Model 450; John Deere, Moline, IL, USA) and mustard was sown with a drop spreader (Series 36; Gandy, Owatonna, MN, USA). At Los Lunas, barley and mustard were sown with a custom-built plot drill. Sowing dates can be found in Table 1. After sowing, cover crops were flood-irrigated 3 to 4

times across their growing seasons (Table 1). Each irrigation was approximately 10 cm deep and saturated the soil. Noncover control plots were routinely hand hoed to remove weeds and maintain weed-free conditions.

One day before cover crop termination, aboveground cover crop biomass was collected. Hand-operated hedge trimmers were used to cut aboveground biomass at the soil surface from two, 0.25-m<sup>2</sup> quadrats. The biomass was placed into paper bags, which were placed in drying ovens at 60 C until a constant weight was maintained. Biomass quantities were subsequently scaled to kg ha<sup>-1</sup>.

Cover crops were terminated by mowing. The mower at Levendecker was a flail mower (Model ORC12; Rhino Agriculture, Lynden, WA, USA), whereas the mower at Los Lunas was a rotary mower (Model HX6; John Deere, Moline, IL, USA). Within 30 to 60 min of mowing, residues were incorporated into the soil to a depth of 15 cm using an offset tandem disc. The offset tandem disc passed through the field one time at Leyendecker and two times at Los Lunas. A lister (Dave Koenig Enterprises Inc., Mesilla Park, NM, USA) was used to create raised beds approximately 25 cm high and 76 cm wide. Plots were subsequently furrow-irrigated to saturate upper soil levels. The sequence of cover crop mowing, disking, listing and irrigation took no more than 5 hr. Three wk after cover crop termination, beds were disked and shaped using a bed shaper, approximately 15% of aboveground cover crop residue remained on the soil surface. Chile pepper ('Sandia') was seeded to a depth of 2 cm at a rate of 5 kg ha<sup>1</sup> using mechanical seeders (at Leyendecker, MaxEmerge Plus, John Deere, Moline, IL, USA; at Los Lunas Cole Planet Jr., Cole Planter Company, Albany, GA, USA). Chile pepper was furrow-irrigated as needed throughout the growing season. At 8 wk after crop seeding, chile pepper stands were thinned by hand to clumps spaced approximately 15 cm apart. Each clump included two to three chile pepper plants. At 8 and 12 wk after crop seeding, nitrogen fertilizer was applied at 78.46 kg ha<sup>-1</sup> through irrigation.

Beginning 3 wk after sowing chile pepper, weed densities were determined every 2 wk for 6 wk. At each observation time, weeds within two permanently established 0.25  $m^2$  quadrats in each plot were identified to the species level, counted, and removed. Coincident with the second weed-counting event (or the first weed-counting event for the Los Lunas 2022 site-year; Table 1), the time required to hand hoe a permanently established 8 m transect within each plot was recorded. An individual laborer hoed all plots within a replicate. After the third timed

hoeing, weeds were controlled as needed by hand hoeing at both sites. At Leyendecker, weeds after the third timed hoeing were also controlled with a postemergence-directed application of pendimethalin at 1.60 kg ha<sup>-1</sup> (Prowl® H<sub>2</sub>O, 0.45 kg ai L<sup>-1</sup>; BASF Corp., Research Triangle Park, NC, USA) that was implemented 8 wk after chile pepper seeding.

At 131 to 145 d after chile pepper seeding (Table 1), all marketable green fruits were hand harvested along an 8 m transect in each plot. Marketable fruit were those which were straight, free of disease, and at least 10 cm long. Peduncles were removed from the fruit during harvesting. Because the USDA determines profit from weight (USDA-NASS 2024), fresh weights of marketable fruits were determined in the field and scaled to kg ha<sup>-1</sup>.

## Amaranthus palmeri study 1: seed germination

After mowing and incorporation of cover crops, but prior to irrigation, soil was collected from each barley and noncover control plot using hand shovels. Soil sampling depth was 15 cm, which was the same depth as cover crop residue incorporation. For each site-year, soil samples were aggregated within a treatment, homogenized to evenly distribute surface residue, and stored in plastic 18.9 L buckets with tight-fitting lids. Lidded buckets were stored indoors at 20 to 25 C for 2 to 4 months. *Amaranthus palmeri* seeds were collected from plants in a fallow field at Leyendecker in September 2021. To do this, inflorescences with mature seeds were removed from plants with hand clippers, and inflorescences were dried under room conditions for 28 d. Seeds were then hand-threshed into paper bags and subjected to repeated sieving before being stored in airtight containers at 4 C.

The effects of barley-amended soil on *A. palmeri* germination were determined with a three-step process. First, seeds were incubated in soil collected from barley or noncover control plots. Next, seeds were removed from soil and subjected to germination assays. Finally, time-integrated measures of seed germination were determined and compared between the treatments.

To incubate seeds in soil, *A. palmeri* seeds were placed in packets fabricated with nylon mesh (No nonsense® nylon knee highs, Kayser-Roth Corp., Greensboro, NC, USA) and sealed using a heat sealer. For each combination of site-year and cover crop treatment, eight seed packets were prepared. Each seed packet contained 35 *A. palmeri* seeds and measured 3 cm by 3 cm. Each seed packet was buried under 30 ml of soil from the selected site-year and cover crop treatment within a 60 ml plastic cup. Immediately after burying seed packets, soil was watered to

saturation by adding 25 ml of deionized water to each plastic cup. Hydrated seedbanks were placed into chambers set to darkness with 12 hr diurnal cycles at 25 C and 20 C. Seed packets were incubated for 28 d. Following incubation, packets were disinterred from soil and *A. palmeri* seeds were recovered. For packets buried in barley-amended soil, 10% of seeds germinated during incubation. For packets buried in soil without barley, 12% of seeds germinated during incubation. These seedlings were discarded.

Germination assays were conducted in 100 mm-diameter Petri plates filled with agar. Agar was created by autoclaving a solution of 7.5 g technical agar and 500 ml deionized water. When cooled slightly, the agar solution was poured into the Petri plates. When agar in plates was completely cool, seeds recovered from incubation were added to plates. Plates were lidded and placed in a germination chamber set to 12 hr photoperiods at 25 C day and 20 C night, which are conditions favorable for germination of *A. palmeri* (Chahal et al. 2015). Germinated seeds with a radicle at least 1 mm in length were counted and removed every 24 h for 14 d. At the end of the 14-d period, crush tests were performed on remaining seeds to test for viability as described in Borza et al. (2007).

Indicators of germination time and speed, as well as germination percentages, were calculated based on the total number of viable seeds per plate. The total number of viable seeds per plate was calculated as the sum of the number of germinated seeds plus the number of viable seeds determined in crush tests. Cumulative total germination data were used to determine germination percentage (GP). For germination time, the following metrics were determined: mean germination number (MGN), number of seeds germinated per day; first germination time (FGT), first d on which a germinated seed was observed; last germination time (LGT), last d on which a germinated seed was observed; the time spread of germination (TSG), the span of time between FGT and LGT; time to 50% germination (t50), d until 50% of maximum cumulative germination percentage in d; and peak germination time (PGT), the d on which the most germination (CVG), percentage of seeds which germinated per day; germination speed corrected (GSC), seeds germinated per d based on 100% germination; and mean germination percentage (MGP), mean germination percentage per day.

## Amaranthus palmeri study 2: seedling development

Barley-amended soil without barley residue on the soil surface was examined for suppression of *A. palmeri* seedling development. Experimental units were conical tubes (2.5 cm diameter x 16 cm depth, RLC3U Ray Leach Conetainers, Stuewe and Sons Inc., Tangent, Oregon, USA) filled with approximately 60 ml of soil from a selected site-year and treatment. Each combination of site-year and cover crop treatment featured eight replications. For a given year, treatments were arranged in a complete randomized design within plant growth chambers set to 12 hr photoperiods at 25 C day and 23 C night. Photosynthetically active radiation within chambers was 132  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. Ten *A. palmeri* seeds were placed on the soil surface of each tube. Surface placement followed by subsequent watering caused shallow burial of seeds, which is beneficial for seedling establishment of *A. palmeri* (Ward et al. 2013).

For the first 9 d of the incubation period, each tube was watered daily with 5 ml deionized water. Tubes that did not contain an *A. palmeri* seedling by the 9th d of incubation were replaced with new tubes filled with fresh soil and seeds. The new tubes were watered daily for the subsequent 9 d. On the 9th d of incubation, seedlings were thinned to 1 seedling tube<sup>-1</sup>. Between the 10th and 28th d of incubation, tubes were watered with 5 ml of deionized water only every other day. On the 28th day of incubation, seedlings were evaluated for height and number of leaves.

# Statistical analyses

All analyses were performed using R Statistical Software (v4.2.2; R Core Team 2022). For Objectives 1 and 2, differences in cover crop biomass, hoeing times, and fruit yield were determined with two-way ANOVA using cover crop treatment and replicate as predictor variables. Preliminary analyses indicated that weed density and hoeing time responses to cover crop treatments differed among site-years; therefore, site-years were analyzed separately. ANOVA assumptions were validated with visual inspections of residuals plotted against fitted values. Treatment differences were determined by carrying out Tukey's HSD post-hoc test with  $\alpha = 0.05$ . Additionally, Welch's two-sample t-test was used to detect differences in biomass between barley or mustard monocultures and the barley or mustard component of the combination treatment. Weed density data for Weed Counts 1, 2, and 3 were analyzed separately to distinguish changes in weed suppression over time. For each observation time, cover crop

treatment effects on weed densities were determined with generalized linear models with negative binomial distributions. These models also included replicate as a predictor variable. Models were developed using the R library *mass* (Venables and Ripley 2002), and model parameter estimates were used to assess possible differences among cover crop treatments. Specifically, parameter estimates with overlapping 90% confidence intervals indicated similarity among cover crop treatments.

Results from Objective 2 indicated that weed density responses to barley-amended soil differed between sites. Thus, sites were analyzed separately for Objective 3. For each site (Leyendecker, Los Lunas) preliminary analyses indicated germination percentages were not affected by interactions between cover crop treatment and year. Thus, years were analyzed together within sites in Objective 3.

The GerminationMetrics R package (v0.1.8; Aravind et al. 2023) was used to determine germination percentages and indicators of germination time and speed for the *A. palmeri* germination study. As a default setting in GerminationMetrics,  $N_t$  is a factor used to describe the total number of seeds tested in an individual germination assay. This default setting was rewritten such that  $N_t$  was replaced with the sum of all germinants and seeds determined to be viable in crush tests – hereafter  $N_v$ . Replacing  $N_t$  with  $N_v$  allowed nonviable seeds to be excluded from consideration when calculating the effects of incubation on subsequent seed germination.

Data from the seedling assay, as well as differences in germination metrics calculated from the germination assay, were analyzed with a two-way ANOVA using cover crop treatment and year as predictor variables. Assumptions for ANOVA were validated with visual inspections of residuals plotted against fitted values. Additionally, marginal means of height and leaf count were determined with the emmeans package (v1.8.9; Lenth 2023).

#### **Results and Discussion**

## **Objective 1: Barley and brown mustard biomass production**

For barley, the mean aboveground biomass at Leyendecker ranged from 5072 to 8367 kg ha<sup>-1</sup> (Table 2), which was within the range previously reported for barley grown in southern New Mexico (Pratt et al. 2022). However, aboveground biomass of barley at Los Lunas was over 12,000 kg ha<sup>-1</sup> less than the values reported by Agarwal et al. (2022a) for barley grown at Los Lunas. Possible reasons for lower amounts of barley biomass in this study compared to Agarwal

et al. (2022a) include differences in amounts of precipitation at the study site during the periods of barley growth. In Agarwal et al. (2022a), the study site received 8.76 and 11.73 of precipitation during barley growing seasons, whereas in this study, the study site received 3.02 and 4.24 cm of precipitation during barley seasons (Anonymous 2024). Additional possible causes for less barley biomass in this study than Agarwal et al (2022a) include differences in final irrigation and cover crop termination dates. Agarwal et al. (2022a) irrigated barley on April 12 and terminated barley on May 30. In this study, the final irrigation at Los Lunas occurred on January 21 and February 20, and cover crops were terminated on April 7 and April 11. The relatively early dates of final irrigation and termination in this study were necessary for chile pepper seeding in accordance with local guidelines for optimized production of this crop (Bosland and Walker 2014). Thus, standard agronomic practices in chile pepper production schedules prevent maximum biomass for barley grown for green manure.

Barley biomass comprised 89% and 75% of the combination treatment biomass in Leyendecker 2022 and Leyendecker 2023, respectively (Table 2). In these site-years, the barley monoculture also yielded significantly more biomass than the mustard monoculture treatment, indicating that Stockford barley can produce more aboveground biomass than Caliente Rojo brown mustard under the same conditions when grown together or separately in southern New Mexico. In Los Lunas 2022, however, the barley component of the combination treatment comprised only 35% of the total biomass, suggesting Stockford barley is not suited for winter cover cropping in central New Mexico. In Los Lunas 2023, Valor barley was chosen to replace Stockford barley, and Valor barley biomass subsequently comprised 71% of the combination treatment.

Brown mustard did not overwinter in Los Lunas 2023, and thus, brown mustard for Los Lunas 2023 was excluded from statistical analyses. Previous reports of aboveground biomass for brown mustard grown at Leyendecker ranged from 5678 and 6282 kg ha<sup>-1</sup> (Nagila et al. 2022) and 5209 to 8962 kg ha<sup>-1</sup> (Bajagain et al. 2024). These previously reported values for brown mustard biomass (Bajagain et al. 2024; Nagila et al. 2022), however, exceeded those for brown mustard biomass in this study in all site-years except Leyendecker 2023 (Table 2). The differences in brown mustard biomass between this and previous studies (Bajagain et al. 2024; Nagila et al. 2022) is consistent with Björkman et al. (2015) and Agarwal et al. (2022b) who observed high levels of inter-annual variability in aboveground biomass for brown mustard

grown with standardized practices. Bajagain et al. (2024) indicated that brown mustard cover crop biomass between 3650 and 5912 kg ha<sup>-1</sup> is required for suppression of weed density in chile pepper. This level of brown mustard biomass was achieved only in Leyendecker 2023.

# Objective 2: Barley and brown mustard cover crop effects on weeds, hand hoeing, and chile pepper yield

Weeds of chile pepper in previously noncover control plots included annual species (Supplementary Fig. 1) that are known to interfere with New Mexico chile pepper production (USDA 2000). Compared to the noncover control, barley reduced weed density in chile pepper more than any other cover crop treatment (Figure 1). In Leyendecker 2022, barley reduced weed density by 60% compared to the noncover control during Weed Count 1 at 32 DAS (Table 1), whereas the mustard and combination treatments had no effect on weed density. In Leyendecker 2023, barley reduced weed density by 80% during Weed Count 1 at 22 DAS, with the mustard and combination treatments reducing weed density by 56% and 49%, respectively (Figure 1). Neither barley nor any other treatment reduced weed density compared to the noncover control during Weed Count 1 in either Los Lunas site-year.

There were no differences in weed density because of cover crop treatment during Weed Count 2 at 35-48 DAS and Weed Count 3 at 49-60 DAS (Table 1) in any site-year. This short-lived reduction in weed density is consistent with a previous study that indicated suppression of weeds by barley residue was greater at 5 wk rather than 10 wk after seeding corn (*Zea mays* L.) (Efthimiadou et al. 2012). Such diminishing effects of cover crop residues on weeds may be a result of the degradation of allelochemicals in the soil over time (Flamini 2012; Teasdale et al. 2007).

Variable effects of barley on weed density between study sites may have reflected differences in the ability of the soil to retain allelochemicals emitted from barley residue. The primary allelochemicals in barley, gramine and hordenine, are positively charged (Lebecque et al. 2018). The ability of soil to retain positively charged particles can be measured by cation exchange capacity (CEC) (Saidi 2012). CEC is positively correlated with the percentage of soil comprised of clay and negatively correlated with the percentage of soil comprised of sand (Khaledian et al. 2017). Accordingly, allelochemicals may not adsorb as strongly to sandy soils than they would to clay soils, due to the former's comparatively lower CEC (Scavo et al. 2019).

The length of time that allelochemicals remain in the soil matrix is not only a product of chemical adsorption, but also leaching via infiltration (Scavo et al. 2019). The macropores characteristic of coarse soil cause more rapid leaching of allelochemicals through the soil profile than the micropores of finer soils (Blum 2006). Further, microbial decomposition of allelochemicals may be accelerated in drained, well-aerated sandy soils (Rietveld et al. 1983). Clay soils therefore may demonstrate stronger retention of allelochemicals, leading to the more pronounced phytotoxic effects on weeds at Leyendecker than observed in the sandy soils of Los Lunas in this study.

Additionally, because barley cultivars generally differ in allelopathic potential (Asghari and Tewari 2007) the difference in barley cultivars may have also contributed to variable effects of barley on weed density. Given that Valor barley was only used in one site-year, more research is needed to quantify the relationship between this barley cultivar and weed density in a subsequent cash crop. Finally, differences among site-years in barley-induced weed suppression could have been partly caused by variation in barley biomass as increased amounts of barley residue incorporated into soil can cause greater levels of weed suppression (Efthimiadou et al. 2012).

At Hoeing Event 1, which took place 35-48 DAS (Table 1), barley decreased hoeing time compared to the noncover control in three of four site-years (Figure 2). For Los Lunas 2023, the barley treatment reduced hoeing time without causing a significant decrease in weed seedling density. This result is similar to results from a previous study that determined false seedbeds can reduce hoeing times in chile pepper without reducing weed densities (Schutte et al. 2021). Barley-induced reductions in hoeing time coincident with barley-induced reductions in weed density is consistent with previous research that indicated weed density can be positively correlated with hoeing time (Melander and Rasmussen 2001).

The combination treatment decreased hoeing time during Hoeing Event 1 compared to the noncover control in Leyendecker 2022 and Los Lunas 2023, whereas brown mustard did not reduce hoeing times at any site-year. Similarity in hoeing times between the brown mustard treatment and the noncover control is consistent with previous research that indicated brown mustard failed to diminish hoeing time in chile pepper (Bajagain et al. 2024).

Yield of chile pepper fruit was not affected by any cover crop treatment in this study (Table 3). In previous a previous study, an oat cover crop incorporated into the soil decreased

yield of transplanted pepper (*Capsicum annuum* L. 'Cleor') compared to a noncover crop control (Campiglia et al. 2014), possibly due to negative effects on soil fertility (Campiglia et al. 2014; McKenzie-Gopsell and Farooque 2023). In this study, however, fertigation applied during the chile pepper growing season may have offset possible reductions in fertility caused by cover crop incorporation.

# Objective 3: Barley-amended soil effects on germination and seedling development of A. palmeri

Germination parameters for *A. palmeri* seeds incubated in barley-amended soil from Los Lunas were similar to those for seeds incubated in noncover control soil from Los Lunas (Table 4). However, for the Leyendecker soils, germination parameters for seeds incubated in the barley-amended soil differed from the seeds incubated in the noncover control soil. This was generally consistent with the field study, in which barley-amended soil led to reduced weed density in Leyendecker, but not Los Lunas.

Neither GP nor MGN of *A. palmeri* seeds differed between barley or noncover control soils collected from Leyendecker. However, *A. palmeri* seeds incubated in the barley-amended soil from Leyendecker germinated for a longer time than seeds incubated in the noncover control. This was indicated by greater values of LGT, TSG, t50, and MGT for seeds incubated in barley-amended soil than noncover control soil from Leyendecker. Additionally, incubation with the barley-amended soil from Leyendecker led to slower speeds of germination — evidenced by greater values of CVG and lower values of GSC — than seeds incubated with the noncover control treatment from Leyendecker. These germination parameter results for seeds incubated in soil from Leyendecker are consistent with previous studies that determined aqueous leachates of barley slowed, but did not prevent, germination of white mustard (*Sinapis alba* L.) (Liu and Lovett 1993a; 1993b). In this study, neither *A. palmeri* height nor leaf count were impacted by cover crop treatment at either location (Table 5), which indicates barley-amended soil did not influence *A. palmeri* seedling development.

When considered together, the results from the germination assay and seedling study suggest the weed suppressive effects of barley in the field occurred prior to seedling emergence. Specifically, the barley cover crops that were mowed and incorporated into the soil prior to chile pepper seeding may have slowed germination of annual weeds. Slowed germination causing later

emergence reduces the competitiveness of weeds in a crop (Rühl et al 2016) and increases susceptibility of seeds and preemergent seedlings to mortality caused by pathogens in soil (Beckstead et al. 2007). Soil-borne pathogens causing mortality to seeds and preemergent seedlings may be more abundant following incorporation of cover crop residue (Kumar et al. 2011, Mohler et al. 2012). The absence of barley effects on *A. palmeri* leaf count and height suggests barley-induced weed suppression in the field did not involve inhibition of seedling development after emergence. This is consistent with Weisberger et al. (2023), who determined that growth of weeds that emerge through a cover crop residue is often not inhibited.

### **Practical Implications**

Crop fields in the southwestern U.S. are susceptible to wind erosion in spring (Nordstrom and Hotta 2004) because large-scale atmospheric phenomena generate dry conditions and strong wind at the soil surface (Dhital et al., 2024). To limit soil loss due to wind, the U.S. Department of Agriculture incentivizes farmers in the southwestern U.S. to grow specific species of cover crops during the period when crop fields are particularly vulnerable to wind erosion (February 1 to April 1) (USDA-NRCS 2024b). Cover crop species that farmers are encouraged to grow include brown mustard and barley. Of these two species, barley may be the better choice for fields rotating into chile pepper. This is because barley, compared to brown mustard, produces more aboveground biomass and provides greater, more consistent, suppression of weeds in chile pepper. Thus, barley, rather than brown mustard, may be the cover crop species that can reduce negative impacts of both wind and weeds in southern New Mexico.

Tactics that reduce hand hoeing in chile pepper are critical to both farm profitability and the continued production of chile pepper in New Mexico (Hawkes et al. 2008). Conventional methods for reducing hand hoeing in chile pepper include preplant incorporated and preemergence applications of soil-residual herbicides. The results of this study suggest preplant and preemergence applications of soil-residual herbicides could be replaced by a barley cover crop that is mowed and incorporated into the soil prior to chile pepper seeding. Similarly, Cornelius and Bradley (2017) determined cereal 51 grown as a cover crop and terminated with glyphosate plus 2,4-D ester inhibited early-season emergence of waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] to the same extent as a preemergence application of soil-residual herbicides in soybean [*Glycine max* (L.) Merr.]. However, before barley cover crops can be

recommended as replacements for soil-residual herbicides in chile pepper, further research is needed to determine the relative capacities of herbicides and barley cover crops to suppress weeds in chile pepper.

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#### **Competing Interests**

Competing interests: The authors declare none.

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Management	Leyendecker	Leyendecker		
activity	2022	2023	Los Lunas 2022	Los Lunas 2023
Cover crop	October 21,	October 21,	September 14, 2021	September 19, 2022
seeding	2021 <sup>a</sup>	2022		
Cover crop	October 22,	October 26,	September 17, 2021	September 20, 2022
irrigation 1	2021	2022		
Cover crop	November 10,	November 11,	September 24, 2021	September 28, 2022
irrigation 2	2021	2022		
Cover crop	February 18	February 6	October 14, 2021	October 26, 2022
irrigation 3				
Cover crop	N/A	N/A	January 21	February 20
irrigation 4				
Cover crop	March 23	April 6	April 7	April 11
termination				
Chile pepper	April 15	April 27	April 28	April 27
seeding				
Weed count 1	May 17 (32) <sup>b</sup>	May 19 (22)	May 24 (26)	May 18 (21)
Weed count 2	June 2 (48)	June 2 (36)	June 7 (40)	June 1 (35)
Weed count 3	June 14 (60)	June 16 (50)	June 30 (63)	June 15 (49)
Hoeing event 1	June 2 (48)	June 2 (36)	May 24 (26)	June 1 (35)
Hoeing event 2	June 14 (60)	June 16 (50)	June 7 (40)	June 15 (49)
Hoeing event 3	June 28 (74)	June 30 (64)	June 30 (63)	June 27 (61)
Harvest	August 31 (138)	September 5	September 7 (132)	September 19 (145)
		(131)		

Table 1. Dates for crop management activities and data collection events for Leyendecker (near Las Cruces, NM) and Los Lunas, NM in 2021-2022 and 2022-2023.

<sup>a</sup> Years for each date correspond to the year in the column heading unless otherwise indicated.

<sup>b</sup> Numbers in parentheses indicate days after chile seeding.

Table 2. Mean aboveground biomass from different cover crop treatments in Leyendecker (near Las Cruces, NM) and Los Lunas, NM before a chile pepper crop in 2022 and 2023. Treatments consisted of a barley monoculture, brown mustard monoculture, barley and mustard combination, or a noncover control.

	Cover crop biomass											
	Leyend	ecker	Leyende	ecker	Lestur		L L					
	2022		2023		Los Lun	as 2022	Los Lunas 2023					
Cover crop	Ira ha <sup>-1</sup>											
treatment	kg ha <sup>-1</sup>											
Barley	5072	$Ac^*$	8367	Ad	1327	Ab	1821	Ad				
Mustard	1625	Bb	6064	Bb	1216	Aa	c					
Combo <sup>a</sup>	3409	В	6719	В	1294	А	1691	В				
Combo mustard <sup>b</sup>	363	а	1707	а	796	a	c					
Combo barley <sup>b</sup>	3017	c	5012	c	498	с	1206	с				

<sup>\*</sup> Within a site-year, differences in capital letters represent statistically significant differences in biomass according to Tukey's HSD test ( $p \le 0.05$ ). Lowercase letters indicate statistically significant differences in combo components vs. total biomass for monocultures (Welch's two-sample t-test;  $p \le 0.05$ ).

<sup>a</sup> Mean biomass refers to the undifferentiated biomass in combo plots regardless of species composition.

<sup>b</sup> "Combo mustard" and "Combo barley" refer to the fraction of total combo biomass constituted of either barley or mustard, respectively

<sup>c</sup> In the Los Lunas 2023 site-year, mustard-treated plots were dominated by triticale ( $\times Triticosecale$  sp.) and thus were considered a crop failure.

Table 3. Mean chile pepper yield after incorporation of different cover crop residues in Leyendecker (near Las Cruces, NM) and Los Lunas, NM in 2022 and 2023. Treatments consisted of a barley monoculture, brown mustard monoculture, barley and mustard combination, or a noncover control.

		Chile pepper fruit yield											
		Leyend 2022 <sup>a</sup>	ecker	Leyendecker 2023		Los Lun	as 2022	Los Lunas 2023					
Cover treatment	crop	kg ha <sup>-1</sup>											
Barley		9331	a <sup>*</sup>	9608	a	12100	а	13060	а				
Mustard		10058	a	10658	а	18444	а	12263	a				
Combo		10606	a	11546	а	14175	a	13719	a				
Noncover		7538	a	10479	a	14406	a	11385	а				

<sup>a</sup> For Leyendecker 2022, Leyendecker 2023, Los Lunas 2022, and Los Lunas 2023, harvest dates occurred on August 31, September 5, September 7, and September 19, respectively.

\* Within a site-year, differences in letters represent statistically significant differences in yield according to Tukey's HSD test ( $p \le 0.05$ ).

Table 4. Means of *Amaranthus palmeri* germination assay parameters following a 28 d incubation period in soils from Leyendecker (near Las Cruces, NM) and Los Lunas, NM amended with or without incorporated barley residue.

	Cover	GP <sup>a</sup>	. <u> </u>	MG	N	FGT		LGT	ſ	TSG	j	t50		MG	T	PGT	Г	CVG		GSC	]
Site	crop	%		seed	IS	d		d		d		d		d		d		% da	y <sup>-1</sup>	time	<sup>-1</sup> (
	treatment			day <sup>-1</sup>	1																t
Leyendecker	Barley	67.7	$a^*$	1.2	a	1.9	a	7.5	a	5.6	a	2.7	a	3.4	a	3.3	a	30.3	a	0.3	A
Leyendecker	Noncover	68.0	a	1.4	a	1.9	a	5.1	b	3.2	b	2.4	b	2.9	b	2.9	a	34.7	b	0.4	B 4
Los Lunas	Barley	62.4	a	1.0	а	2.4	а	6.1	a	3.8	a	2.6	a	3.3	a	2.8	a	30.7	a	0.3	A
Los Lunas	Noncover	54.1	a	0.8	а	2.3	a	5.6	a	3.3	a	2.9	a	3.5	a	3.3	a	30.0	a	0.3	A

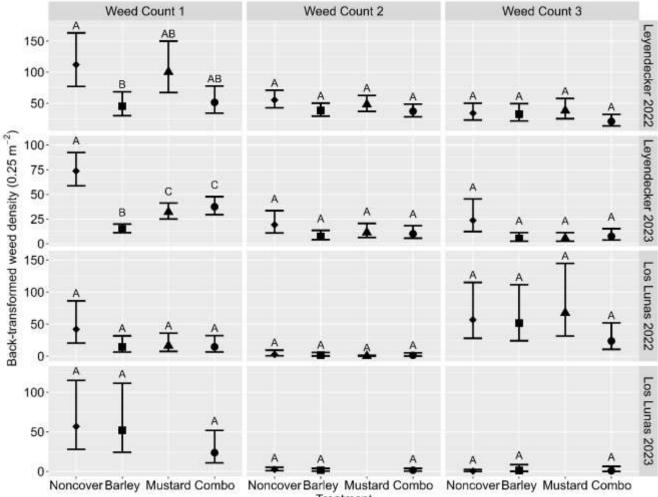
\* Marginal means within a column and site followed by the same letter are not different according to pairwise comparisons with Tukey-adjusted p-values ( $p \le 0.05$ ).

<sup>a</sup> GP = germination percentage; MGN = mean germination number; FGT = first germination time; LGT = last germination time; TSG = time spread of germination; t50 = time to 50% germination; MGT = mean germination time; PGT = peak germination time; CVG = coefficient of velocity of germination; GSC = germination speed corrected; MGP = mean germination percentage.

Table 5. Means of *Ameranthus palmeri* seedling height and leaf count after 28 d of growth in soils from Leyendecker and Los Lunas, NM amended with or without incorporated barley residue. Treatments included soil incorporated with residue from barley cover crops, and soil from the noncover control.

Site	Cover crop	Height		Leaf count			
	treatment	cm					
Leyendecker	Barley	6.66	a <sup>*</sup>	5.94	a		
Leyendecker	Noncover	6.59	a	5.44	a		
Los Lunas	Barley	5.85	a	5.31	a		
Los Lunas	Noncover	7.05	а	6.24	a		

\* Marginal means within a column and site followed by the same letter are not different according to pairwise comparisons with Tukey-adjusted p-values ( $p \le 0.05$ ).



Treatment

Figure 1. Early-season weed density following incorporation of different cover crop residues in chile pepper fields in Leyendecker (near Las Cruces, NM) and Los Lunas, NM. Weed Count 1 occurred 32, 22, 26, and 21 days after chile pepper seeding (DAS) for Leyendecker 2022, Leyendecker 2023, Los Lunas 2022, and Los Lunas 2023, respectively. Weed Count 2 occurred 48, 36, 40, and 35 DAS for Leyendecker 2022, Leyendecker 2023, Los Lunas 2022, and Los Lunas 2023, respectively. Weed Count 3 occurred 60, 50, 63, and 49 DAS for Leyendecker 2022, Leyendecker 2023, Los Lunas 2022, and Los Lunas 2023, respectively. Treatments consisted of a barley monoculture, brown mustard monoculture, barley and mustard combination, or a noncover control. Data points are transformed parameter estimates from generalized linear models with 90% confidence intervals. Different letters within a site-year and Weed Count indicate significant differences in weed density. Because the mustard crop failed in the Los Lunas 2023 site year, it has been omitted from this figure.

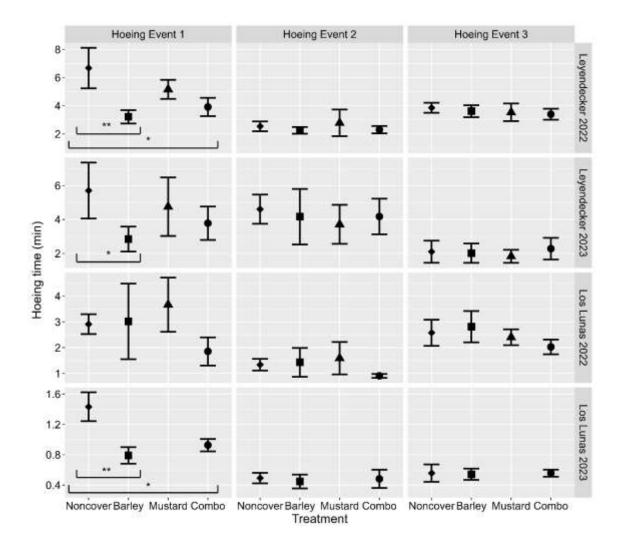


Figure 2. Minutes spent hoeing 8-m transects in chile fields following incorporation of different cover crop residues in Leyendecker and Los Lunas, NM. Hoeing Event 1 took place 48, 36, 26, and 35 days after chile pepper seeding (DAS) for Leyendecker 2022, Leyendecker 2023, Los Lunas 2022, and Los Lunas 2023, respectively. Hoeing Event 2 took place 60, 50, 40, and 49 DAS for Leyendecker 2022, Leyendecker 2023, Los Lunas 2022, and Los Lunas 2023, respectively. Hoeing Event 3 occurred 74, 64, 63, and 61 DAS for Leyendecker 2022, Leyendecker 2022, Leyendecker 2023, respectively. Treatments consisted of a barley monoculture, brown mustard monoculture, barley and mustard combination, or a noncover control. Data points are means (n = 4) with standard errors. Brackets and asterisks indicate results from Tukey's HSD test that determined the effect of soil treatment per site-year and visit; \*p < 0.05, \*\*p < 0.01, and \*\*\*p < 0.001. In Los Lunas 2023, the mustard cover crop failed to establish.