

# Timing Termination of a Biofumigant Cover Crop for Weed Suppression in Chile Pepper

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**ABSTRACT.** Overwinter mustard cover crops incorporated into soil may suppress early-season weeds in chile pepper (*Capsicum annuum*). However, the potential for mustard cover crops to harbor beet leafhoppers (*Circulifer tenellus*) is a concern because beet leafhoppers transmit beet curly top virus to chile pepper. The objectives of this study were to determine the amounts of a biopesticidal compound (sinigrin) added to soil from 'Caliente Rojo' brown mustard (*Brassica juncea*) cover crops ended on three different days before beet leafhopper flights during spring and to determine the effects of the cover crop termination date on weed densities and hand-hoeing times for chile pepper. To address these objectives, a field study was conducted in southern New Mexico. In 2019–20, the cover crop was ended and incorporated into soil 45, 31, and 17 days before beet leafhopper flights. In 2020–21, cover crop termination occurred 36, 22, and 8 days before beet leafhopper flights. Treatments also included a no cover crop control. Cover crop biomass and sinigrin concentrations were determined at each termination. Chile pepper was seeded 28 days after the third termination date. Weed densities and hand-hoeing times were determined 28 and 56 days after chile pepper seeding. In 2019–20, the third termination (17 days before beet leafhopper flights) yielded the maximum cover crop biomass (820 g·m<sup>-2</sup>) and greatest sinigrin addition to soil (274 mmol·m<sup>-2</sup>). However, only the second termination (31 days before beet leafhopper flights) suppressed weeds in chile pepper. In 2020–21, the third termination (8 days before beet leafhopper flights) yielded the maximum cover crop biomass (591 g·m<sup>-2</sup>) and greatest sinigrin addition to soil (213 mmol·m<sup>-2</sup>), and it was the only treatment that suppressed weeds. No cover crop treatment reduced hand-hoeing times. These results indicate that overwinter mustard cover crops can be ended to evade beet leafhopper flights and suppress weeds in chile pepper.

Winter cover crops, as part of an integrated pest management program, can improve

agricultural sustainability and enhance pest management. Cover crops in the mustard family (Brassicaceae) that are mowed and incorporated into the soil with tillage—a process that has been referred to as biofumigation (Matthiessen and Kirkegaard 2006; Tagele et al. 2021)—can suppress plant-parasitic nematodes (Riga 2011) and soil-borne pathogens (Olivier et al. 1999). Biofumigation has also been reported to reduce weeds in potato (*Solanum tuberosum*) in Washington state (Boydston and Hang 1995), strawberry (*Fragaria × ananassa*) in central California (Brennan et al. 2013), pea (*Pisum sativum*) in Washington state (Al-Khatib et al. 1997), and tomato (*Solanum lycopersicum*) in Arkansas (Bangarwa and Norsworthy 2014).

Glucosinolates are cover crop-derived chemical compounds responsible for pest suppression from biofumigation. Upon plant tissue disruption, glucosinolates are enzymatically hydrolyzed to release phytotoxic compounds

including isothiocyanates (Brown and Morra 1996). Pest suppression from isothiocyanates is generally dependent upon their concentration, pest exposure time, and growth stage of the target pest (Teasdale and Taylorson 1986). For weeds, relatively low concentrations of isothiocyanates prevent radicle protrusion but do not kill seeds (Angelini et al. 1998; Leblová-Svobodová and Košťár 1962), whereas relatively high concentrations inhibit physiological processes, thereby suppressing weed seedling emergence and growth (Petersen et al. 2001).

Brown mustard (*Brassica juncea*) is a biofumigant cover crop in which the primary glucosinolate is sinigrin (2-propenyl-glucosinolate) (Bangarwa and Norsworthy 2017). Isothiocyanates released from sinigrin are volatile and dissipate from soil soon after hydrolysis of the parent compound (Gimsing and Kirkegaard 2006). Maximum concentrations of isothiocyanates generally occur within days of sinigrin hydrolysis (Borek et al. 1995), although degradation of sinigrin and retention of isothiocyanates in soil are influenced by factors including soil type and soil moisture (Borek et al. 1995; Tagele et al. 2021; Wood et al. 2020). Given the short-lived nature of sinigrin-derived isothiocyanates in soil, suppression of weeds in a cash crop from brown mustard may require this biofumigant crop to be ended and incorporated into soil soon before planting the cash crop.

Chile pepper (*Capsicum annuum*) is a warm-season crop that is an important component of the agricultural economy in New Mexico. In 2021, chile pepper was the fourth most valuable crop in New Mexico and provided approximately \$44.9 million cash receipts to growers in this state (New Mexico Department of Agriculture 2022). The production of chile pepper in New Mexico involves direct-seeding or transplanting into raised beds between March and May (Bosland and Walker 2014). Fields with raised beds are irrigated 2 to 4 weeks before chile pepper planting and as needed thereafter. Common types of irrigation for chile pepper in New Mexico include furrow irrigation (flood irrigation in the furrows between raised beds) and subsurface drip irrigation (Sanogo and Carpenter 2006). Chile pepper production in New Mexico typically does

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not involve polyethylene mulch, which is different from bell pepper (*Capsicum annuum*) production in other regions of the United States (Dittmar et al. 2022).

Chile pepper is a difficult crop to grow, in part, because maximum yield potential requires a prolonged period during which weeds must be controlled (Amador-Ramirez 2002). Weeds that emerge 9 to 11 weeks after chile pepper seeding can reduce the yield of green fruits by 27% to 38% compared with weed-free conditions (Schroeder 1993). For red fruits, which are green fruits after further maturation on the plant, weed-induced yield reductions can be as great as 76% for weeds emerging 8 to 9 weeks after chile pepper seeding (Schroeder 1992). Current strategies for controlling weeds in chile pepper can involve pre-emergence applications of herbicides, including napropamide, clomazone, or bensulide. Although these herbicides control some weed species for several weeks after a single application (Lanini and Lestrangle 1994), overall weed control is often insufficient for the maximum yield of chile pepper. To address weeds not controlled by residual herbicides applied at planting, chile pepper growers in New Mexico commonly cultivate and hire hand-hoeing crews. Reducing the requirement for hand-hoeing is critical for the continued production of chile pepper in New Mexico because labor costs substantially reduce profitability (Hawkes et al. 2008).

A previous study suggested that early-season weeds in New Mexico chile pepper were suppressed by biofumigation with a brown mustard cover crop that was seeded in fall and ended 20 to 24 d before chile pepper seeding in spring (Nagila et al. 2022). This biofumigation treatment also promoted chile pepper growth under greenhouse conditions by reducing the incidence of *Verticillium* wilt caused by *Verticillium dahliae* (Nagila et al. 2022). Before using or recommending biofumigation with an overwinter brown mustard cover crop, it is prudent to know how this cover crop interacts with an insect that transmits a viral disease to chile pepper. Specifically, technical guidance for biofumigation preceding chile pepper requires knowledge of how overwinter mustard cover crops interact with large, episodic flights of beet leafhoppers (*Circulifer tenellus*) that occur annually in New Mexico.

Beet leafhoppers feed on a wide range of plant species (Cook 1967), including brown mustard (Banuelos et al. 1992). When beet leafhoppers feed on chile pepper, they can transmit beet curly top viruses (viruses in the genus *Curtovirus*) (Chen and Gilbertson 2016), which can cause yield losses up to 50% in New Mexico chile pepper (Creamer et al. 2003). In New Mexico and other regions of the western United States, beet leafhoppers overwinter on agricultural weeds and desert plants (Cook 1967; Davis 2010). Desiccation of these plants initiates flights of beet leafhopper in spring (Davis 2010). Mustard cover crops could attract large numbers of beet leafhoppers to sites for chile pepper production if beet leafhopper flights occur before mustard cover crop termination. Following cover crop termination, these beet leafhoppers could move to, and persist on, plants that occur in unmanaged areas near chile pepper fields in spring (Davis 2010). Thus, influxes of beet leafhoppers caused by mustard cover crops would be detrimental to chile pepper production because they would increase both the local abundance of beet leafhoppers and risk of beet curly top virus transmission to chile pepper.

Although biofumigation for chile pepper requires mustard cover crop termination before the beginning of beet leafhopper flights, ending the mustard cover crop too early may reduce weed control because the pesticidal compounds from mustard biomass are short-lived in soil. Further, early termination may not add enough biopesticide to soil because termination too soon prevents mustard cover crops from obtaining maximum biomass and/or maturing to flowering stages that increase sinigrin concentrations in cover crop plants (Rangkadilok et al. 2002). To better understand the consequences of mustard cover crop termination time on weed control in chile pepper, we ended brown mustard cover crops on different days before beet leafhopper flights and evaluated the amounts of sinigrin added to soil and effects on weed density, hand-hoeing time, and chile pepper crop injury.

## Materials and methods

**FIELD SITE AND EXPERIMENTAL DESIGN.** A field study was conducted at the New Mexico State University

Leyendecker Plant Science Research Center near Las Cruces, NM, USA (lat. 32.204042°N, long. 106.743960°W, elevation 1175 m). The study site was located 100 to 450 m from a network of insect traps that are used for annual beet leafhopper monitoring in southern New Mexico (Lehnhoff and Creamer 2020). Soil at the study site was a Glendale clay loam (fine-silty, mixed, superactive, calcareous, thermic Typic Torrfluvents). The study included two experiment runs (herein referred to as trials), with trial 1 occurring from Fall 2019 to Summer 2020, and trial 2 occurring from Fall 2020 to Summer 2021. During the time between trial 1 and trial 2, all vegetation at the study site was ended before seed production and experimental treatments were spatially rearranged to ensure that treatment locations differed between experimental runs. Before the start of the study, the study site was fallow from Fall 2018 to Summer 2019. In Summer 2018, the study site was used to grow chile pepper in accordance with conventional practices for the region (Bosland and Walker 2014).

Treatments were three mustard cover crop termination times and control with no cover crop. Mustard cover crop termination times were 56, 42, and 28 d before chile pepper seeding. Treatments were initially described as days before chile pepper seeding because days of initiation for beet leafhopper flights were unknown when trials were conducted. When year-specific temporal patterns of beet leafhopper flights were understood, treatments were described as both days before chile pepper seeding and days before beet leafhopper flights. At 56, 42, and 28 d before chile pepper seeding, mustard cover crop plants were at the rosette, stem elongation, and flowering stages, respectively. The least amount of time between cover crop termination and chile pepper seeding was 28 d because this amount of time was needed for the field to dry after the irrigation applied during cover crop termination.

Treatments were arranged in a randomized complete block design with four replications. Experimental units were 48 m long and 4 m wide; they are herein referred to as plots. During growing seasons for the cover crop, plots contained 22 rows of cover crop. Neighboring rows of cover crop were spaced 18 cm apart. During the

growing season for chile pepper, experimental units contained four rows of chile pepper. Chile rows were centrally placed on raised beds that had a width of 0.8 m. Neighboring rows of chile pepper were spaced 1 m apart.

**COVER CROP MANAGEMENT AND CHILE PEPPER SEEDING.** Before sowing the cover crop, fields were prepared with a laser-guided land levelling system (Laser Alignment Inc., Grand Rapids, MI, USA). Dates for field preparation and subsequent management procedures are presented in Table 1. ‘Caliente Rojo’ brown mustard (Caliente Brand™, Stokes Seeds Inc., Holland, MI, USA) was seeded at 7 lb/acre with a mechanical grain drill (Model 450; John Deere, Moline, IL, USA). Within 48 h of seeding, fields were flood-irrigated. Flood irrigations occurred as needed to prevent crop mortality through fall and winter. Each irrigation was approximately 3 inches deep and saturated the soil.

The cover crop treatments were ended using a flail shredder (Model ORC12; RhinoAg Inc., Gibson City, IL, USA). Immediately after shredding, residues were incorporated into soil to the 15-cm depth with two passes of an offset tandem disk (Model 620; John Deere, Moline, IL, USA). Immediately after disking, raised beds were made using a lister (Dave Koenig Enterprises Inc., Mesilla Park, NM, USA). Within 2 h of listing, the furrows between raised beds were flood-irrigated. Each irrigation was approximately 3 inches deep and saturated raised beds.

For plots containing the control with no cover crop, the creation of raised beds and subsequent irrigation coincided with the third termination dates for mustard cover crops (31 Mar 2020 and 1 Apr 2021). On 28 Apr 2020 and 29 Apr 2021, raised beds were lightly disked and shaped using a bed shaper. ‘Big Jim’ chile pepper was seeded at 6 lb/acre to a depth of 1 inch using a mechanical seeder (MaxEmerge® Plus; John Deere, Moline, IL, USA). Chile pepper rows were positioned in central areas of raised beds, with each bed containing one row of chile pepper. Furrow irrigation occurred immediately after seeding and as needed thereafter.

**BEET LEAFHOPPER FLIGHTS.** To measure changes over time in beet leafhopper abundance at the experimental farm, four yellow sticky traps (20 cm × 25 cm; Hummert International, Earth City, MO, USA) were placed 24 inches from the ground surface in four locations across the Leyendecker Plant Science Research Center. One of the four traps was 100 m north of the study site. Two traps were 350 m and 450 m northwest of the study site, and one trap was 450 m south of the study site. The total number of beet leafhoppers on each card was determined every 2 weeks from January to late March, and every week from late March to early July. On the days of collection, new traps were positioned to replace the collected traps.

**SINIGRIN AMENDMENTS TO SOIL.** To determine the amounts of sinigrin added to soil by cover crops ended on

different dates, measurements of cover crop biomass at termination were combined with date-specific measurements of sinigrin concentrations in cover crop biomass. Measurements of cover crop biomass at termination generally followed the procedures of Nagila et al. (2022). Specifically, just before cover crop termination, aboveground biomass of mustard cover crops was clipped at the soil surface and collected from four 0.25-m<sup>2</sup> quadrats (0.5 m width × 0.5 m length) within each plot. The quadrats were evenly spaced along the central long axes of plots. Following collection, biomass samples were oven-dried at 60 °C until they reached a constant weight and then weighed. The weights of the four samples were averaged before performing calculations to determine amounts of sinigrin added to soil and statistical analyses.

To quantify sinigrin in the aboveground and belowground mustard plant biomass, four entire mustard plants were collected from areas near the biomass harvest locations. These plants were placed on dry ice in cold storage containers and transported to the laboratory, where they were stored at –18 °C. Cold-stored plants were crushed to a powder using a mortar and pestle in the presence of liquid nitrogen for 1 min (Doheny-Adams et al. 2017). Sinigrin was extracted from powdered samples following the cold methanol extraction method described by Doheny-Adams et al. (2017). Sinigrin was quantified using high-performance liquid chromatography (HPLC) analyses following the methods of Wood et al. (2020). HPLC was conducted with an Agilent 1100 series HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with a Zorbax column (C18; 4.6 × 100 mm; 3.5 µm). Solvents in the HPLC analyses were 0.02 M tetrabutylammonium bromide and a 70:30 mixture of tetrabutylammonium bromide and acetonitrile. Data were recorded using software (Agilent Software Chemstation V.B.04.01). HPLC data were converted to sinigrin concentrations in plant samples using standard curves produced with five concentrations (1.25, 2.5, 5, 10, and 20 mM) of sinigrin standards (Sigma-Aldrich, St. Louis, MO, USA). Sinigrin concentrations for plants from the same plot were averaged before performing calculations to determine the amounts

**Table 1. Schedule of field operations for the establishment and termination of the cover crop ‘Caliente Rojo’ brown mustard (*Brassica juncea*). Dates for seeding chile pepper (*Capsicum annuum*) are presented. The study was conducted at the New Mexico State University Leyendecker Plant Science Research Center near Las Cruces, NM, USA, from Fall 2019 to Summer 2020 and Fall 2020 to Summer 2021.**

Operation	Date	
	2019–20	2020–21
Disk and laser level	27 Sep 2019	14 Sep 2020
Cover crop seeding	17 Oct 2019	7 Oct 2020
Irrigation	17 Oct 2019	9 Oct 2020
	4 Nov 2019	13 Nov 2020
	31 Jan 2020	6 Jan 2021
First termination <sup>1</sup> of the cover crop	2 Mar 2020	4 Mar 2021
Second termination of the cover crop	17 Mar 2020	18 Mar 2021
Third termination of the cover crop	31 Mar 2020	1 Apr 2021
Shaping of raised beds	28 Apr 2020	29 Apr 2021
Chile pepper seeding	28 Apr 2020	29 Apr 2021

<sup>1</sup> Cover crop termination consisted of the following sequence of four tactics: mowing with a flail shredder; tillage with an offset tandem disk; listing to create raised beds; and flood irrigation in the furrows between raised beds (flood-furrow irrigation).

of sinigrin added to soil and statistical analyses.

Results of HPLC analyses were sinigrin concentrations based on fresh weight. To calculate the amount of sinigrin added to soil during mustard cover crop termination, dry weights of mustard cover crop samples were first multiplied by 10.7, which accounted for the moisture content of freshly harvested mustard biomass (89.3% water). These calculated fresh weights were then multiplied by HPLC-derived sinigrin concentrations.

**WEED DENSITY, HAND-HOEING TIME, AND CROP INJURY FOR CHILE PEPPER.** After chile pepper seeding, plots were segmented to create six contiguous sections along the long axes. Each section was 8 m long and 4 beds wide. Two sections were randomly selected as locations for data collection. To determine weed densities, rectangular quadrats (0.25 m width  $\times$  1 m length) were permanently positioned on central raised beds within each data collection location. Weeds were identified, counted, and removed at 14, 28, 42 and 56 d after chile pepper seeding. At 28 d after seeding and 56 d after seeding, the amounts of time required for one individual to hand-hoe sections of the chile pepper row were determined. Sections of the chile pepper row for hand-hoeing measurements were 8 m long and 1 m wide. Rows that were hand-hoed were central raised beds within data collection locations. An individual hand-hoer was assigned to all plots within a replication.

Chile pepper plants were counted within quadrats (0.25 m width  $\times$  1 m length) at each data collection location at 28 d after seeding. While counting, plants were assessed for visible symptoms of injury from the mustard cover crop. Possible symptoms of injury from the mustard cover crop included leaves lacking turgor and leaf chlorosis. These symptoms of injury were previously observed during a study that assessed mustard seed meal injury on chile pepper (Nagila et al. 2021).

**DATA ANALYSIS.** To determine when beet leafhopper flights began in Spring 2020 and Spring 2021, beet leafhopper abundance data for each observation time were summed across the four traps. Beet leafhopper abundance data were then plotted as functions of observation time and assessed for sharp increases. Sharp increases

were defined as beet leafhopper abundance at least 1000% greater than the abundance of the previous observation time. The first sharp increase for each year was considered the initial beet leafhopper flight for the specific year. The observation date that immediately preceded the first sharp increase was considered the date of beet leafhopper flight initiation.

Statistical analyses were performed with an open-source software program (R version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria). Analyses of variance (ANOVA) and post hoc Tukey tests were used to determine cover crop termination time effects on cover crop biomass and sinigrin concentrations. Preliminary analyses indicated that mustard biomass and sinigrin concentrations differed between the 2019–20 trial and 2020–21 trial. Thus, trials were analyzed separately. For each trial, ANOVA predictor variables were termination time treatment and replicate. Assumptions for the ANOVA were evaluated with visual inspections of residuals plotted against fitted values.

Weed density data were summed to determine cumulative weed density from 0 to 28 d after seeding and 29 to 56 d after seeding. Cover crop treatment effects on weed densities were determined with generalized linear models with negative binomial distributions developed using the R library *mass*. Trials were analyzed separately because preliminary analyses indicated that termination time effects on weed densities differed between the 2019–20 trial and 2020–21 trial. For each trial, predictor variables in generalized linear models were replicate and cover crop treatment including the no cover control. Parameter estimates from generalized linear models were used to assess possible differences among cover crop treatments. Specifically, parameter estimates with overlapping 95% confidence intervals indicated similarity among cover crop treatments. Parameter estimates with 95% confidence intervals that did not overlap indicated cover crop treatments with different weed densities.

To determine cover crop treatment effects on hoeing times and chile pepper stands, ANOVA tests were performed separately for trials. Predictor variables in ANOVA models were

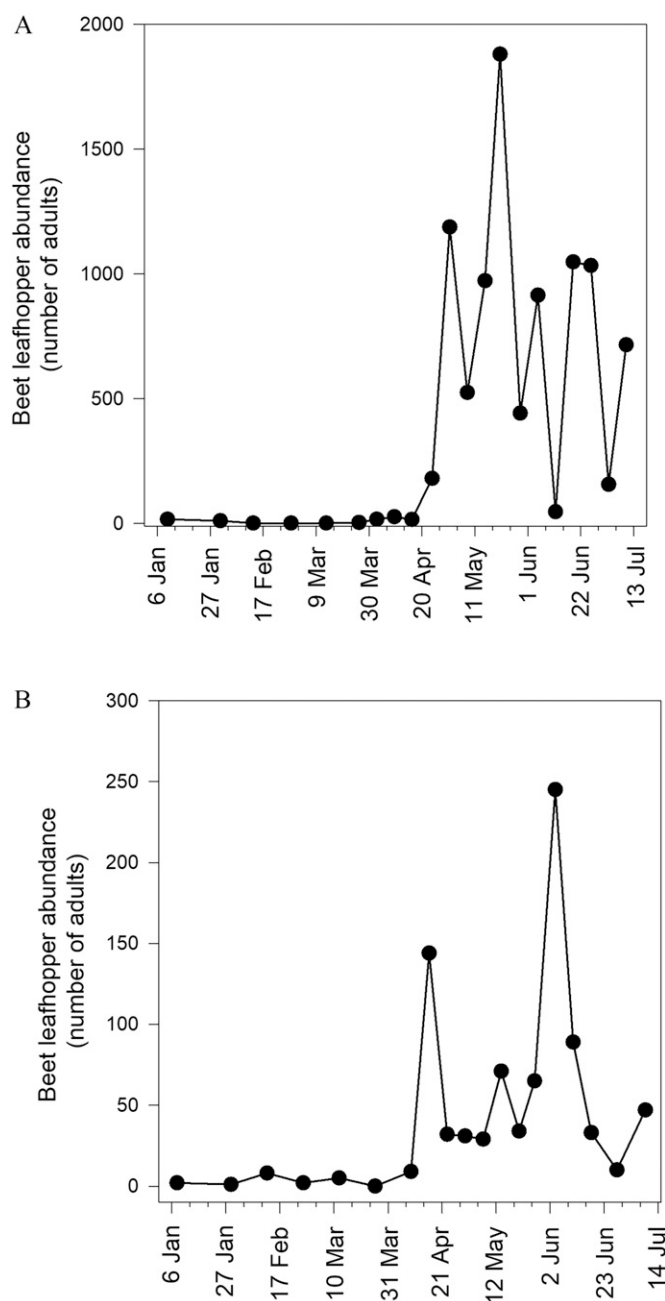
replicate and cover crop treatment. Visual inspections of residuals plotted against fitted values indicated that the log-transformation of response variables was necessary for ANOVA assumptions of the constant variance of errors. Thus, hand-hoeing time and chile stand data were log-transformed before the analyses.

## Results and discussion

**TIMING OF BEET LEAFHOPPER FLIGHTS.** During the 2019–20 trial, few beet leafhoppers were caught on traps between 10 Jan 2020 and 16 Apr 2020 (Fig. 1A). During late April, beet leafhopper abundance increased sharply and then fluctuated across May and June. The beet leafhopper flight for the 2019–20 trial began on 16 Apr 2020. Based on this date, mustard cover crop termination at 56 d, 42 d, and 28 d before chile pepper seeding occurred at 45 d, 31 d and 17 d, respectively, before the initial flight of beet leafhoppers.

During the 2020–21 trial, the number of beet leafhoppers trapped in January, February, and March was low compared with the number of beet leafhoppers trapped in April (Fig. 1B). The initial beet leafhopper flight began on 9 Apr 2021. Based on this date, mustard cover crop termination at 56 d, 42 d, and 28 d before chile pepper seeding occurred at 36 d, 22 d, and 8 d, respectively, before the initial beet leafhopper flight in 2021.

The annual total of beet leafhoppers was less in 2021 than in 2020. Year-to-year variability in beet leafhopper abundance in spring can be attributable to differences in host plant availability (Thomas and Martin 1971) that are partially consequences of annual differences in precipitation during the previous fall (Lehnhoff and Creamer 2020). In fact, levels of precipitation during the previous fall can be used to predict the relative abundance of beet leafhoppers in spring (Lehnhoff and Creamer 2020). Year-to-year variability in dates of initial beet leafhopper flights was associated with yearly differences in levels of precipitation at the study site during spring (Fig. 2). Spring 2021, which was relatively dry, featured an earlier beet leafhopper flight compared with the beet leafhopper flight that occurred in Spring 2020, which was relatively wet. This putative association between



**Fig. 1.** Changes over time in the abundance of beet leafhoppers (*Circulifer tenellus*) at the New Mexico State University Leyendecker Plant Science Research Center near Las Cruces, NM, USA, in 2020 (A) and 2021 (B). For each observation time, abundance is the total number of adults present on four traps positioned across the experimental farm and near the study site.

flight timing and precipitation level is consistent with the underlying causes of beet leafhopper flights. In New Mexico and other regions of the western United States, flights occur because adult beet leafhoppers depart from agricultural weeds and desert plants as this vegetation desiccates in early spring (Cook 1967; Davis 2010). During this study, desiccation of agricultural weeds and desert plants likely occurred earlier during the spring with

little precipitation rather than during the spring with more precipitation.

Although specific dates of initial beet leafhopper flights differed between years, initial flights generally occurred in early-to-mid April. This was consistent with the work of Creamer et al. (2003) and corresponded to increases in soil temperature (Fig. 2), which have been noted for beet leafhoppers in California (Yokomi 1979).

**COVER CROP TERMINATION TIME EFFECTS ON SINIGRIN AMENDMENT TO SOIL.** For all termination times during the 2019–20 trial, sinigrin was primarily found in mustard plant shoots rather than mustard plant roots (Table 2). Sinigrin concentrations in mustard plant shoots were similar among the three termination times, but the above-ground biomass for the third termination (17 d before initial beet leafhopper flight) was greater than the above-ground biomass for the first termination (45 d before initial beet leafhopper flight) and second termination (31 d before initial beet leafhopper flight). Because mustard plants were largest at the third termination, the cover crop ended 17 d before the initial beet leafhopper flight contributed the most sinigrin to the soil.

During the 2020–21 trial, sinigrin was again primarily in mustard plant shoots rather than in roots (Table 2). However, unlike the 2019–20 trial, sinigrin concentrations in shoots of mustard plants differed among termination times. The third termination (8 d before the initial beet leafhopper flight) resulted in a higher concentration of sinigrin in mustard shoots compared with the first termination (36 d before the initial beet leafhopper flight) and second termination (22 d before the initial beet leafhopper flight). Changes over time in the sinigrin concentration could have been caused by changes in soil conditions or plant phenology (Rosa et al. 1996). For example, drought during flowering increases glucosinolate concentrations in oil-seed rape (*Brassica napus*) (Milford and Evans 1991), and high soil organic matter promotes glucosinolate concentrations in turnip (*Brassica rapa*) (Ju et al. 1980). For brown mustard plants beyond seedling stages, sinigrin concentrations increase as plants transition from vegetative to reproductive phases, with the maximum sinigrin concentration occurring 4 weeks after the first flowering (Rangkadilok et al. 2002). During the 2020–21 trial of this study, as a result of increases in both biomass and sinigrin concentration, the mustard cover crop ended 8 d before the initial beet leafhopper flight contributed the most sinigrin to soil.

Sinigrin concentrations during this study were within the range previously reported for brown mustard.

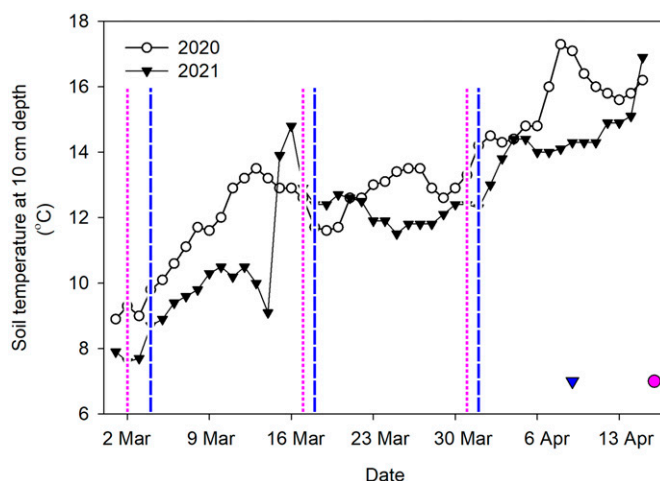


Fig. 2. Daily mean soil temperatures during cover crop termination periods during the 2019–20 trial and 2020–21 trial. Pink dotted vertical lines indicate cover crop termination dates during the 2019–20 trial. Blue dashed vertical lines indicate cover crop termination dates during the 2020–21 trial. The pink circle indicates the date of the initial beet leafhopper (*Circulifer tenellus*) flight in 2020. The blue triangle indicates the date of the initial beet leafhopper flight in 2021. Between the first and second cover crop terminations during the 2019–20 trial, the study site received 3.81 cm of precipitation. Between the second and third cover crop terminations during the 2019–20 trial, the study site received 1.47 cm of precipitation. The study site did not receive precipitation during the cover crop termination period during the 2020–21 trial. Temperature and precipitation data were obtained from a weather station at the New Mexico State University Leyendecker Plant Science Research Center approximately 340 m from the study site (New Mexico State University 2023). ( $1.8 \times ^\circ\text{C}$ ) + 32 =  $^\circ\text{F}$ , 2.54 cm = 1 inch.

Specifically, a previous study indicated that sinigrin concentrations in brown mustard shoots ranged from 5.8 to 59.4  $\mu\text{mol}\cdot\text{g}^{-1}$ , with the sinigrin concentration affected by the brown mustard cultivar and site-year (Bangarwa et al. 2011). During our study, the concentration of sinigrin in roots was lower than that in shoots,

which was also consistent with previous studies (Bangarwa and Norsworthy 2017; Bangarwa et al. 2011) and signifies the importance of increasing shoot biomass to increase sinigrin amendments to soil during biofumigation.

Amounts of mustard cover crop biomass in this study were generally similar to biomass yields for mustard

cover crops in New York (550  $\text{g}\cdot\text{m}^{-2}$ ) (Björkman et al. 2015), Michigan (450  $\text{g}\cdot\text{m}^{-2}$ ) (Björkman et al. 2015), Minnesota (685  $\text{g}\cdot\text{m}^{-2}$ ) (Gieske et al. 2016), and Maine (439  $\text{g}\cdot\text{m}^{-2}$ ) (Haramoto and Gallandt 2005). However, it is important to note that the amount of mustard cover crop biomass for the third termination of the 2019–20 trial (820  $\text{g}\cdot\text{m}^{-2}$ ) was high compared with that previously reported. Yearly variations of biomass in this study might have been caused by factors including weather, insect herbivory, or variation in soil fertility (Björkman et al. 2015). Large amounts of mustard cover crop biomass (>500  $\text{g}\cdot\text{m}^{-2}$ ) are important for effective weed suppression (MacLaren et al. 2019) because not all glucosinolate molecules in mustard biomass are converted to isothiocyanates. For brown mustard, only 25% to 39% of glucosinolates may be converted to isothiocyanates (Bangarwa and Norsworthy 2017), although glucosinolate conversion efficiencies are influenced by environmental conditions and management practices (Bangarwa and Norsworthy 2017; Haramoto and Gallandt 2005; Tagele et al. 2021).

**COVER CROP TERMINATION TIME EFFECTS ON WEEDS, HAND-HOEING, AND CHILE PEPPER.** Wright groundcherry (*Physalis acutifolia*) was the most abundant weed species in chile pepper (Fig. 3). Additional weed species included palmer amaranth (*Amaranthus palmeri*), yellow nutsedge (*Cyperus esculentus*), and junglerice (*Echinochloa*

Table 2. Aboveground biomass and sinigrin concentrations of the cover crop ‘Caliente Rojo’ brown mustard (*Brassica juncea*) ended at different times before both chile pepper seeding (*Capsicum annuum*) and annual flights of beet leafhoppers (*Circulifer tenellus*).

Yr	Date	Termination time		Cover crop characteristics			
		Days before leafhopper flight	Days before chile pepper seeding	Sinigrin in roots	Sinigrin in shoots	Aboveground biomass	Sinigrin amendment to soil <sup>i</sup>
				$\mu\text{mol}\cdot\text{g}^{-1}$ fresh weight (FW) (mean $\pm$ SE) <sup>ii</sup>		$\text{g}\cdot\text{m}^{-2}$ (mean $\pm$ SE) <sup>iii</sup>	$\text{mmol}\cdot\text{m}^{-2}$ (mean $\pm$ SE) <sup>iv</sup>
2020	2 Mar	45	56	$1.4 \pm 0.31^{\text{v}}$ (a)	$31.6 \pm 1.22$ (a)	$340.6 \pm 59.26$ (a)	$115.0 \pm 0.77$ (a)
	17 Mar	31	42	$2.3 \pm 0.21$ (b)	$31.1 \pm 1.02$ (a)	$365.1 \pm 30.70$ (a)	$121.4 \pm 0.33$ (a)
	31 Mar	17	28	$1.6 \pm 0.52$ (a)	$31.3 \pm 0.90$ (a)	$820.1 \pm 76.14$ (b)	$274.5 \pm 0.73$ (b)
2021	4 Mar	36	56	$1.2 \pm 0.61$ (a)	$17.9 \pm 0.90$ (a)	$363.4 \pm 33.85$ (a)	$69.6 \pm 0.32$ (a)
	18 Mar	22	42	$1.5 \pm 0.75$ (a)	$17.4 \pm 1.48$ (a)	$474.7 \pm 37.42$ (a)	$88.3 \pm 0.59$ (a)
	1 Apr	8	28	$1.4 \pm 0.67$ (a)	$33.7 \pm 2.79$ (b)	$591.2 \pm 70.31$ (b)	$213.0 \pm 2.08$ (b)

<sup>i</sup> The amount of sinigrin added to soil is the product of the shoot sinigrin concentration and aboveground biomass. Before the sinigrin calculation, dry aboveground biomass was converted to FW by multiplying by 10.7. This accounted for the moisture content of freshly harvested mustard biomass, which was determined to be 89.3% water.

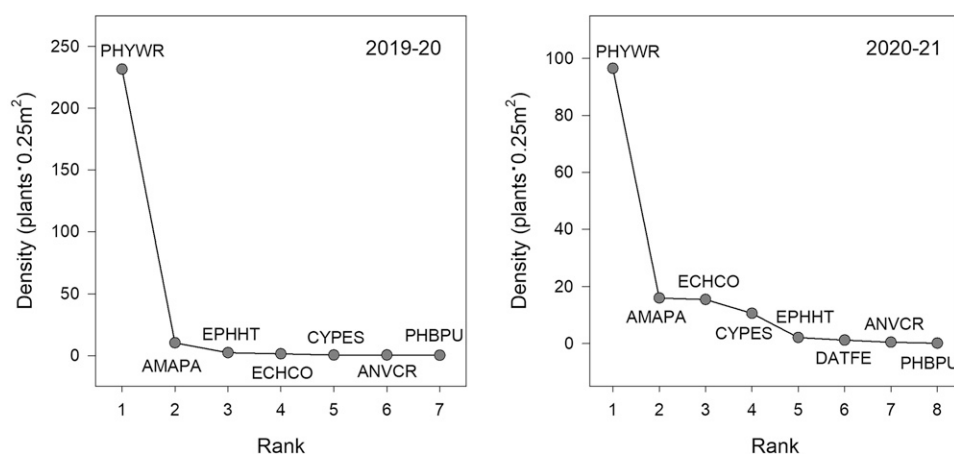
<sup>ii</sup> 1 g = 0.0353 oz.

<sup>iii</sup> 1  $\text{g}\cdot\text{m}^{-2}$  = 0.0295 oz/yd<sup>2</sup>.

<sup>iv</sup> 1 m<sup>2</sup> = 1.1960 yd<sup>2</sup>.

<sup>v</sup> Within 1 year, means within columns that share lowercase letters were not different according to Tukey’s honest significant difference ( $\alpha = 0.05$ ).





**Fig. 3. Rank–abundance curves for weed species present in chile pepper during the 2019–20 trial (A) and 2020–21 trial (B).** For each species, density is the annual total averaged across quadrats in the control with no cover crop. AMAPA = palmer amaranth (*Amaranthus palmeri*); ANVCR = spurred anoda (*Anoda cristata*); CYPES = yellow nutsedge (*Cyperus esculentus*); DATFA = oakleaf datura (*Datura quercifolia*); ECHCO = junglerice (*Echinochloa colona*); EPHHT = ground spurge (*Euphorbia humistrata*); PHBPU = tall morningglory (*Ipomoea purpurea*); PHYWR = wright groundcherry (*Physalis acutifolia*). 1 m<sup>2</sup> = 1.1960 yard<sup>2</sup>.

*colona*). Weeds in this study included species not controlled by residual herbicides currently registered for applications at chile pepper seeding. Specifically, product labels for napropamide, clomazone, and bensulide do not list yellow nutsedge and wright groundcherry as susceptible species (FMC Corporation 2021; Gowan Company 2012; United Phosphorus, Inc. 2016), and yellow nutsedge is known to survive pre-emergence applications of napropamide (Bingham 1977). This absence of options for herbicides effective on weeds during this study emphasizes the need for novel weed control techniques for chile pepper.

Through 28 d after seeding during the 2019–20 trial, there were fewer weeds after the second termination than after the first and third terminations and the control with no cover crop (Fig. 4A). From 29 to 56 d after seeding, the second termination suppressed more weeds than the first and third terminations; however, weed densities did not differ between the second termination and the control with no cover crop from 28 to 56 d after seeding (Fig. 4B). For the 2020–21 trial, only the third termination resulted in fewer weeds than the control with no cover crop through 28 d after seeding (Fig. 4C) and from 29 to 56 d after seeding (Fig. 4D). The first and second terminations did not cause weed suppression in chile pepper during the 2020–21 trial.

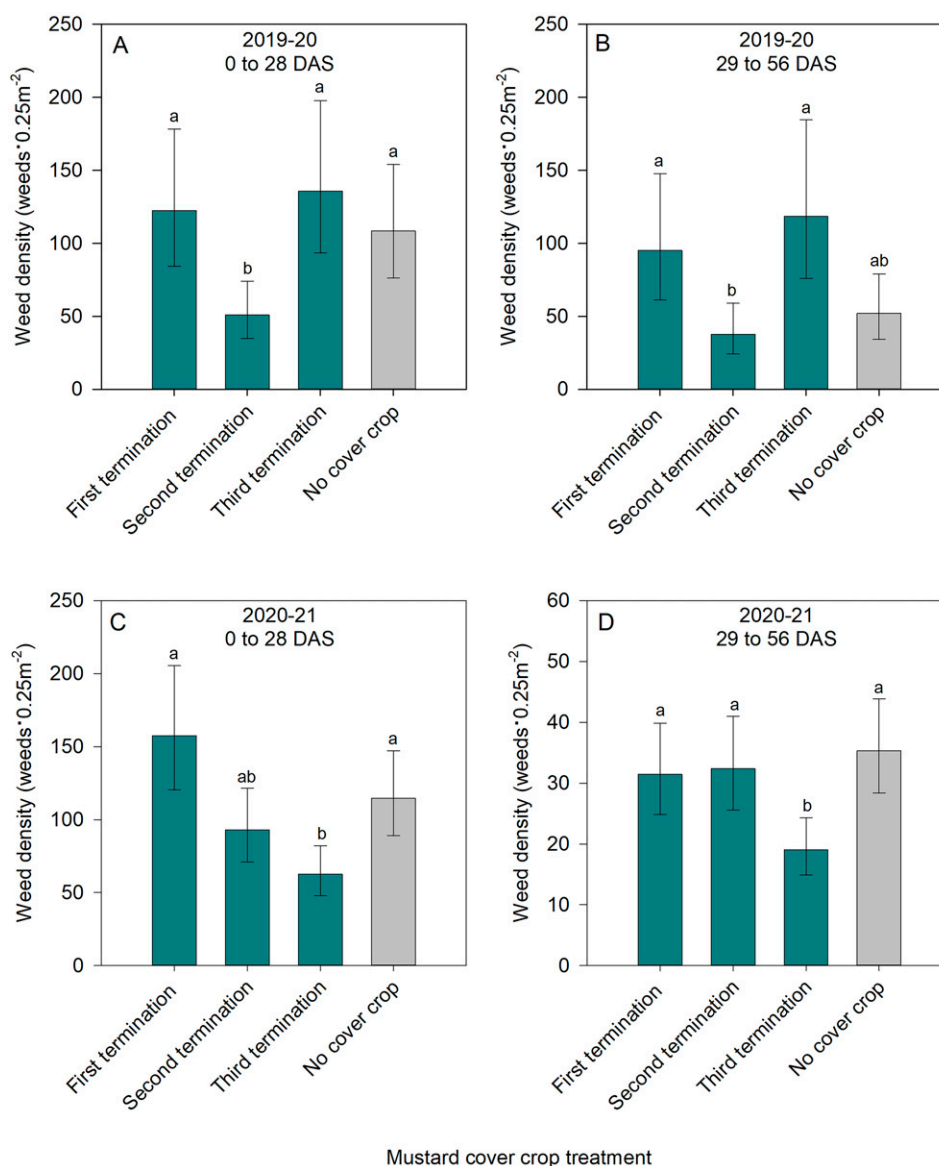
For the 2019–20 trial, weed suppression from the second termination

and no weed suppression from the third termination were unexpected. This was because the third termination contributed the most sinigrin to soil, and because increased glucosinolate production on a per-ground area basis is generally a component of efforts to improve biofumigation efficacy (Kirkegaard and Sarwar 1998). The absence of weed suppression for the third termination during the 2019–20 trial may have been caused by the high amount of biomass for the mustard cover crop. Mustard cover crop biomass incorporated in soil can increase soil nitrogen (Brennan et al. 2013), which can stimulate germination of some plant species (Baskin and Baskin 2014). Consistent with the idea of germination enhanced by cover crop residues in soil, mustard cover crop biomass incorporated in soil has been reported to promote the emergence of weed species such as powell amaranth (*Amaranthus powellii*) and hairy galinsoga (*Galinsoga quadriradiata*) (Björkman et al. 2015). During this study, the promotional effects of cover crop biomass on weed seedling emergence may have offset the inhibitory influence of pesticidal compounds derived from mustard cover crops ended on 31 Mar 2020.

The third termination during the 2020–21 trial had, on average, 27.9% less mustard cover crop biomass than the third termination during the 2019–20 trial. Less than maximum biomass, combined with increased amounts of sinigrin added to soil, may have contributed to greater weed suppression for

the third termination compared with the first and second terminations during the 2020–21 trial. Further, changes over time in soil temperature and soil moisture following cover crop termination may have contributed to differences in weed suppression among termination times during the 2020–21 trial. Soil temperature and soil moisture influence both degradation of cover crop residues (Rosenzweig et al. 2017) and hydrolysis of sinigrin to isothiocyanate (Brown and Morra 1996), with higher temperatures increasing the production of isothiocyanate (Borek et al. 1995). Accordingly, soil warming after termination (Fig. 2) may have strengthened the biofumigation effects on weeds for the third termination in 2020–21.

The lack of weed suppression by the first termination may have been partly attributable to low rates of germination in the weed seedbank. Weed seeds that are quiescent or dormant are less susceptible to isothiocyanates than germinating seeds and seedlings (Leblová-Svobodová and Košťál 1962; Wood et al. 2020). Germination requires specific temperatures (Baskin and Baskin 2014); during this study, soil temperatures might not have supported germination of weed seeds when isothiocyanates were in the soil following cover crop termination in early March. Elevated soil temperature by mid and late March (Fig. 2) could have promoted weed seed germination, making weeds more susceptible to biofumigation for the second and



**Fig. 4.** Densities of weeds in chile pepper following the termination and incorporation the cover crop ‘Caliente Rojo’ brown mustard (*Brassica juncea*). Weed densities were determined twice from 0 to 28 d after chile pepper seeding and twice from 29 to 56 d after seeding. Density data comprise cumulative weeds that emerged from 0 to 28 d after seeding (A, C) and 29 to 56 d after seeding (B, D). The brown mustard cover crop was ended and incorporated in soil at different times before chile pepper seeding. During the 2019–20 trial, termination dates were 2 Mar 2020 (first termination), 17 Mar 2020 (second termination), and 31 Mar 2020 (third termination). These dates were 56 d, 42 d, and 28 d before chile pepper seeding, which corresponded with 45 d, 31 d, and 17 d, respectively, before the annual flight of beet leafhoppers at the study farm. During the 2020–21 trial, termination dates were 4 Mar 2021 (first termination), 18 Mar 2021 (second termination), and 1 Apr 2021 (third termination). These dates were 56 d, 42 d, and 28 d, respectively, before chile pepper seeding and 36 d, 22 d, 8 d, respectively, before the annual flight of beet leafhoppers at the study farm. ‘No cover’ is the control with no cover crop. Bars are parameter estimates with 95% confidence intervals from generalized linear models with negative binomial distributions. Within panels, bars that share lowercase letters are not different according to the overlap of confidence intervals. 1 m<sup>2</sup> = 1.1960 yard<sup>2</sup>.

third terminations compared with the first termination.

Chile pepper stand counts were unaffected by cover crop treatment during the 2019–20 trial ( $F_{3,9} = 2.71$ ;  $P = 0.108$ ) and 2020–21 trial ( $F_{3,9} = 0.35$ ;  $P = 0.794$ ). Additionally, chile pepper plants did not show visible signs of injury from biofumigation.

These results are consistent with those of previous studies that determined biofumigation caused less than 5% crop injury in bell pepper (Norsworthy et al. 2007) and no crop injury in tomato and bell pepper grown with plastic mulch (Bangarwa et al. 2011).

Cover crop treatment did not influence hand-hoeing time at 28 d after

seeding ( $F_{3,9} = 1.47$ ;  $P = 0.281$ ) or 56 d after seeding ( $F_{3,9} = 1.14$ ;  $P = 0.396$ ) during the 2019–20 trial. Similarly, during the 2020–21 trial, cover crop treatment did not influence hand-hoeing time at 28 d after seeding ( $F_{3,9} = 0.38$ ;  $P = 0.770$ ) or 56 d after seeding ( $F_{3,9} = 0.97$ ;  $P = 0.452$ ). These results suggest that



reductions in weed density caused by biofumigation were not adequate for measurable reductions in hand-hoeing time. Further, these results differed from previous research that determined hand-hoeing time was positively correlated with weed density (Melander and Rasmussen 2001). The absence of biofumigation effects on hand-hoeing time might reflect influences on hand-hoeing other than weed density. For example, the time required to hand-hoe is related to the size and position of targeted weeds. Larger weeds in crop rows require more time to hoe than smaller weeds outside of crop rows. Although this study did not determine biofumigation effects on weed size and weed position, hand-hoeing time results suggest that biofumigation did not prevent large weeds or enhance control of weeds in chile pepper rows.

Technical guidance for biofumigation with mustard cover crops includes sprinkler irrigation to retain volatile bio-cidal compounds in the soil after mustard cover crop termination (Duff et al. 2020; Simpson et al. 2010). Sprinkler irrigation is not often used in New Mexico chile pepper production (Sano and Carpenter 2006); therefore, further research of techniques for sealing soil surfaces without sprinkler irrigation may enhance pesticidal efficacy of biofumigation for chile pepper in New Mexico. Biofumigation guidelines also include ending mustard cover crops at flowering stages (Duff et al. 2020). This is because sinigrin concentrations in brown mustard plants are relatively high several weeks after first flowering but before seed maturation (Rangkadi-lok et al. 2002). The results of this study suggest biofumigation for weed suppression will benefit from the development of methods that promote flowering in brown mustard cover crops but prevent very large amounts of brown mustard biomass (aboveground biomass exceeding  $\sim 820 \text{ g}\cdot\text{m}^{-2}$ ).

Another possible method for enhancing the efficacy of biofumigation may involve different mustard species or brown mustard cultivars. A previous study compared levels of biomass and glucosinolates among the following four mustard species and cultivars grown during fall in southern New Mexico: Arcadia broccoli (*Brassica oleracea* var. *italica*); Caliente 61 brown mustard; Caliente 199 brown mustard; and Pacific Gold brown

mustard (Rudolph et al. 2015). Caliente 199 and Pacific Gold exhibited the most biofumigation potential because these brown mustard cultivars produced the most biomass and had the highest glucosinolate concentration (Rudolph et al. 2015). However, 'Caliente 199' brown mustard and 'Pacific Gold' brown mustard were also found to support population growth of southern root-knot nematode (*Meloidogyne incognita*) (Rudolph et al. 2015), which is a plant-parasitic nematode that adversely affects chile pepper (Bosland and Walker 2014). To our knowledge, interactions between southern root-knot nematode and Caliente Rojo—the brown mustard cultivar in this study—have not yet been studied.

## Conclusions

This study determined that biofumigation with an overwinter cover crop can reduce densities of early-season weeds in chile pepper. For chile pepper seeded in April in New Mexico, the optimal time for ending an overwinter mustard cover crop is mid to late March. This period for termination minimizes the likelihood that mustard cover crops will serve as habitat for beet leafhoppers, which typically initiate flights in April. Further research that identifies methods for intensifying biofumigation effects on weeds may lead to strategies that reduce requirements for hand-hoeing in chile pepper. Methods that can prevent or eliminate weeds not controlled by biofumigation include, but are not limited to, pre-emergence and/or postemergence herbicides (Lee and Schroeder 1995), false seedbeds (Schutte et al. 2021), and organic or plastic mulches (Kasirajan and Ngouajio 2012). However, only reducing densities of early-season weeds will prevent yield loss because chile pepper requires extended periods of weed-free conditions for maximum yield potential (Schroeder 1992, 1993). Although managing weeds with biofumigation alone will likely be insufficient, biofumigation with an overwinter mustard cover crop can be a component of management programs that use multiple tactics to manage weeds in chile pepper.

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