Biomass Production of an Overwinter Cover Crop with Biofumigation Properties in New Mexico

Asmita Nagila[1], Soum Sanogo[1], O. John Idowu[2], and
Brian J. Schutte[1]

Keywords. ***Amaranthus palmeri*, *Brassica juncea*, *Capsicum annuum*, chile pepper, Verticillium wilt**

Summary. Soil-borne diseases and weeds can be inhibited by mustard family (Brassicaceae) cover crops that are mowed and incorporated into the soil with tillage—a process referred to as biofumigation. To determine whether a fall-seeded mustard cover crop produces enough biomass to be a biofumigant in spring, this study measured the amount of biomass produced by a mixture of 'Caliente Rojo' brown mustard (*Brassica juncea*) and 'Nemat' arugula (*Eruca sativa*) grown in three commercial fields and a university research farm in southern New Mexico, USA. This study also determined whether the mustard biomass incorporated in the soil inhibits a weed [Palmer amaranth (*Amaranthus palmeri*)], but does not affect a cash crop adversely [chile pepper (*Capsicum annuum*)]. Results indicated that, if the mustard cover crop was seeded before the first frost in fall, mustard cover crops produced biomass in quantities sufficient for biofumigation in spring. Mustard biomass incorporated in the soil reduced the survival and germination of Palmer amaranth seeds. Under greenhouse conditions, chile pepper plants grown in soil with mustard cover crop biomass were larger than chile plants grown in soil without mustard biomass. Chile pepper plants in soil with mustard biomass did not show symptoms of Verticillium wilt (*Verticillium dahliae*), whereas such symptoms were found on about 33% of chile pepper plants in soil without mustard biomass. These results suggest that a fall-seeded mustard cover crop that is tilled into the soil in early spring is a potential pest management technique for chile pepper in New Mexico.

Cover crops are noncash crops grown primarily to protect and enrich soil. In addition to improving soil fertility (Brennan et al. 2013), aggregate stability (Antosh et al. 2020), and organic matter (Agarwal et al. 2022a), some cover crops suppress weeds and soil-borne diseases in subsequent cash crops after the cover crops are ended and incorporated into the soil with tillage (Clark 2007). These "allelopathic green manures" undergo microbial decomposition and release compounds that are toxic to plants and microorganisms (Liebman and Davis 2000).

Cover crops used as allelopathic green manures include several species in the mustard family (Brassicaceae) (Haramoto and Gallandt 2004). The process of using mustard cover crops to suppress soil-borne pests is known as biofumigation (Matthiessen and Kirkegaard 2006). Biofumigation may inhibit emergence and growth of cash crops (Ackroyd and Ngouajio 2011; Haramoto and Gallandt 2005). However, because many allelopathic chemicals from mustard cover crops are volatile and short-lived in soil (Matthiessen and Kirkegaard 2006), potential negative effects of biofumigation can be prevented by not planting cash crops soon after termination and incorporation of mustard cover crops (Ackroyd and Ngouajio 2011; Clark 2007). Relatively low concentrations of mustard-derived allelochemicals in soil at crop seeding can inhibit small-seeded weed species, but not affect emergence of large-seeded crop species because susceptibility to the allelochemicals is inversely related to seed size (Liebman and Davis 2000).

Mustard cover crops suppress pests in crops including potato (*Solanum tuberosum*) in the northwestern (Boydston and Hang 1995) and northeastern United States (Larkin and Griffin 2007), pea (*Pisum sativum*) in the northwestern United States (Al-Khatib et al. 1997), soybean (*Glycine max*) in the central United States (Krishnan et al. 1998; Wen et al. 2017), onion (*Allium cepa*) in the central United States (Wang et al. 2010), and bell pepper (*Capsicum annuum*) in the southeastern United States (Norsworthy et al. 2007). Pest suppression from biofumigation is caused by the enzymatic degradation of chemicals called glucosinolates, which are found in the cover crop biomass (Rask et al. 2000). Degradation of glucosinolates releases compounds toxic to growing plants, but less lethal to dormant seeds or propagules (Angelini et al. 1998; Leblova and Kostir 1962; Neubauer et al. 2014; Petersen et al. 2001). Because some pesticidal compounds derived from glucosinolates are short-lived and volatile (Bangarwa et al. 2011; Haramoto and Gallandt 2004), the pest-suppressing properties of mustard cover crop are maximized by taking measures to retain gaseous compounds in the soil and to end cover crops when weed seeds are germinative (Hansen and Keinath 2013).

The degree of pest suppression from biofumigation depends on the cover crop species, the environment, and the pest species that are targeted. Weed species that are especially inhibited by biofumigation include annual weeds with seeds ~1 mm in diameter (Boydston and Hang 1995; Norsworthy et al. 2007). Such species include

Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
0.3048	ft	m	3.2808
0.0929	ft^2	m^2	10.7639
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
1.1209	lb/acre	kg·ha^{-1}	0.8922
1.6093	mile(s)	km	0.6214
28.3495	oz	g	0.0353
33.9057	oz/yard^2	g·m^{-2}	0.0295
0.8361	yard^2	m^2	1.1960
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

Palmer amaranth (*Amaranthus palmeri*), redroot pigweed (*Amaranthus retroflexus*), and common lambsquarters (*Chenopodium album*). For these weeds, suppression from biofumigation typically occurs early in the growing season (Al-Khatib et al. 1997; Norsworthy et al. 2007; Osipitan et al. 2018; Teasdale 1996). This is because the glucosinolates that release toxic volatiles degrade within days of incorporation of biomass into the soil (Gimsing and Kirkegaard 2006), and the toxic volatiles that suppress weeds dissipate within hours of glucosinolate degradation (Liu et al. 2020).

A substantial amount of cover crop biomass is crucial for pest suppression from biofumigation (McGuire 2016). Previous research suggests that mustard cover crops require 3747 to 7316 lb/acre of dry aboveground biomass at termination to suppress weeds (Al-Khatib et al. 1997). In New Mexico, USA, Rudolph et al. (2015) determined that brown mustard (*Brassica juncea*) cover crops produced up to 12,847 lb/acre of total dry biomass when these biofumigant crops were broadcast-seeded and raked into raised beds in September, fertilized, watered weekly, and ended in November or December. Also in New Mexico, Agarwal et al. (2022b) determined that brown mustard cover crops that were broadcast-seeded on flat ground in October, grown without fertilizer, and irrigated two to four times over 6 months produced up to 9448 lb/acre of aboveground dry biomass by termination in May. Furthermore, Agarwal et al. (2022b) determined that mustard cover crops that were ended and incorporated into the soil reduced the number of weeds that emerged before the seeding of sweet corn (*Zea mays*) in 2 of 3 site-years in New Mexico.

Biofumigation with an overwinter cover crop grown without fertilizer and minimal irrigation is a potential tactic to suppress early-season weeds and soil-borne diseases in chile pepper (*Capsicum annuum*) in New Mexico. However, the timing of chile pepper planting in New Mexico (March and April), combined with the need not to plant a cash crop immediately after termination and incorporation of a mustard cover crop, may force farmers to end mustard cover crops before the cover crops have enough biomass for biofumigation. Thus, an important step toward understanding the possibilities for biofumigation for chile pepper in New Mexico is to quantify biomass produced by a mustard cover crop that is seeded in fall and ended in early spring. Accordingly, the objectives of this study were 1) to determine whether a mustard cover crop that is seeded in fall produces enough biomass for biofumigation in early spring and 2) to determine whether biomass from an overwinter mustard cover crop inhibits Palmer amaranth, but does not affect chile pepper adversely.

Materials and methods

MUSTARD COVER CROP. The mustard cover crop in this study was a proprietary mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula (*Eruca sativa*) (High Performance Seeds, Inc., Moses Lake, WA, USA). 'Caliente Rojo' brown mustard is marketed to contain high concentrations of glucosinolates. 'Nemat' arugula is a trap crop for certain nematodes (*Meloidogyne* sp.). It suppresses population growth of certain nematodes by not allowing nematodes to reproduce (Melakeberhan et al. 2006). Although 'Nemat' arugula was included in the proprietary mixture, nematode responses to mustard cover crops were not within the scope of this study.

SITES AND TREATMENTS. The study was conducted at four sites. Three sites were commercial fields managed by farmer cooperators. The fourth site was a New Mexico State University research farm. The commercial fields were near Columbus, NM, USA (lat. 31.797°N, long. 107.857°W); Deming, NM, USA (lat. 32.225°N, long. 107.775°W); and Las Uvas, NM, USA (lat. 32.605°N, long. 107.350°W). The university research farm was the Leyendecker Plant Science Research Center [hereafter, Leyendecker (lat. 32.202°N, long. 106.743°W)] near Las Cruces, NM, USA. The nearest two study sites were separated by 51 km. At Columbus, the study site featured sandy loam soil (Jal series, fine-loamy, carbonatic, thermic Typic Haplocalcids). At Las Uvas, the study site featured silty clay loam soil (Verhalen series, fine, smectitic, thermic Typic Haplotorrerts), and at Deming, the study site featured loam soil (Gila series, loamy, mixed, superactive, calcareous, thermic Typic Torrifluvents). At Leyendecker, the study field featured clay loam soil (Glendale series, fine-silty, mixed, superactive, calcareous, thermic Typic Torrifluvents).

The mustard cover crop was seeded at 7 lb/acre, as recommended by the seed company (High Performance Seeds, Inc. 2022). At Columbus, the mustard cover crop was seeded into raised beds using a mechanical planter, whereas at Deming, Las Uvas, and Leyendecker, the mustard cover crop was seeded on flat ground using a mechanical grain drill. Raised beds at Columbus were listed rows spaced 38 inches apart and with top ridges smoothed. For each raised bed, there was one row of mustard cover crop. After seeding and throughout the cover crop growing season, fields were irrigated three to four times as needed. At Columbus, Deming, and Las Uvas, fields were irrigated with a subsurface drip. At Leyendecker, the field was flood-irrigated.

At each site, the mustard cover crop was compared against bare ground. These treatments (mustard cover crop, bare ground) were arranged in parallel plots and replicated three times at Columbus, Deming, and Leyendecker. At Las Uvas,

---

Received for publication 6 Jun 2022. Accepted for publication 11 Oct 2022.

[1]Department of Entomology, Plant Pathology and Weed Science, New Mexico State University, 945 College Drive, Las Cruces, NM 88003, USA

[2]Department of Extension Plant Sciences, New Mexico State University, 945 College Drive, Las Cruces, NM 88003, USA

Funding for this research was provided by the Western Sustainable Agriculture Research and Education program (project SW18-059), the National Institute of Food and Agriculture, and the U.S. Department of Agriculture. Additional salaries and research support were provided by state and federal funds appropriated to the New Mexico State University Agricultural Experiment Station.

We gratefully acknowledge the field assistance of Edward Morris and Israel Marquez, as well as the technical assistance from staff at the Leyendecker Plant Science Research Center. We also thank Ivette Guzman and Prashasti Agarwal for reviewing this manuscript, and we express deep gratitude to our farmer collaborators.

This paper is a portion of a thesis submitted by A.N. in fulfillment of a Master of Science degree requirement.

Current address of A.N.: Department of Horticultural Sciences, Texas A&M University, Texas A&M AgriLife Research and Extension Center, 1619 Garner Field Road, Uvalde, TX 78801, USA

B.J.S. is the corresponding author. E-mail: bschutte@nmsu.edu.

https://doi.org/10.21273/HORTTECH05084-22

parallel plots of mustard cover crop and bare ground were replicated twice. Treatment plot dimensions differed among study sites. At Deming, treatment plots were 394 ft long and 40 ft wide. At Leyendecker, plots were 131 ft long and 13 ft wide, and at Columbus plots were 1148 ft long and 59 ft wide. At Las Uvas, treatment plots were 951 ft long and 66 ft wide. Within each mustard cover crop plot, six equally spaced sampling locations were established along the longitudinal axis. Each sampling location was paired with a sampling location in the adjacent bare-ground plot.

To end mustard cover crops, aboveground biomass was mowed and incorporated into the soil by tillage. Tillage programs for termination included furrow plowing (Columbus only), disking, and rotary tilling. Termination concluded with the creation of raised beds for direct-seeded chile pepper. Within 48 h of cover crop termination, study sites were irrigated using a subsurface drip at Columbus, Deming, and Las Uvas, and flood-furrow irrigation at Leyendecker. Table 1 provides information on cover crop seeding and termination dates.

Table 1. Mustard cover crop seeding and termination dates for the four sites in this study.

Site[i]	Cover crop seeding date	Cover crop termination date
Columbus	10 Nov 2018	14 Feb 2019
Deming	29 Sep 2018	22 Feb 2019
Las Uvas	29 Oct 2018	5 Mar 2019
Leyendecker	11 Oct 2018	15 Mar 2019

[i] A cover crop mixture of ‘Caliente Rojo’ brown mustard and ‘Nemat’ arugula was evaluated at commercial fields in New Mexico, USA, near Columbus, Deming, and Las Uvas, and a university research farm (New Mexico State University, Leyendecker Plant Science Research Center) near Las Cruces, NM, USA.

DATA COLLECTION FOR OBJECTIVE 1. Two months after cover crop planting, mustard stand densities and percentages of ground occupied by mustard cover crops were determined with 0.25-m^2 rectangular quadrats at each sampling location (18 sampling locations at Columbus, Deming, and Leyendecker; 12 sampling locations at Las Uvas). Also at each sampling location and just before cover crop termination, aboveground biomass of weeds and cover crop were harvested from 0.25-m^2 quadrats. Weed biomass and cover crop biomass were bagged separately and oven-dried at 65 °C for 72 h.

DATA COLLECTION FOR OBJECTIVE 2. Seeds of Palmer amaranth were collected from Leyendecker in Aug 2018. Seeds were obtained by hand-clipping seed-bearing inflorescences. These inflorescences were dried under room conditions for 14 to 20 d. Dried inflorescences were hand-threshed, and sequential combinations of sieving and forced-air separation were used to separate seeds from chaff. Seeds were then stored in an airtight container at 4 °C. Before use in our study, seeds were assayed for viability using a 1.0% aqueous solution of tetrazolium [2,3,5-triphenyl chloride (Association of Official Seed Analysts 2000)]. Tetrazolium assay results indicated that 96% of the Palmer amaranth seeds were viable at the onset of our study.

Packets (2 × 3 cm) containing 50 Palmer amaranth seeds were made using nylon netting (No-See-Um Netting; Equinox Ltd., Williamsport, PA, USA). The netting was sealed using a heat sealer. Packets were buried at each sampling location after the cover crop was ended, but before the irrigation that occurred after cover crop termination. Packets were buried at a depth of 2 inches. One day before chile pepper seeding, which was 20 to 24 d after packet burial, packets were recovered from the field and brought to the laboratory. At the laboratory, packets were opened and seeds were placed on moistened filter papers in petri plates. Petri plates were placed under a stereoscope and individual seeds were pressed gently using forceps. Seeds that did not collapse under gentle pressure were considered viable and are hereafter referred to as persistent. Responses to gentle pressure have been used for assessments of weed seed viability (Borza et al. 2007; Khan et al. 2022), including assessments of seed viability for Palmer amaranth (Korres et al. 2018). To our knowledge, previous reports for pressure assessments of seed viability did not include information on the amounts of force applied to individual seeds. For information on the amount of force required to rupture weed seedcoats, see Davis et al. (2016).

Persistent seeds were subjected to germination assays conducted in petri plates that were placed in a chamber set to 35/25 °C (day/night) with a 14-h photoperiod. At 2-d intervals for 14 d, germinated seeds were counted and removed. At the conclusion of the 14-d germination assay, seeds that did not germinate were assayed for viability using a 1.0% aqueous solution of tetrazolium. Results from viability assays were used to adjust quantities of persistent seeds determined with forceps and gentle pressure. Specifically, the number of nonviable seeds within each packet was subtracted from the number of persistent seeds determined with forceps and gentle pressure.

After termination of cover crops, but before irrigation, soil from each sampling location in cover crop and bare-ground plots was collected from the top 7 cm using a hand shovel. These soil samples were used in a study conducted in a greenhouse at Leyendecker. Throughout the study, the greenhouse was set to maintain an air temperature of 24 ± 4 °C. Soil from each sampling location in cover crop and bare-ground plots was dispensed into cylindrical plastic pots (diameter, 9 inches; depth, 8.5 inches), which produced pots with and pots without mustard biomass in the soil. After filling, pots were irrigated to field capacity. With no further irrigation, soils were incubated for 4 weeks under greenhouse conditions. After the 4-week incubation period, ‘NM 6–4’ chile pepper plants at the two-leaf stage were transplanted into pots (one plant per pot). Chile pepper plants were watered with a sprinkler canister daily and fertilized once at the 10-leaf stage with 5N–4.4P–8.3K fertilizer. Plants were monitored for symptoms of Verticillium wilt every 7 d. Verticillium wilt is caused by a soil-borne fungus, *Verticillium dahliae*. It is prevalent in chile pepper fields across southern New Mexico (Sanogo and Carpenter 2006) and was observed at study sites before our study. Symptoms of Verticillium wilt include wilting, even when plants receive adequate watering (Fig. 1), followed

Fig. 1. Symptoms of Verticillium wilt on a chile pepper plant grown in a greenhouse. Soil supporting this plant was watered to saturation daily.

by foliar chlorosis and premature senescence (Goldberg 2010).

The greenhouse study was ended 60 d after transplanting, which is when chile pepper plants started bearing fruit. Data collected at termination included plant height, fresh and dry biomass of shoots, and dry biomass of roots. Before collecting data on roots, plants were placed on a 10-mm mesh screen and roots were washed carefully with water. Root and shoot dry weights were determined after biomass was dried in an oven for 72 h at 65 °C.

Data analysis. All statistical analyses were performed using R (version 4.1.0; R Foundation for Statistical Computing, Vienna, Austria). For objective 1, site effects on mustard stand densities and ground coverage at 2 months after seeding were determined with analysis of variance (ANOVA) followed by tests for least significant difference. ANOVA was also used to assess site effects on mustard cover crop biomass at termination. For presentation purposes, data on mustard cover crop biomass were converted to pounds per acre because this unit of yield is commonly used in management guides for cover crops (Clark 2007). To determine site effects on weed biomass at cover crop termination, data were analyzed with a Kruskal-Wallis test followed by post hoc Conover tests for nonparametric data using the R library *PMCMRplus*. For objective 2, quantities of persistent Palmer amaranth seeds were converted to percentages of seeds buried. Quantities of germinated seeds were converted to percentages of persistent seeds. Percentage data for seed persistence and germination were analyzed with generalized linear mixed models fitted with a binomial distribution using the R library *lme4*. Models were developed separately for each site. In these models, treatment (mustard cover crop, bare ground) was the fixed effect; the sampling location within a replication was the random effect. For the greenhouse study, data on chile pepper plant size were sorted by study site and analyzed separately with paired *t* tests. These tests compared plants grown in soil with mustard biomass against plants grown in soil without mustard biomass.

Results and discussion

Objective 1: Determine whether a mustard cover crop seeded in fall produces enough biomass for biofumigation in early spring. At 2 months after cover crop planting, mustard stand densities and ground coverage differed among study sites (Table 2). Mustard stand densities were greatest at Leyendecker (241 plants/m^2) and lowest at Las Uvas (32 plants/m^2). Mustard stand densities at Columbus (85 plants/m^2) and Deming (106 plants/m^2) were less than the mustard stand density at Leyendecker but greater than the mustard stand density at Las Uvas. The percentage of ground covered by the mustard cover crop at 2 months after cover crop seeding was greatest at Deming (85%) and Leyendecker (89%), and lowest at Las Uvas (5%). The percentage of ground covered by the mustard cover crop at Columbus (50%) was less than the percentage of ground covered at Deming and Leyendecker, but greater than the percentage of ground covered at Las Uvas. Low stand density and low ground coverage for the mustard cover crop at Las Uvas was associated with frost shortly after seeding. Moderate stand density and moderate ground coverage for the mustard cover crop at Columbus corresponded with late planting; however, the Columbus site did not receive frost shortly after cover crop seeding.

At termination, mustard biomass differed among sites (Table 3). The mustard cover crop at Leyendecker

Table 2. Stand densities and groundcover for a cover crop mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula at 2 moths after cover crop seeding.

Site[i]	Stand density,[ii] plants/m^2 (mean ± *SE*)	Ground cover,[iii] % (mean ± *SE*)
Columbus	85 ± 12 b[iv]	50 ± 6 b
Deming	106 ± 5 b	85 ± 2 a
Las Uvas	32 ± 5 c	5 ± 3 c
Leyendecker	241 ± 14 a	89 ± 2 a

[i] Sites included commercial fields in New Mexico, USA, near Columbus, Deming, and Las Uvas, and a university research farm (New Mexico State University, Leyendecker Plant Science Research Center) near Las Cruces, NM, USA. For Columbus, Deming, and Leyendecker, *n* = 18; for Las Uvas, *n* = 12.

[ii] 1 seedling/m^2 = 0.8361 seedling/$yard^2$.

[iii] Percentage of ground occupied by mustard cover crops determined with 0.25-m^2 (0.299-$yard^2$) rectangular quadrats.

[iv] Means within a column followed by the same letter are not different according to Fisher's protected least significant difference tests ($P \leq 0.05$).

Table 3. Dry aboveground biomass for a cover crop and weeds co-occurring in quadrats at cover crop termination.

	Cover crop [ii] biomass at termination		
Site[i]	$g \cdot m^{-2}$ (mean ± *SE*)[iii]	Converted mean (lb/acre)[iii]	Weed biomass at termination, $g \cdot m^{-2}$ (mean ± *SE*)
Columbus	525 ± 33.4 ab[iv]	4,684	4.2 ± 1.87 b
Deming	463 ± 17.7 b	4,131	0.1 ± 0.07 a
Las Uvas	125 ± 13.1 c	1,115	5.0 ± 3.14 b
Leyendecker	598 ± 30.2 a	5,335	0 ± 0 a

[i] The study was conducted in commercial fields in New Mexico, USA near Columbus, Deming, and Las Uvas, and at a university research farm (New Mexico State University, Leyendecker Plant Science Research Center) near Las Cruces, NM, USA. For Columbus, Deming, and Leyendecker, n = 18; for Las Uvas, n = 12.

[ii] The cover crop was a mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula.

[iii] For presentation purposes, data on mustard cover crop biomass were converted to pounds per acre; $1\ g \cdot m^{-2} = 0.0295\ oz/yard^2$, $1\ lb/acre = 1.1209\ kg \cdot ha^{-1}$.

[iv] Means within a column followed by the same letter are not different according to Fisher's protected least significant difference test [$P \leq 0.05$ (mustard biomass)] or Conover's test that followed a Kruskal-Wallis test [$P \leq 0.05$ (weed biomass)].

produced the maximum amount of biomass in this study (598 $g \cdot m^{-2}$); the mustard cover crop at Las Uvas produced the least amount of biomass (125 $g \cdot m^{-2}$). For three sites (Columbus, Deming, and Leyendecker), the range of mustard cover crop aboveground biomass at termination was comparable to previous reports of mustard cover crop biomass production in regions outside of New Mexico. For example, brown mustard and white mustard (*Sinapis alba*) seeded at 8.9 lb/acre in fall and spring in the U.S. Great Lakes region had ~1605 to 4730 lb/acre of dry aboveground biomass at termination (Björkman et al. 2015). Brown mustard seeded at 7.1 lb/acre in summer in France had ~1965 to 3390 lb/acre of dry aboveground biomass at termination (Motisi et al. 2009). However, a mixture of brown mustard and white mustard seeded at 19.6 lb/acre in fall in California had ~9800 lb/acre of dry aboveground biomass at termination in early spring (Brennan and Smith 2005), suggesting that the cover crop seeding and production procedures in our study did not sustain maximum levels of mustard cover crop biomass.

Weed biomass at cover crop termination was lower at Deming (0.1 $g \cdot m^{-2}$) and Leyendecker (0 $g \cdot m^{-2}$) than Columbus (4.2 $g \cdot m^{-2}$) and Las Uvas (5.0 $g \cdot m^{-2}$; Table 3). Deming and Leyendecker also featured relatively high levels of cover crop ground coverage at 2 months after cover crop seeding (Table 2), which suggests early-season measurements of cover crop ground coverage foreshadow cover crop suppression of co-occurring weeds at termination. At sites where the mustard cover crop was well established (Columbus, Deming, Leyendecker), weed biomass at cover crop termination was less than 1% of mustard biomass. These results were generally consistent with previous studies that reported mustard cover crops were more competitive than weeds (Björkman et al. 2015; Brennan and Smith 2005).

In a previous study in Washington state, white mustard produced 3747 to 4014 lb/acre of aboveground dry biomass by the time of termination (Al-Khatib et al. 1997). After incorporation into the soil, the white mustard biomass reduced weed density by 17% at 30 d after planting pea. In the same study, rapeseed (*Brassica napus*) produced 5620 to 7315 lb/acre of dry biomass, and after incorporation into the soil, the rapeseed biomass reduced weed density by 34% in pea (Al-Khatib et al. 1997). In potato, biofumigation with 3658 to 5175 lb/acre of dry rapeseed biomass reduced weed densities by 73% to 85% (Al-Khatib et al. 1997). These results from a previous study (Al-Khatib et al. 1997) suggest that mustard cover crops require 3658 to 7315 lb/acre of dry aboveground biomass at termination to suppress pests in subsequent cash crops. According to this literature-based estimate for biomass requirements, and considering the results of our study, a fall-seeded mustard cover crop in southern New Mexico produces enough biomass for biofumigation in early spring, provided the mustard cover crop does not experience frost shortly after seeding.

OBJECTIVE 2: DETERMINE WHETHER BIOMASS FROM AN OVERWINTER MUSTARD COVER CROP INHIBITS A WEED, BUT DOES NOT AFFECT CHILE PEPPER PLANTS ADVERSELY. At sites where the mustard cover crop was well established (Columbus, Deming, and Leyendecker), incorporated mustard biomass reduced the number of viable Palmer amaranth seeds in the soil (Fig. 2A). Palmer amaranth seeds that persisted in soil with mustard biomass had lower rates of germination than seeds retrieved from the soil without mustard biomass (Fig. 2B), which suggests that mustard cover crop biomass induced secondary dormancy of Palmer amaranth seeds. These results are consistent with previous studies that indicated 1) compounds derived from glucosinolates strongly suppressed Palmer amaranth seedling emergence (Norsworthy and Meehan 2005) and 2) germination of redroot pigweed was completely inhibited by pesticidal compounds analogous to compounds derived from decaying mustard biomass (Teasdale and Taylorson 1986).

Under greenhouse conditions, chile pepper plants grown in soil with mustard biomass were larger than chile pepper plants grown in soil without mustard biomass (Table 4). Mustard-induced increases in chile pepper plant size were especially prominent in roots, as root dry weights were up to 160% greater in soil with mustard biomass compared with soil without mustard biomass. Mechanisms by which mustard cover crop biomass could have enhanced chile pepper plant growth include increased soil nitrogen (Brennan et al. 2013; Weinert et al. 2002), increased soil organic matter (Agarwal et al. 2022a), reduced soil pH (Rudolph et al. 2015), and suppression of soil-borne pathogens (Larkin and Griffin 2007), including *V. dahliae* (Subbarao and Hubbard 1996). Suppression of soil-borne pathogens may have been an important causal factor for enhanced plant growth in our study because chile pepper plants in soil with mustard biomass did not exhibit symptoms of Verticillium wilt, whereas Verticillium wilt symptoms were prevalent in chile pepper plants grown in soil without mustard biomass (Table 4).

The general absence of phytotoxicity from mustard biomass in our study was

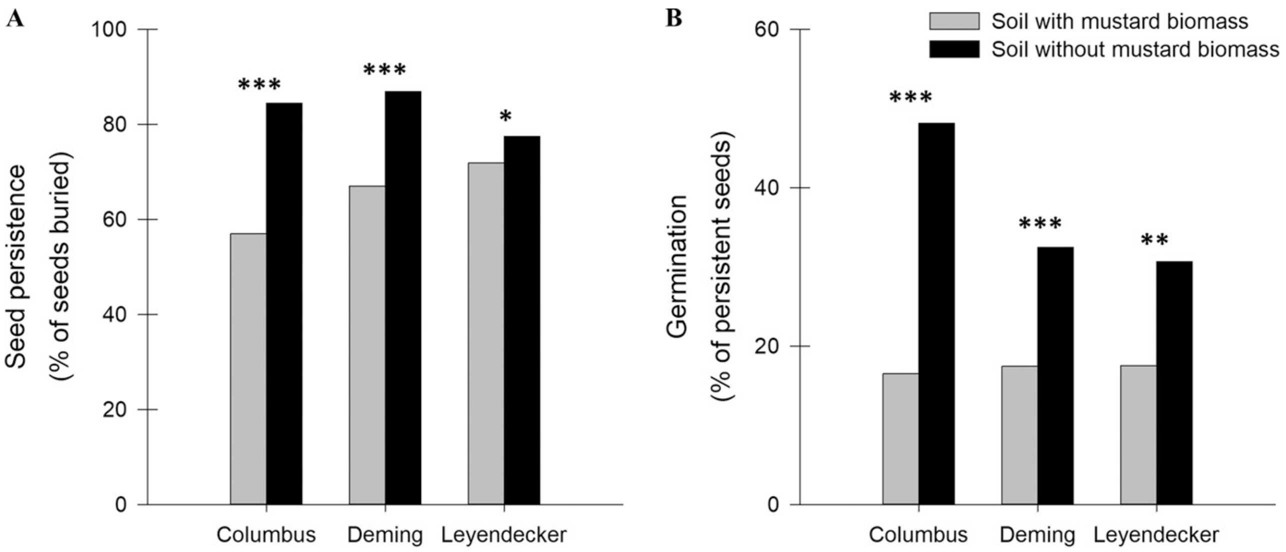

Fig. 2. Mustard biomass effects on (A) persistence and (B) germination of Palmer amaranth seeds at three sites in southern New Mexico, USA (Columbus, Deming, and Leyendecker). At each site, a cover crop mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula was grown and ended with a sequence of mowing and disking. After disking, Palmer amaranth seeds in mesh packets were buried in the soil with and without biomass from the mustard cover crop. After 20 to 24 d, mesh packets were recovered and seeds tested for viability and germination. Bars are means (n = 18). Symbols above data points indicate results from F tests that determined the effects of soil treatment within a site; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

consistent with previous studies that indicated radish (*Raphanus sativus*) biomass did not inhibit the emergence of lettuce [*Lactuca sativa* (Lawley et al. 2012)], and white mustard biomass promoted the growth of onion and celery [*Apium graveolens* (Wang et al. 2010)]. However, greenhouse results in our study were somewhat inconsistent with results from a field study by Rudolph et al. (2015). Rudolph et al. (2015) determined that brown mustard cover crops seeded in September and ended in November or December did not influence vegetative biomass of chile pepper that was seeded the following April or May, although two brown mustard cultivars (Caliente 199 and Pacific Gold) increased fruit yield for the second of two harvests in one of two experimental runs. Inconsistent results for mustard effects on chile pepper between the results of the study of Rudolph et al. (2015) and our study may reflect inherent environmental differences that complicate comparisons between field and greenhouse studies. Also, inconsistent results between Rudolph et al. (2015) and our study may have been caused by differences in termination times for the fall-seeded mustard cover crops. In our study, fall-seeded mustard cover crops were ended in February or March, whereas in Rudolph et al. (2015), fall-seeded mustard cover crops were ended in November or December. Thus, the inconsistent results between Rudolph et al. (2015) and our study suggest that promotion of chile pepper growth with a biofumigant cover crop is more likely to occur when the cover crop is ended 20 to 24 d before chile pepper seeding, rather than 115 to 134 d before chile pepper seeding.

The promotional effects of mustard cover crop biomass on chile pepper plant size, combined with the inhibitory effects of mustard cover crop biomass on Palmer amaranth seed persistence and

Table 4. Shoot fresh weight, shoot dry weight, root dry weight, and Verticillium wilt incidence for chile pepper plants grown in different soils under greenhouse conditions.

Soil source site[i]	Soil type	Chile plants 60 d after transplanting,[ii] g[iii] (mean ± *SE*)			
		Fresh shoot	Dry shoot	Dry root	Verticillium wilt incidence[iv] (% of plants)
Columbus	Without mustard biomass	32.9 ± 2.3 a[v]	5.0 ± 0.3 a	1.9 ± 0.2 a	22
	With mustard biomass[v]	41.1 ± 2.5 b	5.7 ± 0.4 a	2.8 ± 0.2 b	0
Deming	Without mustard biomass	35.8 ± 5.4 a	6.5 ± 0.9 a	2.8 ± 0.5 a	33.3
	With mustard biomass	61.9 ± 6.4 b	10.9 ± 1.1 b	5.5 ± 0.7 b	0
Leyendecker	Without mustard biomass	11.8 ± 2.0 a	1.8 ± 0.2 a	0.5 ± 0.1 a	12.5
	With mustard biomass	17.7 ± 2.4 b	3.2 ± 0.4 b	1.3 ± 0.1 b	0

[i] Soil was collected from commercial fields in New Mexico, USA near Columbus and Deming, and a university research farm (New Mexico State University, Leyendecker Plant Science Research Center) near Las Cruces, NM, USA. At each site, a cover crop mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula was grown and ended with a sequence of mowing and disking. Soil was collected after disking.

[ii] Two-leaf–stage chile pepper plants were transplanted into pots containing field soil with or without mustard biomass.

[iii] 1 g = 0.0353 oz.

[iv] Symptoms of Verticillium wilt included wilting, foliar chlorosis, and senescence. Verticillium wilt data are percentages of all plants in the indicated combination of soil type and site. Soil without mustard biomass was obtained from bare-ground plots that were adjacent to plots with the mustard cover crop.

[v] Means (n = 18) within a column and site followed by the same letter are not different according to paired t tests ($P \leq 0.05$).

seed germination, suggest that biofumigation possibly provides at least two benefits to chile pepper production: 1) enhanced crop growth and 2) suppression of specific crop pests. This two-part hypothesis needs to be confirmed with further field studies. In addition to the cover crop mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula, cultivars and species in the mustard family that might be used for biofumigation in southern New Mexico include 'Caliente 199' brown mustard. A previous study in New Mexico indicated that an overwinter cover crop of 'Caliente 199' brown mustard improved soil quality by increasing the diameter of stable soil aggregates (Antosh et al. 2020). Another previous study in New Mexico determined that 'Caliente 199' brown mustard generally had greater concentrations of glucosinolates than 'Caliente 61' brown mustard and 'Arcadia' broccoli (*Brassica oleracea* var. *italica*), with glucosinolate concentrations in 'Caliente 199' brown mustard significantly greater in 1 of 2 years (Rudolph et al. 2015). Future studies should compare mustard cultivars, including Caliente Rojo and Caliente 199 brown mustards, for their potential as biofumigant cover crops supporting chile pepper production in New Mexico.

Possible benefits from biofumigation must be balanced against potential hazards from the mustard cover crop. Notably, brown mustard cover crops could increase population densities of southern root-knot nematode [*Meloidogyne incognita* (Rudolph et al. 2015)]—a chile pepper pest that reduces fruit yield. Also, mustard family plants in the southwestern United States are hosts to *Beet curly top virus* and its vector, the beet leafhopper (*Circulifer tenellus*) (Creamer et al. 2003, 2005). If beet leafhoppers on a mustard cover crop persist in the local environment after the cover crop is ended, these leafhoppers may transmit the virus to cause curly top disease in chile pepper. Direct evidence of *Beet curly top virus* transfer from mustard cover crops to chile pepper via beet leafhopper is lacking, but further research is needed.

Conclusion

If seeded several weeks before the first frost in fall, a cover crop mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula produces enough biomass for biofumigation in early spring in southern New Mexico, USA. Thus, biofumigation with a cover crop mixture of 'Caliente Rojo' brown mustard and 'Nemat' arugula could be a pest management technique for chile pepper in New Mexico.

References

Ackroyd, V.J. and M. Ngouajio. 2011. Brassicaceae cover crops affect seed germination and seedling establishment in cucurbit crops. HortTechnology 215:525–532, https://doi.org/10.21273/HORTTECH.21.5.525.

Agarwal, P., E.A. Lehnhoff, R.L. Steiner, and O.J. Idowu. 2022a. Cover crops enhance soil properties in arid agroecosytem despite limited irrigation. Agronomy (Basel) 12:1235, https://doi.org/10.3390/agronomy12051235.

Agarwal, P., B.J. Schutte, O.J. Idowu, R.L. Steiner, and E.A. Lehnhoff. 2022b. Weed suppression versus water use: Efficacy of cover crops in water-limited agroecosystems. Weed Res. 62:24–37, https://doi.org/10.1111/wre.12519.

Al-Khatib, K., C. Libbey, and R. Boydston. 1997. Weed suppression with *Brassica* green manure crops in green pea. Weed Sci. 45:439–445, https://doi.org/10.1017/s0043174500093139.

Angelini, L., L. Lazzeri, S. Galletti, A. Cozzani, M. Macchia, and S. Palmieri. 1998. Antigerminative activity of three glucosinolate-derived products generated by myrosinase hydrolysis. Seed Sci. Technol. 26:771–779.

Antosh, E., J. Idowu, B. Schutte, and E. Lehnhoff. 2020. Winter cover crops effects on soil properties and sweet corn yield in semiarid irrigated systems. Agron. J. 112:92–106, https://doi.org/10.1002/agj2.20055.

Association of Official Seed Analysts. 2000. Tetrazolium testing handbook, contribution no. 29 to the handbook on seed testing (1st ed). Association of Official Seed Analysts, Stillwater, OK, USA.

Bangarwa, S.K., J.K. Norsworthy, J.D. Mattice, and E.E. Gbur. 2011. Glucosinolate and isothiocyanate production from Brassicaceae cover crops in a plasticulture production system. Weed Sci. 59:247–254, https://doi.org/10.1614/ws-d-10-00137.1.

Björkman, T., C. Lowry, J.W. Shail, D.C. Brainard, D.S. Anderson, and J.B. Masiunas. 2015. Mustard cover crops for biomass production and weed suppression in the Great Lakes region. Agron. J. 107:1235–1249, https://doi.org/10.2134/agronj14.0461.

Borza, J.K., P.R. Westerman, and M. Liebman. 2007. Comparing estimates of seed viability in three foxtail (*Setaria*) species using the imbibed seed crush test with and without additional tetrazolium testing. Weed Technol. 21:518–522, https://doi.org/10.1614/WT-06-110.

Boydston, R.A. and A. Hang. 1995. Rapeseed (*Brassica napus*) green manure crop suppresses weeds in potato (*Solanum tuberosum*). Weed Technol. 9:669–675, https://doi.org/10.1017/S0890037X00024039.

Brennan, E.B., N.S. Boyd, and R.F. Smith. 2013. Winter cover crop seeding rate and variety effects during eight years of organic vegetables: III. Cover crop residue quality and nitrogen mineralization. Agron. J. 105:171–182, https://doi.org/10.2134/agronj2012.0258.

Brennan, E.B. and R.F. Smith. 2005. Winter cover crop growth and weed suppression on the central coast of California. Weed Technol. 19:1017–1024, https://doi.org/10.1614/wt-04-246r1.1.

Clark, A. 2007. Managing cover crops profitably (3rd ed). Sustainable Agriculture Research and Education handbook series book 9. U.S. Department of Agriculture, College Park, MD, USA.

Creamer, R.J., J. Carpenter, and J. Rascon. 2003. Incidence of the beet leafhopper, *Circulifer tenellus* (Homoptera: Cicadellidae) in New Mexico chile. Southwestern Entomologist 28:177–182.

Creamer, R., H. Hubble, and A. Lewis. 2005. Curtovirus infection of chile pepper in New Mexico. Plant Dis. 89:480–486, https://doi.org/10.1094/pd-89-0480.

Davis, A.S., X.H. Fu, B.J. Schutte, M.A. Berhow, and J.W. Dalling. 2016. Interspecific variation in persistence of buried weed seeds follows trade-offs among physiological, chemical, and physical defenses. Ecol. Evol. 6:6836–6845, https://doi.org/10.1002/ece3.2415.

Gimsing, A.L. and J.A. Kirkegaard. 2006. Glucosinolate and isothiocyanate concentration in soil following incorporation of *Brassica* biofumigants. Soil Biol. Biochem. 38:2255–2264, https://doi.org/10.1016/j.soilbio.2006.01.024.

Goldberg NP. 2010. Verticillium wilt of chile peppers. Coop. Ext. Serv. College Agric. Environ. Sci. New Mex. State Univ. Guide H-250.

Hansen, Z.R. and A.P. Keinath. 2013. Increased pepper yields following incorporation of biofumigation cover crops and the effects on soilborne pathogen populations and pepper diseases. Appl. Soil Ecol. 63:67–77, https://doi.org/10.1016/j.apsoil.2012.09.007.

Haramoto, E.R. and E.R. Gallandt. 2004. *Brassica* cover cropping for weed management: A review. Renew. Agric. Food Syst. 19:187–198, https://doi.org/10.1079/rafs200490.

Haramoto, E.R. and E.R. Gallandt. 2005. *Brassica* cover cropping: I. Effects on weed and crop establishment. Weed Sci. 53:695–701, https://doi.org/10.1614/WS-04-162R.1.

High Performance Seeds, Inc. 2022. Mustards and arugula blends. https://www.hpseeds.com/products. [accessed 26 Jul 2022].

Khan, A.M., A. Mobli, J.A. Werth, and B.S. Chauhan. 2022. Germination and seed persistence of *Amaranthus retroflexus* and *Amaranthus viridis*: Two emerging weeds in Australian cotton and other summer crops. PLoS One 17:e0263798, https://doi.org/10.1371/journal.pone.0263798.

Korres, N.E., J.K. Norsworthy, B.G. Young, D.B. Reynolds, W.G. Johnson, S.P. Conley, R.J. Smeda, T.C. Mueller, D.J. Spaunhorst, K.L. Gage, M. Loux, G.R. Kruger, and M.V. Bagavathiannan. 2018. Seedbank persistence of Palmer amaranth (*Amaranthus palmeri*) and waterhemp (*Amaranthus tuberculatus*) across diverse geographical regions in the United States. Weed Sci. 66:446–456, https://doi.org/10.1017/wsc.2018.27.

Krishnan, G., D.L. Holshouser, and S.L. Nissen. 1998. Weed control in soybean (*Glycine max*) with green manure crops. Weed Technol. 12:97–102.

Larkin, R.P. and T.S. Griffin. 2007. Control of soilborne potato diseases using *Brassica* green manures. Crop Prot. 26:1067–1077, https://doi.org/10.1016/j.cropro.2006.10.004.

Lawley, Y.E., J.R. Teasdale, and R.R. Weil. 2012. The mechanism for weed suppression by a forage radish cover crop. Agron. J. 104:205–214, https://doi.org/10.2134/agronj2011.0128.

Leblova, S. and J. Kostir. 1962. Action of isothiocyanates on germinating plants. Experientia 18:554–555, https://doi.org/10.1007/bf02172173.

Liebman, M. and A.S. Davis. 2000. Integration of soil, crop and weed management in low-external-input farming systems. Weed Res. 40:27–47.

Liu, J., X. Wang, W. Fang, D. Yan, D. Han, B. Huang, Y. Zhang, Y. Li, C. Ouyang, A. Cao, and Q. Wang. 2020. Soil properties, presence of microorganisms, application dose, soil moisture and temperature influence the degradation of allyl isothiocyanate in soil. Chemosphere 224:125540, https://doi.org/10.1016/j.chemosphere.2019.125540.

Matthiessen, J.N. and J.A. Kirkegaard. 2006. Biofumigation and enhanced biodegradation: Opportunity and challenge in soilborne pest and disease management. Crit. Rev. Plant Sci. 25:235–265, https://doi.org/10.1080/07352680600611543.

McGuire, A. 2016. Using green manures in potato cropping systems. Washington State Univ. Bull. FS218E. http://pubs.cahnrs.wsu.edu/publications/wp-content/uploads/sites/2/publications/FS218E.pdf. [accessed 13 May 2022].

Melakeberhan, H., A. Xu, A. Kravchenko, S. Mennan, and E. Riga. 2006. Potential use of arugula (*Eruca sativa* L.) as a trap crop for *Meloidogyne hapla*. Nematology 8:793–799, https://doi.org/10.1163/156854106778877884.

Motisi, N., F. Montfort, V. Faloya, P. Lucas, and T. Doré. 2009. Growing *Brassica juncea* as a cover crop, then incorporating its residues provide complementary control of Rhizoctonia root rot of sugar beet. Field Crops Res. 113:238–245, https://doi.org/10.1016/j.fcr.2009.05.011.

Neubauer, C., B. Heitmann, and C. Muller. 2014. Biofumigation potential of Brassicaceae cultivars to *Verticillium dahliae*. Eur. J. Plant Pathol. 140:341–352, https://doi.org/10.1007/s10658-014-0467-9.

Norsworthy, J.K., M.S. Malik, P. Jha, and M.B. Riley. 2007. Suppression of *Digitaria sanguinalis* and *Amaranthus palmeri* using autumn-sown glucosinolate-producing cover crops in organically grown bell pepper. Weed Res. 47:425–432, https://doi.org/10.1111/j.1365-3180.2007.00586.x.

Norsworthy, J.K. and J.T. Meehan. 2005. Use of isothiocyanates for suppression of Palmer amaranth (*Amaranthus palmeri*), pitted morningglory (*Ipomoea lacunosa*), and yellow nutsedge (*Cyperus esculentus*). Weed Sci. 53:884–890, https://doi.org/10.1614/ws-05-056r.1.

Osipitan, O.A., J.A. Dille, Y. Assefa, and S.Z. Knezevic. 2018. Cover crop for early season weed suppression in crops: Systematic review and meta-analysis. Agron. J. 110:2211–2221, https://doi.org/10.2134/agronj2017.12.0752.

Petersen, J., R. Belz, F. Walker, and K. Hurle. 2001. Weed suppression by release of isothiocyanates from turnip-rape mulch. Agron. J. 93:37–43, https://doi.org/10.2134/agronj2001.93137x.

Rask, L., E. Andreasson, B. Ekbom, S. Eriksson, B. Pontoppidan, and J. Meijer. 2000. Myrosinase: Gene family evolution and herbivore defense in Brassicaceae. Plant Mol. Biol. 42:93–113, https://doi.org/10.1023/a:1006380021658.

Rudolph, R.E.C., C. Sams, R. Steiner, S.H. Thomas, S. Walker, and M.E. Uchanski. 2015. Biofumigation performance of four *Brassica* crops in a green chile pepper (*Capsicum annuum*) rotation system in southern New Mexico. HortScience 50:247–253, https://doi.org/10.21273/HORTSCI.50.2.247.

Sanogo, S. and J. Carpenter. 2006. Incidence of Phytophthora blight and Verticillium wilt within chile pepper fields in New Mexico. Plant Dis. 90:291–296, https://doi.org/10.1094/PD-90-0291.

Subbarao, K.V. and J.C. Hubbard. 1996. Interactive effects of broccoli residue and temperature on *Verticillium dahliae* microsclerotia in soil and on wilt in cauliflower. Phytopathology 86:1303–1310.

Teasdale, J.R. 1996. Contribution of cover crops to weed management in sustainable agricultural systems. J. Prod. Agric. 9: 475–479, https://doi.org/10.2134/jpa1996.0475.

Teasdale, J.R. and R.B. Taylorson. 1986. Weed seed responses to methyl isothiocyanate and metham. Weed Sci. 34:520–524, https://doi.org/10.1017/S0043174500067357.

Wang, G.Y., M. Ngouajio, and K.S. Charles. 2010. *Brassica* biofumigants improve onion (*Allium cepa* L.) and celery (*Apium graveolens*) production systems. J. Sustain. Agric. 34:2–14, https://doi.org/10.1080/10440040903396516.

Wen, L., S. Lee-Marzano, L.M. Ortiz-Ribbing, J. Gruver, G.L. Hartman, and D.M. Eastburn. 2017. Suppression of soilborne diseases of soybean with cover crops. Plant Dis. 101:1918–1928, https://doi.org/10.1094/pdis-07-16-1067-re.

Weinert, T.L., W.L. Pan, M.R. Moneymaker, G.S. Santo, and R.G. Stevens. 2002. Nitrogen recycling by nonleguminous winter cover crops to reduce leaching in potato rotations. Agron. J. 94:365–372, https://doi.org/10.2134/agronj2002.3650.