Author for correspondence:
Brian J. Schutte, Associate Professor, Department of Entomology, Plant Pathology and Weed Science; New Mexico State University, 945 College Avenue, Las Cruces, NM 88003.
Email: bschutte@nmsu.edu

Water availability influences the inhibitory effects of mustard seed meal on Palmer amaranth (*Amaranthus palmeri*) and *Verticillium dahliae*

Joseph B. Wood[1], Brian J. Schutte[2], Ivette Guzman[3] and Soum Sanogo[4]

[1]Graduate Student; Department of Entomology, Plant Pathology and Weed Science; New Mexico State University, Las Cruces, NM, USA; [2]Associate Professor, Department of Entomology, Plant Pathology and Weed Science; New Mexico State University, Las Cruces, NM, USA; [3]Assistant Professor, Department of Plant and Environmental Sciences; New Mexico State University, Las Cruces, NM, USA and [4]Professor; Department of Entomology, Plant Pathology and Weed Science; New Mexico State University, Las Cruces, NM, USA

Abstract

Palmer amaranth, an annual weed, and *Verticillium dahliae*, a fungal pathogen, can substantially reduce chile pepper yield. On the basis of the results of this study, we clarified implementation strategies for a potential management tactic for Palmer amaranth and *V. dahliae* in chile pepper: mustard seed meal (MSM). The objectives were to (1) determine MSM effects on Palmer amaranth seedbanks under different moisture levels, (2) measure glucosinolate degradation in soil hydrated to saturation and field capacity, and (3) determine the effects of decreasing moisture availability on MSM control of Palmer amaranth and *V. dahliae*. To address objective 1, seedbanks with and without MSM were hydrated to levels expected to both inhibit and promote germination (flooded, saturated, −0.03, −0.6 MPa, respectively). For objective 2, soil columns with MSM were held at different moisture levels and sampled over time. For objective 3, Palmer amaranth seeds were incubated with and without MSM, and with polyethylene glycol (PEG) solutions comprising a range of water potentials (0, −0.03, −0.6, −1.0, and −2.0 MPa). These PEG solutions were also used to hydrate MSM in agar plates with plugs of *V. dahliae*. All experiments were performed in growth chambers with temperatures and light conditions conducive to Palmer amaranth germination and *V. dahliae* mycelial growth. MSM-induced mortality in Palmer amaranth seedbanks was greater in soil at field capacity than in saturated soil and flooded soil; however, rates of glucosinolate degradation were greatest in saturated soil. Decreasing water availability progressively decreased the efficacy of MSM on Palmer amaranth because MSM was ineffective on nongerminated seeds. When incubated with PEG solutions with water potentials of 0, −0.03, and −0.6 MPa, MSM stopped growth of *V. dahliae*; however, MSM-induced control of *V. dahliae* was reduced by water potentials of −1.0 and −2.0 MPa. The results of this study indicate soils hydrated to field capacity maximize MSM-induced control of Palmer amaranth and *V. dahliae*.

Introduction

Mustard seed meal (MSM) is the solid residue of rape (*Brassica napus* L.), brown mustard [*B. juncea* (L.) Czern] or white mustard (*Sinapis alba* L.) seeds after they have been pressed for oil. When MSM is incorporated into soil that is subsequently hydrated, MSM releases chemicals that have microbicidal, insecticidal (Brown et al. 1991; Delaquis and Sholberg 1997; Dufour et al. 2015; Sarwar et al. 1998; Smolińska et al. 1997), herbicidal (Boydston et al. 2011; Rice et al. 2007; Vaughn et al. 2006), and nematicidal (Donkin et al. 1995) properties. Thus, MSM is a promising tool to simultaneously manage weeds, insect pests, and soilborne diseases that adversely affect crop production.

Pest suppression from MSM is caused by the enzymatic breakdown of glucosinolates, which are a class of secondary metabolites found in most plants in the Brassicaceae (Rask et al. 2000). Hydrolysis of glucosinolates by the enzyme myrosinase (thioglucoside glucohydrolase E.C. 3.2.3.1) releases pesticidal, volatile chemicals, including isothiocyanates and thiocyanates. Enzymatic catalysis of glucosinolates is greater in a waterlogged soil (i.e., soil with a thin film of water on the surface) compared with soil hydrated to field capacity (at water potential of −0.03 MPa) (Morra and Kirkegaard 2002). Although waterlogging promotes glucosinolate hydrolysis, the pesticidal efficacy of MSM may not be greater in waterlogged soils than in soils at lower levels of hydration. This is because for MSM to control pests effectively, hydrolysis

of glucosinolates must take place when pests are at life stages susceptible to glucosinolate breakdown products (De Cauwer et al. 2019).

Seed dormancy is a critical stage in the life cycle of many weeds because it inhibits germination under conditions otherwise suitable for germination and promotes seed persistence in soil (Vleeshouwers et al. 1995). For small-seeded weeds, susceptibility to MSM increases as the percentage of dormant seeds in a population decreases (Lefebvre et al. 2018). This is consistent with previous research that indicated glucosinolate degradation products at low concentrations prevent radicle protrusion but do not directly kill seeds (Angelini et al. 1998; Leblová-Svobodová and Koštíř 1962; Peterson et al. 2001). If dormant or nongerminable seeds are not susceptible to glucosinolate degradation products, soil moisture conditions that inhibit germination but promote enzymatic hydrolysis of glucosinolates would negate the efficacy of MSM. Although this hypothesis is compelling, glucosinolate hydrolysis and MSM pesticidal activity have not been studied across soil moisture levels that differ in their ability to stimulate or inhibit weed seed germination, to our knowledge. Clarifying relationships among soil moisture levels, rates of glucosinolate hydrolysis, and MSM pesticidal activity will support the development of sustainable strategies for irrigations that occur immediately after the incorporation of MSM into soil.

Chile pepper is an important, irrigated crop in the U.S. Southwest (Bosland 2015). However, chile pepper can be difficult to grow because it is susceptible to weeds (Amador-Ramirez 2002) and soilborne diseases (Sanogo 2003). Consequently, chile pepper production in the U.S. Southwest will benefit from novel management tactics that simultaneously target weeds and soilborne diseases.

Palmer amaranth is a common, summer annual weed in chile pepper fields in the U.S. Southwest (Skaggs et al 2000) and can substantially reduce chile pepper yield (Schroeder 1992). It can grow 5 to 7 cm d^{-1}, making Palmer amaranth an aggressive competitor for light, water, and nutrient resources (Ehleringer 1983). Palmer amaranth seeds are small (1 to 2 mm diameter) and capable of persistence under waterlogged conditions (Schutte et al. 2016). In the absence of competition, Palmer amaranth plants can produce 200,000 to 600,000 seeds plant^{-1} (Keeley et al. 1987), and plants growing in competition with crops can produce between 1,800 and 91,000 seeds plant^{-1} (Massinga et al. 2001). Although Palmer amaranth is a highly competitive weed with prolific reproductive capacity, its emergence is suppressed by products of glucosinolate hydrolysis (Norsworthy and Meehan 2005).

Verticillium wilt (caused by *Verticillium dahliae*) is a common soilborne disease in chile pepper in the U.S. Southwest (Sanogo and Carpenter 2006; Skaggs et al. 2000). Current strategies for managing soilborne diseases include the use of chemical soil fumigants and biofungicides in combination with cultural tactics including crop rotation and soil-water management to avoid prolonged saturation. These strategies do not always adequately reduce soilborne diseases, and as a result, Verticillium wilt can cause substantial yield loss in chile pepper fields. MSM treatments inhibit *V. dahliae* growth and reduce Verticillium wilt disease incidence (Neubauer et al. 2015; Smolińska et al. 2010). Thus, MSM amendments may be an additional effective tool for managing Verticillium wilt in chile pepper.

Seeking information to guide irrigation after MSM amendments for chile pepper, we conducted experiments to determine if waterlogged soils lead to ineffective MSM applications by inhibiting weed seed germination but promoting glucosinolate hydrolysis. We also conducted experiments to determine if decreasing soil moisture inhibits herbicidal and fungicidal activities of MSM. The objectives of this study were to (1) determine the effects of MSM on Palmer amaranth soil seedbanks under high soil moisture, (2) measure glucosinolate degradation in soil hydrated to saturation and field capacity, and (3) determine the effects of decreasing moisture availability on MSM control of Palmer amaranth and *V. dahliae*.

Materials and Methods

Biological Materials

MSM derived from brown mustard seed was purchased from a commercial supplier in September 2017 (Pescadero Gold; Farm Fuel Inc. Watsonville, CA). Experiments were performed from September 2018 through March 2019. When not in use, MSM was stored in airtight plastic bags at room temperature. The primary glucosinolate in brown mustard seed meal is sinigrin (Rice et al. 2007; Rothlisberger et al. 2012). Just prior to the beginning of the experiment, the sinigrin concentration of MSM was 168 μmol g^{-1}. This was determined using gas chromatography and mass spectrometry (Heaney et al. 1988).

Seeds of Palmer amaranth were collected from the New Mexico State University (NMSU), Leyendecker Plant Science Research Center (32.198°N, 106.748°W) in September 2017. Mature Palmer amaranth inflorescences were clipped from plants, removed from the field, and hand-thrashed with sequential combinations of sieving and forced-air separation. Collected seeds were stored in an airtight container at 4 C.

V. dahliae was isolated from chile pepper plants grown on a farm located in Doña Ana County, New Mexico (32.341°N, 106.824°W). *V. dahliae* cultures were maintained on Czapek-Dox agar (CDA) at room temperature. CDA was prepared using a method adapted from Hawke and Lazarovits (1994). Specifically, 35 g of CDA broth (Sigma-Aldrich, St. Louis, MO) was mixed with 15 g of agar, 1 mL of streptomycin sulfate (50 mg L^{-1}), and 1 L of distilled water. This CDA solution was autoclaved and poured into Petri dishes (100 × 15 mm).

MSM Effects on Palmer Amaranth Soil Seedbanks Under High Moisture

The effects of MSM rate (0 and 4,400 kg ha^{-1}) and soil moisture level (flooded, −0.03 MPa, and saturated, −0.6 MPa) combinations on Palmer amaranth seed survival were examined in a completely randomized, factorially designed experiment with four replicates. This experiment was conducted twice.

Experimental units were seedbank mesocosms designed to maintain a desired soil water potential. Mesocosms (herein called "cores") were constructed with polyvinyl chloride (PVC) pipes (6-cm diameter, 7-cm height). To one end of each pipe, cheesecloth (grade 40) was attached to hold soil but allow for subirrigation. A Vinton sandy loam soil (Thermic Typic Torrifluvents, pH 7.9, 1.6% organic matter) collected from the NMSU Los Lunas Agricultural Science Center (34.766°N, 106.762°W) was the medium for the experiment. This soil was passed through a 2-mm sieve to remove all rocks and debris before being used to fill the cores. For MSM-treated cores, soil was mixed with MSM at a rate equivalent to 4,400 kg ha^{-1}, which is an application rate recommended by the supplier and suppresses soil pathogens (Wang and Mazzola 2019). Control cores contained no MSM.

Palmer amaranth was seeded in each core at a depth of 1 cm to create a density of 50 seeds per seedbank.

All cores were subirrigated to saturation. Then, for flooded cores, additional water was added to create 1 cm of standing water on the soil surface. For −0.03 and −0.6 MPa cores, saturated cores were dried to the weight corresponding with the desired soil water potential. Drying requirements for different soil-water potentials were established using a function for the relationship between soil gravimetric moisture content and soil-water potential. This function was developed using a soil pressure-plate extractor to establish a calibration curve for soil-water potential (Dane et al 2002). Once desired moisture levels were reached, the cores were wrapped with a single layer of low-density polyethylene film (Glad Cling Wrap; The Glad Products Company, Oakland, CA). This seedbank study system maintains desired soil moisture levels for prolonged periods (Schutte et al. 2016).

Hydrated cores were placed in a growth chamber set to 35 C day/25 C night and a 12-hr photoperiod—conditions favorable for germination of Palmer amaranth (Willis et al. 2014). After 10 d, the cores were unwrapped and all emerged seedlings were counted. The soil from each core was then individually sieved using a 500-μm sieve to collect all nongerminated Palmer amaranth seeds. Recovered seeds were assayed for viability using a 0.6% aqueous solution of 2,3,5-triphenyl-tetrazolium chloride (Peters 2000). Viable seeds were classified as "persistent." Rates of persistence were expressed as percentages of seeds buried.

The number of seeds and/or pre-emergent seedlings terminated by MSM (hereafter, "MSM-induced mortality") was determined for every replication and moisture level, using Equation 1:

$$\text{MSM} - \text{induced mortality}_{rl} = 100 \times \frac{[(E_{crl} + V_{crl}) - (E_{mrl} + V_{mrl})]}{50} \quad [1]$$

where E_{crl} is the number of emerged seedlings in cores without MSM for replicate r and moisture level l, V_{crl} is the number of viable seeds recovered from cores without MSM for replicate r and moisture level l, E_{mrl} is the number of emerged seedlings in cores with MSM for replicate r and moisture level l, and V_{mrl} is the number of viable seeds recovered from cores with MSM for replicate r and moisture level l.

Data analyses were performed with the statistical software R, version 3.5.2 (The R Foundation for Statistical Computing, http://www.r-project.org). Levene tests for homogeneity of variances, performed with the R library *car*, indicated equal variances among runs. Accordingly, data from runs were combined. Generalized linear mixed models for the binary response variables MSM-induced mortality and seed persistence were developed using the R library *lme4*. In these models, fixed effects included moisture level, and random effects included run and replication within each run. The R library *multcomp* was used to generate Tukey all-pairs comparisons for the factor "moisture level."

Glucosinolate Degradation in Soil Hydrated to Saturation and Field Capacity

In a completely randomized experiment that was conducted twice, the effect of factorial combinations of three soil moisture levels (i.e., saturated, field capacity [−0.03 MPa], and dry) and two MSM levels (i.e., 0 and 20% [wt/wt]) on glucosinolate degradation was assessed.

Experimental units were cores similar to those described for objective 1. The medium for cores was a Vinton sandy loam soil (Thermic Typic Torrifluvents, pH 7.9, 1.6% organic matter) collected from the NMSU Los Lunas Agricultural Science Center. Soil was passed through a 2-mm sieve; mixed with MSM, if required; and poured into PVC pipes (6-cm diameter, 7-cm tall). Each core contained 100 g of soil and, if needed, 20 g of MSM. Cores were subirrigated to saturation and then dried to the weight corresponding to desired soil-water potential. Dry cores were not subirrigated. Once desired moisture levels were reached, the cores were wrapped with a single layer of low-density polyethylene film and placed in a growth chamber set to 30 C.

Cores were destructively sampled at 0, 3, 6, 9, 12, 24, and 48 h after reaching the desired moisture level. Samples were collected using a method adapted from Doheny-Adams et al. (2017). Specifically, soil was sampled from the middle of each core using a hole borer to extract 1 cm³ of soil. Then, soil samples were placed into 2-mL Eppendorf tubes, immediately flash-frozen in liquid nitrogen, and then mixed with cold (−20 C) 80% methanol. Once the methanol was added, samples were vortexed for 1 min to break up soil pellets. The samples were then placed in a refrigerator at 4 C for 30 min. Afterward, the samples were vortexed for 30 sec and then stored at 4 C for another 30 min. Samples were then centrifuged at 8,000 rpm for 10 min. Supernatants were filtered through 0.20-μm syringe filters and transferred into 2-mL high-performance liquid chromatography (HPLC) vials. These vials were stored at −20 C for 2 to 52 h.

The HPLC analyses were conducted using an Agilent 1100 series (Agilent Technologies, Santa Clara, CA) with a Zorbax column (C18; 4.6 × 100 mm, 3.5 μm). HPLC methods for quantifying sinigrin followed those of Doheny-Adams et al. (2017). Solvents were 0.02 M tetrabutylammonium bromide (A) and a 70:30 mixture of tetrabutylammonium bromide and acetonitrile (B). The initial concentration started at 0% B and increased to 100% B over 30 min, holding for 5 min, and then returning to the initial concentration for another 10 min. Injection volume was 5.0 μL and flow rate was set to 1 mL min⁻¹. The column temperature was maintained at 25 C, and detection was conducted using a photodiode array detector set to record at 229 nm. Agilent software ChemStation (V.B.04.01) was used to record all data. Sinigrin standards (Sigma-Aldrich) of 1.25, 2.5, 5, 10, and 20 mM were prepared in triplicate and analyzed with the extracted samples.

Sinigrin concentrations for each moisture level and run were expressed as proportions of concentrations at 0 h. Mean concentrations were plotted as functions of incubation time, and SigmaPlot (Systat Software, Inc. San Jose, CA) was used to fit two-parameter, single exponential decay curves to the data. To test the null hypotheses that regressions for different runs and moisture levels estimated the same population regression model, we used the following F test (Equation 2) for coincidental regression (Zar 1999):

$$F = \frac{\frac{SS_t - SS_p}{(m+1) - (k-1)}}{\frac{SS_p}{DF_p}} \quad [2]$$

where SS_t is the total residual sums of squares of the combined data sets, SS_p is the pooled residual sums of squares, m is the number of independent variables, k is the number of regressions being compared, and DF_p is the pooled degrees of freedom of the individual regressions.

Decreasing Moisture Availability Effects on MSM Control of Palmer Amaranth and *V. dahlia*

In this experiment, we used germination assays conducted in Petri plates (100 × 15 mm). Water potentials for germination assay solutions were manipulated with polyethylene glycol 8000 (PEG) following methods described by Steuter et al. (1981). This experiment was conducted twice; on each occasion, a completely randomized design with four replicates was used. Treatments were factorial combinations of MSM amount (0, 1.2, and 2.4 g MSM, equivalent to 0, 2,200, and 4,400 kg ha^{-1}, respectively) and germination-assay water potential (0, −0.03, −0.6, −1.0, and −2.0 MPa).

Petri plates were first filled with the appropriate amount of MSM. Control dishes contained no MSM. A blotter paper (Anchor Paper, St. Paul MN) was then placed on top of the MSM and saturated with 5 mL of water or PEG solution. Fifty Palmer amaranth seeds were placed on each blotter paper in a grid pattern. Petri dishes were then sealed with Parafilm (Bemis Co., Neenah, WI) and placed in a growth chamber at 30 C. A steady, rather than fluctuating, temperature was used to maintain constant water potential of the PEG solutions (Steuter et al. 1981). After 21 d, the Petri dishes were unwrapped and all germinated seeds were counted. All nongerminated seeds were tested for viability using the tetrazolium chloride stain test (Peters 2000). Pesticidal activity potential for Palmer amaranth (PAP_{Palmer}) was determined using Equation 3:

$$PAP_{Palmer} = 100 \times \left(\frac{[G_{crl} − G_{mrl}]}{G_{cr0MPa}} \right) \quad [3]$$

where G_{crl} is the number of germinated seeds in plates without MSM for replicate r and water potential l, G_{mrl} is the number of germinated seeds in plates with MSM for replicate r and water potential l, and G_{cr0MPA} is the number of germinated seeds in plates without MSM and the water potential treatment 0 MPa for replicate r.

Because germination assay solutions of varying water potential were in contact with Palmer amaranth seeds and MSM, PAP_{Palmer} results indicated the effects of moisture availability on both Palmer amaranth seed germination and MSM hydrolysis. To understand how decreasing moisture affects only hydrolysis of MSM, plugs of *V. dahlia* were plated on CDA and suspended over mixtures of MSM and different PEG solutions. Specifically, CDA plates (described under *Biological Materials*) were inoculated with a 1-cm diameter plug of *V. dahliae* and then inverted over a container with MSM and 50 mL of PEG solution or water. Containers were sealed with Parafilm and placed in an incubator at 25 C with no supplemental lighting. This experiment was conducted twice. For each run, the experimental design was a completely randomized design with four replications. Treatments were factorial combinations of MSM amount (0, 1.2, and 2.4 g MSM; the last two equivalent to 2,200 and 4,400 kg MSM ha^{-1}, respectively) and MSM solution water potential (0, −0.03, −0.6, −1.0, and −2.0 MPa).

Mycelial growth of *V. dahliae* was measured at 14 d after the start of incubation over MSM and treatment solutions. Pesticidal activity potential for *V. dahliae* (PAP_{Vert}) was determined by Equation 4:

$$PAP_{Vert} = 100 \times \left(\frac{[D_{crl} − D_{mrl}]}{D_{crl}} \right) \quad [4]$$

where D_{crl} is the growth across the diameter of *V. dahliae* mycelia in Petri plates suspended above solutions without MSM for replicate

r and water potential l, and D_{mrl} is the diameter growth of *V. dahliae* mycelia in Petri plates suspended above solutions with MSM for replicate r and water potential l.

Levene tests for homogeneity of variances indicated equal variances among runs. However, among water potential treatments, PAP_{Palmer} and PAP_{Vert} data severely violated the assumption of variance homogeneity. Data transformations did not sufficiently improve variance homogeneity. Therefore, these data were analyzed with nonparametric procedures using the R library *PMCMRPlus*. Friedman tests with post hoc Conover tests were conducted to compare PAP_{Palmer} and PAP_{Vert} among moisture levels.

Results and Discussion

MSM Effects on Palmer Amaranth Soil Seedbanks Under High Moisture

MSM-induced mortality in Palmer amaranth seedbanks was 17% greater in soil hydrated to field capacity than in saturated soil and flooded soil (Figure 1). Low rates of MSM-induced mortality in saturated and flooded soils coincided with higher rates of seed persistence, compared with soil at field capacity (Table 1). High rates of persistence in flooded and saturated soils were consistent with previous research that found increased persistence of Palmer amaranth seedbanks under saturated and flooded conditions (Schutte et al. 2016). MSM did not influence rates of emergence (Table 1), which were conditioned by soil moisture level. Seedbanks hydrated to −0.03 and −0.6 MPa generally produced more seedlings than flooded and saturated seedbanks. Although MSM did not influence emergence, MSM did reduce persistence in seedbanks hydrated to −0.03 MPa and −0.6 MPa.

When saturated or flooded, seedbanks, as used in this study, have reduced levels of oxygen (Schutte et al. 2016), which can induce secondary dormancy in seeds of many weed species (Baskin and Baskin 2014). Dormant seeds are less vulnerable to MSM because germination facilitates MSM-induced mortality (Lefebvre et al. 2018). In our study, low levels of MSM-induced mortality and high levels of persistence in saturated and flooded soil were likely caused by inhibited germination resulting from reduced oxygen availability.

Germination promotes MSM-induced mortality because the final stage of germination (i.e., radicle protrusion) removes the physical barrier of the seed coat and potentially allows isothiocyanate to infiltrate the seed. Isothiocyanates readily react with thiols and amines (Dufour et al. 2015), as well as cysteine residues to inhibit adenosine triphosphate binding sites (Breier and Ziegelhoffer 2000). High concentrations of isothiocyanates stall the metabolic processes associated with seedling growth (Angelini et al. 1998; Leblová-Svobodová and Koštíř 1962), leaving the seedling susceptible to degradation from soil microbes (Dalling et al. 2011). Moisture levels that are optimum for germination differ among species (Werle et al. 2014) and can be conditioned by soil temperature (Kebreab and Murdoch 1999). Accordingly, soil moisture and temperature conditions that promote MSM effects on Palmer amaranth may not be the same for other weed species in the seedbank.

Sinigrin Degradation in Soil Hydrated to Saturation and Field Capacity

Dry soil amended with MSM contained 98% of initial sinigrin content after 48 h of incubation (data not shown), indicating that sinigrin was stable in dry soil. For saturated soil, the *F* test for coincidental regression indicated that individual regressions for
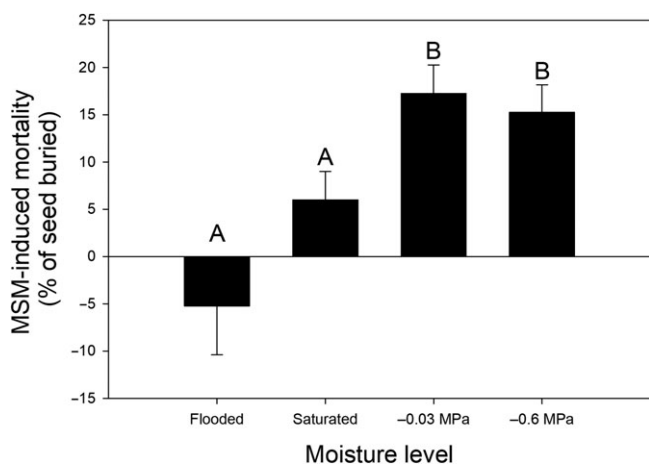
Table 1. Emergence and persistence percentages for Palmer amaranth seedbanks hydrated to specific moisture levels and subjected to MSM treatments.

MSM treatment[a]	Moisture level	Emergence	Persistence
		Mean (SE) (N = 8)[b]	
		--------------%--------------	
No MSM	Flooded	1.5 ± 1.05 a	70.0 ± 3.81 ab
	Saturated	0.3 ± 0.25 a	79.3 ± 2.27
	−0.03 MPa	9.8 ± 1.91 b	73.0 ± 2.78 ab
	−0.6 MPa	14.8 ± 2.95	67.5 ± 4.42 b
With MSM[a]	Flooded	2.3 ± 1.55 a	72.0 ± 3.21 ab
	Saturated	0.3 ± 0.25 a	75.3 ± 3.05 ab
	−0.03 MPa	11.5 ± 5.49 b	54.3 ± 6.25 c
	−0.6 MPa	12.7 ± 4.05 b	53.3 ± 4.46 c

[a]Abbreviation: MSM, mustard seed meal.
[b]Within a column, means with the same letter are not significantly different at P ≤ 0.05.
[c]MSM was added to soil at a rate equivalent to 4,400 kg ha^{-1}.

Table 2. Germination percentages for Palmer amaranth seeds in Petri plates with MSM and aqueous solutions with different water potentials.

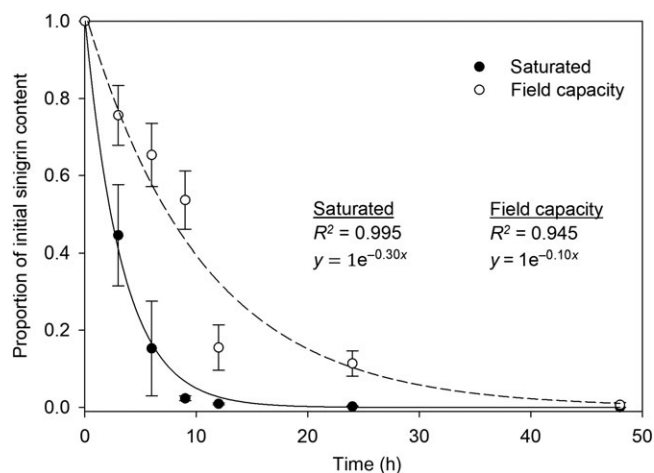| Germination-assay solution water potential[a]	Palmer amaranth germination[b,c,d]			
	No MSM	1.2 g MSM	2.4 g MSM	PAP$_{Palmer}$[e]
	-----------------%-----------------			%
0 MPa	16.3 ± 1.69 a	0	0	100.0 a
−0.03 MPa	11.5 ± 1.22 b	0	0	70.8 b
−0.6 MPa	7.8 ± 1.70 c	0	0	47.7 c
−1.0 MPa	3.8 ± 1.12 d	0	0	23.1 d
−2.0 MPa	0 ± 0 e	0	0	0.0 e

[a]Water potential treatments were created with polyethylene glycol solutions.
[b]Data are reported as mean ± SE (N = 8).
[c]Within a column, means with the same letter are not significantly different according to Conover test (α = 0.05).
[d]Abbreviations: MSM, mustard seed meal; PAP$_{Palmer}$, pesticidal activity potential for Palmer amaranth.
[e]Germination data were used to calculate the pesticidal activity potential for MSM on Palmer amaranth, PAP$_{Palmer}$ (Equation 3).

Figure 1. Mustard seed meal (MSM)-induced mortality in Palmer amaranth soil seedbanks hydrated to specific moisture levels and incubated for 14 d at 35 C/25 C day/night cycles with 12-h photoperiods. MSM was added to soil at a rate equivalent to 4,400 kg ha^{-1}. Bars are means with SEs (N = 8). Bars with the same letter are not different at P ≤ 0.05.

Figure 2. Exponential decay curves for sinigrin derived from mustard seed meal in soil hydrated to saturation (solid line) or field capacity (dashed line). Experimental units were incubated in a growth chamber at 30 C. Data points are means with SEs (N = 6).

experimental runs estimated the same population ($F_{2,10}$ = 1.75; P = 0.223), which meant there was no difference in the sinigrin decay curve between the two runs. Similarly, experimental run did not affect the decay curve for sinigrin in soil hydrated to field capacity ($F_{2,10}$ = 0.38; P = 0.693). Accordingly, and for both moisture treatments, data from the two runs were combined.

The F test for coincidental regression indicated that individual regressions for soil moisture treatments estimated different populations ($F_{2,10}$ = 28; P < 0.05); thus, decay curves for sinigrin were developed separately for saturated and field capacity soil. Saturated soil had a higher rate of sinigrin hydrolysis than soil at field capacity, as shown by a more rapid decrease in sinigrin concentration in saturated soil (Figure 2). Sinigrin was depleted after 24 h in saturated soil, whereas in field-capacity soil, sinigrin was depleted up to 24 h after that of the waterlogged soil. The breakdown of sinigrin corresponds with isothiocyanate production (Rask et al. 2000). Previous research indicated that isothiocyanate formation from MSM-treated soil was greater in waterlogged soil than in soil at field capacity (Morra and Kirkegaard 2002). Thus, the results from our study were consistent with those from previous research (Morra and Kirkegaard 2002).

Decreasing Moisture-Availability Effects on MSM Control of Palmer Amaranth and V. dahliae

Palmer amaranth seeds incubated in the presence of MSM failed to complete germination, whereas Palmer amaranth seeds without MSM completed germination at rates conditioned by the water potential of the germination assay solution (Table 2). At 0 MPa, 16% of Palmer amaranth seeds germinated, whereas at −1.0 MPa, only 4% of Palmer amaranth seeds germinated. At −2.0 MPa, no seeds germinated. Because fewer seeds germinated as available water diminished, PAP$_{Palmer}$ (Equation 3) decreased with decreasing water potential in the germination assay solution.

When incubated in PEG solutions with water potentials of 0 MPa, −0.03 MPa, and −0.6 MPa, both rates of MSM (1.2 g and 2.4 g MSM) inhibited growth of V. dahliae (Table 3; Figure 3). However, when incubated in PEG solutions with water potentials of −1.0 MPa and −2.0 MPa, MSM effects on V. dahliae growth were conditioned by MSM rate. For the PEG solution with the water potential −1.0 MPa, V. dahliae suspended above 1.2 g MSM grew 2.2 cm in diameter after 14 d of incubation, whereas V. dahliae suspended above 2.4 g MSM was unable to grow. For the PEG solution with the water potential of −2.0 MPa, V. dahliae suspended above 1.2 g

Table 3. Mycelial growth of *Verticillium dahliae* in Czapek-Dox agar after 14 d of incubation above factorial combinations of MSM amounts and MSM-solution water potentials.

MSM solution water potential (MPa)[a]	Mycelial growth of *V. dahlia*[b,c]			PAP_{Vert}[d]	
	No MSM	1.2 g MSM	2.4 g MSM	1.2 g MSM	2.4 g MSM
	————————————— cm —————————————			———————— % —————————	
0	4.93 ± 0.233 b	0 a	0 a	100.0 a	100.0 a
−0.03	4.99 ± 0.204 b	0 a	0 a	100.0 a	100.0 a
−0.6	5.20 ± 0.132 b	0 a	0 a	100.0 a	100.0 a
−1.0	4.93 ± 0.172 c	2.23 ± 0.357 b	0 a	54.8 b	100.0 a
−2.0	5.36 ± 0.222 c	3.01 ± 0.647 b	0.55 ± 0.328 a	43.8 b	89.7 b

[a]Abbreviations: MSM, mustard seed meal; PAP_{Vert}, pesticidal activity potential for *Verticillium dahlia*.
[b]Data are reported as mean ± SE (N = 8).
[c]Means within a column that share the same letter are not different at P ≤ 0.05.
[d]Mycelial growth data were used to calculate the pesticidal activity potential for MSM on *V. dahliae*, PAP_{Vert} (refer to Equation 4). PAP_{Vert} was separately calculated for 1.2-g and 2.4-g MSM treatments.
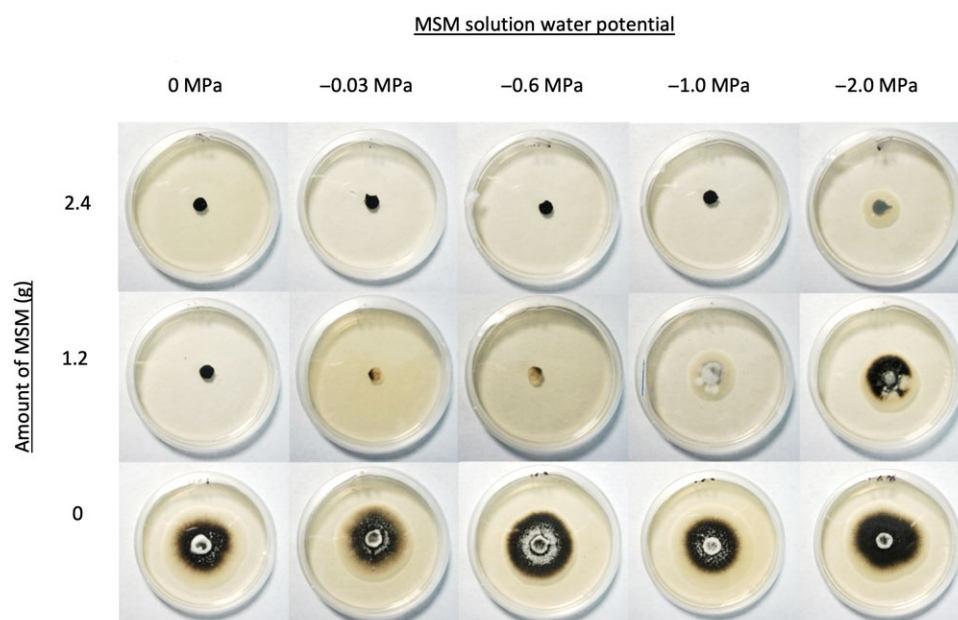
Figure 3. Images of *Verticillium dahliae* cultures after 14 d of incubation above factorial combinations of mustard seed meal (MSM) amounts and MSM-solution water potentials. At the beginning of the incubation period, cultures featured *V. dahlia* plugs (1-cm diameter) centrally located in Petri plates (inner diameter, 8.8 cm) with Czapek-Dox agar. *V. dahliae* plugs did not enlarge when incubated above 0, −0.03, and −0.6 MPa solutions containing 1.2 g MSM; and 0, −0.03, −0.6, and −1.0 MPa solutions containing 2.4 g of MSM.

MSM grew 3.0 cm in diameter, whereas *V. dahliae* suspended above 2.4 g MSM grew 0.6 cm in diameter. *V. dahliae* suspended above no MSM grew approximately 5 cm in diameter at all moisture levels. For each MSM rate, PAP_{Vert} decreased with decreased moisture availability. The decrease in PAP_{Vert} (Equation 4) was likely due to decreased sinigrin hydrolysis at lower moisture levels, which reduced isothiocyanate formation and allowed *V. dahliae* to grow. These results were consistent with previous research that determined isothiocyanate formation from MSM decreased as soil-water level decreased (Wang and Mazzola 2019).

Implications for Management

A previous field study determined preplant incorporated (PPI) applications of MSM reduced Palmer amaranth densities and hand-hoeing requirements during the first 4 wk of the chile pepper season (Wood 2019). After this period, there was no effect of PPI applications of MSM on weed densities and hoeing requirements. Accordingly, PPI applications of MSM are potential tactics for managing early-season weeds in chile pepper fields in the U.S. Southwest. However, for MSM to become a sustainable component of cropland pest management programs in this water-limited region, irrigations after MSM amendments must not reduce the pesticidal potential of MSM but maximize MSM-induced control of weeds and soilborne diseases.

Morra and Kirkegaard (2002) suggested irrigation as a method to reduce loss of volatile isothiocyanates from soil amended with brassicaceous plant residues. We propose that moisture conditions intended to trap isothiocyanates should also support germination and growth of targeted pests. By irrigating to create conditions that simultaneously enhance pest susceptibility to MSM and capture of pesticidal compounds derived from MSM, farmers can obtain maximum efficacies for MSM applications.

MSM control of Palmer amaranth is greatest in soil hydrated to field capacity and hindered in soil hydrated to only −1.0 MPa and saturated soil. MSM-induced reductions in weed emergence

depend on the concentration of isothiocyanate formed during the enzymatic catalysis of glucosinolates (Aliki et al. 2014). Enzymatic breakdown of glucosinolates requires a sufficient amount of water (Wang and Mazzola 2019). The results of this study, specifically the PAP_{Vert} results, indicate a water potential of −1.0 MPa inhibits the enzymatic breakdown of sinigrin and production of pesticidal compounds from MSM. A water potential of −1.0 MPa also inhibits Palmer amaranth germination, thereby reducing the potential for MSM to control this weed species. In saturated soil, the pesticidal properties of MSM are wasted because Palmer amaranth seed germination is inhibited, but gluocosinolates are rapidly and irreversibly degraded. Thus, saturated soils should be avoided after MSM amendments intended to target Palmer amaranth.

  Although the results of this study indicated hydration to field capacity is optimal for MSM-induced, PRE control of Palmer amaranth and *V. dahliae*, these results need to be validated in the field before recommendations can be made. In addition, more research is required to determine the minimum length of time soil needs to hydrate after MSM applications. Previous research determined that pesticidal compounds from degradation of sinigrin in soil reached maximum concentrations within 24 h of hydration, and subsequent declines of sinigrin-derived pesticidal compounds differed among soil types (Borek et al. 1994). Accordingly, additional research seeking to clarify minimum durations of hydration after MSM applications will need to account for the effects of soil type, as well as soil pH and soil temperature, which also influence enzymatic hydrolysis of sinigrin (Al-Turki and Dick 2003; Borek et al. 1994). Improved understanding of the relationships among soil characteristics, moisture, and persistence of sinigrin-derived pesticidal compounds will facilitate the development of models that allow farmers in the U.S. Southwest to use MSM without unnecessary irrigation, thereby conserving regional water resources.

References

Aliki HM, Reade JP, Back MA (2014) Effects of concentrations of *Brassica napus* (L.) water extracts on the germination and growth of weed species. Allelopathy J 34:287–297

Al-Turki A, Dick WA (2003) Myrosinase activity in soil. Soil Sci Soc Am J 67:139–145

Amador-Ramirez MD (2002) Critical period of weed control in transplanted chilli pepper. Weed Res 42:203–209

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

Baskin CC, Baskin JM (2014) Seeds: Ecology, Biogeography, and, Evolution of Dormancy and Germination. 2nd edn. San Diego, CA: Elsevier. 787 p

Borek V, Morra MJ, Brown PD, McCaffrey JP (1994) Allelochemicals produced during sinigrin decomposition in soil. J Agric Food Chem 42:1030–1034

Bosland PW (2015) The history, development, and importance of the New Mexican pod-type chile pepper to the United States and world food industry. Plant Breed Rev 39:283–324

Boydston RA, Morra MJ, Borek V, Clayton L, Vaughn SF (2011) Onion and weed response to mustard (*Sinapis alba*) seed meal. Weed Sci 59:546–552

Breier A, Ziegelhoffer A (2000) "Lysine is the Lord", thought some scientists in regard to the group interacting with fluorescein isothiocyanate in ATP-binding sites of P-type ATPases. But, is it not cysteine? (Minireview). Gen Physiol and Biophys 19:253–264

Brown PD, Morra MJ, McCaffrey JP, Auld DL, Williams L (1991) Allelochemicals produced during glucosinolate degradation in soil. J Chem Eco 17:2021–2034

Dalling JW, Davis AS, Schutte BJ, Elizabeth Arnold A (2011) Seed survival in soil: interacting effects of predation, dormancy and the soil microbial community. J Ecol 99:89–95

Dane JH, Hopmans JW, Topp GC (2002) Pressure plate extractor. Pages 688–690 *in* Methods of Soil Analysis. Part 4. SSSA book series 5. Madison, WI: Soil Science Society of America

De Cauwer B, Vanbesien J, De Ryck S, Reheul D (2019) Impact of *Brassica juncea* biofumigation on viability of propagules of pernicious weed species. Weed Res 59:209–221

Delaquis PJ, Sholberg PL (1997) Antimicrobial activity of gaseous allyl isothiocyanate. J Food Protect 60:943–947

Doheny-Adams T, Redeker K, Kittipol V, Bancroft I, Hartley SE (2017) Development of an efficient glucosinolate extraction method. Plant Methods 13:17

Donkin SG, Eiteman MA, Williams PL (1995) Toxicity of glucosinolates and their enzymatic decomposition products to *Caenorhabditis elegans*. J Nematol 27:258–262

Dufour V, Stahl M, Baysse C (2015) The antibacterial properties of isothiocyanates. Microbiology 161:229–243

Ehleringer J (1983) Ecophysiology of *Amaranthus palmeri*, a Sonoran Desert summer annual. Oecologia 57:107–112

Hawke MA, Lazarovits G (1994) Production and manipulation of individual microsclerotia of *Verticillium dahliae* for use in studies of survival. Phytopathology 84:883–890

Heaney RK, Spinks EA, Fenwick, GR (1988) Improved method for the determination of the total glucosinolate content of rapeseed by determination of enzymically released glucose. Analyst 113:1515–1518

Kebreab E, Murdoch AJ (1999) Modelling the effects of water stress and temperature on germination rate of *Orobanche aegyptiaca* seeds. J Exp Bot 50:655–664

Keeley PE, Carter CH, Thullen RJ (1987) Influence of planting date on growth of Palmer amaranth (*Amaranthus palmeri*). Weed Sci 35:199–204

Leblová-Svobodová S, Koštíř J (1962) Action of isothiocyanates on germinating plants. Experientia 18:554–555

Lefebvre M, Leblanc ML, Watson AK (2018) Seed dormancy and seed morphology related to weed susceptibility to biofumigation. Weed Sci 66:199–214

Massinga, RA, Currie RS, Horak MJ, Boyer J (2001) Interference of Palmer amaranth in corn. Weed Sci 49:202–208

Morra MJ, Kirkegaard JA (2002) Isothiocyanate release from soil-incorporated Brassica tissues. Soil Biol Biochem 34:1683–1690

Neubauer C, Hüntemann K, Heitmann B, Müller C (2015) Suppression of *Verticillium dahliae* by glucosinolate-containing seed meal amendments. Eur J Plant Pathol 142:239–249

Norsworthy JK, Meehan JT (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

Peters J (2000) Tetrazolium Testing Handbook. Lincoln, NE: Association of Official Seed Analysts. Pp 2–18

Peterson J, Belz R, Walker F, Hurle, K (2001) Weed suppression by release of isothiocyanates from turnip-rape mulch. Agron J 93:37–43

Rask L, Andréasson E, Ekbom B, Eriksson S, Pontoppidan B, Meijer J (2000). Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol Biol 42:93–114

Rice AR, Johnson-Maynard JL, Thill DC, Morra MJ (2007) Vegetable crop emergence and weed control following amendment with different Brassicaceae seed meals. Renew Agr Food Syst 22:204–212

Rothlisberger KL, Hons FM, Gentry TJ, Senseman SA (2012) Oilseed meal effects on the emergence and survival of crop and weed species. Appl Environ Soil Sci 2012:1–10

Sanogo S, Carpenter J (2006) Incidence of Phytophthora blight and Verticillium wilt within chile pepper fields in New Mexico. Plant Dis 90:291–296

Sanogo S (2003) Chile pepper and the threat of wilt diseases. Plant Health Progress 4:23

Sarwar M, Kirkegaard JA, Wong PTW, Desmarchelier J (1998) Biofumigation potential of brassicas. Plant Soil 201:103–112

Schroeder J (1992) Oxyfluorfen for directed postemergence weed control in chile peppers (*Capsicum annuum*). Weed Technol 6:1010–1014

Schutte BJ, Klypin N, Shukla MK (2016) Influence of irrigation timing on disturbance-induced reductions in soil seedbank density. Weed Sci 64:613–623

Skaggs R, Decker MF, VanLeeuwen D (2000) A Survey of Southern New Mexico Chile Producers: Production, Practices and Problems. Las Cruces, NM: New Mexico State University, Agricultural Experiment Station. p 68

Smolińska U, Kowalska B, Kowalczyk W, Horbowicz M (2010) Effect of rape and mustard seed meals on Verticillium wilt of pepper. Veg Crop Res Bull 73:119–132

Smolińska U, Morra MJ, Knudsen GR, Brown PD (1997) Toxicity of glucosinolate degradation products from *Brassica napus* seed meal toward *Aphanomyces euteiches* f. sp. *pisi*. Phytopathology 87:77–82

Steuter AA, Mozafar A, Goodin JR (1981) Water potential of aqueous polyethylene glycol. Plant Physiol 67:64–67

Vaughn SF, Palmquist DE, Duval SM, Berhow MA (2006) Herbicidal activity of glucosinolate-containing seedmeals. Weed Sci 54:743–748

Vleeshouwers LM, Bouwmeester HJ, Karssen CM (1995) Redefining seed dormancy: an attempt to integrate physiology and ecology. J Ecol 83:1031–1037

Wang L, Mazzola M (2019) Effect of soil physical conditions on emission of allyl isothiocyanate and subsequent microbial inhibition in response to Brassicaceae seed meal amendment. Plant Dis 103:846–852

Werle R, Sandell LD, Buhler DD, Hartzler RG, Lindquist JL (2014) Predicting emergence of 23 annual weed species. Weed Sci 62:267–279

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R; NESCent Germination Working Group (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytol 203:300–309

Wood JB (2019) Mustard Seed Meal Suppression of Palmer amaranth (*Amaranthus palmeri*) and *Verticillium dahlia* in Chile Pepper. MS thesis. Las Cruces, NM: New Mexico State University. 61 p

Zar JH (1999) Biostatistical Analysis. New Delhi, India: Pearson Education India