EFFECTS OF OILSEED MEALS ON THE GERMINATION, GROWTH, AND SURVIVAL OF CROP AND WEED SPECIES

A Thesis

by

KATIE LYNN ROTHLISBERGER

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2010

Major Subject: Soil Science

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Approved by:

Co-Chairs of Committee,	Frank M. Hons
	Terry J. Gentry
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	Scott A. Senseman
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ABSTRACT

Effects of Oilseed Meals on the Germination, Growth, and Survival of Crop and Weed Species. (December 2010)

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Oilseed crops are being widely evaluated for potential biodiesel production. Seed meal (SM) remaining after extracting oil may have use as a bioherbicide or organic fertilizer. *Brassicaceae* SM often contains glucosinolates that can hydrolyze into biologically active compounds. *Jatropha curcas* SM does not contain glucosinolates but contains curcin, a known phytotoxin (toxalbumin). A 14-d greenhouse study was conducted to determine how *Sinapis alba* (white mustard, WM), *Brassica juncea* (Indian mustard, IM), *Camelina sativa* (camelina) and *Jatropha curcas* (jatropha) applied to soil at varying application rates and incubation times affected seed germination and seedling survival of cotton [*Gossypium hirsutum* (L.)], sorghum [*Sorghum bicolor* (L.) Moench], Johnsongrass (*Sorghum halepense*), and redroot pigweed (*Amaranthus retroflexus*). Seed meals were analyzed for the presence of glucosinolates, and were applied at 0, 0.5, 1.0 or 2.5% (w/w) to Darco fine sand soil and incubated for 1, 7 or 14 d prior to planting. With the weed species, germination and survival was most reduced by 2.5% WM SM incubated 1d for Johnsongrass and 14 d for redroot pigweed. Cotton and sorghum seedlings showed strong negative responses to WM SM applications of 2.5% at any

incubation time. All crops and weed species were most inhibited by 2.5% application with any SM, but incubation days varied. Seed meals of each species showed negative results dependent on the incubation day, but overall, WM and camelina SMs were most detrimental compared to IM and jatropha. A second greenhouse study was conducted to determine the availability of nutrients in SMs (WM and IM) to cotton and sorghum compared to inorganic fertilization. Seed meals were applied at 1.0 and 2.5% (w/w) and initially incubated for 35 days prior to planting. Emergence of both species was so poor that treatments were incubated for an additional 21 d and replanted. Application rates of 2.5% WM and IM SMs reduced sorghum heights and biomass, but only WM had a negative effect on cotton yield. However, the higher of the SM application rates provided greater levels of nutrients compared to the fertilized treatment and control. Results suggested that the type, rate, and timing of SM applications should be considered before land-applying SMs in organic cropping systems in order to successfully manage weeds while producing a profitable crop.

DEDICATION

To my mother

ACKNOWLEDGEMENTS

I would like to thank Frank Hons, Terry Gentry, Scott Senseman, and Tom Boutton for guiding me through my Masters program and providing me with more than an outstanding education. I have had the opportunity to gain life-long lessons and knowledge through both academic and social experiences. I would also like to thank Jason Wight, Emily Hollister, Kathy Carson, Heidi Mjelde, Landon Crotwell and Joe Storlien for their endless help with my research. My friends and family have been the greatest support and for that I would like to thank my mother and father, Clay, Kyle, Michele, and Pat.

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

CHAPTER I

INTRODUCTION

Research involving oilseed crops for biodiesel production has increased due to greater needs for renewable energy sources. Biodiesel is an EPA – approved renewable fuel that can be produced from oilseed crops. The oil extracted from seed is chemically reacted with an alcohol, such as methanol, to form chemical compounds known as fatty acid methyl esters, or "biodiesel". The oil contained in the seed is most often extracted mechanically using a screw-press. The residue remaining after oil extraction is referred to as either a press cake or seed meal (SM). In order for biodiesel production to be economically and environmentally sustainable, feasible and profitable means of byproduct (SM) disposal and/or usage must also be developed. Utilization in organic agricultural production systems offers a possible solution.

Organic farming systems were the norm prior to the advent of widespread use of synthetic fertilizers, herbicides, and insecticides beginning in the 1950s. Today's organic farms must adhere to rather strict guidelines in order for products to be sold as organic. Organically produced food is grown and processed using natural fertilizers and pesticides. Certain oilseed species have been reported to have allelopathic properties, with the SM potentially acting as a natural pesticide when incorporated into soil.

This thesis follows the style of Soil Biology and Biochemistry.

The main objective of this research was to determine the potential effects of *Sinapis alba* (white mustard, WM)*, Brassica juncea* (Indian mustard, IM)*, Jatropha curcas* (jatropha) and *Camelina sativa* (camelina) SMs added to soil at varying application rates and incubation times on the germination and survival of crop and weed species. Additional studies focused on the identification and quantification of biologically active compounds present within meals and the effects of added SMs on soil chemical characteristics, such as nutrient availability and pH.

CHAPTER II

LITERATURE REVIEW

Oilseeds

Oilseeds that contain large quantities of oil have the potential to produce significant renewable fuels. Such oilseeds include: soybean [*Glycine max* (L.) Merr.], canola and rapeseed (*Brassica napus)*, Indian mustard (*Brassica juncea*), white mustard (*Sinapis alba*), physic nut or jatropha (*Jatropha curcas*), camelina (*Camelina sativa*) and castor bean (*Ricinus communis*). *Brassicaceae* oilseeds have been reported to contain 30 to 40% oil by weight (Snyder et al., 2009), while jatropha seeds contain a similar range of 30 to 37% oil by weight (Rao et al., 2008). Recent interest in jatropha is due primarily to its purported ability to grow on marginal lands. Therefore, its cultivation would be less likely to displace food-producing crops (King et al., 2009). Jatropha and generally all oilseeds are rich in protein, containing a good balance of amino acids.

Many oilseed meals, such as from soybean, have been used as additives in animal feed because of their high nutrient content, but certain plants within the *Brassicaceae* family cannot be used in the same manner because of growth-inhibiting chemicals. Upon enzymatic hydration by myrosinase, a number of allelochemicals are produced in some *Brassicaceae* species as secondary biologically active compounds of glucosinolates, which are β-thioglucosides with a sulphonated oxime moiety and a variable side-chain derived from amino acids (Mithen, 2001). The enzyme myrosinase is physically separated from the glucosinolates until the plant tissue is disrupted, such as in

crushing (Gimsing and Kirkegaard, 2009). Glucosinolates are grouped as either aliphatic, aromatic, or indolyl based on the nature of their side chain. Individual SMs in combination with environmental conditions such as pH, moisture levels, Fe^{2+} concentration, and the presence of coenzymes, determine which hydrolysis products will form. Potential allelochemicals include: isothiocyanates (ITCs), ionic thiocyanates (SCN^-), nitriles, and oxazolidinethiones (OZT).

Glucosinolate-containing SMs incorporated into soil have been reported to have herbicidal, insecticidal, nematicidal and fungicidal effects (Hansson et al., 2008). A field study by Rice et al. (2007) showed that WM, IM, and rapeseed SMs significantly reduced redroot pigweed (*Amaranthus retroflexus* L.) biomass by 59 – 93% compared to the control. A greenhouse study by Ju et al. (1983) reported that SCN^- liberated from WM SM inhibited the growth of tobacco (*Nicotiana tabacum* L. cv. Delhi 76) and bean (*Phaseolus vulgaris* L. cv. Contender) crops. Though not in the mustard family, jatropha SM also contains toxic compounds such as curcin, a toxalbumin, and other equally negative substances such as phorbol esters (King et al., 2009). Thus, oilseed meals may potentially be applied to agricultural soils as organic sources of nutrients and/or organic pesticides.

Seed Meal Characterization

Nutrient Concentrations

Nutrient cycling is a continuous process of competing mineralization and immobilization reactions. Nutrient availability and cycling within the soil is related to both the quality and quantity of organic matter (OM) already present in the soil plus these same attributes in organic materials that may be added. Microorganisms mineralize plant unavailable organic nutrients to usable inorganic forms for plant uptake. Nutrients in SMs will be mineralized when incorporated into soil, but rates generally have not been well documented.

Paul and Solaiman (2004) compared the additions of different sources of OM with changes in soil mineral nitrogen (N). Mustard oil cake (MOC), when compared to sugarcane trash, press mud and cow dung, had the greatest concentrations of carbon (C) and N (44.6% and 5.5%, respectively), the narrowest C:N ratio (8:1) and the smallest lignin concentration (5.3%). Nitrogen is the predominant nutrient deficiency in soils with respect to microbes and plants (Havlin et al., 2005). A sufficient amount of N and a narrow C:N ratio in the OM source must normally be present in order for N mineralization to occur. Mustard oil cakes possessed both properties. Nitrogen released from the soil amended with MOC produced the highest amount of mineral N, with concentrations throughout the 84-d incubation period ranging from 106 to 170 mg N kg^{-1} soil. Sugarcane trash had lower levels of N mineralization than the control, indicating immobilization.

Snyder et al. (2009) characterized *Brassicaceae* SMs as averaging 50% C, 5.9% N and 1.3% P by weight. The C:N ratios of the SMs (IM, 8.2:1; rapeseed, 8.7:1; WM, 8.2:1) were similar and averaged 8.4:1. A study by Rice et al. (2007) quantified similar N concentrations and C:N ratios in SMs of IM (5.6% N, 8.5:1), rapeseed (5.3% N, 9.1:1), and WM (5.8% N, 8.1:1).

A literature review by Achten et al. (2008) examined a complete process of jatropha biodiesel production and use. Typically, a SM with high concentrations of protein (58.1%), such as jatropha, would be used as animal fodder, but jatropha SM cannot be used in this manner due to its phytotoxic properties. However, the SM may be valuable as an organic, soil nutrient source, as it contains more nutrients than either chicken or cattle manure (Francis et al., 2005). Makkar et al. (2008) found jatropha SM to be high in protein while conducting a study to assess the possibility of the SM as animal feed. The authors of this study recovered higher and lower amounts of protein concentrates depending on the extraction and precipitation conditions. The highest levels of protein (334 g kg^{-1}) were extracted at pH 10 and 60° C and precipitated at pH 4. In the same conditions, defatted SM contained 294.7 g kg^{-1} protein. The authors suggested that a product of high protein concentration with sufficiently high recoveries could be obtained from *J. curcas* SM. Even though nutrient and protein concentrations are sufficient, the toxicity makes it unsuitable for animal feed, but there is the possibility that it will be acceptable as an agricultural soil amendment.

Chemical Profile

Depending on the forms of glucosinolate present, *Brassicaceae* SMs have individually unique chemical profiles. Initial concentrations of glucosinolates within the SM may also vary among oilseed species. Rice et al. (2007) reported total glucosinolate concentrations of 166 and 126 μmol g^{-1} of WM and IM meals, respectively. Hansson et al. (2008) measured similar glucosinolate concentrations (163.9 μmol g^{-1} WM SM and 153.2 μmol g^{-1} IM SM). Greater concentrations of glucosinolates present will potentially lead to greater allelochemical production and consequently higher levels of toxicity. Schuster and Friedt (1998) found camelina, also a member of the *Brassicaceae* family, to have a total glucosinolate concentration between 13.2 and 36.2 μmol g^{-1} dry seeds. The lower concentration in camelina seed would suggest much less potential toxicity.

Seed meals may contain numerous forms of glucosinolates, each at varying concentrations, but most SMs have a primary form. According to the findings of Rice et al. (2007) and Hansson et al. (2008) WM SM is dominated by 4-hydroxybenzyl glucosinolate (glucosinalbin) ranging from 148.1 to 156.8 μmol g^{-1} SM, whereas IM SM is dominated by 2-propenyl glucosinolate (sinigrin) (123.8 to 152.0 μmol g^{-1} SM). Vaughn et al. (2006) also found that IM SM primarily contains 2-propenyl glucosinolate at an average concentration of 147.2 μmol g^{-1} SM. Borek and Morra (2005) reported that 4-hydroxybenzyl glucosinolate constituted approximately 93% of the total glucosinolate content in WM meal. Both Schuster and Friedt (1998) and Matthaus and Angelini (2003) found the primary glucosinolate in camelina to be glucoamelinin (10-methylsulfinyldecyl glucosinolate).

The dominant form of glucosinolate found in SM is a likely indicator of the hydrolysis product that will be liberated. Glucosinolates are preserved in the SM until the addition of water initiates a hydrolysis reaction with myrosinase that produces biocidal and phytotoxic products (Morra, 2004), including: isothiocyanates (ITCs), ionic thiocyanates (SCN^-), nitriles, and oxazolidinethiones (OZT) (Fig. 1). Studies have shown that both water content (Gimsing et al. 2006, 2007) and temperature (Price et al., 2005) are positively correlated with the concentration of isothiocyanates liberated from glucosinolate hydrolysis. Glucosinalbin is predicted to produce SCN^- (Borek and Morra, 2005; Hansson et al., 2008; Rice et al., 2007), which is a known phytotoxin and is likely the major allelochemical responsible for weed suppression after soil amendment with WM (Borek and Morra, 2005; Ju et al., 1983). Ionic thiocyanates can also be produced from other species in the *Brassicaceae* family (Hansson et al., 2008). Thiocyanates are not as easily degraded as isothiocyanates; therefore, their effect may persist in the soil for a longer duration (Vaughn et al, 2006; Rice et al., 2007). The toxicity of isothiocyanates has been known since the early twentieth century (Walker et al., 1937).

Unlike glucosinolates, isothiocyanates are hydrophobic and are primarily sorbed by soil OM (Gimsing and Kirkegaard, 2009). Research shows that in the presence of OM-rich soil, the toxicity of isothiocyanates is lower than in the presence of soils with less OM because sorbed isothiocyanates are less bioactive (Brown and Morra, 1997; Matthiessen and Shackleton, 2005). Isothiocyanates, depending on the length of their side chain, can volatilize, but generally are less volatile than other synthetic soil

fumigants (Brown and Morra, 1997). Price et al. (2005) determined that increasing soil temperature increased the volatilization of isothiocyanates.

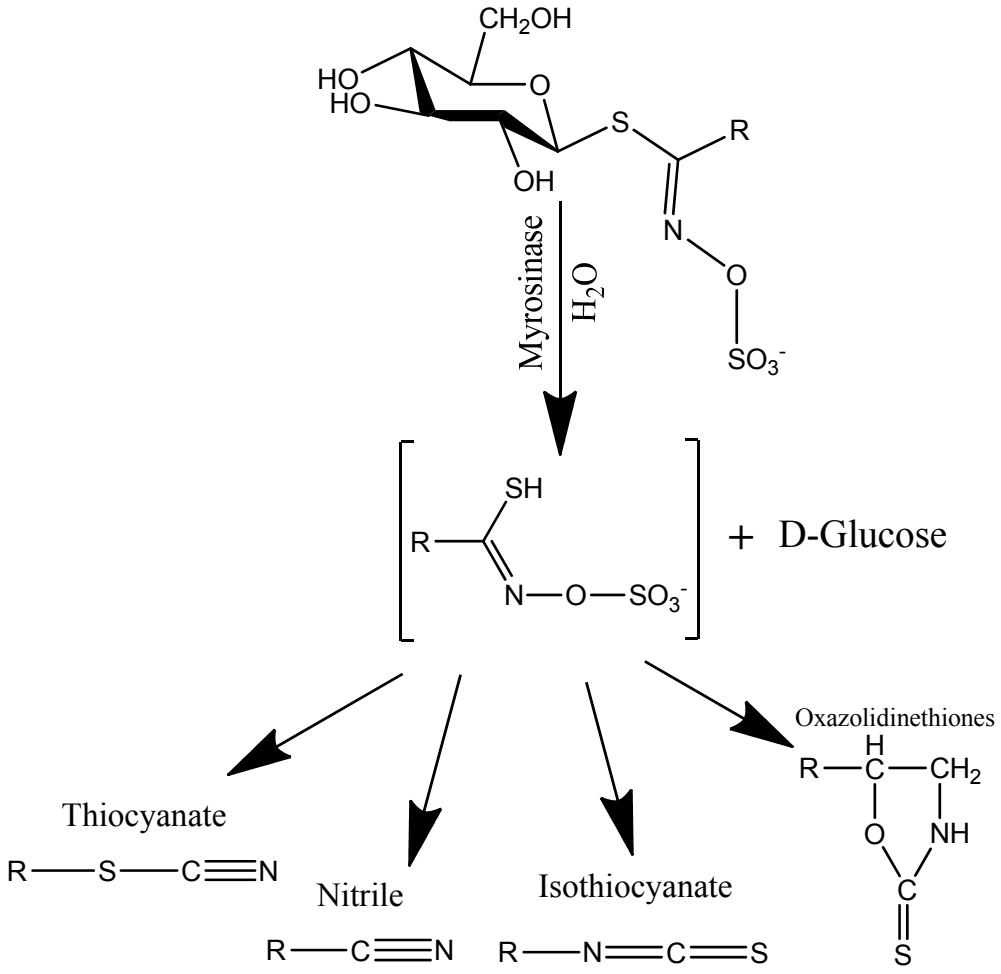

Fig. 1. Glucosinolate reaction and major hydrolysis products. Letter R refers to the type of organic side chain.

Jatropha seed contain a variety of toxins and antinutrients, with curcin and phorbol esters being the primary toxic contributors. A review by King et al. (2009) described curcin as a ribosome inactivating protein (RIPs), which depurinates rRNA, consequently preventing protein synthesis. Curcin is classified as a type-I RIP, whereas ricin from castor bean (*Ricinus communis)* is a type-II RIP. The two types differ in that type-II RIPs contain a carbohydrate binding lectin B-chain. Type-I RIPs, lacking the lectin domain, have LD_{50} values over 1000-fold higher than those observed for type-II RIPs in whole animal (mouse) models (Barbieri et al., 1993).

Phorbol esters can be defined as polycyclic compounds in which two hydroxyl groups on neighboring carbon atoms are esterified to fatty acids (Goel et al., 2007). An analysis by Makkar et al. (1998) revealed that edible varieties of jatropha seed do not contain phorbol esters, leading to the conclusion that these compounds are a source of toxicity within seed exhibiting toxic traits. Phorbol esters are skin-irritants, as well as potent tumor promoters. The biological activity of phorbol esters is highly structure specific (Goel et al., 2007). The conditions with the highest protein recovery reported by Makkar et al. (2008) measured 32.5% of protein isolate to be phorbol esters. The authors predicted that phorbol ester levels of 1.48 mg g^{-1} in the protein concentrate obtained from the SM and 0.5 mg g^{-1} from the defatted SM will cause toxicity in animals.

Ionic Thiocyanate

The objectives of a recent study by Hansson et al. (2008) were to quantify SCN^- liberated in field soil amended with WM, IM and rapeseed SMs. The authors found that an application of 2 t ha^{-1} of WM SM produced greater amounts of SCN^- than 1 t ha^{-1} of WM, IM and rapeseed SMs. Ionic thiocyanate mobility occurred predominately between 0 and 10 cm of soil depth and almost completely degraded after 44 days. Indian mustard and rapeseed SM amendments had much lower concentrations of SCN^- than WM, but followed the same mobility patterns.

The initial product, 4-hydroxylbenzyl isothiocyanate, from white mustard SM is highly unstable and will hydrolyze to SCN^-. Borek and Morra (2005) conducted a pH stability study by incubating partially purified SM extract containing 4-hydroxybenzyl isothiocyanate dissolved in eight different buffers ranging from pH 3.0 to 6.5. A 1-mL sample was withdrawn during the incubation from the buffered reaction solution with a syringe and injected into a Waters Integrity HPLC system. A plot of the natural logarithm of the normalized concentration vs. time produced a straight line that was used to obtain the half-lives of 4-hydroxybenzyl isothiocyanate at different pH values. The shortest half-life (6 min) of 4-hydroxybenzyl isothiocyanate was at pH 6.5, increasing to half-lives of 16, 49, 100, 195, 270, 312, and 321 min with decreasing pH values of 6.0, 5.5, 5.0, 4.5, 4.0, 3.5, and 3.0, respectively. These results suggest that the rate of hydrolysis is at least partially controlled by pH.

The pH-stability results led the above authors to question the quantity of SCN^- produced at pH values relevant to expected pH values of agricultural soils. White

mustard SM was incubated with deionized water and buffer solutions ranging from pH 4 to 7. Their results showed that SCN^- production was slowest at pH 4, but that final concentrations at 48 h varied from 143 μmol g^{-1} at pH 6 to 166 μmol g^{-1} in deionized water at pH 7. Based on the concentration of 4-hydroxybenzyl glucosinolate in the meal and the assumption that there is a complete 1:1 stoichiometric conversion to SCN^- (Hansson et al., 2008), approximately 152 μmol SCN^- g^{-1} of SM was expected. Borek and Morra (2005) concluded that in 48 h and with pH values between 4.0 and 7.0, the majority of 4-hydroxybenzyl glucosinolate should be converted to SCN^-.

Germination Inhibition

Isothiocyanates are extremely reactive compounds which react with nucleophilic groups like sulphydryl groups and disulfide bonds and amines present on OM (Borek at al., 1996). The basis of their general toxicity to organisms and for potential biofumigation stems from this reactivity with proteins (Gimsing and Kirkegaard, 2009).

Crop Suppression

Ionic thiocyanates are capable of inhibiting the germination of dormant seeds. A greenhouse study by Ju et al. (1983) indicated that SCN^-, liberated from WM, inhibited the growth of tobacco and bean. A germination study by Vaughn and Boydston (1997) found that allyl-isothiocyanate released by IM tissues was as effective as the commercial soil fumigant methyl isothiocyanate at inhibiting germination of several crop species. These studies focused on the allelopathic properties of *Brassicaceae* tissues rather than

their SMs. More recent studies have reported germination inhibition by land applying *Brassicaceae* oilseed meals [Boydston et al. (2008); Hansson et al. (2008); Rice et al. (2007)].

Hansson et al. (2008) recognized the potential phytotoxicity of SCN^- by determining inhibitory effects on emergence of carrot (*Daucus carota*) seed planted 15, 22, 29, and 36 days post WM SM treatments of 1 and 2 t ha^{-1}. For this study, the authors examined SCN^- concentrations in the upper 5 cm of the soil profile where carrot emergence occurred, and found that emergence decreased with increasing concentrations of SCN^-. The authors predicted that the moisture regime during the study facilitated leaching of SCN^- below the zone of seed germination, which otherwise may have resulted in greater phytotoxic effects on the crop.

In order for oilseed meals to be used most efficiently, planting dates and meal treatments must be synchronized so that weed control is maximized but crop injury is reduced. Rice et al. (2007) determined the impact of 1 and 3% SM applications of IM, rapeseed, and WM on crop emergence in growth chamber and field studies. The growth chamber study determined the impact of WM and IM meals on lettuce (*Lactuca sativa* L.) germination. Organic soil was amended with 3% rapeseed, 3% WM, or no SM addition by surface applying each week for six weeks. One week after the last meal application, lettuce seeds were planted. Rapeseed treatment had lower seedling emergence than the no meal treatment when lettuce was planted one or two weeks after SM amendments, but seed planted three to six weeks after meal amendment had higher emergence than the no meal treatment. Lettuce seed emergence in WM amended soil

was 3 to 17 % of the emergence from an untreated control for seed planted from one to four weeks after SM application, implying that WM SM can inhibit lettuce emergence if it is planted less than five weeks after meal application.

The field portion of the Rice et al. (2007) study was conducted over a two-year period. First year treatments included 1 and 3% SM applications of rapeseed and IM, and a no SM control. In order to eliminate any residual effects of the SM from year one into the second year, plots were relocated approximately 20 m from the previously established plots. The SM at rates of 1 (503 g SM $plot^{-1}$) and 3% (1509 g SM $plot^{-1}$) were applied in a single application and incorporated to a 3-cm depth with a rototiller. Lettuce and beet (*Beta vulgaris*) seed were planted in year one 14 days after meal application, and again 14 days after initial planting due to extremely poor germination rates. In the second year, seed were planted 28 days after SM application. In year one, meal-amended plots were irrigated using a drip system prior to planting.

For year one, average crop population counts taken 22 days after the second planting were significantly higher in 1% IM and 3% rapeseed treatments than in the no-meal treatment. Unlike year one, average plant emergence in year two was significantly higher in the no-meal treatment than in any SM treatment. Crop emergence was inhibited by 58% in the 3% IM treatment relative to other SMs. The results from year one are consistent with the results of the growth chamber study for rapeseed that determined no significant germination inhibition after two weeks of incubation. The authors could not entirely explain the variation between years, but they recognized that there were major differences between years one and two (climatic condition, type of

irrigation system, and the physical location of the plots). The field results indicated that crop emergence can be reduced by high-glucosinolate containing SMs, but that environmental conditions influence the formation and retention of biologically active secondary compounds.

Snyder et al. (2009) determined the effects of *Brassicaceae* SMs with differing glucosinolate profiles on carrot emergence. Treatments included SMs of IM, rapeseed, and WM applied to field plots at rates of 1 and 2 t ha^{-1}. White mustard treatments had the most substantial emergence inhibition. Only the higher 2 t ha^{-1} rate of rapeseed SM produced similar inhibition to that recorded for both rates of WM.

Weed Suppression

Rice et al. (2007) in their study also determined weed control by collecting and weighing the aboveground weed biomass in their field plots. In year one, redroot pigweed and common lambsquarters (*Chenopodium album* L.) were the dominant species present in all treatments. All SM treatments significantly reduced redroot pigweed biomass by 59 – 93% compared to the no-meal treatment. Biomass of common lambsquarters ranged from 0.01 g m^{-2} in the 3% rapeseed treatment to 1.18 g m^{-2} in the no-meal treatment. No difference in weed biomass between SM treatments and the no-meal treatment in the second harvest of year one indicated that weed control following SM applications was short-term. Redroot pigweed was the dominant weed in the first harvest of year two with 74% suppression within the 3% IM treatment relative to the no-meal treatment. By the second harvest, however, a 52% increase in redroot pigweed

biomass was observed in the 3% IM treatment compared to the no-meal treatment. Data indicated that SM application may result in higher weed biomass later in the season and that repeated SM applications during the growing season might be necessary.

To evaluate the response of weeds to WM SM applied to the soil surface, Boydston et al. (2008) planted 20 seeds of common chickweed (*Stellaria media*) and annual bluegrass (*Poa annua)* in containers with transplanted ornamentals. White mustard SM was then surface applied at rates of 0, 113, 225, and 450 g m^{-2}. Seed meal inhibited emergence of both annual bluegrass and common chickweed at all rates of application. Annual bluegrass seedling counts were most reduced by the 225 and 450 g m^{-2} rates (86 and 98%, respectively). No visual phytotoxicity symptoms were observed on any of the ornamentals tested throughout the eight week period after SM application.

Hoagland et al. (2008) conducted a study that compared the effects of SM amendments (IM, rapeseed and WM) on the biomass of broadleaf and grass weed species. Rapeseed amendments resulted in greater grass biomass in comparison to all other treatments. Broadleaf biomass was significantly ($P<0.05$) lower compared to the control when treated with WM SM, while rapeseed and IM amendments resulted in increased broadleaf biomass. Hoagland et al. (2008) concluded that a reduction or increase in weed biomass is strongly dependent on the type of SM.

Organic Fertilizer

While research has shown the efficacy of SMs as biopesticides to control weeds (Boydston et al., 2008; Hoagland et al., 2008; Rice et al., 2007; Vaughn et al., 2006), nematodes (Walker, 1996), pathogens (Mazzola et al., 2001), and even crop inhibition (Rice et al., 2007), few studies have investigated SMs from different oilseed species as organic nutrient sources for agricultural crops. As mentioned above, SMs contain between 5 and 6% N and as much as 1.3% P by weight and have C:N ratios of approximately 8:1, thus showing the potential to be utilized as organic sources of nutrients in agricultural production systems. Francis et al. (2005) determined jatropha SM to contain 4.4 to 6.4% N, 2.1 to 3.0% P, 0.9 to 1.7% K, 0.6 to 0.7% Ca and 1.3 to 1.4% Mg.

Kucke (1993) documented that rapeseed meal applied as a fertilizer for wheat (*Triticum spp.*), barley (*Hordeum spp.*) and sugar beets (*Beta vulgaris*) increased N uptake and improved yields and crop quality. When used as soil amendments, rapeseed meals increased soil total N and N concentrations in apple (*Malus domesitca* Borkh.) leaves in both greenhouse and orchard settings (Mazzola et al., 2001). Snyder et al. (2009) detected higher carrot (*Daucus carota*) shoot N uptake with all Brassica SM treatments compared to that of the control and observed no effects on carrot quality, but detected yield increases due to greater N availability during the second year of the study. Approximately 55 to 81% of total N in Brassica SM was mineralized during a 96-d carrot growing season (Snyder et al., 2009).

Methods of Chemical Analysis for Seed Meal Toxins

Seed meals can be analyzed for a series of compounds that are markers for potential toxicity. These compounds include glucosinolates, intermediate isothiocyanates, and thiocyanates. In order to identify and quantify these compounds, researchers have successfully used both gas and liquid chromatography methods with an assortment of detectors.

Desulfonated Glucosinolates

Past experiments have successfully identified intact and desulphonated glucosinolates from both SM and soil/SM mixtures by using high performance liquid chromatography (HPLC). Glucosinolate SM extraction as described by the International Organization of Standardization (ISO, 1992) is similar to methods used by Borek and Morra (2005) and Gimsing and Kirkegaard (2006), but with slight differences. The general extraction method for glucosinolates includes combining defatted SM with boiling 70% methanol, addition of an internal standard, shaking intermittently while heating in a hot water bath (70º C) and then after cooling, centrifuging in order to precipitate SM. The main difference between methods was the internal standard reference compound that was used. Borek and Morra (2005) used 4-methoxybenzyl glucosinolate while Gimsing and Kirkegaard (2006) used benzyl glucosinolate. Desulphonation of extracts was achieved by applying glucosinolate supernatant to a DEAE-Sulphadex anion exchanger and allowed to freely drain. A buffer (pH 4 to 5) along with a sulfatase enzyme solution was added to the column and allowed to stand

overnight, after which time the samples were eluted into HPLC autosampler vials by rinsing the column using deionized water. Gimsing and Kirkegaard (2006) utilized an HPLC module coupled with a variable wavelength absorbance detector, while Borek and Morra (2005) detected desulphoglucosinolates by a photodiode array detector, though both detectors were set to 229 nm. For both experiments, separation was performed on a C-18 reversed phase column.

Additional steps are required when extracting glucosinolates from soil amended with SM. Gimsing et al. (2005) successfully extracted and measured glucosinolates from field soil. The same methanol additions and centrifugation processes were followed as above, but after centrifugation the supernatant was filtered through a 0.22 μm syringe-driven nylon filter. An additional portion of methanol was added to the soil, followed by shaking, centrifuging, filtering the supernatants, and finally combining the two filtrates.

Isothiocyanates

Results from Borek and Morra (2005) demonstrated the instability of 4-hydroxybenzyl isothiocyanates in aqueous media. Additional similar attempts with water have also been unsuccessful, leading Kjaer and Rubinstein (1954) to trap the compound by continuously extracting into ether containing aniline. The isothiocyanate was then converted to a phenylthiourea derivative. Morra and Kirkegaard (2002) based their procedure of isolating isothiocyanates on reacting the unstable compounds with 1,2-benzenedithiol (BDT) to produce 1,3-benzodithiole-2-thione, which is stable and amendable to ultraviolet spectrometric detection at 365 nm (Zhang et al., 1992).

Following the process of Morra and Kirkegaard (2002), field soil samples were added to cold methanol, shaken and centrifuged prior to passing the methanol extract through a 0.2 μm syringe filter to obtain a clear filtrate for isothiocyanate derivatisation. A subsample of the clear filtrate containing isothiocyanates was placed in a HPLC autosampler vial containing 100 mM K_2HPO_4 buffer (pH 8.6) and 35 mM 1,2-benzenedithiol/1% mercaptoethanol solution. The vials were capped, inverted several times to mix the solutions and then incubated at 65 ºC in a water bath. The isothiocyanates were quantified using a HPLC module coupled to a 486 UV/Vis detector set at 365 nm. A calibration curve was constructed with the use of derivatised phenylethyl isothiocyanate as an external standard.

CHAPTER III

SEED MEAL EFFECTS ON CROP AND WEED GERMINATION AND SURVIVAL

Introduction

Glucosinolate-containing seed meals (SM) incorporated into soil have been reported to have herbicidal, insecticidal, nematicidal and fungicidal effects (Hansson et al., 2008). A field study by Rice et al. (2007) showed that *Sinapis alba* (white mustard, WM), *Brassica juncea* (Indian mustard, IM), and *Brassica napus* (rapeseed) SMs significantly reduced redroot pigweed (*Amaranthus retroflexus* L.) biomass by 59 – 93% compared to the control. A greenhouse study by Ju et al. (1983) reported that SCN^-, liberated from WM SM, inhibited the growth of tobacco (*Nicotiana tabacum* L. cv. Delhi 76) and bean (*Phaseolus vulgaris* L. cv. Contender). Though not in the mustard family, *Jatropha curcas* (jatropha) SM also contains toxic compounds such as curcin, a toxalbumin, and other equally negative substances such as phorbol esters (King et al., 2009).

Oilseed meals may potentially be applied to agricultural soils as sources of organic nutrients and/or organic pesticides. However, concerns arise from the harmful effects that crop species may potentially experience from the SMs used in this manner. The main objective of this research was to determine the potential effects of WM, IM, camelina (*Camelina sativa*), and jatropha SMs added to soil at varying application rates and incubation times on the germination and survival of both crops and weeds.

Materials and Methods

Greenhouse studies were conducted using soil collected from the Texas AgriLife Research and Extension Center near Overton, TX. Soil at this site was characterized as a Darco loamy fine sand (loamy, siliceous, semiactive, thermic Grossarenic Paleudults) with a pH of 5.6. The soil was air dried for approximately 21 days, thoroughly mixed and stored until further use. This soil was chosen due to its sandy texture and low native fertility.

Oilseed species chosen for this study were *Sinapis alba* cv. Ida Gold (L.A. Hearne Seeds, Monterey County, CA), *Brassica juncea* cv. Pacific Gold (L.A. Hearne Seeds, Monterey County, CA), *Jatropha curcas*, and *Camelina sativa*. Jatropha fruit was dehulled by hand prior to seed pressing. A motor driven screw press operating at 95-100° C was used to extract the oil from seed. The oil constituted approximately 20 to 30% of the seed by weight, and approximately 90 to 95% of the total oil content was extracted. The SMs were stored at temperatures below 0° C until incorporation into soil. Both the soil and SMs were analyzed for total organic C and total N by a combustion procedure (McGeehan and Naylor, 1988; Schulte and Hopkins, 1996; Storer, 1984). The soil was analyzed for extractable P, K, Ca, Mg, and S by Mehlich III (Mehlich, 1978; Mehlich, 1984) and analysis by ICP, micronutrients (Cu, Fe, Mn, and Zn) by extraction with DTPA-TEA, followed by ICP analysis (Lindsay and Norvell, 1978), and extractable NO_3-N by cadmium reduction following extraction by 1 N KCl (Keeney and Nelson, 1982). Seed meal mineral concentrations (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) were determined by ICP analysis of nitric acid digests. The electrical

conductivity of the soil was determined in a 1:2 soil:water extract using deionized water with the actual determination made using a conductivity probe (Rhoades, 1982). Soil texture was determined using the hydrometer procedure (Day, 1965)

A germination and survival study was conducted in a temperature controlled glasshouse using cotton [*Gossypium hirsutum* (L.)], sorghum [*Sorghum bicolor* (L.) Moench], Johnsongrass (*Sorghum halepense*), and redroot pigweed (*Amaranthus retroflexus*) as the crop and weed species. The study was established as a complete factorial within a completely randomized design with four replications of 36 treatment combinations, including: SM source (WM, IM, camelina, and jatropha), application rate [0.5, 1.0, and 2.5% on dry weight basis (g/g)] and incubation time (1, 7, and 14 d prior to planting). Before mixing with soil, SMs were finely crushed using a mortar and pestle. Approximately 340 g of soil-SM mixture were added to ~500-ml growth cups and incubated for the designated times at 32-35° C in the glass house. The soil was not disturbed other than at planting. The gravimetric water content of mixtures was kept constant at 0.24 g/g by weighing and adding distilled water daily. Unamended soil was used as the control treatment for each crop or weed species.

On 29 July 2009, ten sorghum or cotton, 50 pigweed, or 100 Johnsongrass seed were planted into each individual treatment replication. The number of seed planted was based on the percentage of live viable seed for the crops or weeds. Data collection began the first day following planting and continued on a daily basis for 14 days. Seed were considered germinated when emerged from the soil surface. On the 14th and final day of data collection, survival counts were made based on the number of viable seedlings

present within each replicate. Viable seedlings were defined as having a well developed root and shoot system and as being at a comparable growth stage relative to the controls. Seed of certain species, especially cotton and sorghum, sometimes germinated, but did not survive. Soil samples were collected on day 14 from 2.5% SM and control treatments from pots planted to cotton, dried at 60 °C, ground, and analyzed for total C, total N and extractable NO_3-N, P, K, Ca, Mg, S, and micronutrients by the same methods described earlier.

Statistical Analysis

Relative germination was calculated as the percentage of seed germinated in SM treatments relative to those germinated in controls. Relative survival was based on the number of viable seedlings in treatments as a percent of control seedlings. Statistical analysis was conducted using SAS version 9.2. The effects of main factors and their interactions on crop and weed germination and survival were analyzed using a mixed analysis of variance (ANOVA) procedure at a significance level of $p < 0.05$. Means from significant main and interaction effects were separated using Fisher's protected LSD.

Results

Soil and SM Characteristics

Results showed the Darco soil to be deficient in NO_3^--N, P, K, and Mg. The soil was sufficient in Ca, S, and Cu, and somewhat high in Fe, Zn, and Mn (Table 1). This

sandy soil has an EC value of 37 μmhos cm^{-1}; therefore, its salinity effects were negligible. Having a sandy texture (79.3% sand, 14.2% clay and 6.5% silt), the Darco soil also had a low buffering capacity for nutrients and pH.

Compositional analysis of SMs indicated that these materials may potentially supply significant amounts of nutrients for plant growth (Table 1). White mustard, IM and camelina SMs had similar concentrations of total C and N (40 to 50% and 5%, respectively). Total N was slightly less in jatropha SM.

Table 1
Total nutrient concentrations of oilseed meals and total C and N and extractable nutrients in Darco soil

		Soil	Oilseed Meal			
Concentration		Darco	White Mustard	Indian Mustard	Camelina	Jatropha
Organic C	%	0.37	49.17	50.35	44.88	47.58
Total N	%	0.08	5.09	5.00	5.36	3.46
C:N	–	4.6	9.7	10.1	8.4	13.8
NO_3 - N	mg kg^{-1}	7.9	–	–	–	–
P	mg kg^{-1}	28	8848	11818	8695	8058
K	mg kg^{-1}	42	11014	11368	14978	15397
Ca	mg kg^{-1}	191	6341	6092	6832	11470
Mg	mg kg^{-1}	26	3473	4470	4270	4748
S	mg kg^{-1}	14	–	–	–	–
Na	mg kg^{-1}	97	493	588	550	1291
Fe	mg kg^{-1}	15.1	40.1	47.0	45.2	40.1
Zn	mg kg^{-1}	1.8	65.1	68.1	65.4	30.6
Mn	mg kg^{-1}	7.5	35.9	57.7	64.6	35.9
Cu	mg kg^{-1}	0.2	9.9	10.2	14.5	15.9

Table 2
Soil nutrient concentrations at the end of the 14-d germination study from pots planted to cotton and receiving 2.5% seed meal

	Incubation	TN	TC	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn
Seed Meal	d	---------%	---------	----------	----------	----------	----------	----------mg kg^{-1}	----------	----------	----------	----------
Control	1	0.063	0.439	43	72	229	40	406	4	2528	2	31
	7	0.069	0.442	57	85	283	41	487	6	3200	2	35
	14	0.069	0.459	38	82	228	39	407	4	2092	2	31
White Mustard												
	1	0.144	0.899	92	175	279	77	400	6	2205	2	29
	7	0.134	0.822	109	186	325	67	561	6	3306	2	38
	14	0.120	0.702	114	187	306	67	499	6	2350	2	38
Indian Mustard												
	1	0.138	1.041	150	217	361	96	537	6	2114	2	36
	7	0.136	0.811	124	207	354	92	535	7	2812	2	36
	14	0.098	0.961	84	228	372	92	448	4	2088	3	34
Jatropha												
	1	0.102	0.866	72	189	317	80	427	4	2435	2	29
	7	0.121	1.025	90	240	373	84	528	6	2279	2	32
	14	0.104	0.605	106	179	295	83	455	5	2161	2	33
Camelina												
	1	0.145	1.027	127	319	353	98	523	6	2425	2	36
	7	0.083	0.658	118	223	413	82	548	7	3024	2	35
	14	0.110	0.716	102	210	344	80	567	6	3442	2	41

TN and TC denote total N and total C, respectively.

Carbon:Nitrogen ratios ranged from 8.4 to 10.1 for glucosinolate containing SMs and was 13.8 for jatropha SM. Phosphorus concentration of IM SM was higher at 1.18% compared to the other three meals that averaged 0.85% P. Potassium concentration of jatropha SM was 1.54%, which was greater than the average of the three remaining SMs at 1.25%. Nutrient concentrations of SMs were comparable to values of Snyder et al. (2009) who reported *Brassicaceae* SMs to average 50% C, 5.9% N and 1.3% P by weight.

Soil analyses after the end of the 14 d study for all 2.5% SM treatments planted to cotton averaged 0.84% TC and 0.12% TN (Table 2). Seed meal treatments for 1 d incubation ranged from 0.10 to 0.14% TN, with jatropha resulting in the lowest value. Control treatments averaged 50% less than SM treatments for TN and TC. All seed meal treatments, except with jatropha, decreased in TN from 1 to 14 d incubation. All treatments resulted in less TC after 14 d incubation compared to 1 d, except for the control. Phosphorus averaged 107 mg kg^{-1} among SMs, with IM SM at 1 d incubation resulting in the highest concentration (150 mg kg^{-1}). The controls ranged from 38 to 57 mg P kg^{-1} soil. Across all SM treatments, K averaged 213 mg kg^{-1}, Ca averaged 340 mg kg^{-1} and Mg averaged 83 mg kg^{-1}. Potassium, Ca, and Mg concentrations were less in the controls compared to SMs. Calcium and Mg were both highest in IM SM treatments.

Johnsongrass

Main effects. Within each main factor (SM source, application rate, and incubation time), observed effects were significant for both relative germination and

survival of Johnsongrass (Table 3). Rate exhibited the most significant effect on germination, while all three main effects were highly significant ($p<0.001$) for survival. Camelina and WM SM resulted in significantly lower germination (78.8 and 79.0% of the control, respectively) compared with jatropha SM (91.0%) (Fig. 2). Jatropha SM applied at 0.5% had a relative germination greater than 100% (113.7%) because the germination rate of this treatment was greater than the germination of the control. Relative survival of Johnsongrass seedlings in WM treatments was also significantly less (60.4%) than with any of the other three SMs (92.3 - 94.9%).

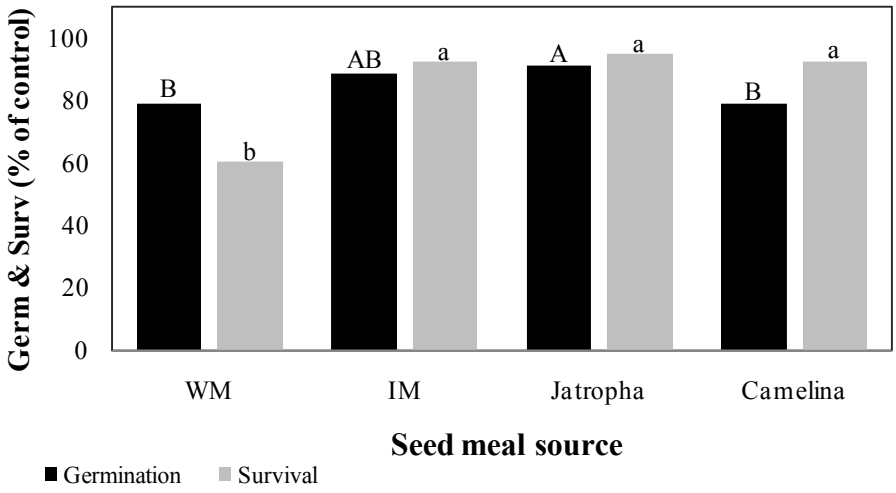

Fig. 2. Main effect of 'seed meal source' on Johnsongrass germination (germ) and survival (surv). Means within germination or survival followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. Uppercase letters separate germination means and lowercase letters separate survival means. WM and IM denote white mustard and Indian mustard, respectively.

Table 3
ANOVA results for the main and interactive effects of seed meal source, application rate, and incubation time on cotton, sorghum, Johnsongrass, and pigweed germination (germ) and survival (surv)

	Cotton		Sorghum		Johnsongrass		Pigweed	
	germ	surv	germ	surv	germ	surv	germ	surv
Effect	p-value							
SM	<.0001	0.0349	0.6148	<.0001	0.0283	<.0001	0.2307	0.0024
Rate	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
SM*Rate	0.0541	0.2411	0.8481	0.0031	0.0315	0.0374	0.0899	0.0018
Incubation	0.1191	<.0001	0.0266	0.007	0.0185	<.0001	<.0001	<.0001
SM*Incubation	<.0001	0.0182	0.0009	0.1825	0.2107	<.0001	0.0017	0.0095
Rate*Incubation	0.0041	0.0001	0.0059	0.3865	0.0056	0.0285	0.0002	0.0715
SM*Rate*Incubation	0.3804	0.0433	0.0084	0.0428	<.0001	0.0029	0.0978	0.0008

SM denotes seed meal source.

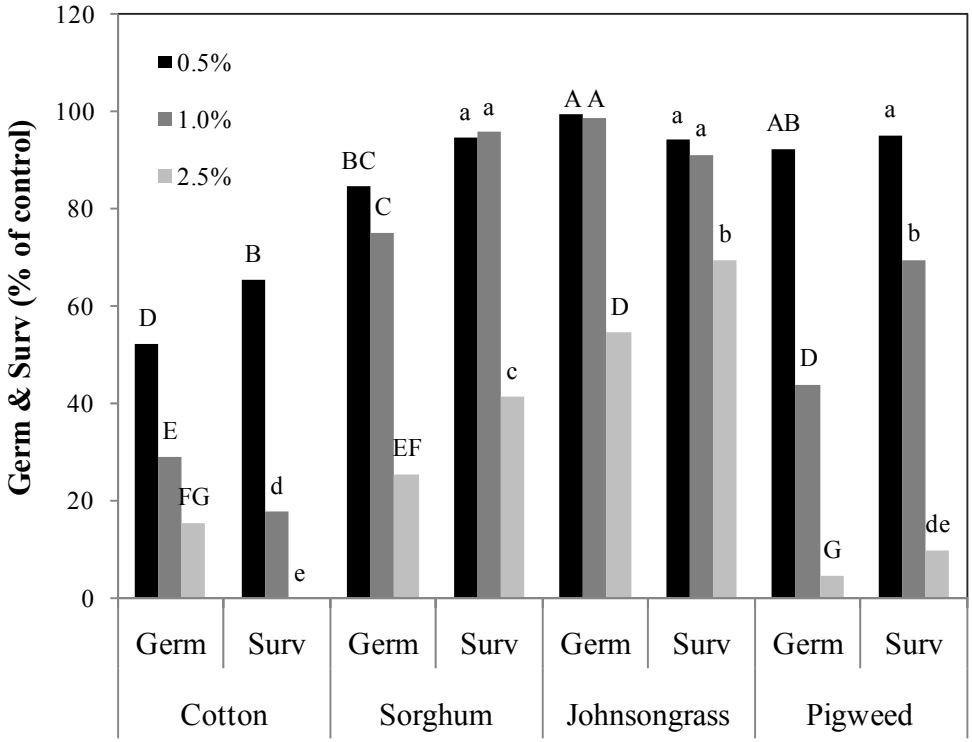

Fig. 3. Seed meal rate effect on cotton, sorghum, Johnsongrass, and pigweed germination (germ) and survival (surv). Means followed by the same letter are not different at P<0.05 by Fisher's protected LSD. Uppercase letters separate germination means and lowercase letters separate survival means.

Seed meals added at 2.5% resulted in both the lowest germination and survival of Johnsongrass (Fig. 3). Johnsongrass, pigweed, cotton and sorghum all showed significantly less germination and survival with a SM application rate of 2.5%. Incubation time exhibited significantly different effects on relative germination and survival of Johnsongrass (Fig. 4). The 7-d incubation resulted in significantly less relative germination than when incubated for 14 d (78.0 and 90.8%, respectively), but not 1 d (84.5%). However, the 1-d incubation did result in significantly less relative survival than either 7 or 14 d (67.0, 91.9, and 96.2%, respectively).

Interactive effects. Johnsongrass showed to be the hardier weed and more resistant than the two crops to phytotoxins in SMs (Fig. 3). The treatment combination that was most effective at suppressing Johnsongrass germination was 2.5% WM SM incubated for 7 d (16.4% of control) (Fig. 5). Seedling survival was most affected by 2.5% WM SM applied only 1 d prior to planting (4.4% of control). The relative survival of Johnsongrass seedlings with the latter treatment was significantly less than for any other treatment combination, other than 1.0% WM incubated 1 day (14.6% of control) (Fig. 5). Figure 4 represents the two-way interactive effect of SM source and incubation time on Johnsongrass and Figure 5 represents the 3-way interaction effects.

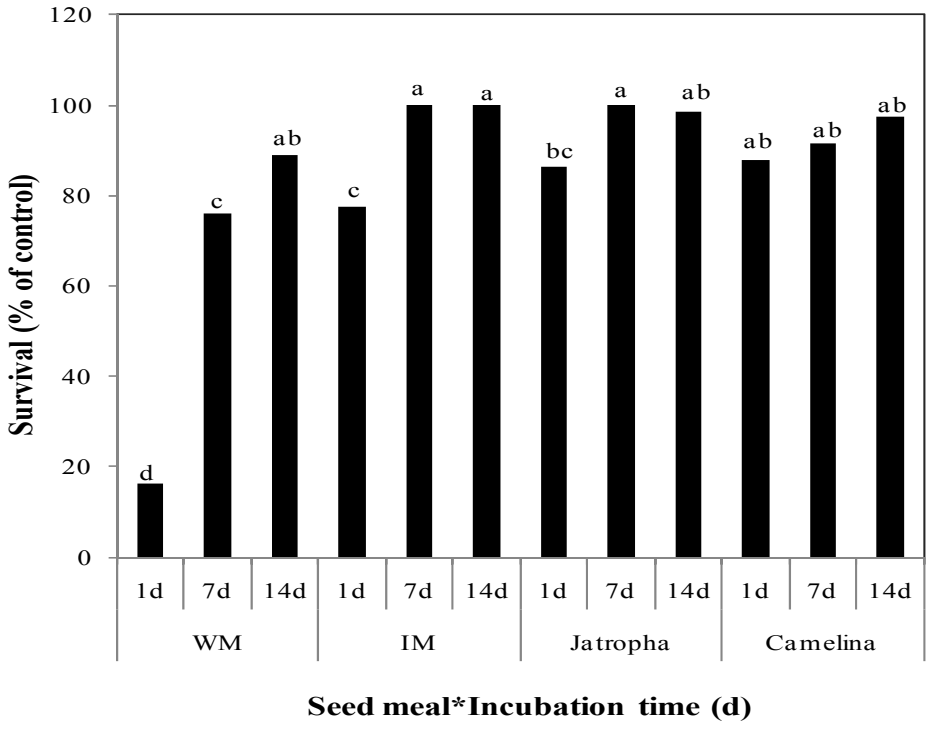

Fig. 4. Interactive effects of 'seed meal source and incubation time' on Johnsongrass survival. Survival means followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

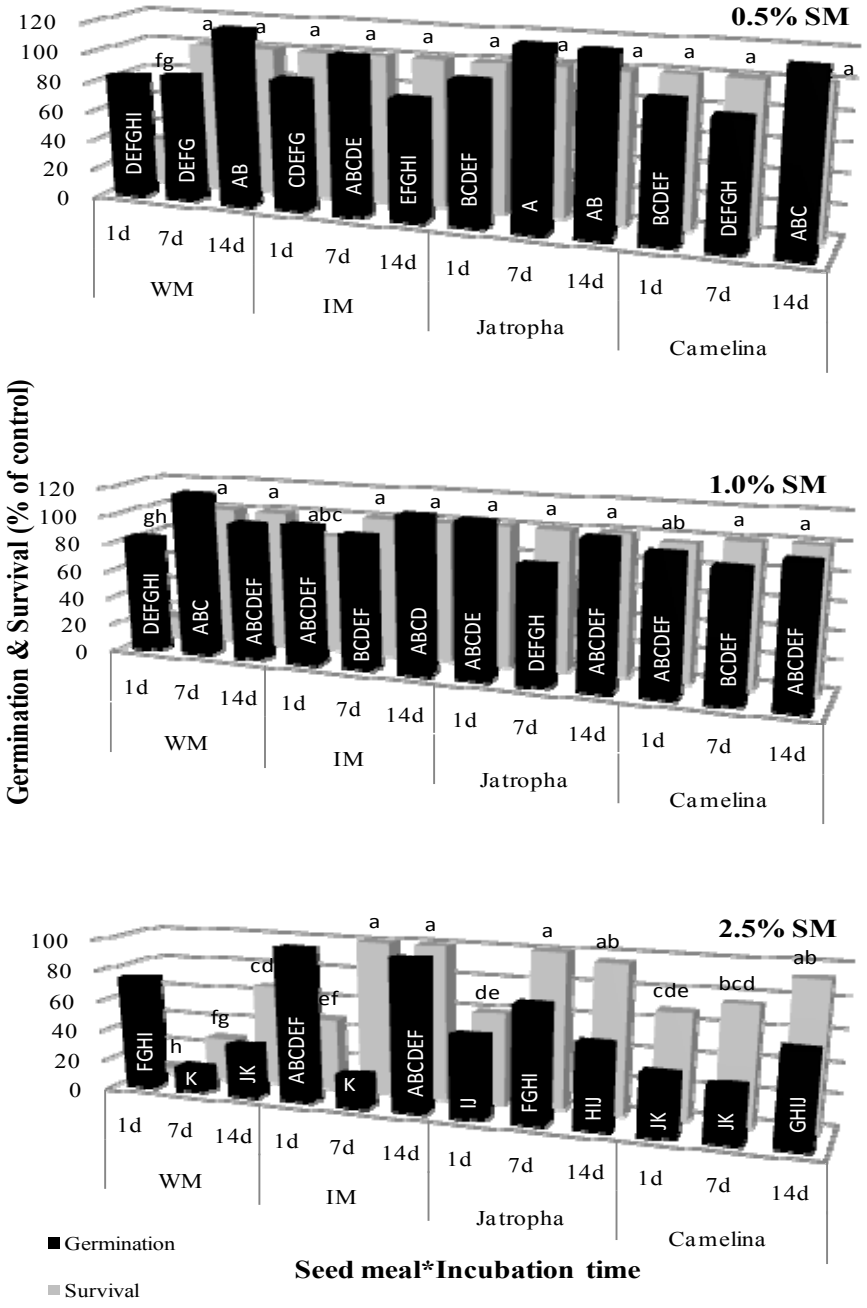

Fig. 5. Three-way interaction of 'seed meal source, application rate and incubation time' on Johnsongrass germination and survival. Means within germination (uppercase letters) or survival (lowercase letters) across application rate followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

Pigweed

Seed meal source did not affect relative germination of pigweed, but did significantly influence relative survival (Table 3). Camelina and WM SMs significantly reduced pigweed survival compared with IM (48.9, 54.2, and 70.1% of control, respectively) (Fig. 6). Pigweed seed and seedlings were extremely sensitive to SM treatments applied at 2.5% (Table 3, Fig. 3).

Incubation times of 1 and 7 d produced significantly lower relative germination and survival percentages relative to 14 d (33.6, 24.2, and 83.3% of control for germination, respectively and 56.0, 46.3, and 72.5% of control for survival, respectively) (Fig. 7). Relative germination and survival were 0% for all 2.5% treatments, with the exception of IM SM incubated for 14 d (49.0% of control for germination and 100% of control for survival) and WM SM incubated for 1 d (7.0% of control for germination and 18.8% of control for survival) (Fig. 8). Numerically, relative survival of seedlings in treatments of 2.5% WM SM applied 14 d before planting were less than all other 2.5% treatments, but statistically there were no significant differences (Table 3, Fig. 8).

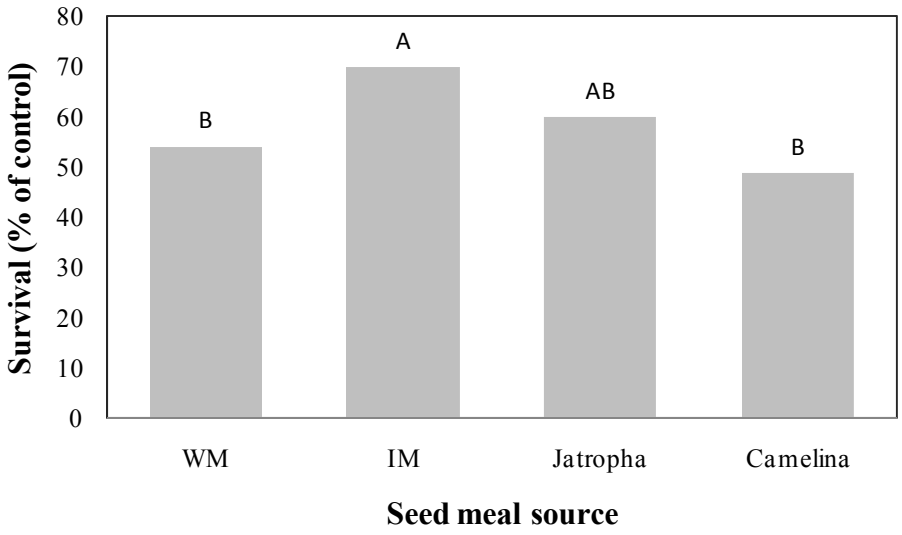

Fig. 6. Main effect of 'seed meal source' on pigweed survival. Means within survival followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

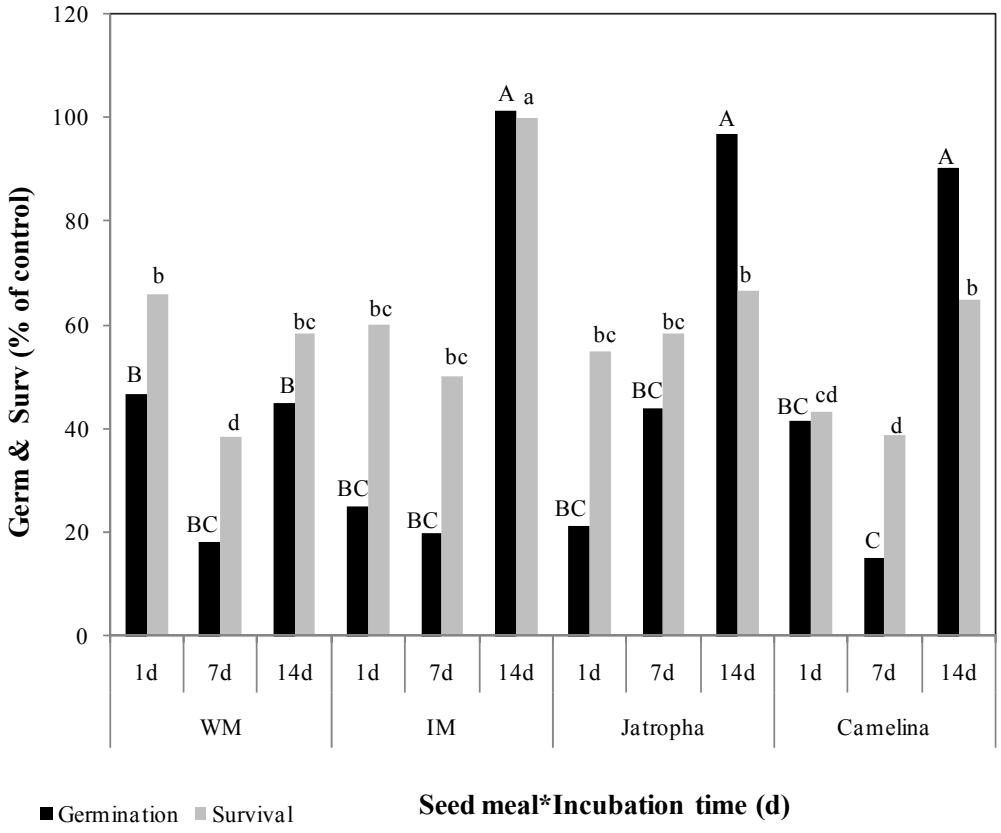

Fig. 7. Interactive effects of 'seed meal source and incubation time' on pigweed germination (uppercase letters) and survival (lowercase letters). Means within germination or survival followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

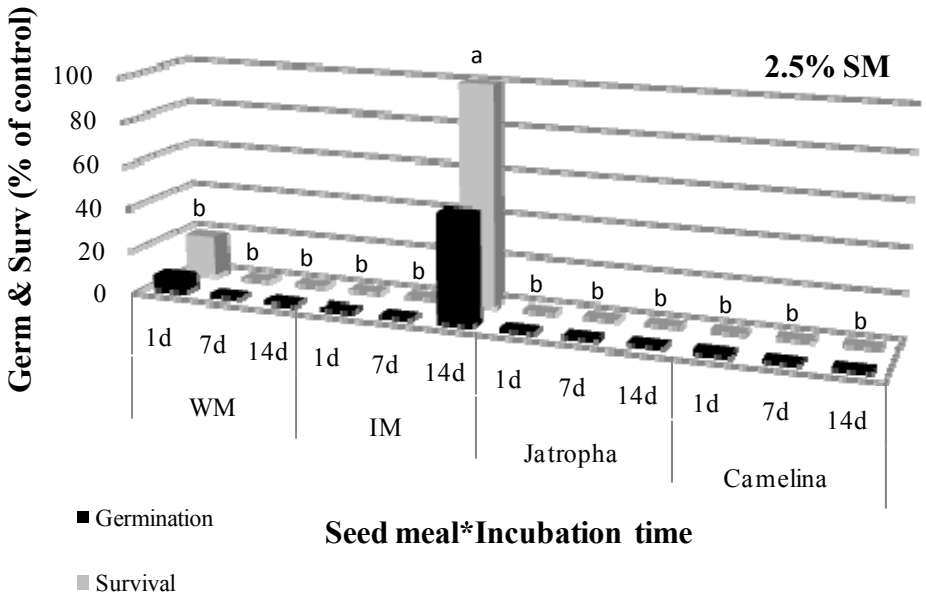

Fig. 8. Interaction of 'SM source, 2.5% application and incubation time' on pigweed germination and survival. Means within survival followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. Germination was not significant. WM and IM denote white mustard and Indian mustard, respectively.

Cotton

Main effects. Of the three main effects, incubation time was the only one that did not show significant treatment effects on germination of cotton seed (Table 3). Camelina SM resulted in significantly lower germination (15.7%) than WM (51.4%) and jatropha (35.5%), but not IM (26.9%) (Fig. 9). Seedling survival showed somewhat different results, with camelina treatments again showing numerically the lowest survival (17.1%), but being only significantly less compared to treatments with jatropha (38.3%), which resulted in the highest survival percentage (Fig. 9).

As with both weed species, treatment combinations including 2.5% SM exhibited significantly reduced cotton germination and seedling survival (Fig. 3). Incubation time significantly altered seedling survival, but not germination (Table 3). One-d incubation prior to planting had the most negative impact on seedling survival, but not germination (Fig. 10). The longer incubation time of 14 d increased seedling survival to 46.4%, but relative germination was still only 31.9% for this incubation treatment. This result likely indicates the necessity for SM incubation longer than 14 d prior to planting cotton.

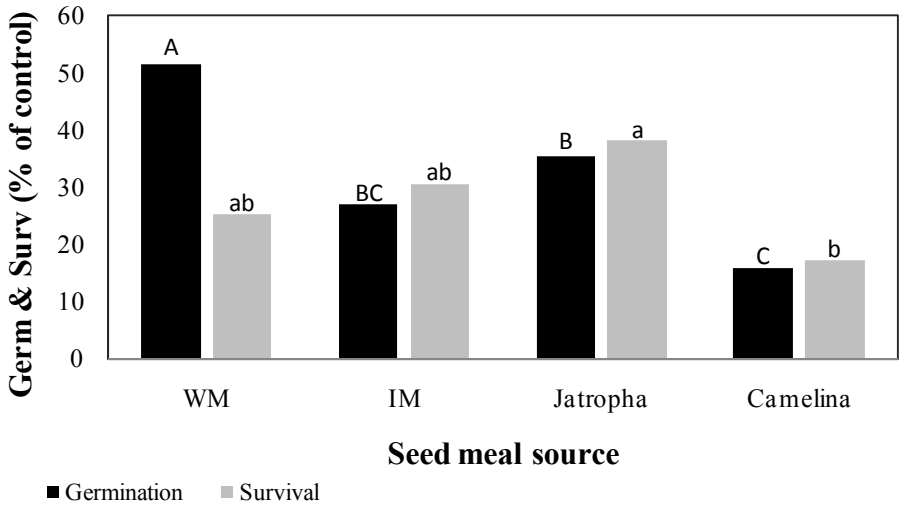

Fig. 9. Main effect of 'seed meal source' on cotton germination (uppercase letters) and survival (lowercase letters). Means within germination or survival followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

Interactive effects. The two -way interaction of ‘seed meal source and application rate’ was not significant for either relative germination or survival of cotton (Table 3). From the two-way interaction of ‘seed meal source and incubation time’ (Table 3, Fig. 10), which was significant for both germination and survival, relative rates of glucosinolate hydrolysis may be inferred. Hydrolysis of glucosinolates in WM SM based on germination apparently increased over the incubation period, decreased with IM, was not affected by time for jatropha, and showed greatest toxicity at 7 d for camelina. White mustard SM applied 1 d prior to planting resulted in the highest germination rate (86.8%) relative to other treatments, but the survival rate of the seedlings was poor (17.7%) (Fig. 10). Longer incubation periods of WM SM resulted in decreased germination, but increased seedling survival. The most negative effects on cotton germination and survival with IM SM were observed with 1 d incubation (11.4% germination and 9.8% survival), while camelina and jatropha SMs were most detrimental at 7-d incubation (Fig. 10).

The three-way interaction of ‘seed meal source, application rate, and incubation time’ was not significant for cotton germination, and only slightly for survival (Table 3). White mustard applied at 2.5% and incubated for 1 d resulted in significantly higher cotton germination (94.7%) compared to any other treatment of 2.5% SM (0-36.8%) (Fig. 11). Relative survival of seedlings in this treatment, however, failed to be significantly different than WM added at 2.5% and incubated for 7 or 14 d. The treatment most effective at suppressing Johnsongrass and pigweed growth, 2.5% WM SM at 1 or 7 d incubation, also resulted in 0% survival of cotton seedlings (Fig. 11).

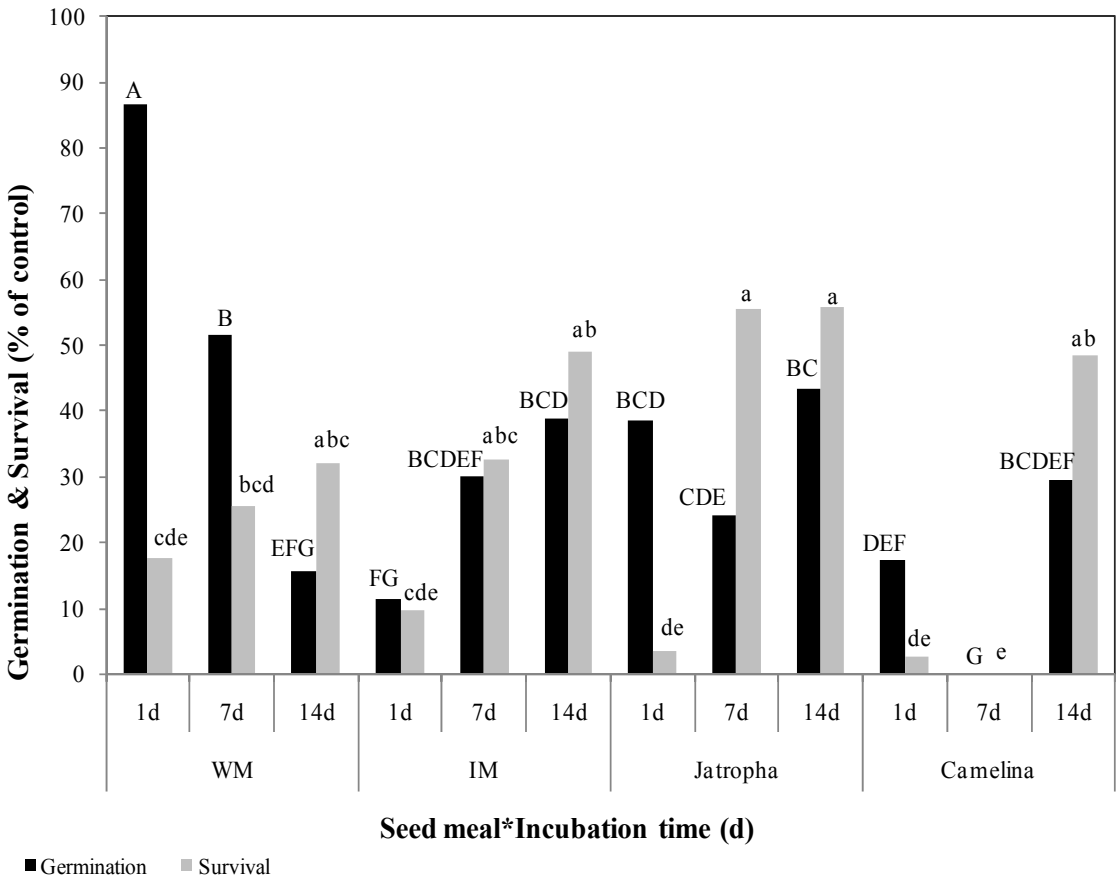

Fig. 10. Interactive effect of 'seed meal source and incubation time' on cotton germination and survival. Germination (uppercase letters) or survival (lowercase letters) means followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

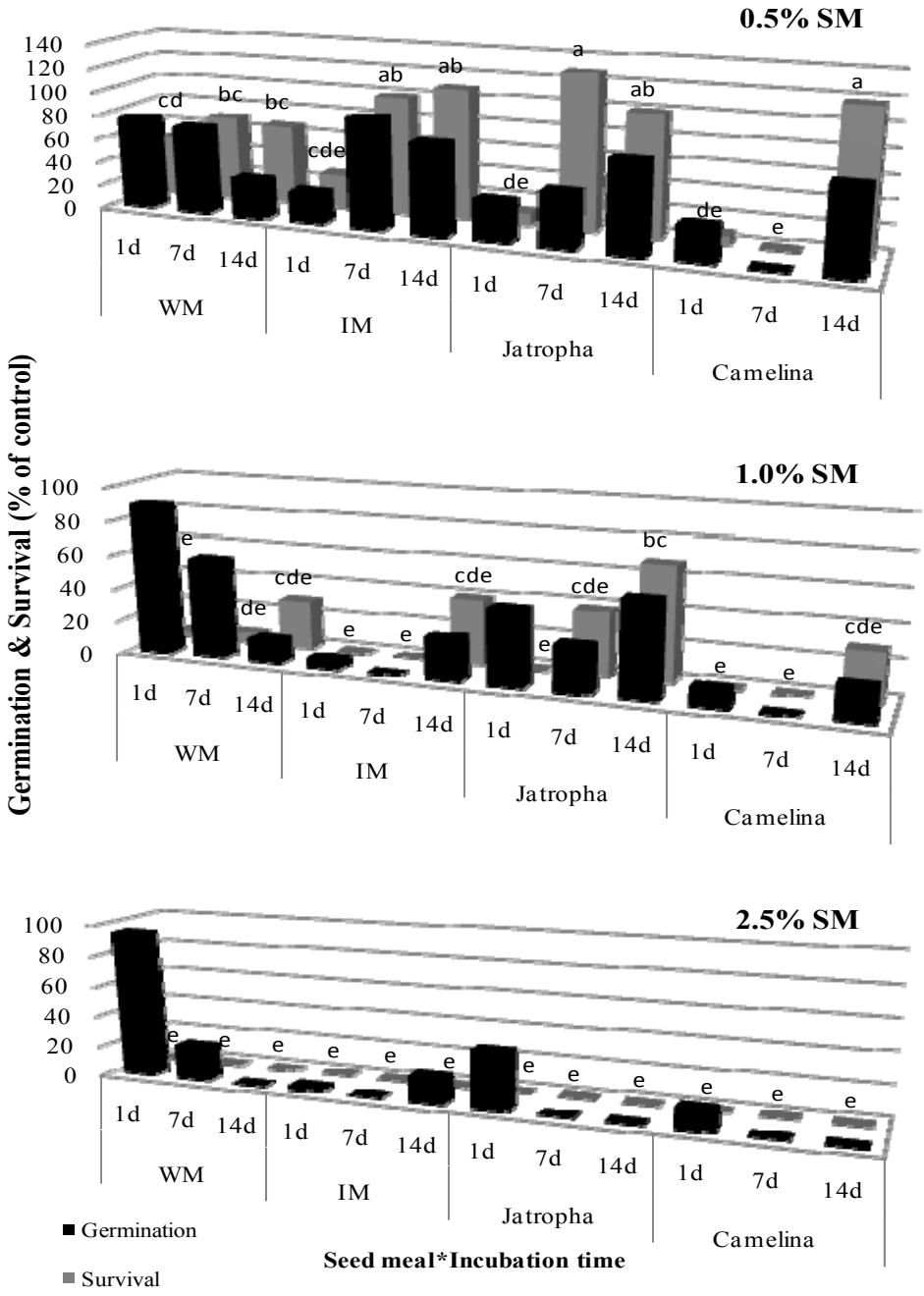

Fig. 11. Three-way interaction of 'SM source, application rate and incubation time' on cotton germination and survival. Means within survival across application rates followed by the same letter are not different at P<0.05 by Fisher's protected LSD. Germination was not significant. WM and IM denote white mustard and Indian mustard, respectively. Sorghum

Main effects. Of the three main effects, SM source was the only one not significant for sorghum germination, but all three were significant for seedling survival (Table 3). Sorghum seedling survival was significantly less when treated with WM SM (56.6%) relative to all other SMs (82.1%-88.3%) (Fig. 12). Application of 2.5% SM resulted in both significantly reduced germination and seedling survival (25.6 and 41.5%, respectively) compared to other rates (75.1-84.6% germination and 94.8-95.8% survival) (Fig. 3).

Interactive effects. The three-way interaction was significant for both relative germination and survival (Table 3). As with cotton, germination of sorghum planted in treatments with WM SM decreased with increasing incubation time, while survival increased from 1 to 7 d of incubation (Fig. 13). WM SM at 2.5% and 1-d incubation had significantly greater relative germination (75.9%) than any other 2.5% SM treatment combination (2.9 - 45.7%) (Fig. 13). No treatment combinations were able to completely inhibit germination, but all treatments containing 2.5% WM SM resulted in 0% relative survival (Fig. 13).

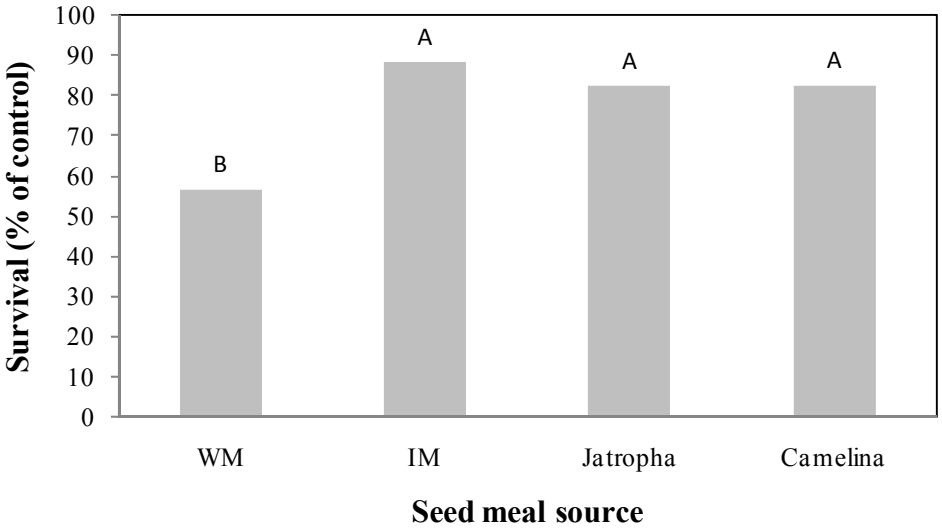

Fig. 12. Main effect of 'seed meal source' on sorghum survival. Means followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

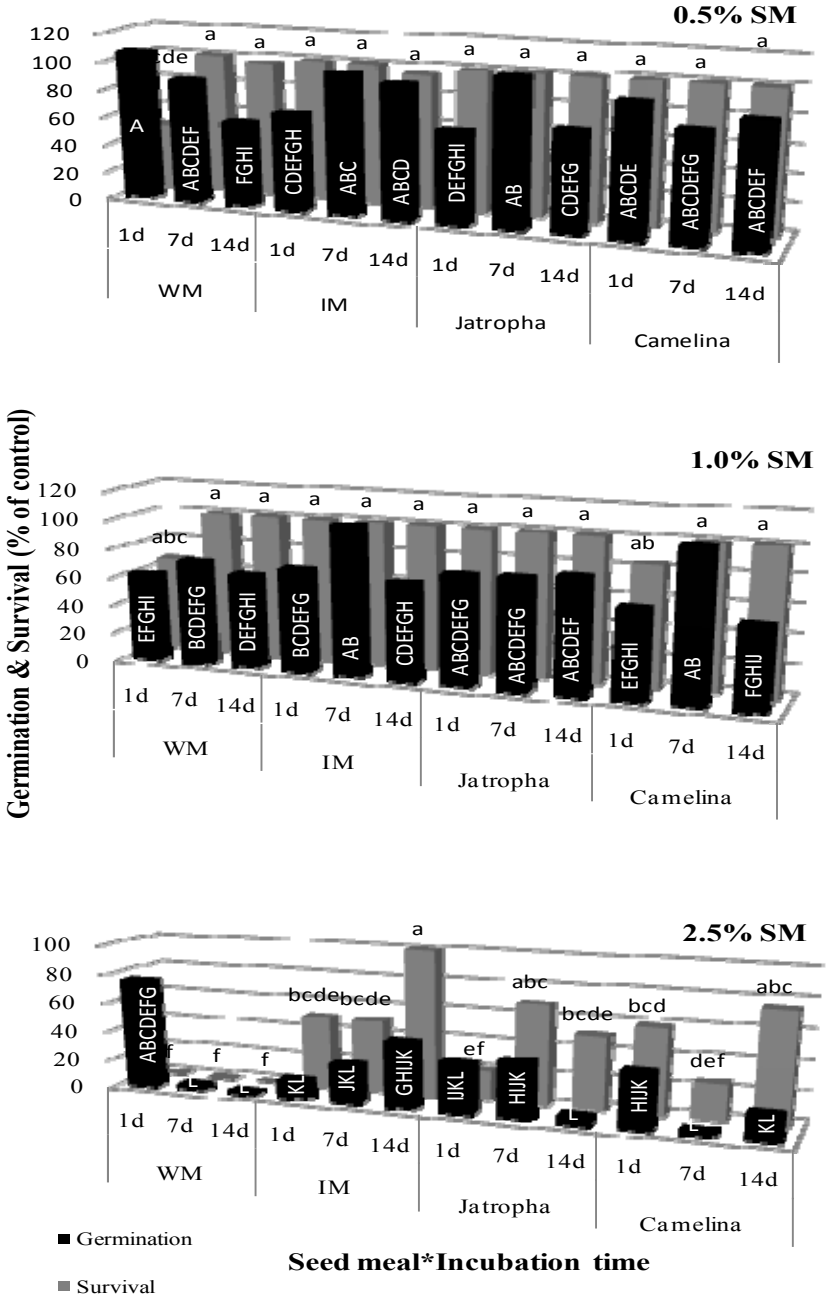

Fig. 13. Three-way interaction of 'seed meal source, application rate and incubation time' on sorghum germination and survival. Means within germination (uppercase letters) or survival (lowercase letters) and across application rate followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

Discussion

The use of oilseed meals as soil amendments has several potential benefits, but there are also possible detriments. Primarily, SMs might serve to replenish soil organic matter (SOM) in cropping systems where, for instance, stover has been removed for use as biofuel feedstocks. Used in this manner, meals from certain oilseeds have the potential to add significant organic C and nutrients to soil, while controlling or inhibiting weed growth. In order to suppress weeds, WM SM should be applied at rates between 1 and 2.5%, which will also supply a substantial amount of N (1120 - 2800 kg N ha^{-1}). Nitrogen applied in excess to soils and not synchronous with plant uptake may be lost from the system and could pose significant environmental risks. Seed meals applied at appropriate rates do contain nutrient concentrations capable of potentially enhancing the productivity of low nutrient soils.

As mentioned above, WM SM applied to soil at 2.5% and incubated for 1 or 7 d prior to planting was most inhibitory to Johnsongrass, which was the most tolerant of species tested. While relative germination of Johnsongrass was significantly higher in treatments of '1% white mustard incubated for 1-d' compared to the most inhibitory treatment, relative survival of seedlings in this treatment failed to be significantly different than with the 2.5% SM application. It is likely that an application rate ranging from 1 to 2.5% SM would be adequate to suppress weed growth.

Pigweed germination and survival was suppressed by all SM treatments of 2.5%, excluding IM SM incubated for 14 d. It is hypothesized that after 14 d of incubation the toxicity associated with IM SM dissipated sufficiently so that its inhibitory effects were

reduced compared to other SMs. These results are in contrast to results reported by Rice et al. (2007), who found that IM SM applied at 3% was the only SM of the three studied (WM, IM, and rapeseed) to suppress pigweed biomass compared to the no-meal treatment.

The treatment combination of '2.5% WM SM with 7 or 14 d incubation' prior to planting was extremely detrimental to cotton and sorghum in our study indicating that this SM likely must be incubated for a longer period of time before planting agricultural crops. Previous studies have shown the phytotoxin associated with WM, SCN^-, decreased to almost background concentrations after 44 d at an application rate of 2 t ha^{-1} (Hansson et al., 2008). Phytotoxin dissipation in soil is highly dependent on SM application rates, microbial activity, glucosinolate release efficiency and rate of reaction.

Due to the decrease in cotton seed germination from 1 to 14 d of incubation when planted in WM SM treatments, the rate of glucosinolate hydrolysis in this SM was assumed to be slower relative to the other SMs. Glucosinolates in IM SM apparently had the fastest rate of reaction since cotton seed germination was lowest for treatments with 1 day incubation. Isothiocyanate concentrations of IM and rapeseed tissues have been shown to be highest 24 hrs after incorporation and then dropped to less than half of the maximum in 72 hrs (Morra and Kirkegaard, 2002). Other studies have reported SCN^- to have a longer half-life in soil compared with 2-propenyl isothiocyanate, the major phytotoxin produced from IM (Borek et al., 1995; Brown and Morra, 1993). Research has shown that 60% of SCN^- remained after 6 days (Brown and Morra, 1993), whereas the average half-life of 2-propenyl ITC in six different soils was 48 h (Borek et al.,

1995). The rate of glucosinolate hydrolysis and ITC persistence are dependent on many soil and environmental factors and for this reason are somewhat unpredictable, but they appear to be a feasible means of determining the point at which phytotoxins are at maximum concentrations and consequently, most detrimental to plant viability.

CHAPTER IV

SEED MEAL EFFECTS ON SOIL PH

Introduction

Extreme soil pH, whether acidic or alkaline, can be detrimental to plant growth and productivity. Darco soil has a native pH of ~5.6, which is approaching the lower limit for the growth of cotton and sorghum. This soil type also has a low buffering capacity, making it highly susceptible to changes in pH. Due to the results observed in the germination and survival study, it was deemed necessary to determine if changes in soil pH with seed meal (SM) addition caused germination and survival inhibition. The main objective of this study was to determine changes in soil pH affected by SM amendments.

Materials and Methods

Seed meals of *Sinapis alba* (white mustard, WM), *Brassica juncea* (Indian mustard, IM), *Jatropha curcas* (jatropha), and *Camelina sativa* (camelina) were applied to 140 g of Darco (Loamy, siliceous, semiactive, thermic Grossarenic Paleudults) and Branyon (Fine, smectic, thermic Udic Haplusterts) soils at rates of 0, 0.5% and 2.5% (g/g) and were replicated three times. Branyon soils are clayey with a much higher buffering capacity than Darco soils and a native pH of 7.88. The gravimetric water content of the mixtures was maintained on a daily basis at 0.23 and 0.35 g g^{-1} soil for Darco and Branyon soil, respectively, by adding distilled water when differences in

weight were measured. The SM/soil mixtures were incubated at 32.5° C for 30 d in a thermostatically controlled incubator.

Soil pH of SM mixtures was determined in a 1:2 soil: deionized water suspension after the 30-d incubation. Samples were stirred and allowed to equilibrate for 30 minutes after adding water. A Corning model 440 pH meter coupled with a hydrogen selective electrode was used to determine pH.

Statistical Analysis

The change in pH was calculated by the difference between the pH of the SM treated soil and the control soil without SM additions. Statistical analysis was conducted using SAS version 9.2. The effects of main factors and their interactions on change in soil pH were analyzed using a mixed analysis of variance (ANOVA) procedure at a significance level of $p < 0.05$. Significant main and interaction means were separated using Fisher's protected LSD.

Results

Differences were detected between Darco and Branyon series soils ($p<0.0001$). The Darco soil pH increased by 0.22 to 1.5 units compared to the controls receiving no SM amendments; however, Branyon soil pH decreased by 0.14 to 0.74 units compared to the control (Fig. 14).

Seed meal source was not significant ($p=0.4643$), but the interaction effect of 'soil and SM source' was significant ($p=0.0041$). The rate effect was highly significant

($p<0.0001$) for Darco and Branyon soils with 2.5% applications averaging changes of 1.34 and 0.60 units, respectively, and 0.5% applications averaging changes of 0.40 and 0.18 units, respectively. Even though SM source was not significant, the interaction of 'SM source and application rate' showed differences ($p=0.0345$). The three way interaction of 'soil, SM source, and application rate' was not significant ($p=0.0957$) for either soil.

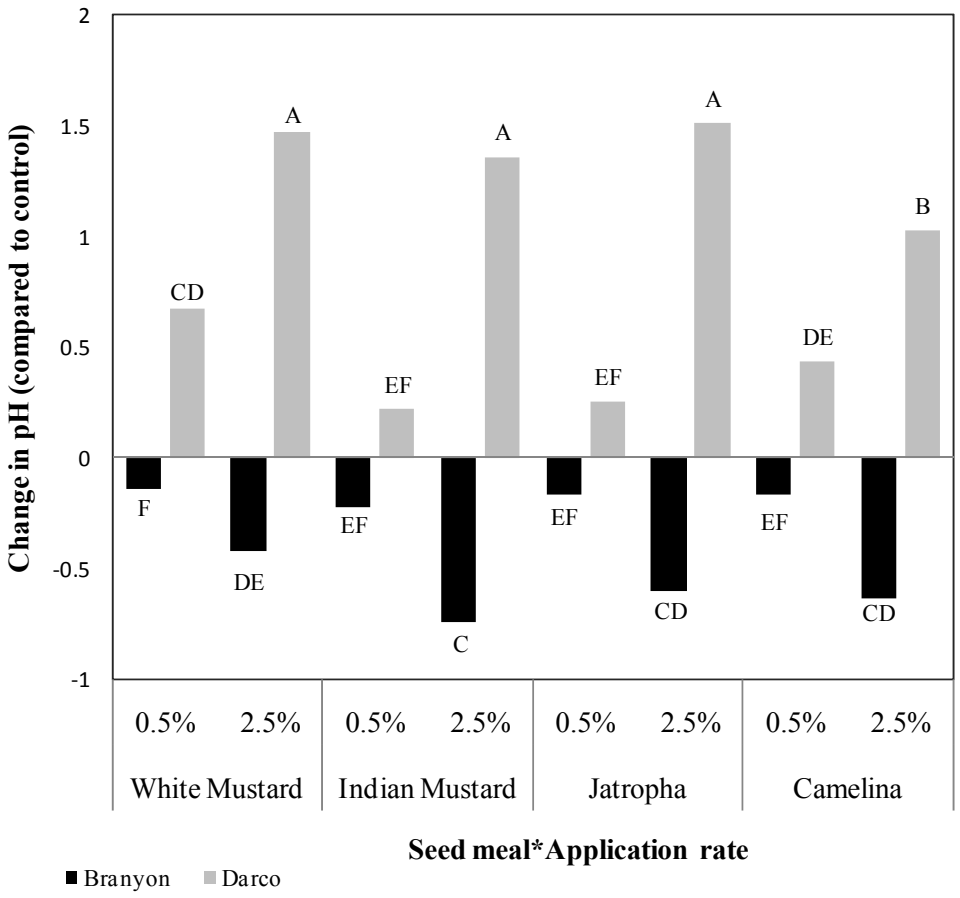

Fig. 14. Effect of 'seed meal source and application rate' on soil pH. Branyon and Darco are the soil series used. Absolute values of means followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD.

Discussion

Soil pH changes were dependent on both soil characteristics and the amount of SM added. The pH of both soils moved towards neutrality with an increase of SM from 0.5% to 2.5%. When 2.5% SM (56,000 kg SM ha^{-1} 15 cm) was applied to poorly buffered Darco soil, the pH of the soil system slowly changed to a pH higher than both the soil and SM amendments, which range from 5.0 (WM SM) to 7.0 (jatropha SM). Sorghum sludge has also been reported to increase the pH of Darco soil by 1.3 units with an application rate of 90 Mg sludge ha^{-1} (Vincent, 1989).

Branyon soil has a much higher buffering capacity compared to Darco soil; therefore, it was expected that the soil pH would not be altered to the degree of the Darco soil. It is feasible to suggest that Branyon soil decreased in pH due to the acidic nature of the SM. Regardless of why the change occurred it most likely resulted in positive effects on soil processes, such as an increase in microbial growth, thereby increasing mineralization and N availability.

CHAPTER V

GLUCOSINOLATE IDENTIFICATION AND QUANTIFICATION BY HPLC

Introduction

Most oilseed meals originating from a species of the *Brassicaceae* family contain compounds known as glucosinolates. These sulfur and nitrogen containing compounds are initially nontoxic, but in the presence of myrosinase, hydrolyze into compounds potentially toxic to plants and animals. Therefore, glucosinolate concentrations in these seed meals (SM) are measures of potential total toxicity. High performance liquid chromatography (HPLC) has been successfully used to identify and quantify desulfoglucosinolates (Borek and Morra, 2005; Gimsing and Kirkegaard, 2006).

Materials and Methods

Glucosinolate concentrations in SMs were determined using methods similar to those found in the International Organization of Standardization (ISO, 1992), but with a few modifications. Seed meals of *Sinapis alba* (white mustard, WM), *Brassica juncea* (Indian mustard, IM), and *Camelina sativa* (camelina) were first defatted with one extraction and two rinses of petroleum ether by vacuum filtration using a Büchner funnel. Defatted SM (300 mg) was weighed into 50-mL centrifuge tubes to which 500 mg of 5-mm glass beads (Borek and Morra, 2005) were added and then immediately vortexed. A hot (70 °C) 70% methanol:H_2O solution (10 mL) was added to the samples that were then placed in a hot water bath for 20 minutes and vortexed intermittently.

After heating and mixing, the samples were centrifuged and the supernatant was collected. An additional extraction was performed similar to above, but with 5 mL of hot methanol rather than 10 mL. The extracts were combined and 2 mL were added to a 0.6 mL plug of DEAE Sephadex A-25 anion exchanger and allowed to freely drain. The poly-prep chromatography columns (purchased from BioRad) were then rinsed with 1 mL of deionized water and finally with two aliquots of 1 mL 0.02M sodium acetate buffer (pH 4.5). Sulfatase solution (100 µL) was added to the columns and allowed to sit overnight (16 hrs). Desulfoglucosinolates were eluted with 3 consecutive 1 mL volumes of deionized water.

Samples were immediately separated and quantified using an HPLC with a Waters 600s System Controller, 717 autosampler and 996 photodiode array detector. The system was equipped with a Waters 3.5 µm Symmetry Shied RP8 column (2.1 x 150 mm). Mobile phases flowed at 0.3 mL/min and compounds were separated using an acetonitrile gradient starting at 2% and increasing to 95%. Expected retention behavior, such as time and sequence, and absorbance spectra were used to identify glucosinolate peaks (Wathelet et al., 2004). A calibration curve was constructed using sinigrin monohydrate (purchased from Science Lab) as an external standard for IM samples and as an internal standard for WM and camelina samples.

Results

Glucosinolate extracts from SMs can be utilized as an indicator of the potential biocidal activity that may be produced when *Brassicaceae* SMs are incorporated into soil. Each SM in this study was determined to have its own individual glucosinolate profile. The dominant glucosinolate compound found in WM SM was 4-hydroxybenzyl glucosinolate (glucosinalbin or sinalbin) at a concentration of 149.59 μmol g^{-1} and a standard deviation of 2.29 μmol g^{-1} (Fig. 15). Indian Mustard SM contained several compounds with the dominant one being 2-propenyl glucosinolate (sinigrin) at a concentration of 159.1 ±15.9 μmol g^{-1} (Fig. 15). The calibration curve generated using sinigrin monohydrate produced a coefficient of determination (r^2) value of 0.96 (Fig. 16). Camelina SM contained three dominant compounds with the most prominent being 10-methylsufinyldecyl (12.20 ± 7.51 μmol g^{-1}) (Fig. 15).

	White mustard	Indian mustard	Camelina
	----------μmol g^{-1} SM----------		
Dominant glucosinolate	149.59 ± 2.29 p-hydroxybenzyl (Sinalbin)	159.10 ± 15.90 2-propenyl (Sinigrin)	12.20 ± 7.51 10-methylsulfinyldecyl (Glucocamelinin)

Fig. 15. Glucosinolate compounds and concentrations determined in oilseed meals.

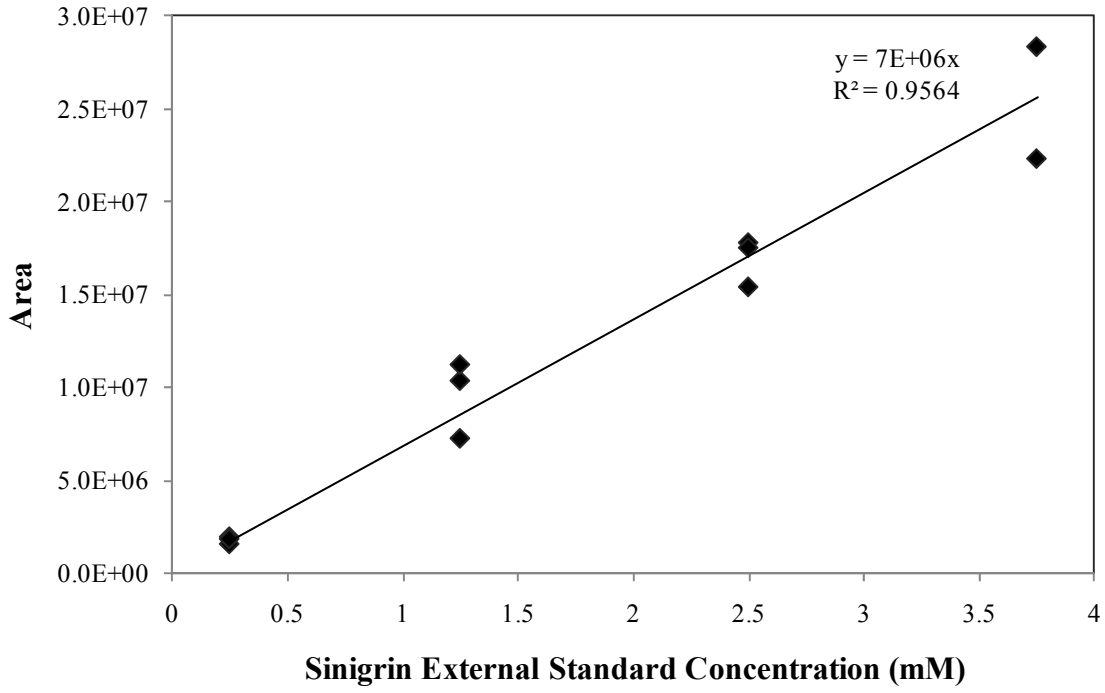

Fig. 16. Sinigrin monohydrate standard concentration curve as determined by high performance liquid chromatography equipped with photodiode array detection (HPLC-PDA).

Discussion

Glucosinolates were found in all SMs sampled, but the side groups and concentrations varied between species. The side group is largely responsible for the product that is formed after glucosinolate hydrolysis. Glucosinalbin or sinalbin, the primary glucosinolate within WM SM will potentially produce, through a series of reactions, thiocyanate (SCN^-). Borek and Morra (2005) stated that applying WM SM to soil along with the addition of sufficient water is expected to produce an amount of SCN^- stoichiometrically equivalent to the amount of 4-hydroxybenzyl glucosinolate in the SM. If this complete conversion assumption is true, it is expected that SCN^- concentrations should reach approximately 150 μmol g^{-1} of SM. These results are very similar to those of Borek and Morra (2005) who estimated SCN^- to be approximately 152 μmol g^{-1} of SM.

Indian mustard SM contains low concentrations of SCN^- yielding glucosinolates, but instead contains high levels of isothiocyanate yielding glucosinolates, such as sinigrin. Vaughn et al. (2006) found IM SM to contain 2-propenyl glucosinolate at an average concentration of 147.2 μmol g^{-1} SM, while the sinigrin concentration measured in our IM SM was 159.1 ± 15.9 μmol g^{-1} SM.

The concentration of the major glucosinolate, glucoamelinin, contained in camelina SM was much lower than glucosinolate concentrations of both WM and IM SMs. The results from the germination study (Chapter III) would suggest that camelina SM had either equal or greater amounts of glucosinolates as WM and IM SM due to suppressed germination. However, since it did not, other sources of toxicity must have

been present. Schuster and Friedt (1998) also found glucoamelinin to be the most dominant of three glucosinolates found within camelina SM and at a concentration of approximately 15.5 μmol g^{-1}. While glucosinolate quantification is not an exact measure of toxicity within SM, it has generally shown to be an acceptable estimation of potential toxicity.

CHAPTER VI

SEED MEAL EFFECTS ON NUTRIENT AVAILABILITY AND PLANT GROWTH

Introduction

Oilseed meals (SM) would most commonly be utilized as animal feed, but due to toxic properties mustard meals are not usable in this manner. For this reason, research has been devoted to using mustard SM, to control agricultural pests (Boydston et al., 2008; Hansson et al., 2008; Mazzola et al., 2001; Rice et al., 2006; Snyder et al., 2009; Vaughn et al., 2006). Plants in the *Brassicaceae* family contain glucosinolates, which are β-thioglucosides with a sulphonated oxime moiety and a variable side-chain derived from amino acids (Mithen, 2001). Upon enzymatic hydration by addition of water and myrosinase in the seed, glucosinolates produce a number of allelochemicals as secondary biologically active compounds (Brown and Morra, 1997; Fenwick et al., 1983; Gimsing et al., 2005; Matthiesen and Kirkegaard, 2006; Vaughn et al., 2006). While research has shown the efficacy of SMs as biopesticides to control weeds (Boydston et al., 2008; Hansson et al., 2008; Rice et al., 2007; Vaughn et al., 2006), nematodes (Walker, 1996) and pathogens (Mazzola et al., 2001), few studies have compared SMs to inorganic fertilizer as nutrient sources for agricultural crops.

Snyder et al. (2009) characterized *Brassicaceae* SMs to average 50% carbon (C), 5.9% nitrogen (N) and 1.3% phosphorus (P) by weight. The C:N ratios of the SMs [*Sinapis alba* (white mustard, WM), 8.2:1; *Brassica napus* (rapeseed), 8.7:1; *Brassica juncea* (Indian mustard,IM), 8.2:1] were similar and averaged 8.4:1. A study by Rice et

al. (2007) quantified similar N concentrations and C:N ratios in SMs of IM (5.6% N, 8.5:1), rapeseed (5.3% N, 9.1:1), and WM (5.8% N, 8.1:1). Therefore, SMs may have the potential to be utilized as organic sources of nutrients in agricultural production systems. Kucke (1993) documented rapeseed meal applied as a fertilizer for wheat (*Triticum* spp.), barley (*Hordeum* spp.) and sugar beets (*Beta vulgaris*) to increase N uptake and improve yields and crop quality. When used as soil amendments, rapeseed meals increased total soil N and N concentrations in apple (*Malus domestica* Borkh.) leaves in both greenhouse and orchard settings (Mazzola et al., 2001). Snyder et al. (2009) detected higher carrot (*Daucus carota*) shoot N uptake with all Brassica SM treatments compared to that of the control and observed no effects on carrot quality, but did see increases in yield due to enhanced N availability during the second year of the study. Snyder et al. (2009) reported that Brassica SM mineralized approximately 55 to 81% of its total N during a 96-d carrot growing season. The objective of this study was to determine the effects of WM and IM SMs on plant growth and nutrient uptake by cotton and sorghum when planted to soil amended with WM and IM SMs.

Materials and Methods

Based on the results of the germination study, treatments (SM, application rate, and incubation time) were chosen that should theoretically maximize crop dry matter production. Cotton and sorghum were seeded into SM-treated soils and monitored for germination, biomass production, and nutrient uptake from soil.

A nutrient uptake study was conducted in a temperature controlled glasshouse using cotton and sorghum as crop species. Treatments included soil applications of WM and IM SMs at 1.0 and 2.5% and compared with fertilizer applications of chemical grade reagents, including NH_4NO_3 (448 kg N ha^{-1} 15cm), $CaHPO_4 \cdot 2H_2O$ (168 kg P ha^{-1} 15cm), and KCl (336 kg K ha^{-1} 15cm). Unamended soil was utilized as the control treatment. All treatments, including the unamended soil, received additions of $CaCl_2 \cdot 2H_2O$ (336 kg Ca ha^{-1} 15cm) and $MgSO_4$ (168 kg Mg ha^{-1} 15cm). The Darco soil that was used for this portion of the research is naturally low in Ca and Mg. The study used a completely randomized design with four replications of each treatment.

Oilseeds used in this study were pressed using a motor driven screw press in order to extract oil. Seed meal was collected and stored at temperatures below -18° C prior to use. Soil and SM mixtures were added to 3.8 L pots. Each pot contained 2 kg of air-dried Darco soil, to which either applications of SM or fertilizer were made, except for control treatments. Prior to mixing with soil, SM was crushed using a mortar and pestle. Applications of SM were on a dry weight basis (g/g). Seed meal treatments of 1.0 and 2.5% were prepared by adding 20 g and 50 g, respectively, of crushed SM. Fertilizer treatments received 1.14 g NH_4NO_3, 0.83g $CaHPO_4 \cdot 2H_2O$, and 0.58 g KCl per pot. All treatments, as well as controls, received 1.10 g $CaCl_2 \cdot 2H_2O$ and 0.74 g $MgSO_4$. Seed meals and fertilizers were thoroughly mixed with soil and then added to pots.

To each pot, 480 mL of distilled water was added at the start of the experiment. The gravimetric water content was maintained at 0.238 g/g by adding water to a constant weight on a daily basis. Perlite was applied to soil surfaces to avoid surface crusting and

erosion of the surface when watering. The pots were left to incubate for 35 d at a temperature of ~32° C before planting. Soil mixtures were not disturbed throughout the incubation.

Seed of cotton (5 seed/pot) and sorghum (6 seed/pot) was planted at the end of the incubation. Soil samples were taken for nutrient analysis immediately before planting seeds. Total C, total N, and extractable macro- and micronutrients were analyzed utilizing the same procedures mentioned previously (Chapter III). Sub-samples of soil were taken for nitrite (NO_2^--N) analysis which was extracted using 1 N KCl (Keeney and Nelson, 1982) and measured spectrophotometrically. Samples were obtained by collecting a complete top to bottom core through the depth of the soil mixtures using a 1.0-cm diameter metal tube. Two samples were taken from each pot and combined. Samples for nutrient analysis were dried at ~65° C and then ground using a mortar and pestle to pass a 2-mm sieve. Once samples were taken, the top 2.54 cm of soil were removed from each pot, seed were evenly dispersed, and then the soil was carefully replaced.

Germination measurements began the day following planting, and then were taken daily for a period of 14 d. Cotton germination at this time was very poor and plants that did emerge were harvested and left to decompose on the soil surface. The soil and SM mixtures were incubated for an additional 21 days and then pots were replanted as previously described. Germination data were collected for another 14-d period. Fourteen- d after planting, sorghum and cotton were thinned to the one most

representative plant per pot. Additional information that was collected every 7 d for a period of 28 d included plant height and the number of leaves and nodes per plant.

Plants were grown for approximately six weeks. Following the last day of data collection, plants were harvested (23 July 2010). Cotton plants were harvested at the soil surface, while sorghum was harvested 5 cm above the soil surface, so that the plants might ratoon. After harvest, soil samples were taken as previously described for nutrient analysis. Wet weights of plant biomass per pot were determined, with plants subsequently dried at 60° C until a steady dry weight was reached. Samples were finely ground through a Wiley mill and then a ring and puck mill and analyzed for total C and N and macro/micro nutrients as described previously for SM analyses (Chapter III). Five cotton seeds were subsequently replanted into each pot that previously contained cotton. Sorghum seed were replanted into any sorghum replicates that did not have a living plant after the first harvest. Germination was recorded for two weeks for both cotton and sorghum that were replanted. Plant growth measurements as described above were again taken weekly for six weeks, with plants harvested and analyzed as previously described.

Dry matter yields and nutrient uptake were compared within harvest and for the total of the two harvests. Nutrient uptake was calculated as the product of plant dry weight and elemental concentration. Statistical analysis was conducted using SAS 9.2. Significant ($P<0.05$) treatment effects were determined using mixed ANOVA with differences separated by Fisher's protected LSD.

Results

Germination, Plant Height and Yield

The first planting on 12 May 2010, 35 d after incorporating SM into soil, resulted in such poor germination (data not shown) that the few plants that emerged were removed and left to decompose on the soil surface. Controls and SM mixtures were subsequently incubated for an additional 21 days before cotton and sorghum were replanted on 2 June 2010. Germination and survival for the second planting showed no significant differences between treatments (Table 4). Numerically, 2.5% WM treatments resulted in lower cotton germination and survival than any other treatment (Table 4). Sorghum survival was most inhibited by both 2.5% WM and IM SM (Table 4).

Table 4
Relative germination and survival values on 16 June 2010 of cotton and sorghum as a percent of the control and ANOVA results

	Cotton		Sorghum	
	Germination	Survival	Germination	Survival
Treatment	----------% of control----------			
1.0% WM	90	100	94	100
2.5% WM	75	81	100	85
1.0% IM	90	100	94	92
2.5% IM	100	100	100	82
Fertilizer	95	100	94	100
p value (0.05)	0.2038	0.4449	0.9762	0.3287

WM and IM denote white mustard and Indian mustard, respectively.

Table 5
ANOVA results of each plant height measurement that was taken for cotton and sorghum

	Days after planting					
	14	21	28	35	42	49
	p - value					
Cotton	0.0008	0.0024	0.0146	0.03	0.0458	0.0446
Sorghum	<.0001	0.0007	0.0005	0.0009	0.0007	0.0106

Plant heights throughout the first growing season on each measurement date were significantly different between treatments for both cotton and sorghum (Table 5). On day 14, 2.5% WM SM resulted in significantly shorter cotton plants compared to all other treatments and remained that way until day 28 when they became statistically similar to controls. Cotton in 2.5% WM and control treatments were similar and shorter than all other treatments from day 28 to the end of the study (Fig. 17).

Sorghum height trends varied somewhat from that of cotton (Fig. 18). Not only did 2.5% WM SM negatively affect sorghum plant height, but so did the 2.5% IM SM treatment. The plants grown in these treatments, and especially 2.5% WM, were much shorter than any of the other plants (Fig. 18). Plant heights tended to be similar for the fertilizer and 1% SM treatments throughout the growth period. Decreased heights with 2.5% SM indicated that detrimental effects on sorghum growth from these amendments were still occurring more than two months following SM application.

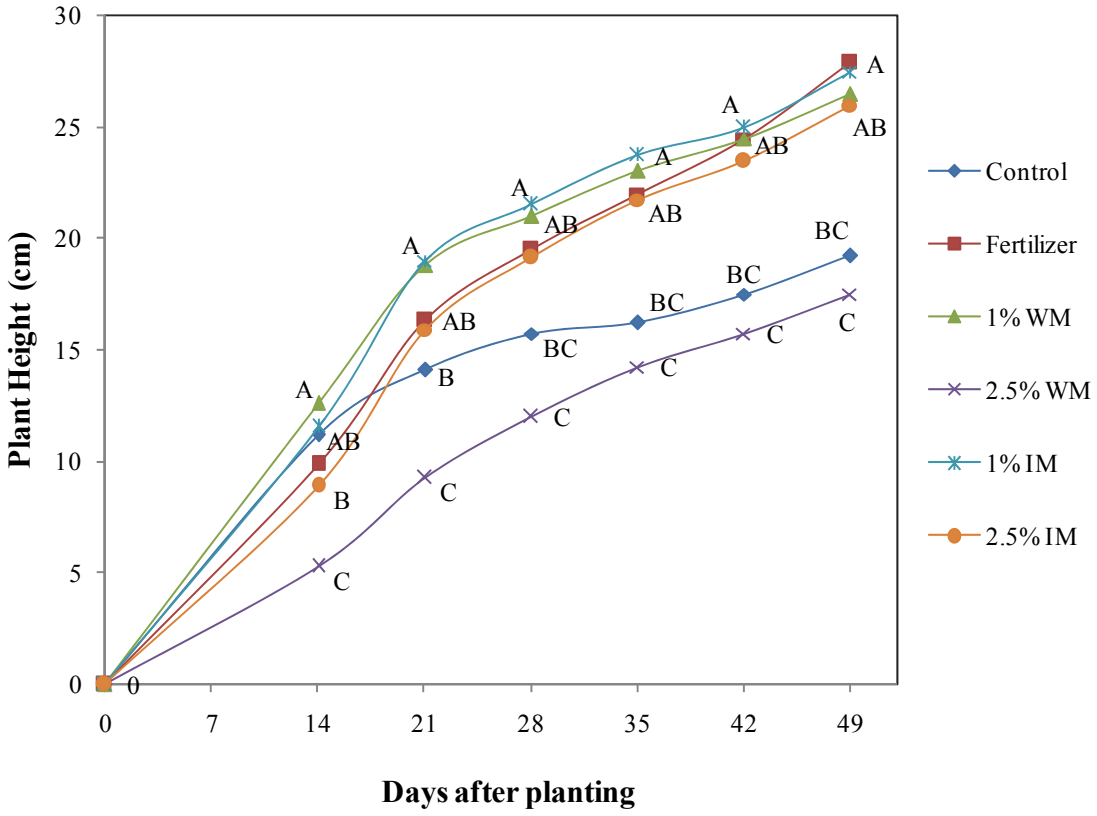

Fig. 17. Cotton plant heights over a 49-d period prior to the first harvest. Means within measurement date followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

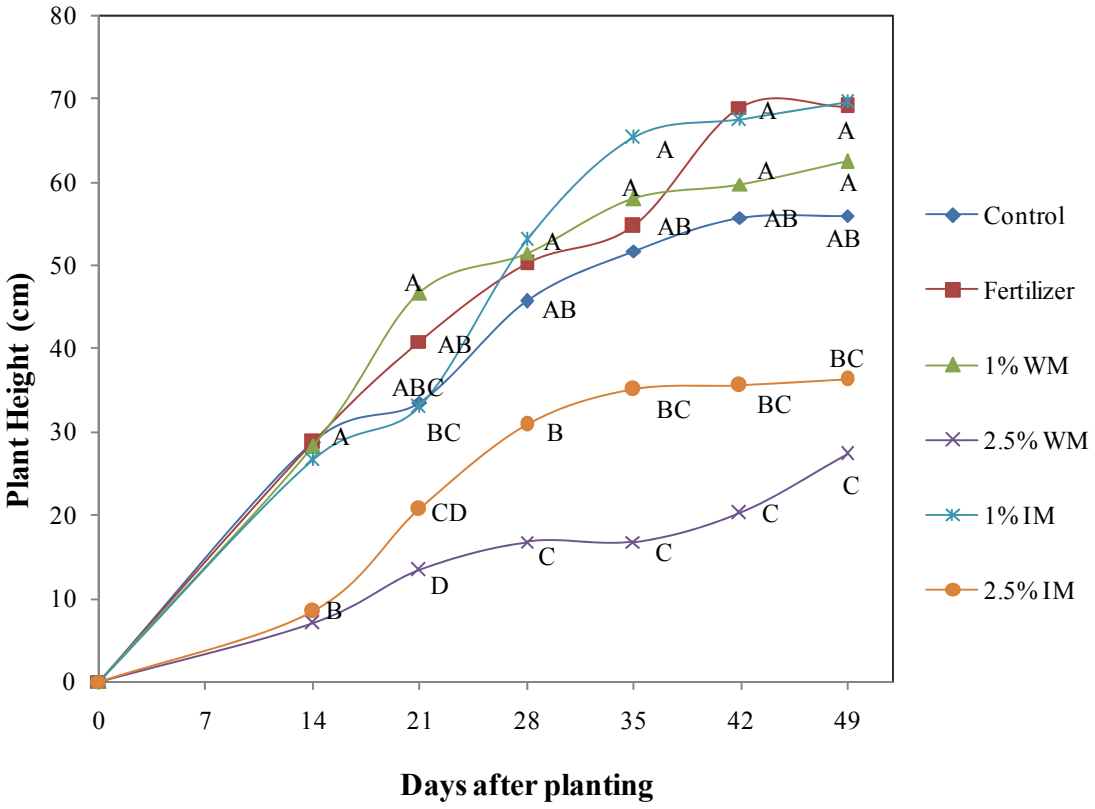

Fig. 18. Sorghum plant heights over a 49-d period prior to the first harvest. Means within measurement date followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

Cotton plants prior to the second harvest followed a very similar growth pattern to that of the first harvest (Figs. 17 and 19). Statistically, differences in height were not seen until 49 d after planting (Fig. 19). Prior to harvesting, plants in 2.5% WM treatments were significantly shorter than SM and fertilizer treatments. Both applications of IM and 1.0% WM SMs were significantly similar to the fertilizer treatment.

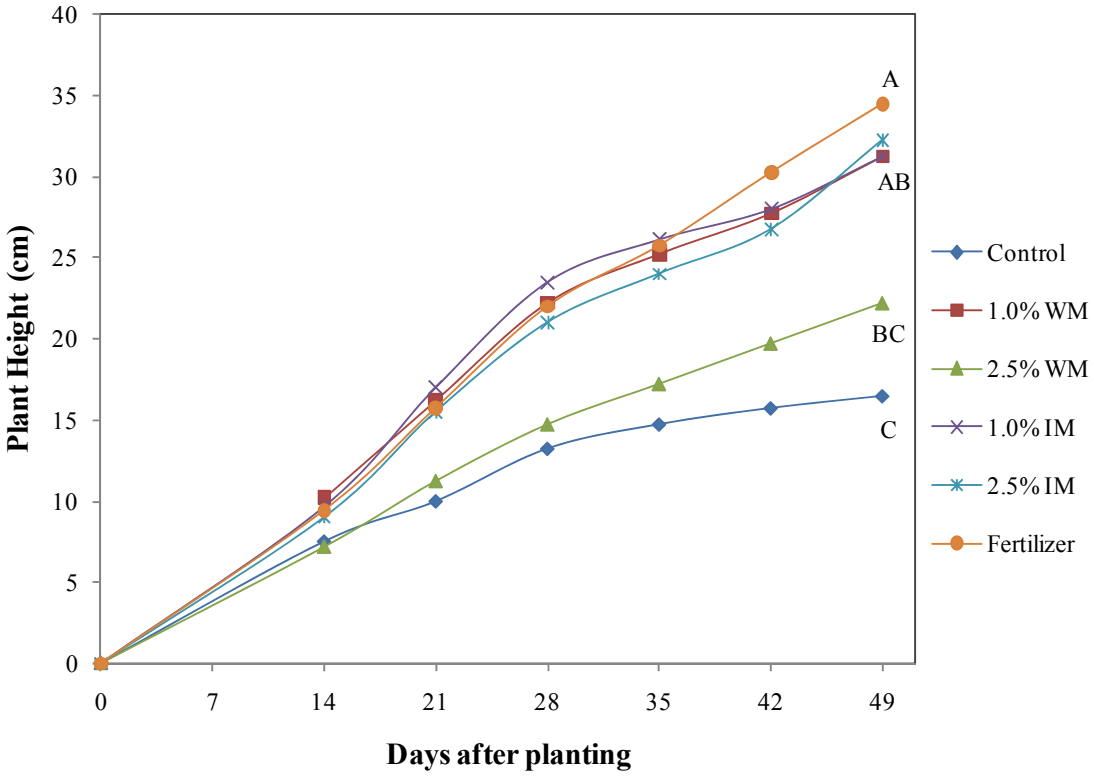

Fig. 19. Cotton plant heights over a 49-d period prior to the second harvest. Means within measurement date followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

The ratoon or second crop of sorghum followed similar growth patterns as the first crop, but heights of the second harvest exceeded that of the first for most treatments (Figs. 18 and 20). For each measurement date, sorghum planted in 2.5% WM SM was significantly shorter than all treatments, including the control (Fig. 20). At the end of the 49-d growth period, IM and 1.0% WM SMs resulted in similar heights to that of fertilizer treatments, but were not different from that of the control.

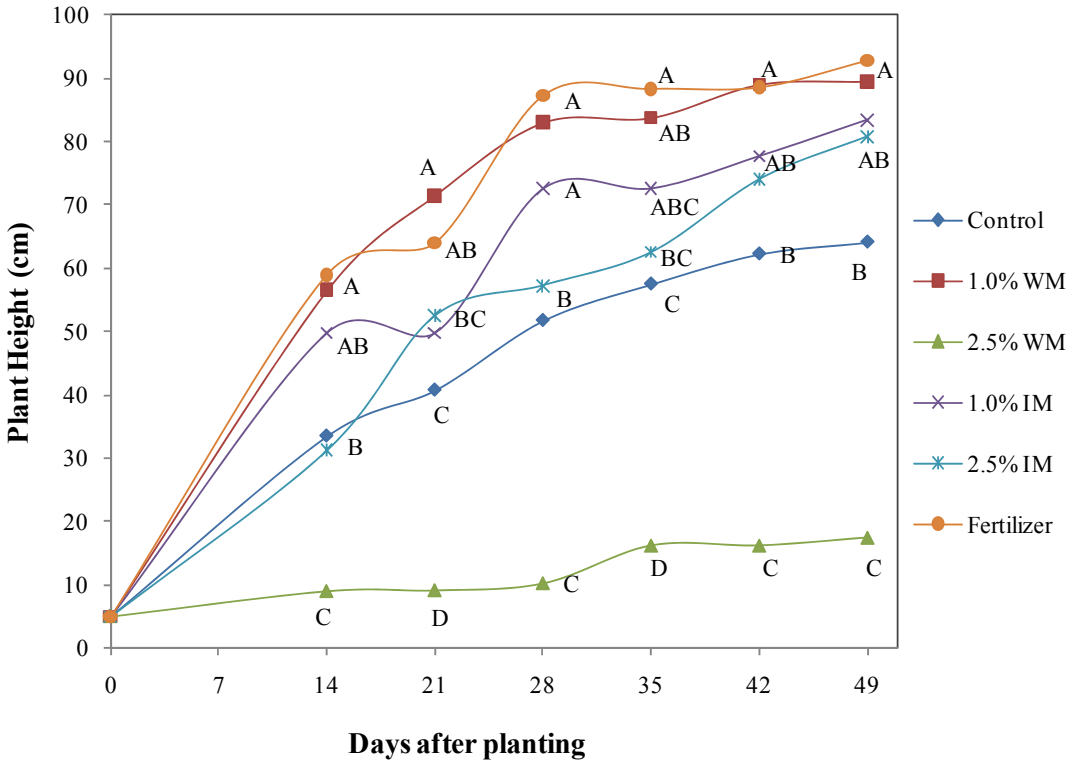

Fig. 20. Sorghum plant heights over a 49-d period prior to the second harvest. Means within measurement date followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

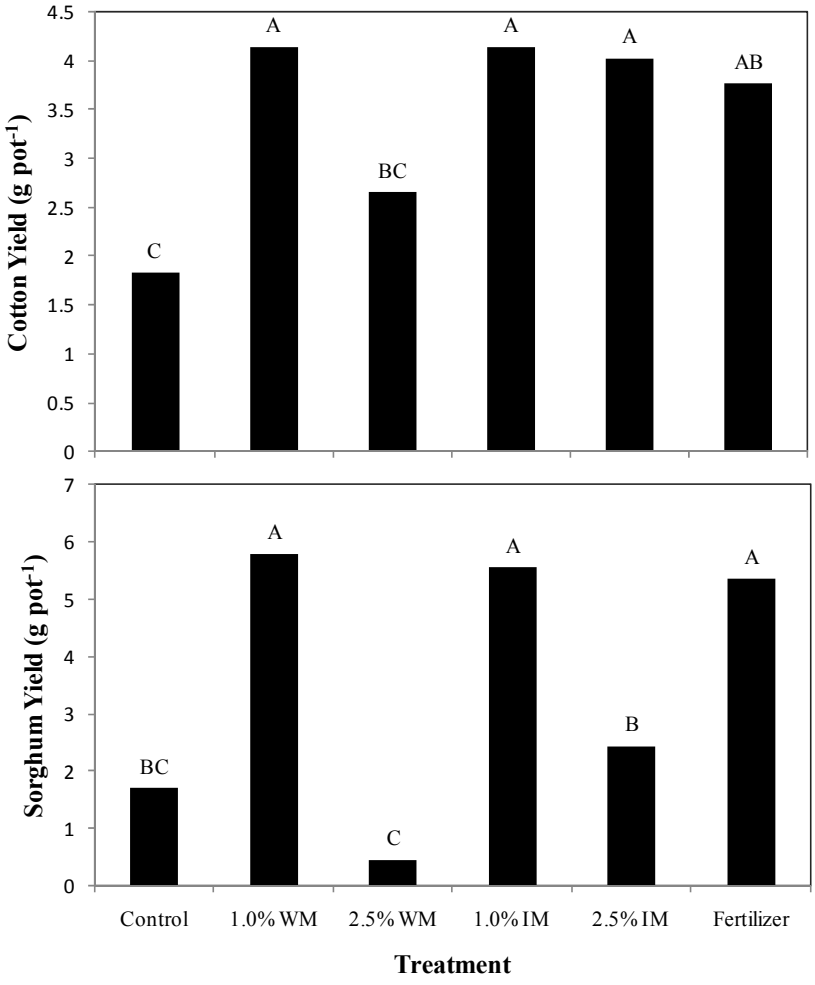

Fig. 21. Aboveground cotton and sorghum biomass yields from the first harvest. Means within cotton or sorghum followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

Treatments significantly affected aboveground biomass yields from the first harvest of both cotton and sorghum ($p = 0.0037$ and $p < 0.0001$, respectively). Dry matter yields followed plant height results for both crops. Cotton yield was lowest with the control and 2.5% WM SM treatments (Fig. 21). Seed meal applications of 1.0% produced similar biomass compared to the fertilized treatment and these were all greater than controls for both cotton and sorghum (Fig. 21). White mustard applied at 2.5% resulted in the lowest sorghum biomass, again indicating the longer-term negative effects from this treatment.

Aboveground biomass from the second harvest of both cotton and sorghum followed the same trends as the first harvest, but slight increases were seen with 2.5% SM applications (Figs. 21 and 22). Cotton biomass from fertilizer treatments was similar to all treatments but 2.5% WM SM and the control (Fig. 22). Unlike the first harvest, 2.5% WM SM treatments of the second harvest resulted in statistically greater biomass than the control. Sorghum biomass of the second harvest in 2.5% WM treatments was similar to the control. Biomass production in 2.5% IM increased dramatically from the first to the second harvest. This SM treatment did not affect sorghum biomass compared to the fertilizer treatments.

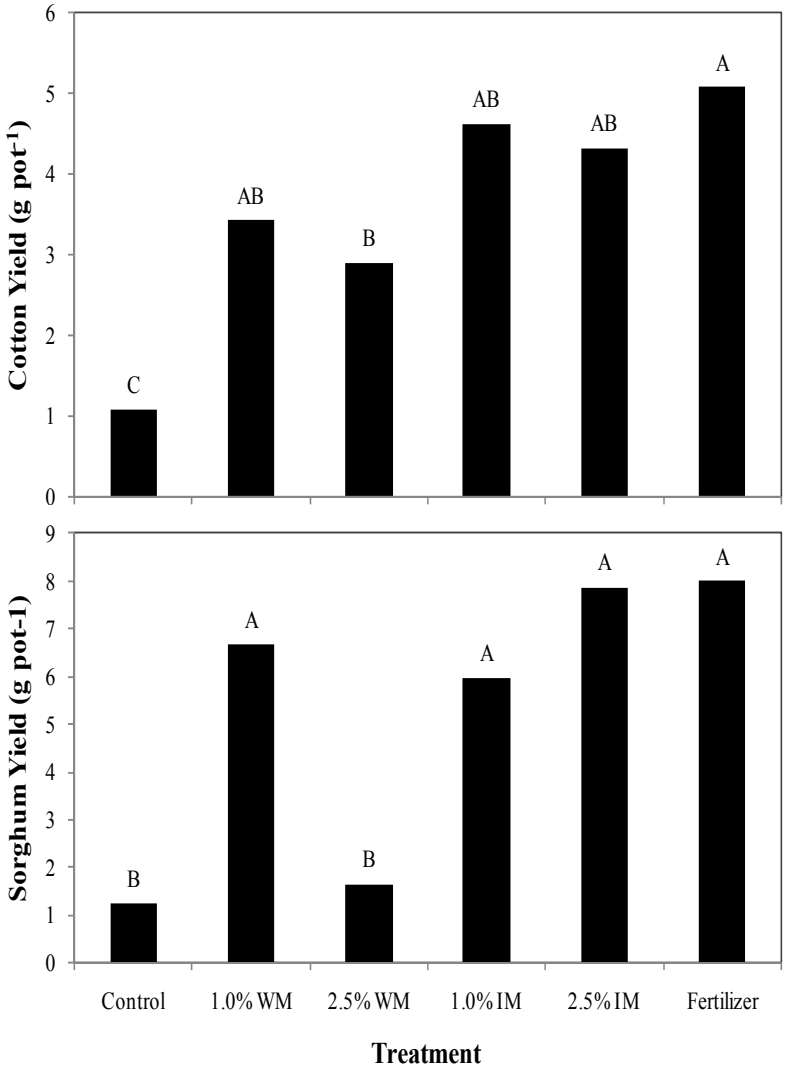

Fig. 22. Aboveground cotton and sorghum biomass yields from the second harvest. Means within cotton or sorghum followed by the same letter are not different at $P<0.05$ by Fisher's protected LSD. WM and IM denote white mustard and Indian mustard, respectively.

Plant Nutrient Concentrations and Uptake

For the first harvest of cotton and sorghum, treatment differences were noted for all plant nutrient concentrations and uptake (Table 6 & 7). For all nutrient parameters, treatments generally resulted in values statistically higher than the controls. Nitrogen uptake in cotton was highest with 2.5% SM, followed by those with 1.0% SM (Table 6). Sorghum N uptake was suppressed by 2.5% WM, but not any other SM treatments, which were all higher than the fertilizer treatment (Table 7). Even though uptake was low, the N concentration for sorghum grown in 2.5% WM SM was higher than any other treatment (Table 7). The very low biomass produced by this treatment, however, resulted in the low N uptake.

Phosphorus concentrations in cotton plants ranged from 1889 mg kg^{-1} (control) to 9225 and 10505 mg kg^{-1} (2.5% WM and 2.5% IM, respectively), and 3495 mg kg^{-1} for the fertilized treatment (Table 6) Similarly to cotton, 2.5% SM treatments resulted in statistically higher P concentrations within sorghum (Table 7). Seed meals applied at 2.5% also resulted in the highest potassium (K) concentrations for both cotton and sorghum, while K uptake was low for sorghum with 2.5% WM SM, but not cotton (Tables 6 & 7). Calcium (Ca) and magnesium (Mg) concentrations and uptake were lowest with 2.5% SMs, but in most cases results for 1.0% SM treatments were higher than the fertilized treatment. Sulfur (S) concentrations in cotton and sorghum plants were generally much higher in 2.5% SM treatments compared to all other treatments, and the 1.0% SM treatments were always higher than the fertilizer treatment (Tables 6 & 7).

Table 6
Cotton tissue nutrient concentrations and uptake from the first harvest

	TC	TN	P	K	Ca	Mg	S
Treatment	----------%----------		----------mg kg^{-1}----------				
Concentrations							
Control	40.96 b	1.28 d	1889 d	9846 d	16282 b	4915 c	3484 d
1.0% WM	42.17 a	3.99 bc	3439 c	20146 c	10739 cd	5524 bc	7942 c
2.5% WM	40.84 bc	5.35 a	9225 b	29869 a	8771 de	4867 c	12524 a
1.0% IM	41.87 a	4.13 b	4218 c	21076 c	11181 c	6306 a	7416 c
2.5% IM	41.00 b	5.26 a	10505 a	29177 a	7315 e	4932 c	9355 b
Fertilizer	40.34 c	3.74 c	3495 c	24944 b	20674 a	6256 ab	4099 d
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	0.0019	<.0001
	---------g pot^{-1}---------		----------mg pot^{-1}----------				
Shoot Uptake							
Control	0.75 b	0.02 d	3.5 d	18.0 d	30.0 c	9.0 d	6.4 d
1.0% WM	1.75 a	0.16 bc	14.3 c	82.8 c	44.4 b	22.8 ab	32.8 b
2.5% WM	1.44 a	0.19 ab	32.6 b	104.9 ab	30.7 c	17.2 c	44.3 a
1.0% IM	1.74 a	0.17 b	17.4 c	87.3 bc	46.0 b	26.0 a	30.6 b
2.5% IM	1.65 a	0.21 a	42.1 a	117.3 a	29.5 c	19.8 bc	38.1 ab
Fertilizer	1.52 a	0.14 c	13.2 c	94.1 bc	77.8 a	23.5 a	15.4 c
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Means within element concentration or uptake followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM, IM, TC and TN denote white mustard, Indian mustard, total C and total N, respectively.

Table 7
Sorghum tissue nutrient concentrations and uptake from the first harvest

	TC	TN	P	K	Ca	Mg	S
Treatment	----------%----------		----------mg kg^{-1}----------				
Concentrations							
Control	42.00 ab	0.71 d	1127 d	13360 d	10201 b	3947 b	813 d
1.0% WM	42.31 a	2.76 c	3226 c	19727 c	6766 c	6322 a	2753 b
2.5% WM	39.41 d	4.77 a	17903 a	27839 ab	3443 d	2735 b	4271 a
1.0% IM	41.88 ab	3.31 b	5620 b	22323 bc	6874 c	6633 a	2798 b
2.5% IM	40.59 c	4.41 a	15877 a	33474 a	3865 d	4122 b	4587 a
Fertilizer	41.81 b	2.65 c	3004 cd	23416 bc	13011 a	3673 b	1837 c
p-value	<.0001	<.0001	<.0001	0.0003	<.0001	0.0001	<.0001
	--------g pot^{-1}---------		----------mg pot^{-1}----------				
Shoot Uptake							
Control	0.72 c	0.01 c	1.9 d	22.7 b	17.3 c	6.7 c	1.4 c
1.0% WM	2.45 a	0.16 ab	18.6 c	113.5 a	39.1 b	36.2 a	15.9 a
2.5% WM	0.36 c	0.04 c	16.5 c	26.2 b	3.2 c	2.5 c	4.0 c
1.0% IM	2.32 a	0.18 a	30.4 b	121.6 a	37.8 b	37.2 a	15.3 a
2.5% IM	1.32 b	0.14 ab	50.3 a	106.8 a	13.1 c	14.2 bc	15.5 a
Fertilizer	2.24 a	0.14 b	16.1 c	125.7 a	69.8 a	19.9 b	9.8 b
p-value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Means within element concentration or uptake followed by the same letter are not different at P<0.05 by Fisher's protected LSD. WM, IM, TC and TN denote white mustard, Indian mustard, total C and total N, respectively.

Soil Nutrients

Darco soil analysis before SM amendments showed deficiencies in NO_3^--N, P, K, and Mg. The soil was sufficient in Ca, S, and Cu, and somewhat high in Fe, Zn, and Mn (Table 1; see Chapter III). Seed meals of WM, IM and camelina had similar concentrations of total C and N (45-50% and 5%, respectively), while total N was slightly less in jatropha SM (3.46%). Phosphorus concentrations ranged from 0.81to 1.18%, with IM containing the highest concentration compared to the other three SMs.

Jatropha contained the highest concentration of K at 1.54%, but WM (1.10%), IM (1.14%), and camelina (1.50%) were not much less.

Prior to planting cotton and sorghum on 12 May 2010, which was 35 d after treatments were initiated, soil nitrate (NO_3^--N) concentrations ranged from ~3.2 mg kg^{-1} in 2.5% SM treated soil to 6.6 mg kg^{-1} soil in controls to as high as 27.4 mg kg^{-1} with 1% SM, and ~63.0 mg kg^{-1} fertilizer treated soil (Tables 8 & 9). The 1.0% SM treatments averaged 17.9 mg NO_3-N kg^{-1} soil for WM and 25.1 mg kg^{-1} for IM SM (Tables 8 & 9). Due to such low NO_3^--N levels in soil treated with 2.5% SM, a nitrite (NO_2^--N) analysis was performed to help determine if organic N in 2.5% SM treatments was not being mineralized, or if it was mineralized, was it not being nitrified. Results showed <0.01 mg NO_2^--N kg^{-1} soil in both fertilizer and control treatments, but significantly higher concentrations were found in WM and IM treated soil (0.273 and 0.210 mg kg^{-1} soil, respectively). Although these NO_2^--N values were higher, they may not have been high enough to indicate significantly inhibited nitrification.

Total carbon did not show significant differences between treatments at any sampling time, but there were significant differences among treatments for TN, except after the second harvest of cotton (Tables 8 & 9). For all SM treatments, TN concentrations were higher (0.06 – 0.11%) than both the fertilizer (0.05 – 0.08%) and control (0.05 – 0.08%) treatments.

Table 8
Soil nutrient concentrations in samples taken preplant and after the first and second harvests of cotton

	TC	TN	NO_3^--N	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu
Treatment	----------%----------		----------mg kg^{-1}----------									
Pre-plant (35 d)												
Control	0.39	0.07 c	6.6 d	35 c	48 d	257 c	51 d	48 d	16.9 a	1.3 c	10.7 c	12.9
1.0% WM	0.39	0.08 b	19.5 c	70 b	116 c	290 bc	80 c	126 c	16.6 a	1.4 bc	26.2 a	11.7
2.5% WM	0.41	0.09 b	3.3 d	170 a	229 b	325 ab	104 b	258 b	16.1 a	1.9 a	28.8 a	23.3
1.0% IM	0.42	0.09 b	27.4 b	84 b	111 c	280 bc	90 bc	112 c	16.8 a	1.3 c	21.6 b	12.9
2.5% IM	0.46	0.11 a	3.1 d	201 a	308 a	360 a	142 a	367 a	19.9 a	1.5 b	17.6 b	20.4
Fertilizer	0.40	0.08 bc	51.8 a	77 b	140 c	300 bc	52 d	37 d	10.1 b	1.5 b	12.8 c	11.5
p-value	0.3786	0.0004	<.0001	<.0001	<.0001	0.0273	<.0001	<.0001	0.0078	<.0001	<.0001	0.228
Harvest 1 (105 d)												
Control	0.48	0.06 c	5.3 d	27 d	34 d	262	60 c	25 d	12.8 b	1.5 c	7.3 d	2.7
1.0% WM	0.57	0.08 abc	17.1 cd	97 c	76 c	361	93 bc	109 c	19.1 a	1.7 bc	28.5 b	4.7
2.5% WM	0.57	0.10 a	62.7 a	190 b	224 a	353	141 a	345 a	13.3 b	2.5 a	39.1 a	3.8
1.0% IM	0.44	0.08 abc	24.4 bcd	123 c	95 c	267	109 ab	90 cd	19.4 a	1.2 d	12.3 c	3.4
2.5% IM	0.46	0.10 ab	34.2 bc	251 a	204 a	360	142 a	241 b	10.7 b	2.0 b	27.9 b	3.8
Fertilizer	0.45	0.07 bc	48.2 ab	96 c	151 b	327	64 c	36 d	11.8 b	1.7 bc	13.6 c	3.7
p-value	0.0596	0.0112	0.0024	<.0001	<.0001	0.5902	0.0005	<.0001	<.0001	<.0001	<.0001	0.3389
Harvest 2 (140 d)												
Control	0.45	0.05	1.9	38 d	32 b	234 c	55 de	25 b	14.1 b	1.2 e	14.5 b	0.2
1.0% WM	0.44	0.06	2.9	98 c	46 b	226 c	77 cd	87 b	20.5 a	1.7 bc	31.3 a	0.2
2.5% WM	0.52	0.09	36.1	180 b	175 a	281 ab	127 b	310 a	11.7 b	2.2 a	34.5 a	0.2
1.0% IM	0.48	0.06	3.4	112 c	55 b	336 bc	83 c	95 b	18.7 a	1.3 de	17.7 b	0.2
2.5% IM	0.43	0.07	16.3	260 a	152 a	296 a	171 a	300 a	12.4 b	1.9 b	31.1 a	0.2
Fertilizer	0.42	0.05	7.5	90 c	78 b	276 bc	49 e	28 b	13.8 b	1.5 cd	15.5 b	0.2
p-value	0.8665	0.1091	0.0567	<.0001	<.0001	0.0026	<.0001	<.0001	<.0001	<.0001	<.0001	0.3964

LSD values were calculated using Fisher's protected LSD at P<0.05. Means within a sampling and nutrient followed by the same letter are not different. WM, IM, TC and TN denote white mustard, Indian mustard, total carbon and total nitrogen, respectively.

Table 9
Soil nutrient concentrations in samples taken preplant and after the first and second harvests of sorghum

	TC	TN	NO_3^--N	P	K	Ca	Mg	S	Fe	Zn	Mn	Cu
Treatment	----------%----------		----------mg kg^{-1}----------									
Pre-plant (35 d)												
Control	0.38	0.08 cd	6.7 cd	30 d	38 d	263 c	51 c	43 c	14.7 ab	1.4 b	13.9 c	6.6
1.0% WM	0.41	0.12 b	16.3 bc	89 c	118 c	311 abc	92 b	135 b	13.1 bc	1.5 b	29.3 a	9.6
2.5% WM	0.53	0.15 a	3.0 d	172 b	242 a	365 a	126 a	319 a	13.7 ab	1.7 a	27.7 a	7.5
1.0% IM	0.35	0.07 d	22.7 b	107 c	121 c	297 bc	98 b	116 b	11.5 c	1.2 c	21.8 b	8.9
2.5% IM	0.45	0.09 c	3.6 d	240 a	265 a	349 ab	126 a	268 a	15.4 a	1.4 b	15.8 c	14.7
Fertilizer	0.44	0.07 d	63.0 a	98 c	159 b	307 abc	57 c	41 c	9.2 d	1.4 b	14.9 c	7.2
p-value	0.0626	<.0001	<.0001	<.0001	<.0001	0.0238	<.0001	<.0001	0.0002	0.0003	<.0001	0.4919
Harvest (105 d)												
Control	0.43	0.07 d	3.3 d	26 d	15 d	210 e	43 c	22 d	15.0 b	1.2 c	7.7 d	2.2
1.0% WM	0.55	0.09 bc	5.2 d	87 c	28 cd	279 cd	60 bc	97 c	22.0 a	1.3 bc	23.7 b	1.8
2.5% WM	0.55	0.12 a	74.4 a	193 b	255 a	339 b	142 a	303 a	12.6 b	2.0 a	32.0 a	2.7
1.0% IM	0.48	0.08 cd	9.1 cd	101 c	26 cd	267 d	74 b	86 c	20.3 a	1.2 bc	16.0 c	2.8
2.5% IM	0.51	0.10 b	35.0 b	277 a	184 b	410 a	145 a	233 b	12.2 b	1.4 b	24.8 b	3.1
Fertilizer	0.45	0.08 cd	27.3 bc	92 c	61 c	325 bc	56 bc	32 d	13.0 b	1.3 bc	14.2 c	2.1
p-value	0.0956	<.0001	0.0031	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.2923
Ratoon Harvest (140 d)												
Control	0.37	0.04 c	2.9 b	33 c	23 b	273 bc	50 b	29 c	17.4 bc	1.1 c	18.2 c	0.3 a
1.0% WM	0.49	0.06 b	2.6 b	73 b	20 b	199 d	38 b	79 c	24.0 a	1.2 c	23.6 b	0.2 bc
2.5% WM	0.47	0.09 a	69.2 a	184 a	187 a	350 a	119 a	301 a	12.3 d	2.1 a	32.2 a	0.2 c
1.0% IM	0.48	0.06 b	2.2 b	89 b	22 b	206 cd	42 b	65 c	23.4 a	1.0 c	18.6 c	0.3 ab
2.5% IM	0.44	0.07 b	10.1 b	191 a	68 b	304 ab	97 a	194 b	19.6 ab	1.4 b	24.9 b	0.2 bc
Fertilizer	0.42	0.05 c	3.8 b	73 b	26 b	221 cd	30 b	26 c	14.7 cd	1.0 c	15.3 c	0.2 bc
p-value	0.1037	<.0001	<.0001	<.0001	0.0001	0.0019	<.0001	<.0001	0.0002	<.0001	<.0001	0.0439

LSD values were calculated using Fisher's protected LSD at $P<0.05$. Means within a sampling and nutrient followed by the same letter are not different. WM, IM, TC and TN denote white mustard, Indian mustard, total carbon and total nitrogen, respectively.

All SM treatments showed higher extractable phosphorus (P) concentrations than the control for preplant, post-harvest 1, and post-harvest 2 samples for both cotton and sorghum. Extractable P was also greater in 2.5 vs. 1.0% SM treatments for all three time periods for both crops, implying that mineralization may not have been suppressed by the higher SM application. Soil P concentrations varied little over time for the various treatments, possibly because uptake was limited compared to nutrients like N and K (Tables 6 and 7). Calcium, Mg, and S followed similar trends as observed with P.

Soil samples taken on 23 July 2010 after the first cotton harvest showed increased NO_3^--N in 2.5% SM treatments (34.2 – 62.7 mg kg^{-1} soil), but a slight decrease with 1.0% additions (17.2 – 24.4 mg kg^{-1} soil) compared to preplant samples (Table 8). Nitrate –N concentrations after the first harvest were also higher in the 2.5% WM (62.7 mg kg^{-1} soil) than in the fertilizer treatment (48.3 mg kg^{-1} soil) or any other treatment at this time. Soil total N decreased from preplant to first harvest for all treatments, except 2.5% WM (Table 8). Extractable soil P was greater in all SM treatments compared with the control, and 2.5% SM additions exhibited higher P levels than the fertilizer treatment (Table 8).

After the first harvest, soil planted to sorghum showed the same trends for NO_3^--N compared to soil planted to cotton (Tables 8 & 9). After 15 weeks, the 2.5% WM treatment contained 74.4 mg NO_3^--N kg^{-1} soil compared to 27.3 mg kg^{-1} fertilized soil (Table 9). Total N ranged from 0.073% in controls to 0.118% in 2.5% WM. There were no significant treatment differences for TC at any time period.

Soil analyses after the second cotton harvest resulted in no significant differences between treatments for TC, TN and NO_3^--N, but all other test parameters were significant (Table 8). Even though TN and NO_3^--N were not significant, the highest concentrations were in the 2.5% WM treatment compared to any other treatment (Table 8). The same trend was observed for P and K. Both 1.0 and 2.5% SM treatments resulted in higher concentrations of P than the controls and fertilizer treatments. Potassium levels in 1.0% SM treatments were not higher than in fertilizer treated soil after the second harvest.

After harvesting the ratoon, or second, crop of sorghum, significant differences were noted for all parameters, except TC (Table 9). Total N and NO_3^--N were highest in 2.5% WM SM treatments, and all SM treatments were higher in TN compared to the fertilizer treatment. Soils with 1.0% SM were not higher than fertilizer treated soil in NO_3^--N. Phosphorus and K were also highest in 2.5% SM treatments, but 1.0% SM additions compared to the fertilizer treatment were not higher in K.

Discussion

Germination, Plant Height and Yield

Based on results from the initial germination study and other literature (Hansson et al., 2008; Rice et al., 2007), it was unexpected that cotton and sorghum germination and survival would have been inhibited after incubating SMs for 35 d. Throughout this initial incubation period, dormant weed seed were observed germinating in the control and fertilizer treatments, but this was not the case for most replicates of 2.5% IM SM

and all replicates of 2.5% WM SM. The probable cause of germination inhibition was glucosinolate produced toxins, such as 2-propenyl isothiocyanate and ionic thiocyanate (SCN^-), produced from the SMs of IM and WM, respectively.

Increased cotton and sorghum germination and survival after the second planting, which extended incubation to 56 days, suggested that potential toxicity levels in SMs were decreasing by this time. However, sufficient residual toxicity apparently was present in the 2.5% WM SM treatment for cotton and 2.5% WM and IM treatments for sorghum to significantly decrease plant growth. These treatments resulted in the means for plant height and yield to be negatively affected by the replications that did not produce viable plants. Even though overall treatment means were lower for 2.5% WM SM compared to fertilizer and other SMs, the growth rate for the viable plants followed a similar trend to those planted with fertilizer or 1.0% SM treatments (Figs. 17 & 18). Seed meal applied at 1.0% did not have negative effects on plant growth; therefore it is likely that an application rate between 1.0 and 2.5% will not only control weeds, but should also enhance the growth of crops. Aboveground biomass production of cotton and sorghum increased for 2.5% WM and IM SMs from the first harvest to the second. The residual toxicity from these SMs must have decreased during the first growing period to cause an increase in production. It is recommended that SM applied at 2.5% or greater be allowed a period of time longer than 56 days for toxins to degrade.

Soil and Plant Nutrients

Darco soil is low or deficient in several macro and micronutrients and also has a low buffering capacity; thus making it highly susceptible to changes in nutrient concentrations by SM amendments. Seed meals when added in large enough amounts contain adequate nutrients to satisfy requirements for plant growth. Sorghum stover contained 0.43% N and 1200 mg P kg^{-1} stover (Powell et al., 1991), which is much lower compared to N and P concentrations in SMs. Both N and P are often limiting to plant growth in soil systems; therefore, SMs may be one way to offset these limitations.

Similar to the report by Brown and Morra (2009), there was evidence in our study of nitrification inhibition in pre-plant soil samples taken 35 days after SM amendments were added to soil, especially with 2.5% SM application. The NO_3^--N levels in samples receiving 1.0% SM were significantly higher than those receiving 2.5% SM additions (Tables 8 & 9), which normally should not be the case. In comparison soil NO_3^--N levels after the first and second harvests were generally greater with 2.5% than 1.0% SM additions. The inhibition that was occurring at 35 days apparently was no longer an issue 21 days later. Soil NO_2^--N concentrations with 2.5% SM applications were many times greater than those in fertilizer or control treatments. Typically, NO_2^- is a transient, intermediate product of the nitrification process; therefore, an increased concentration of NO_2^- in soil may have further indicated that nitrification was suppressed in 2.5% SM treatments.

Total soil C was not affected by SM application when compared to both the control and fertilizer treatments. Total N, conversely, was affected by SM addition. Seed

meal treatments exhibited higher levels of TN at all sampling times compared to the fertilizer treatment. The drastic increase in soil NO_3^--N concentration in 2.5% SM treatments after the first harvest, implied that N mineralization and nitrification had increased compared to the initial incubation period. During this same period, NO_3^--N did not increase in 1.0% SM treatments, suggesting that the N mineralized within this time period was taken up by plants or immobilized by microorganisms.

Treatment was significant for plant nutrient concentrations and uptake. Plants grown in SM-amended soil, compared to fertilized soil, had higher levels of TN, P and K, but not Ca or Mg. Sulfur concentrations were statistically higher in plants from SM–amended compared to fertilized soil, but more specifically plants from 2.5% SM additions were higher in S than those from 1.0% SM treatments. This is most likely due to the high levels of S, which originated from the glucosinolate molecules in the SM material. Seed meals, even applied at the 1.0% rate, can result in significant loading of nutrients to the soil, thus increasing the potential for more available nutrients for plant uptake compared to the fertilized treatment. Applying SM at a 1.0% rate is equivalent to 1120 kg N ha^{-1}, 210 kg P ha^{-1} and 290 kg K ha^{-1}. Seed meals can potentially be used as organic sources of nutrients for plant growth, but negative consequences may arise with over-loading the soil with nutrients when added at high rates.

CHAPTER VII

SEED MEAL EFFECTS ON SOIL BACTERIAL AND FUNGAL COMMUNITIES

Introduction

Microorganisms, specifically microflora, are responsible for a vast majority of processes occurring in the soil, such as soil formation, organic matter (OM) decomposition, nutrient cycling, and mutualistic interactions with plants. These processes are largely controlled by bacteria, fungi and archaea. In soil microbial communities, bacteria are generally most prevalent in number (10^8 individuals g^{-1} soil) and tend to rapidly metabolize the sugars, starches, and simple proteins in soil OM (Wollum, 1998; Alexander, 1998). Fungal organisms are usually much less in number (10^4 to 10^6 g^{-1} soil), compared to bacteria, but are important agents in soil mineralization processes (Wollum, 1998). Together with bacteria, fungi are primary actors in the decomposition of cellulose, hemicelluloses, and pectin in plant cell walls. Ligin, a much more recalcitrant material, can be degraded by fungi, as well (Morton, 1998).

Gimsing et al. (2006) indicated the involvement of microorganisms in seed meal (SM) mineralization and glucosinolate degradation by sterilizing soil, after which no degradation took place. During glucosinolate degradation, isothiocyanates are produced and made available to plants and microorganisms. The germination study (Chapter III) reported in this thesis showed that plants are highly sensitive to the toxicity generated from SM, but it is unclear as to the effect it might have on the soil microbial community.

The objective of this study was to determine the effects of SM application on soil bacterial and fungal communities during SM degradation.

Materials and Methods

Soil Sampling

This study was conducted in conjunction with the nutrient uptake study (Chapter VI) in a temperature controlled glasshouse. A Darco sandy loam was amended with treatments of *Sinapis alba* (white mustard, WM) and *Brassica juncea* (Indian mustard, IM) SMs at 1.0 and 2.5% (w/w) and fertilizer applications of chemical grade reagents, including NH_4NO_3 (448 kg N ha^{-1} 15cm), $CaHPO_4 \bullet 2H_2O$ (168 kg P ha^{-1} 15cm), and KCl (336 kg K ha^{-1} 15cm). Unamended soil was utilized as the control treatment. All treatments, including the unamended soil, received additions of $CaCl_2 \bullet 2H_2O$ (336 kg Ca ha^{-1} 15cm) and $MgSO_4$ (168 kg Mg ha^{-1} 15cm). The study was a completely randomized design with 4 replications of each treatment.

Treatments were prepared on 7 April 2010 and allowed to incubate at ~32° C for the duration of the experiment. To each pot, 480 mL of distilled water was added at the start of the experiment. The gravimetric water content was maintained at 0.238 g/g by adding water to a constant weight on a daily basis. Soil samples were taken for microbial analysis every 35 days for 20 weeks and were obtained by collecting a complete top to bottom core through the depth of the soil mixtures using a 1.0-cm diameter metal tube. Two samples were taken from each pot, combined and immediately placed in a -80° C freezer.

DNA Extraction and Purification

Soil samples extracted and analyzed were those taken on 12 May 2010 (pre-plant; 35 d after amendment) and 23 July 2010 at plant harvest 1 (105 d after amendment). DNA extractions were performed using a lysozyme-modified version of the manufacturer's protocol (Hollister et al., 2010) of a PowerMax soil DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). Thawed soil samples (10 g) along with 15 mL of bead solution were added to each bead-beating tube, after which samples were vortexed for 5 min. Lysozyme was added at a final concentration of 1 mg mL^{-1} and then samples were incubated in a water bath (37° C) for 1 h. Following lysozyme treatment, solution "C1" was added and samples were incubated for an additional 30 min at 65° C. The remainder of the extraction was conducted following the manufacturer's protocol. Following elution, DNA samples were concentrated by an ethanol precipitation and quantified using a NanoDrop ND-1000 spectophotometer (NanoDrop Technologies, Wilmington, DE, USA) and a Quant-iT Picogreen dsDNA assay kit (Invitrogen Corp, Carlsbad, CA, USA).

Community Quantitative PCR (qPCR)

Based upon methods and results of Fierer et al. (2005), Boyle et al. (2008), and Hollister et al. (2010), community qPCR assays were used to evaluate relative abundances of bacteria and fungi in SM and fertilizer treated soil. Assays were performed in triplicate, using an Eppendorf Mastercycler® ep Realplex thermal cycler (Eppendorf, Hamburg, Germany). Reactions (10 µL) contained: 4.5 µL 2.5x

RealMasterMix with 20x SYBR solution (5Prime, Inc., Gaithersburg, MD, USA), 1.0 μL BSA (10 mg mL^{-1}), 0.5 μL of each primer (10 μM), 2.5 μL molecular-grade water, and 1.0 μL template DNA (2.5 ng μL^{-1}). Bacterial primer sets were Eub338 and Eub518 (Fierer et al., 2005) and fungal primer sets included 5.8S and ITS1F (Boyle et al., 2008). Thermocycling began with an initial denaturation at 95° C for 15 min and was followed by 40 cycles of 95° C for 1 min, 53° C for 30 s, and 70° C for 1.5 min.

Plasmid standards for bacteria were generated from the genomic DNA of *Escherichia coli* DH10B (pUC19) (obtained from Carlos Gonzales, Texas A&M University). Fungal plasmid standards were generated from the genomic DNA of *Neurospora crassa* 74-OR23-1VA (obtained from Heather Wilkinson, Texas A&M University). Gene fragments were amplified using a FailSafe PCR kit (Epicenter Biotechnologies, Madison, WI, USA), their corresponding qPCR primer sets, and the amplification conditions described above. Fragments were then cloned into a pGEM-T easy cloning kit (Promega), and plasmids from successful transformants were extracted using a Wizard SV Miniprep kit (Promega). Plasmids were set up in a dilution series ranging from 5 x 10^{-3} ng to 5 x 10^{-7} ng of DNA $reaction^{-1}$.

Calculations and Statistical Analysis

Target copy numbers for each reaction were calculated using a standard curve generated by the log of bacterial or fungal copy numbers and the calculated threshold cycle value ($r^2 \geq 0.94$ in all cases). DNA copy numbers were originally represented as copy number per 2.5 ng DNA. Conversion of results to DNA copy number g^{-1} soil was

conducted using DNA amounts (ng uL^{-1}) quantified with a Picogreen assay and the purification volume for each sample. DNA extractions were performed with 10 g soil.

Statistical analyses were performed using SAS version 9.2. Quantitative PCR data were analyzed by mixed analysis of variance (ANOVA). Significant effects (main and interaction) were separated using Fisher's protected LSD at the $P<0.05$ level.

Results

Bacterial gene copy numbers 35 d after amendment application and prior to planting the first crop did not show significant effects between treatments ($p=0.2617$), but values ranged from 5.01×10^8 copies g^{-1} soil in the fertilizer treatment to 1.09×10^{10} and 2.53×10^{10} copies g^{-1} soil in 2.5% WM and 2.5% IM treatments, respectively (Fig. 23). The number of bacterial copies in all treatments, other than the fertilizer treatment, was numerically greater than in the control; this was also observed at plant harvest (105 d after amendment addition). From 35 d to 105 d, bacterial copy numbers decreased for all treatments, with the greatest decreases occurring with 2.5% WM and 2.5% IM additions (Fig. 23).

Significant differences between treatments for fungal gene copy numbers were observed at the 35- and 105-d samplings ($p=0.0122$ and $p=0.0053$, respectively). For all treatments and both sampling dates, bacteria were numerically dominant to fungi (Fig. 24).While bacterial copy numbers decreased between sampling dates, the majority of treatments experienced an increase in fungal copy numbers. Seed meals applied at 2.5% experienced a decrease in fungal copy numbers from 35 d to 105 d, but 1.0% SM

increased (Fig. 24). Pre-plant fungal copy numbers ranged from 2.00 x 10^7 copies g^{-1} soil in fertilizer treatments to 1.79 x 10^8 copies g^{-1} soil in 2.5% WM. Conversely, at harvest 2.5% WM showed the least number of fungal copies (1.85 x 10^7 copies g^{-1} soil).

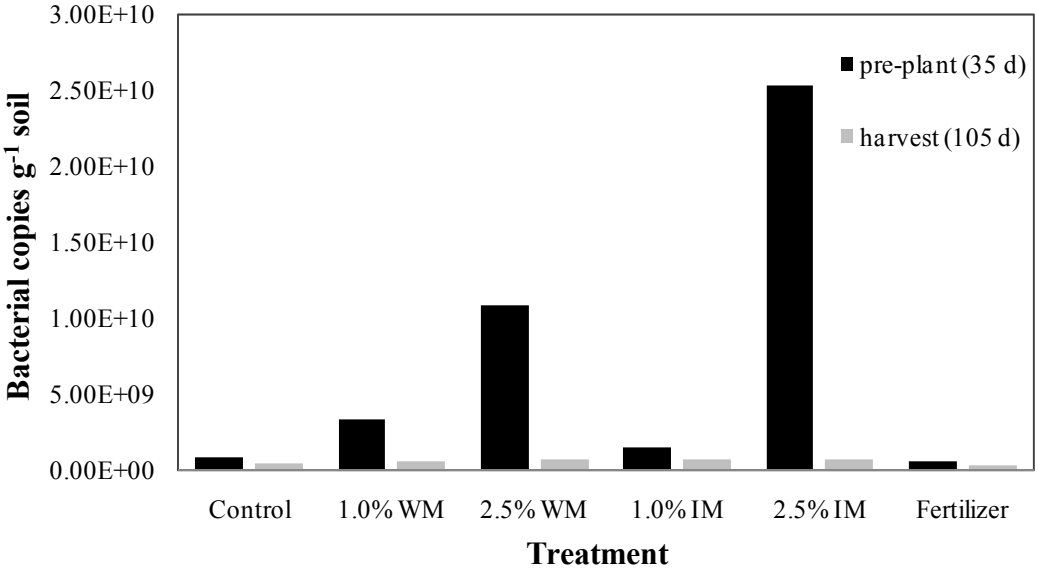

Fig. 23. Bacterial copy number for treatments at preplant and harvest 1. Means were not significant.

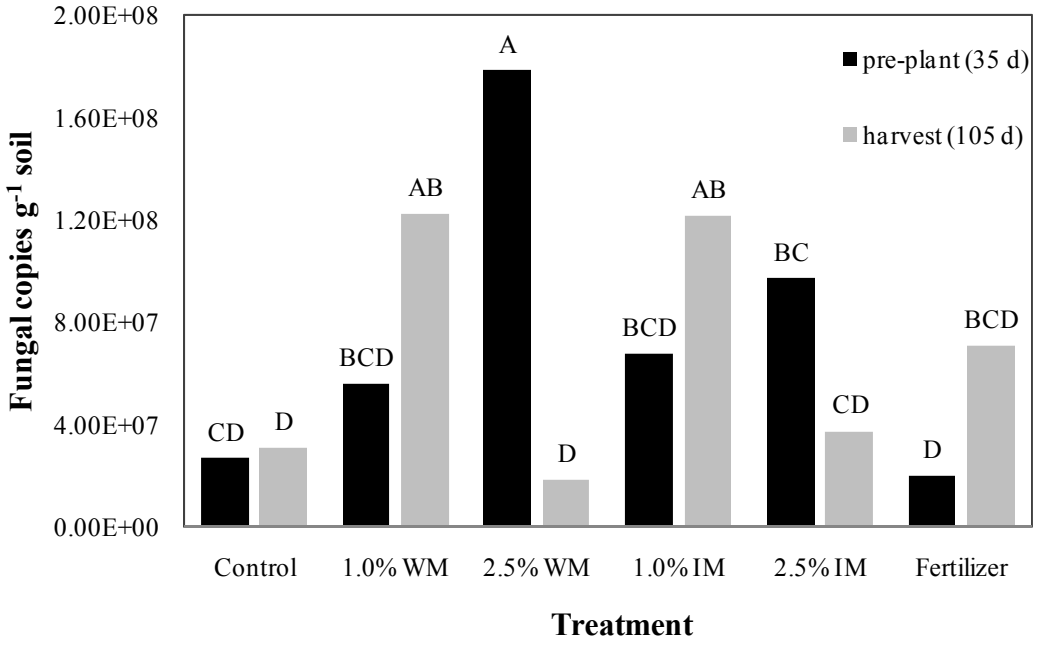

Fig. 24. Fungal copy number for treatments at preplant and harvest 1. Means followed by the same letter are not different by Fisher's protected LSD at $P<0.05$.

Discussion

Seed meal amendments applied at 2.5% added the greatest amount of organic material to the soil; therefore, it was expected that bacterial numbers would be greatest for the higher SM amendments compared to all other treatments. White mustard SM applications resulted in lower bacterial numbers, compared to IM SM, which is most likely due to ionic thiocyanate (SCN^-) inhibition on microorganisms. White mustard SM was reported to contain greater amounts of SCN^- liberating glucosinolates compared to IM SM (see Chapter V). Also, SCN^- is not as easily degraded as isothiocyanates;

therefore, their effect may persist in the soil for a longer duration (Vaughn et al, 2006; Rice et al., 2007).

Fungi are responsible for the mineralization of more recalcitrant OM, so it is expected that fungal copy numbers would increase while bacterial copy numbers decreased from 35 to 105 d. Seed meals applied at 1.0% resulted in a decrease in bacterial copies and an increase in fungal copies; thus, one might assume that fungi are more tolerant to the toxins produced from SM compared to bacteria. Treatments that did not increase fungal copy numbers included 2.5% WM and IM SM treatments. Fungi were likely inhibited by toxins produced by these SM treatments. The WM treatment exhibited a greater decrease in fungal numbers over the 70 d time period compared to IM. Greater concentrations of SCN^- in WM SM were a probable cause of greater inhibition in not only bacteria, but fungi. It is likely that toxicity was still an issue even 105 d after amendments were added as evidenced by not only microbial inhibition, but also plant inhibition.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Mechanical weed control is a commonly used practice in organic farming systems, but is not always feasible, successful or economical. The germination and survival study demonstrated the ability of oilseed meals to suppress and, in some cases, control Johnsongrass and redroot pigweed growth by as much as 96%. The most tolerant of weed species in this study, Johnsongrass, was most suppressed with 2.5% WM incubated for 1 or 7 d. Survival of Johnsongrass was similarly negative for both 1.0 and 2.5% WM incubated for 1 d. Seed meal applications of 2.5% were also most detrimental to the germination of pigweed, regardless of the SM source. The SM applications most effective at controlling Johnsongrass and pigweed, were also inhibitory to cotton and sorghum. While weed suppression is achievable, such factors as SM source, application rate and incubation time prior to growing agronomic crops must be optimized to control weeds without harming crops.

There were initial concerns that SM applications may have acidified the Darco soil enough to potentially injure crops, but this was not the case. Both acidic Darco and alkaline Branyon soils approached a neutral pH level with SM amendments. This result supported the hypothesis that glucosinolate hydrolysis products, and not changes in soil pH, were the primary cause of germination and survival inhibition.

The *Brassicaceae* SMs were determined to have individually unique glucosinolate profiles. White mustard SM was dominated by 4-hydroxybenzyl or

sinalbin, which is the precursor of thiocyanate (SCN^-). Sinigrin (2-propenyl glucosinolate) was the main compound isolated in IM SM. Camelina SM was slightly different in that it did not contain just one dominant compound but three. The prominent compound of the three was 10-methylsulfinyldecyl (glucoamelinin). The concentrations of dominant glucosinolates found in WM and IM SM were much higher than the combined concentration measured in camelina SM. This suggests that camelina SM must have additional compounds, besides isothiocyanate producing glucosinolates, to inhibit seed germination and seedling survival to degrees similar to WM SM. The germination and survival inhibition results suggest WM to be more toxic to plants than IM, even though WM and IM contain nearly equal concentrations of glucosinolates. The apparent toxicity levels assessed from the germination study may be attributed to, not only the glucosinolate hydrolysis product formed, but also the persistence of that product in the soil.

Seed meals pose potential benefits as soil amendments to suppress weeds and enhance nutrient availability in the soil, but if applied too close to planting, may be inhibitory to plant growth and production. Aboveground biomass production and plant height of both cotton and sorghum were affected by SMs applied at 2.5% during the first growth period. The increase in biomass from the first harvest to the second harvest with 2.5% WM and IM SMs indicated that toxicity effects were beginning to diminish. The negative results of 2.5% SM applications were not evident with 1.0% applications. Seed meal applied at 1.0% resulted in similar growth and production compared to the fertilizer

treatment. Also, the second harvest of sorghum produced similar biomass yield with 2.5% IM SM compared to that of the fertilizer treatment.

While plant growth was affected, nutrient availability did not seem to be altered, except for lowered NO_3^--N concentrations in preplant soil samples with 2.5% SM treatments. After the first harvest, NO_3^--N levels of these treatments were greater than in any other treatment. This suggests possible inhibition of nitrification. Total soil N and extractable P were either similar or greater in 1.0 and 2.5% SM treatments compared to the fertilized treatment. Plants grown in SM-amended soil, compared to fertilized soil, had higher levels of TN, P and K. Nutrient uptake levels in 2.5% SM-amended soil were hindered due to the low biomass production in these treatments.

Applying SMs at rates as low as 1.0% (1120 kg N ha^{-1}, 210 kg P ha^{-1} and 290 kg K ha^{-1}) may lead to significant nutrient loading, thus increasing the potential for more available nutrients for plant uptake and decreasing the potential for plant growth inhibition if incubated for a sufficient length of time. Seed meal applied at a 1.0% rate should control weed growth prior to planting crops. An incubation period of at least 56 d, which is proposed to prevent negative growth effects, should be ideal for high concentrations of plant available N to be present in the soil. Residual N levels after the first harvest presented an opportunity to capitalize on a ratoon crop, while improving N use efficiency. With additional research, SM utilization in organic farming systems may be an effective way to utilize byproducts of biodiesel production.

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