EFFECTIVENESS OF MUSTARD SHORT-CYCLE COVER CROPS FOR MANAGEMENT OF *PHYTOPHTHORA CAPSICI* AND *FUSARIUM* SPP. IN CUCURBITS

BY

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THESIS

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ABSTRACT

This research was conducted to determine the effectiveness of mustard *Brassica juncea* L. 'Florida Broadleaf' (FBL) and *Sinapis alba* L. 'Tilney' for management of *Phytophthora capsici* and *Fusarium* spp. in cucurbits. To accomplish this goal laboratory, greenhouse, and field studies were conducted during 2008-2010.

In the laboratory studies, mustard extracts were tested for inhibition of colony growth, sporangia production, zoospore germination, and oospore germination of four *P. capsici* isolates (PC-1, PC-2, PC-3, and PC-4). In addition, the mustard extracts were tested for inhibition of colony growth of *F. oxysporum* (F-2 and F-3) and *F. solani* (F-1) isolates. The FBL extract significantly reduced the colony growth of all four isolates of *P. capsici* compared to that of control treatment. In addition, FBL extract significantly reduced the sporangia production of PC-1 isolate of *P. capsici* compared to that of control treatment. FBL and Tilney extracts significantly reduced sporangia production of PC-3 isolate of *P. capsici*. The FBL extract significantly reduced germination of *P. capsici* oospores compared to that of control treatment. None of the mustard extracts significantly affected zoospore germination of any of the isolates of *P. capsici*. The FBL+Tilney and Tilney extracts significantly reduced colony growth of F-3 isolate of *F. oxysporum* and F-1 isolate of *F. solani*, respectively, but colony growth of other *Fusarium* isolates were not affected.

In the greenhouse trial, thirteen glucosinolates were detected and quantified in both FBL and Tilney mustard cultivars. FBL roots had significantly lower total glucosinolate content (μmol/g) than FBL foliage. Conversely, Tilney roots had significantly higher glucosinolate content (μmol/g) than Tilney foliage. Mean total glucosinolate content (μmol/g) was not

significantly different for FBL and Tilney cultivars. Sinigrin was the major glucosinolate detected in both FBL and Tilney cultivars.

Mustard extracts were tested against *P. capsici* crown infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin in the greenhouse. None of the 'Eureka' cucumber seedlings were infected by *P. capsici*. However, all of the 'Magic Lantern' pumpkin seedlings were infected. The FBL+Tilney and FBL extracts significantly reduced *P. capsici* crown infection in 'Dickinson' pumpkin seedlings compared to the control.

Four field trials, including 2008-spring, 2008-fall, 2009-spring, and 2010-spring were conducted in two fields in Tazewell County near Pekin, Illinois. Both fields had naturally infested soils with *P. capsici* and a history of Phytophthora blight. Similar to the greenhouse trial, 13 glucosinolates were also detected and quantified in the field grown FBL and Tilney cultivars. Foliage of FBL and Tilney did not significantly differ in total glucosinolate content (μmol/g) compared to that of roots. Mean total glucosinolate content (μmol/g) was also not significantly different for FBL and Tilney cultivars in the field trials. Sinigrin and glucoalyssin were the major glucosinolates detected in FBL and Tilney cultivars, respectively.

No glucosinolates were detected in soil samples collected after incorporation of mustard plants into the soil. This was likely due to hydrolysis of glucosinolates in the soil samples at higher temperatures during the transit of soil samples from the fields to the laboratory.

Biomass (gram fresh weight/m^2) of mustard plants collected from 1 m^2 area of field plots was determined. Mean biomass was the lowest in 2008-fall and the highest in 2008-spring field trials. Mean biomass of FBL and Tilney mustard cultivars was not significantly different from each other in any of the trials. Mean glucosinolate content in mustard plants incorporated to 1 m^2 area of the plots (μmol/ m^2) was the lowest in 2008-fall and the highest in 2008-spring trials.

In the field trials, following incorporation of mustard plants, 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin were seeded and monitored for *P. capsici* and *Fusarium* spp. infection on the plants. No *Fusarium* infection was detected on any of the cucurbit plants. Also, *P. capsici* did not infect any of the 'Eureka' cucumber seedlings and vines in the field. None of the mustard treatments significantly reduced vine- and fruit-infection by *P. capsici* in the cucurbit crops tested. Soil samples were collected before and after incorporation of mustards to determine density of *P. capsici* oospores and *Fusarium* spp. colony forming units (cfu). The FBL+Tilney treatment significantly reduced oospore density of *P. capsici* and cfu count of *Fusarium* spp. in the plots incorporated with mustard plants compared to the same plots before incorporation of mustard plants. Mustard incorporation into the soil did not affect seed germination of the cucurbit crops used in this study.

To My Family

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CHAPTER 1

LITERATURE   REVIEW

Cucumber and pumpkin production

Cucumber (*Cucumis sativus* L.) and pumpkin (*Cucurbita* spp.) are among the major vegetable crops grown in the United States (US) for fresh market and processing. In 2009, field area planted to cucumbers was more than 60,000 hectares, with fruit yields exceeding 900,000 metric tons (USDA/NASS, 2009). Florida and Michigan are the leading states in fresh and processing cucumber production, respectively (USDA/NASS, 2009). Jack-o-lantern and processing pumpkin field was 19,300 ha in 2009, with a fruit yield of 473,000 metric tons (USDA/NASS, 2009). Illinois is the leading state in pumpkin production with over 5,700 ha and 217,000 metric tons of yield (USDA/NASS, 2009). Illinois produces and processes more than 90% of the US processing pumpkins.

Phytophthora blight of cucumber and pumpkin

Phytophthora capsici is among the major pathogens that cause significant crop loss in cucumber and pumpkin fields. *P. capsici* is an oomycete pathogen and was described by Leonian in 1922 in New Mexico (Ristaino and Johnston, 1999). In recent years, this pathogen has become a serious threat to cucurbit production in the US and worldwide (Babadoost and Zitter, 2009; Hausbeck and Lamour, 2004; Islam, et al. 2004). This pathogen can infect cucurbit plants at any growth stage causing up to 100% crop loss. *P. capsici* infects seedlings, leaves, vines, and fruits of cucumber and pumpkin causing damping-off, foliar blight, and fruit rot (Babadoost, 2000). Infection of fruit usually starts on the side of the fruit contacting the soil. Fruit symptoms start

with the appearance of water-soaked lesions on the fruit surface, which soon becomes covered by white cottony mycelium and sporangiophores. Gevens et al. (2006) reported that severity of cucumber fruit infection by *P. capsici* decreases with increasing age of the fruit. Leaf symptoms caused by *P. capsici* appear as water-soaked, olive green lesions, which gradually become necrotic (Babadoost, 2000). Vine infection of pumpkin by *P. capsici* also results in water-soaked lesions, which girdles the vine leading to wilting of the foliage above the point of infection (Babadoost and Zitter, 2009; Zitter, 1989).

Characteristics of *Phytophthora capsici*

P. capsici produces hyphae, which are without cross walls (coenocytic). It produces sporangiophores, sporangia, and zoospores (asexual reproductive bodies) and antheridia, oogonia, and oospores (sexual reproductive bodies). *P. capsici* produces abundant sporangia on culture media at 20-25°C (Erwin and Ribeiro, 1996; Islam et al., 2004). Under favorable conditions of moisture and temperature, sporangia produce zoospores. In the moist soil, sporangia release zoospores into the soil. *P. capsici* also forms chlamydospores (thick-walled cells), which survive in soil under unfavorable environmental conditions (Erwin and Ribeiro, 1996). However, not all isolates of *P. capsici* are capable of forming chlamydospores (Ristaino, 1990).

P. capsici is a heterothallic oomycete requiring two different mating types (A1 and A2) for oospore production. Both of the mating types can produce antheridia (male reproductive structure) and oogonia (female reproductive structure). Oospores of *P. capsici* undergo a maturation phase during which nuclei fuses before oospores germinate (Erwin and Ribeiro, 1996). *P. capsici* oospore germination was reported to increase with age (Satour and Butler,

1968). Lower rate of germination was reported for oospores produced in dark conditions but exposed to light for a week before harvesting from the culture medium, compared to the oospores under continuous dark conditions until harvested from the plates (Hord and Ristaino, 1991). The best temperature for *P. capsici* oospore germination was reported to be 24°C (Hord and Ristaino, 1991).

Disease cycle and epidemiology of Phytophthora blight

P. capsici overwinters in soil as oospores and as mycelia in infected plant debris (Erwin and Ribeiro, 1996). Oopores can survive in the soil for more than three years, while survival of mycelia does not exceed four months (Babadoost and Pavon, 2009; French-Monar et al., 2007). Under warm and wet conditions, *P. capsici* oospores in the soil germinate to form sporangia and sporangia produce zoospores. Spread of sporangia occurs by water and by aerial dispersion (Ristaino and Johnston, 1999). Plants may be infected by sporangia directly or by zoospores via direct contact or through splashing irrigation and rainfall (Erwin and Ribeiro, 1996; Granke et al., 2009; Zitter, 1989). Zoospores, released into saturated soil with water, swim and infect the plants and produce mycelia, sporangiophores, and sporangia on the infected parts. The life cycle of *P. capsici* is repeated in every few days and disease spreads rapidly. *P. capsici* can grow at a temperature range of 7-37°C and sporangia and zoospores are abundantly produced at a temperature range of 27-32°C (Roberts et al., 2000). Surviving mycelia on plant debris, in the soil, also cause infection in cucurbit crops. Mycelia grow to form sporangia, which produce zoospores to infect plants.

Management of *Phytophthora capsici* infection in the field

P. capsici is a devastating pathogen and can cause complete crop loss in cucurbits (Babadoost, 2000). No single method provides adequate protection against this pathogen (Babadoost and Islam, 2003; Hausbeck and Lamour, 2004; Hwang and Kim, 1995). Currently, pathogen exclusion, crop sanitation, crop rotation, moisture management, and application of fungicides are used to reduce the severity of infection caused by *P. capsici* (Hausbeck and Lamour, 2004; Islam et al., 2002).

Pathogen exclusion is the best approach to manage Phytophthora blight. The crops should be planted in the field with no history of Phytophthora blight. Also, preventing *P. capsici* from moving into the field should be considered. Farm equipment and irrigation water can introduce *P. capsici* from an infested field to an uninfested field. It was reported that Phytophthora blight in a cucurbit field was initiated with waste water infested with *P. capsici* from cucurbit processing stations (Hausbeck and Lamour, 2004). Crop sanitation can reduce the severity and spread of the disease within a field and between fields. Scouting for occurrence of *P. capsici* infection, removal of infected crop residues after harvest, and control of weed hosts will reduce the incidence of Phytophthora blight in the field. Crop rotations with non-host crops can reduce the inoculum build-up, eventually leading to reduction in the *P. capsici* infection. Since *P. capsici* oospores can survive 3-4 years in soil without host crops, management of Phytophthora blight is possible with at least three years of rotation to non-host crops (Babadoost and Pavon, 2009).

Soil saturation with irrigation water for 5-6 hours was reported to induce sporangia production in *P. capsici* (Bernhardt and Grogan, 1982). If *P. capsici* is present in the soil, well-drained fields are a better option for cucurbit production than poorly drained fields (Lamour and

Hausbeck, 2002). In drip-irrigated fields, *P. capsici* infection has been reported to increase with the increase in frequency of irrigation (Ristaino et al., 1992). Controlled irrigation without yield reduction can minimize the occurrence of Phytophthora blight (Lamour and Hausbeck, 2001; Lamour and Hausbeck, 2003).

There is no cucurbit cultivar available with measurable resistance to *P. capsici* (Babadoost and Islam, 2003; Gevens et al. 2006). Biological control, using antagonistic microorganisms to manage *P. capsici* infection in the field has also been explored. For example, compost amended with *Trichoderma hamatum* 382 was used as potting mix to control *P. capsici* infection on cucumber (Khan et al., 2004). However, no biocontrol agent is currently available for effective control of Phytophthora blight in cucurbits.

Fungicide application is another approach to reduce the severity of *P. capsici* infection in cucurbits. Some of the fungicides were effective in reducing *P. capsici* infection in the field (Islam et al., 2002). Mefenoxam, a systemic fungicide, used as seed-treatment, controlled pre- and post- emergence damping-off caused by *P. capsici* in cucurbits (Babadoost and Islam, 2003). Several fungicides, either sprayed or applied through drip irrigation were effective in reducing severity of Phytophthora blight in cucurbit fields (Hausbeck and Lamour, 2004; Holmes et al., 2000; Shishkoff and McGrath, 1999; Stevenson, et al., 2001).

Fusarium infection of cucumber and pumpkin

Fusarium oxysporum f.sp. *cucumerinum* and *Fusarium solani* f.sp. *cucurbitae,* cause vascular wilt of cucumber and crown and foot rot of pumpkin, respectively (Owen 1955; Zitter, 1998). Infection of *F. oxysporum* f.sp. *cucumerinum* on cucumber is a serious problem worldwide (Booth, 1971; Owen, 1955). Two races of *F. solani* f.sp. *cucurbitae* (Fsc-1 and Fsc-2)

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have been identified. Fsc-1 infects root, crown, and fruit, while Fsc-2 mostly infects cucurbit fruit (Elmer et al., 2007; Mehl and Epstein, 2007; Toussoun and Snyder, 1961).

Symptoms of Fusarium infection on cucumber and pumpkin

In wet conditions, *F. oxysporum* f.sp. *cucumerinum* infect lower leaves of cucumber and infection proceeds to upper leaves. Under highly conducive conditions for disease development, root infection may also occur (Fletcher and Kingham, 1966). Similarly, symptoms of Fusarium crown and fruit rot caused by *F. solani* f.sp. *cucurbitae* are prominent in wet conditions. Common symptoms are plant wilting, distinct necrotic crown, and occasional tap root infection (Zitter, 1998). Under high inoculum density, infected leaves of pumpkin become dull, gray-green and wilting starts from the older leaves gradually proceeding to younger leaves and entire plant die shortly (Egel and Martyn, 2007).

Disease cycle

Disease cycles of both *F. oxysporum* f.sp. *cucumerinum* and *F. solani* f.sp. *cucurbitae* are similar. Both of these pathogens survive in the soil as chlamydospores up to three years (Nash and Alexander, 1965; Zitter, 1998). Chlamydospores germinate under favorable environmental conditions and penetrate into the cucurbit tissues and produce macroconidia and microconidia on the host surface (Jones and Epstein, 1990). Macroconidia produced in sporodochia, are 3- to 7-celled, with a blunt apical cell and a foot-shaped basal cell. Microconidia are oval or ellipsoid, 1- to 2-celled, and produced abundantly on long monophialide false heads (Leslie et al., 2006). Both of these pathogens can infect seeds (Toussoun and Snyder, 1961).

Management of Fusarium diseases of cucumber and pumpkin

Managing Fusarium diseases in cucurbit crops is a difficult task as these pathogens can survive in the soil for more than three years. Crop rotation, controlled irrigation, soil fumigation, and fungicide application have been used to reduce the severity of Fusarium diseases in the field (Egel and Martyn, 2007; Zitter, 1998). Crop rotations for 3 to 7 years have been advised in several reports, against Fusarium infection (Davis et al., 2005; Egel and Martyn, 2007; Mehl and Epstein, 2007; Sumner, 1994; Toussoun and Snyder, 1961). Prolonged period of soil wetness is one of the important factors for the development and spread of the Fusarium wilt in the field (Zitter, 1998). Proper soil drainage and controlled irrigation are the key factors for management of Fusarium diseases in the field.

Soil fumigation using methyl bromide is effective against many fungal pathogens including *Fusarium* spp. Due to the restriction in methyl bromide use, researchers are trying to find alternative fumigants that can effectively control soil-borne plant pathogens, including *Fusarium* spp. Fumigant combinations like 1,3-dichloropropene and chloropicrin (Pic), has been reported to be effective against *Fusarium* spp. in the field (Santos, 2007). Fungicides are being used against *F. solani* f.sp. *cucurbitae* with varying degree of effectiveness. Mehl and Epstein (2007) reported that fungicide seed-treatment was not effective against *F. solani* f.sp. *cucurbitae* because the pathogen is in the seed.

Biofumigation as a tool to control *P. capsici* and *Fusarium* spp. in cucurbits

Biofumigation refers to the release of toxic gaseous materials from wounded tissues of Brassicaceae plant family (Kirkegaard et al., 1993). Wounded Brassica tissues release chemicals known as glucosinolates, which undergo hydrolysis in presence of the enzyme myrosinase to

form toxic substances like nitriles, isothiocyanates, and thiocyanates (Cole, 1976; Delaquis and Sholberg, 1997; Rosa et al., 1997).

Glucosinolates are stable and water soluble compounds, which act naturally as plant defense compounds (Bending and Lincoln, 1999). They are β-thioglucocide-N-hydroxy-sulfates and a β-D-thioglucose moiety containing aliphatic, aromatic or heterocyclic side chain (Fahey et al., 2001; Olivier et al., 1999). More than 100 different glucosinolates have been described based on side chain group differences (Fenwick et al., 1983). More than 20 different glucosinolates have been identified in the genus *Brassica* which are grouped into aliphatic, aromatic, and heterocyclic (indole) glucosinolates (Kirkegaard and Sarwar, 1998). Myrosianse (β-thioglucosidase glucohydrase) is an enzyme present in the myrosin cells or vacuoles inside Brassica plants (Bones et al., 1991; Olivier et al, 1999). In natural conditions, glucosinolates are separated from myrosinase inside the plant tissues. When the tissues break down, myrosinase comes in contact with glucosinolate leading to production of toxic antimicrobial substances. Production of antimicrobial substances from glucosonolate-myrosinase reaction depends on the glucosinolate side chains (Gardiner et al., 1999). It has been reported that isothiocyanates and nitriles are the major products of enzymatic decomposition of glucosinolates (Borek et al., 1995). More than 50 different isothiocyanates are produced by enzymatic hydrolysis of Brassica plants (Ettlinger and Lundeen, 1957; Kjaer, 1973).

Gimsing et al. (2005) reported that glucosinolates are mobile in the soil and easily extractable. Glucosinolates were detected for more than one week in the soil after incorporation of Brassica tissues (Gimsing and Kirkegaard, 2006). Intact plant tissues buried in the soil released limited quantity of glucosinolates compared to wounded plant tissues (Morra and Kirkegaard, 2002). Researchers also identified that soil microorganisms including some fungi

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and bacteria were capable of producing the myrosinase enzyme (Rask, et al., 2000; Sakorn et al., 2002). This trend was further demonstrated by production of isothiocyanates from benzyl glucosinolate added to soil without adding myrosinase along with benzyl glucosinolate into the soil (Gimsing et al., 2007).

Brassica crops were reported to have fungicidal activities (Brown and Morra, 2005). Residues of *B. oleracea* L. var. *capitata* L. (green cabbage) affected the growth of *Pythium ultimum,* and *Sclerotium rolfsii* (Gamliel and Stapleton, 1993). *B. juncea* and *S. alba* suppressed the germination of *P. deliense* Meurs and *P. ultimum* Trow. var. *ultimum* from soil samples (Lazzeri and Manici, 2001). *B. juncea* has been reported to inhibit the hyphal growth of *P. ultimum* and *Rhizoctonia solani* (Snapp et al., 2007). Manici et al. (1997) reported that glucosinolates isolated from *B. juncea* and *S. alba* when mixed with myrosinase from *S. alba*, were toxic to fungi including *Fusarium culmorum, F. oxysporum* and *R. solani.* Dunne et al. (2003) reported that *B. juncea* effectively suppressed the growth of *Phytophthora cactorum,* and *P. cinnamomi*.

The main goal of this study was to determine the effectiveness of mustard short-cycle cover crops in managing *P. capsici* and *Fusarium spp*. in cucurbit fields. Specific objectives were:

1. To determine specific glucosinolates present in the mustards and their quantity in roots and foliage.
2. To determine the effect of mustard extracts on colony growth, sporangial production, zoospore germination and oospore germination of *P. capsici* and colony growth of *F. oxysporum* and *F. solani* in the laboratory.

3. To determine the effectiveness of mustard extracts on controlling *P. capsici* crown infection in the greenhouse.

4. To compare the effectiveness of the planting season of mustards on controlling *P. capsici* and *Fusarium* spp. in the field.

5. To determine the efficacy of mustard treatments combined with fungicide applications for managing *P. capsici* and *Fusarium* spp. in the field.

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CHAPTER 2

QUANTIFICATION OF GLUCOSINOLATES IN MUSTARD PLANTS AND SOIL

Brassica juncea L. 'Florida Broadleaf' (FBL) and *Sinapis alba* L. 'Tilney' mustard plants were grown in the greenhouse and field trials. Glucosinolates were quantified from the roots and foliage of 35-days-old mustard plants collected from the greenhouse trial, using high performance liquid chromatography (HPLC). Similarly, glucosinolates were quantified from the roots and foliage of the mustard plants collected immediately before their incorporation into the soil in the field trials. In addition, glucosinolates were quantified from the soil samples collected from the field trials after incorporation of mustard plants into the soil.

Materials and Methods

Collection and freeze-drying of greenhouse grown mustards

In the greenhouse, FBL, Tilney, and FBL+Tilney mustard treatments were grown in a completely randomized design (CRD) with three replications. A sterilized mix of soil:peat:perlite (1:1:1) was used to grow the mustard plants in the greenhouse trial. Mustard plants were growing at 24ºC under 12 to 14 hours of continuous light until they were harvested, 35 days after sowing seeds to analyze for glucosinolates. Mustard plants were washed to remove soil particles adhering to the roots. The plants were cut and separated into roots and foliage and were frozen in liquid nitrogen. These frozen mustard tissues were immediately stored at -80ºC until the samples were taken out freeze-dried. After freeze-drying, mustard tissues were ground using a coffee grinder (Mr. Coffee, IDS 55) and immediately transferred back to -80ºC freezer. These ground

mustard tissues were analyzed to quantify glucosinolates using high performance liquid chromatography (HPLC).

Field trials

Four field trials were conducted in Tazewell County near Pekin, Illinois. The field trials were, 2008-spring, 2008-fall, 2009-spring, and 2010-spring. In each field trial, FBL, Tilney, and FBL+Tilney mustard treatments and a control (no mustard) were arranged in a randomized complete block design (RCBD) with three replications. In each plot of FBL, Tilney, and FBL+Tilney, 806, 1345, and 1135 g of mustard seed, respectively, was sown using a seed broadcaster and covered with a steel bar harrow. In the 2008-spring trial, mustard seeds were sown on 28 April and mustard plants were collected on 13 June (45 days after sowing the seeds). FBL mustard plants were at pre-bloom growth stage and Tilney mustard plants were at full-bloom, when mustard plants were collected. In the 2008-fall trial, mustard seeds were sown on 22 September. Mustard plants were collected on 23 October (30 days after sowing seeds). Both FBL and Tilney were at pre-bloom growth stage, when mustard samples were collected. In 2009-spring trial, seeds were sown on 9 June and mustard plants were collected on 15 July (35 days after sowing mustard seeds). FBL plants were at pre-bloom growth stage and Tilney mustard plants were at full-bloom, when mustard samples were collected. In 2010-spring trial, seeds were sown on 26 April and mustard plants were collected on 1 July (65 days after sowing seeds). Both FBL and Tilney mustard plants were at full-bloom, when mustard plants were collected.

Collection and freeze-drying of field grown mustards

In each field trial, mustard plants were collected from the field immediately before the plants were incorporated into the soil. Three mustard plant samples (each from 1 m^2 area) were collected from each plot and average fresh biomass (grams/m^2) was determined. From each mustard treatment, a subsample was used for quantification of glucosinolates.

Mustard plants collected were washed to remove soil particles from roots. They were cut and separated into roots and foliage. Root and foliage tissues were frozen immediately in liquid nitrogen and stored at -80ºC freezer until the samples were freeze-dried. After freeze-drying, mustard tissues were ground using the coffee grinder and immediately transferred back to -80ºC freezer. These ground mustard tissues were analyzed to quantify glucosinolates using HPLC.

Soil sample collection from the field trials

Soil samples were collected from 2008-spring, 2009-spring, and 2010-spring field trials after incorporation of mustard plants into the soil. Soil samples were not collected from 2008-fall field trial where mustard plants were left to overwinter in the field. By the early spring of 2009, mustard plants were dead and almost completely disintegrated. Using a soil core sampler (with a diameter of 1.8 cm), soil samples were collected from 10-15 cm depth. Three soil samples were collected from each plot after plants were incorporated using shallow disking and field cultivator. In 2008-spring trial, soil samples were collected within thirty minutes after incorporation of the plants. In 2009-spring and 2010-spring trials, soil samples were collected one day after incorporation of plants.

Quantification of glucosinolates in the mustard samples and soil samples

Mustard samples from greenhouse and field, also the soil samples, were analyzed to quantify glucosinolates according to the procedure described by Kushad et al. (2004). The type and amount of glucosinolates, were estimated based on retention times and response factors developed for desulfoglucosinolates in the standardized and certified rapeseed reference material BCR 367 (Commission of the European Community Bureau of References, Brussels, Belgium).

Data analysis

The general linear models (GLM) procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC) was used for the data analysis. Biomass data of mustard plants collected from the field trials were not normally distributed. These data were log-transformed (base 10) to normalize the data distribution. Fisher's protected least significant difference (LSD) ($P=0.05$) was used to compare the mean biomass of the mustard treatments in the field trials. Overall mean biomass of four field trials were compared with each other. Also, overall mean biomass of each treatment (FBL, Tilney, and FBL+Tilney) in the trials were compared with others.

Overall mean total glucosinolate content (μmol/g) in the whole plants, roots and shoots of FBL, Tilney, and FBL+Tilney in the trials were compared using Fisher's protected LSD. Glucosinolate content in the mustard plants incorporated into 1 m^2 area of the plots (μmol/m^2) for each mustard treatment was determined for each field trial. This was done by multiplying total glucosinolate content in a gram of fresh tissue (μmol/g) of each mustard treatment from the field and its biomass. Glucosinolate content (μmol/m^2) data were not normally distributed. Therefore the data were log-transformed (base 10) to normalize the data distribution. Mean values of total glucosinolate content (μmol/ m^2) of the four different field trials were compared.

Mean value of total glucosinolate content (µmol/ m^2) of each treatment (FBL, Tilney, and FBL+Tilney) was compared with others.

Results

Biomass

Mean biomass of FBL, Tilney, FBL+Tilney treatments in four field trials were 2,016.66, 2,042.50, and 1,874.17 g/m^2, respectively. Mean biomass of 2008-spring, 2008-fall, 2009-spring, and 2010-spring trials were 2,965.56, 211.11, 1,975.56, and 2,785.89 g/m^2, respectively. In 2008-fall trial, all of the mustard treatments had lower biomass compared to other field trials (Table 2.1).

Glucosinolate content of mustard cultivars in the greenhouse

Mean total glucosinolate content (µmol/g) of foliage of FBL mustard cultivar was higher than that of roots (Table 2.2). But, in Tilney mustard cultivar, glucosinolate content (µmol/g) of roots were higher than that of foliage (Table 2.2). Average glucosinolate content of foliage and roots together in FBL, Tilney and FBL+Tilney were 49.53, 33.64, and 35.20 µmol/g, respectively. Average glucosinolate content of roots and foliage in three treatments (FBL, Tilney and FBL+Tilney) were 25.77 and 53.08 µmol/g, respectively.

Glucosinolate content of mustard cultivars in the field

Glucosinolate content (µmol/m^2 area) of mustard treatments in 2008-fall trial were the lowest and were significantly lower than 50% of the total number of treatments (Table 2.3). The lowest glucosinolate content (31.19 µmol/m^2 area) was in FBL+Tilney treatment in 2008-fall

trial and the highest glucosinolate content (3,204.26 μmol/m^2 area) was in FBL+Tilney treatment in 2009-spring trial. Average glucosinolate content in the fresh tissues of FBL, Tilney, and FBL+Tilney treatments in four field trials were 1,240.99, 916.75, and 1,417.63 μmol/m^2, respectively. Average glucosinolate content of 2008-spring, 2008-fall, 2009-spring, and 2010-spring trials were 1,636.80, 286.59, 1,482.09, and 1,552.73 μmol/m^2, respectively.

Mean total glucosinolate content (μmol/g) of roots and foliage in the field trials were not significantly different from each other, except that of roots and foliage of FBL+Tilney treatment in 2009-spring trial (Table 2.4). Mean total glucosinolate content (μmol/g) of FBL, Tilney and FBL+Tilney over four field trials were 5.996, 4.313, and 6.174, respectively, which were not significantly different from each other.

Glucosinolate profiles of FBL and Tilney

Thirteen glucosinolates each were extracted and identified in both FBL and Tilney mustard cultivars from greenhouse and field trials (Table 2.5). Glucoiberin was detected only in FBL and Tilney cultivars grown in greenhouse trial and gluconapin was detected only in FBL and Tilney cultivars grown in the fields. In the greenhouse trial, 4-methoxyglucobrassicin, glucobrassicanapin, gluconapoleiferin, gluconasturtiin, glucoraphanin, and sinigrin were the major glucosinolates (≥2 μmol/g) detected in FBL mustard (Fig. 2.1). 4-methoxyglucobrassicin, glucobrassicanapin, glucobrassicin, gluconasturtiin, and sinigrin were the major glucosinolates (≥2 μmol/g) detected in Tilney mustard. Sinigrin was detected in high quantity in both FBL and Tilney in the greenhouse. Mean total glucosinolate content of FBL and Tilney were not significantly different from each other (Fig. 2.1)

In the field trials, glucobrassicanapin, gluconapin, gluconasturtiin, and sinigrin were the major glucosinolates ($\geq$0.5 µmol/g) found in FBL mustard. 4-hydroxyglucobrassicin, glucoalyssin, glucobrassicanapin, and gluconapin were the major glucosinolates ($\geq$0.5 µmol/g) detected in Tilney mustard (Fig. 2.2). Sinigrin and glucoalyssin were detected in highest quantities (>2 µmol/g) compared to other glucosinolates in FBL and Tilney mustard cultivars, respectively. Mean total glucosinolate content of FBL and Tilney were not significantly different from each other (Fig. 2.2). Total glucosinolates of mustard plants in greenhouse trial (41.59 µmol/g) was significantly higher than that of field trials (5.33 µmol/g) (Fig. 2.3). Mean total glucosinolate content (µmol/g) of FBL and Tilney foliages in the greenhouse trial were significantly higher than that of FBL and Tilney foliages in field trials (Fig 2.4). Similarly, mean total glucosinolate content (µmol/g) of FBL and Tilney roots in the greenhouse trial were significantly higher than that of FBL and Tilney roots in field trials (Fig 2.4).

Glucosinolate content of soil samples

Glucosinolates were not detected in any of the soil samples collected from the fields after mustard incorporation during 2008-2010 field trials.

Discussion

The lowest biomass was in the 2008-fall trial. Mustard plants in 2008-fall trial were not growing as fast as the mustard plants of same age in other field trials. Field temperature might be the main reason for the slow growth of plants in 2008-fall trial. For the growth and development of *B. juncea* and *S. alba*, the plants need certain threshold level of heat units represented as growing degree days (GDD) (Miller et al., 2001). For each field trial, accumulated growing

degree day (GDD) were determined by summing up GDD of each day for which mustard plants were present in the field (calculated at base 50°F). The accumulated GDD were 573, 340, 820, and 1182 in 2008-spring, 2008-fall, 2009-spring, and 2010-spring trials, respectively. For the 2008-fall grown mustards, the low GDD might have reduced the growth of the plants in the field.

In the spring field trials, FBL mustard showed more horizontal growth than vertical growth until the plants were 45 days old. However, Tilney mustard showed more vertical growth throughout the period of 45 days than FBL. In the period of 45 days, majority of FBL mustard foliage were leaves. However, both stem and leaves equally contributed to the foliage of the Tilney mustard. After 65 days of growth, FBL and Tilney showed smaller leaves compared to 45 days. Height of both FBL and Tilney were also similar by 65 days. In general, biomass of FBL and Tilney increased with age until 45 days. The FBL mustard had higher biomass (g/m^2) than that of Tilney mustard in 45 days old plants. FBL mustard plants harvested after 65 days had less biomass than that of Tilney mustard plants. In the field trials, morphological differences and growth characteristics of FBL and Tilney might have contributed to the difference in their biomass. Additional studies needed to be done to determine the biomass of FBL and Tilney at different growth stages.

Biomass influenced glucosinolate content ($\mu mol/m^2$) in the mustard plants incorporated into soil. Higher biomass of 2008-spring, 2009-spring, and 2010-spring trials compared to 2008-fall trial contributed to higher glucosinolate content ($\mu mol/m^2$) in the mustard plants incorporated in those trials compared to 2008-fall trial. Considering the glucosinolate content ($\mu mol/m^2$), the best time to incorporate FBL mustard cultivar was between 45-65 days. Also, the best time to incorporate Tilney mustard cultivar was between 35-45 days. Thus, cultivars with dense biomass when incorporated into soil could release higher amounts of glucosinolates.

Glucosinolate contents (µmol/g) of both FBL and Tilney mustard cultivars were significantly higher in the greenhouse trials than the field trials. This finding does not agree with the study by Antonious et al. (2009), who reported that field grown *B. juncea* had higher total glucosinolate content (µmol/g) compared to greenhouse. The difference between our study and the report by Antonious et al. (2009) could be due to the difference in temperature at which mustard plants were grown. Rosa et al. (1998) reported that a temperature change from 20ºC to 30ºC significantly increased the content of total glucosinolates in the leaves of *B. oleracia* var. *capitata* 'Duchy F1' (cabbage). In our greenhouse experiment, the temperature was set at 24ºC and in the field trials the average atmospheric temperatures were 20, 16, 23 and 19ºC in 2008-spring, 2008-fall, 2009-spring and 2010-spring, respectively. Also, He et al. (2003) reported up to 50% decrease in total glucosinolate content with increasing age in the foliage of *B. juncea* grown in the field. They reported that total glucosinolate content per unit weight of plants was constantly decreasing after the seedling stage until the mustard plants were harvested. In our study, glucosinolate content of the plants in greenhouse trial was measured 35 days after sowing seeds, when the plants were in pre-bloom stage; while plants grown in the field were from pre-bloom to full-bloom stages. This result indicated that temperature, growth stage of plant, plant species, and unknown factors might affect production of glucosinolates in Brassica species. Further investigations are needed to determine all factors affecting glucosinolate production of Brassica plants used as cover crops.

In our greenhouse and field trials, overall glucosinolate content (µmol/g) in the foliage was higher than that of the roots of the mustard plants. This finding is not in agreement with the findings of other researchers (Rosa et al., 1998, Van Dam et al., 2009) who reported that root had higher glucosinolate content than the shoots in Brassica plants. The difference might be due to

the different species of Brassicas used by these researchers in their experiments compared to *B. juncea* L. 'Florida Broadleaf' and *S. alba* L. 'Tilney', used in our experiments.

The glucosinolates found in FBL and Tilney were the same as reported by other researchers in *B. juncea* and *S. alba* (Kirkegaard and Sarwar, 1998). They also reported that in the field, sinigrin, gluconapin, glucobrassicanapin and gluconasturtiin were the major glucosinolates in *B. juncea* and glucobrassicanapin was the major one in *S. alba.* Sinigrin was found in very high quantities in both FBL and Tilney in our greenhouse trial. Rosa et al. (1998) also found high quantity of sinigrin in *B. oleracea* var. *capitata*. These findings suggest that sinigrin is one of the most common glucosinolates in Brassica species.

Gimsing and Kirkegaard (2006) were able to identify 13% of the glucosinolates present in the mustard plants in the soil thirty minutes after mustard incorporation. However, in our field trials, glucosinolates were not detected in any of the soil samples collected from the fields after mustard incorporation. This might be because the soil samples collected in our study were not stored at low temperature during transition from field to laboratory. The collected soil samples in our studies were kept at >20ºC for more than an hour after collection. This might have resulted in hydrolysis of glucosinolates during transit.

Fall field trial was not repeated during 2009 and 2010 seasons. In 2008-fall trial, fields were only available from the late September. In October 2008, the temperature was mostly below 16ºC. Plants were killed by the frost in early November 2008. As a result of all these factors, sufficient biomass was not produced during 2008-fall season. Thus, fall trial of mustards was discontinued after 2008 season.

TABLES

Table 2.1. Mean biomass of mustard plants in 1 m^2 area in the field trials.

| Trial | Mean biomass (grams/m^2) | | |
	Florida Broadleaf (FBL)	Tilney	FBL+Tilney
2008-spring	3793.33 a[*]	2790.00 ab	2313.33 bc
2008-fall	260.00 d	200.00 d	173.33 d
2009-spring	1163.33 cd	2260.00 bc	2503.33 abc
2010-spring	2850.00 ab	2920.00 ab	2506.67 ab

[*] Values within the rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according to Fisher's protected LSD.

Table 2.2. Glucosinolate content in one gram of freeze-dried tissue of roots and foliage of Florida Broadleaf (FBL), Tilney, and FBL+Tilney mustard treatments in the greenhouse trial.

| Mustard cultivar | Mean total glucosinolate content (µmol/g) | | |
	Root	Foliage	LSD[*]
FBL	16.00	83.05	21.6
Tilney	42.00	25.29	13.7
FBL+Tilney	19.30	50.90	19.3
LSD[*]	15.3	17.6	

[*] Least significant difference.

Table 2.3. Glucosinolate content of the mustard plants in 1 m^2 area in the field trials.

Trial	Florida Broadleaf (FBL)	Tilney	FBL+Tilney
2008-spring	1744.93 cd[*]	2008.80 bc	1155.67 de
2008-fall	179.40 fg	76.00 g	31.19 g
2009-spring	360.63 fg	881.40 ef	3204.26 a
2010-spring	2679.00 ab	700.80 efg	1278.40 cde

[*] Values within the rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according to Fisher's protected LSD.

Table 2.4. Glucosinolate content in one gram of freeze-dried tissue of roots and foliage of Florida Broadleaf (FBL), Tilney, and FBL+Tilney mustard treatments in the field trials.

| Mustard treatment	Trial	Root	Foliage	LSD[x]
		Mean total glucosinolate content (μmol/g)		
FBL	2008-spring	2.0767	7.0714	NS[y]
	2008-fall	7.5120	6.3305	NS
	2009-spring	3.6198	2.6566	NS
	2010-spring	7.6459	11.0559	NS
`				
	Mean	5.2136	6.7786	NS
Tilney	2008-spring	7.8469	6.4893	NS
	2008-fall	2.8985	4.7001	NS
	2009-spring	2.0616	5.7612	NS
	2010-spring	1.0212	3.7293	NS
	Mean	3.4571	5.1698	NS
FBL+Tilney	2008-spring	2.3600	7.6700	NS
	2008-fall	1.9800	1.6800	NS
	2009-spring	7.9667 b	17.5000 a	5.97[z]
	2010-spring	2.3000	7.9333	NS
	Mean	3.6517	8.6958	NS

[x] Least significant difference.

[y] Not significant.

[z] Values within the row followed by same letter are not significantly different ($P=0.05$) according to Fisher's protected LSD.

Table 2.5. Glucosinolates detected from Florida Broadleaf and Tilney mustard plants in greenhouse and field trials.

| Trivial name	Chemical name	Chemical class
4-hydroxyglucobrassicin	4-hyroxy-3-indolylmethyl	Indolyl
4-methoxyglucobrassicin	4-methoxy-3-indolylmethyl	Indolyl
Epiprogoitrin	2-hydroxy-3-butenyl	Aliphatic
Glucoalyssin	5-methylsulphinylpropyl	Aliphatic
Glucoiberin	3-methylsulphinylpropyl	Aliphatic
Glucobrassicanapin	4-pentenyl	Aliphatic
Glucobrassicin	3-indolylmethyl	Indolyl
Gluconapin	3-butenyl	Aliphatic
Gluconapoleiferin	2-hydroxy-4-pentenyl	Aliphatic
Gluconasturtiin	2-phenylethyl	Aromatic
Glucoraphanin	4-methylsulphinylbutyl	Aliphatic
Neoglucobrassicin	1-methoxy-3-indolylmethyl	Indolyl
Progoitrin	2-hydroxy-3-butenyl	Aliphatic
Sinigrin	2-propenyl	Aliphatic
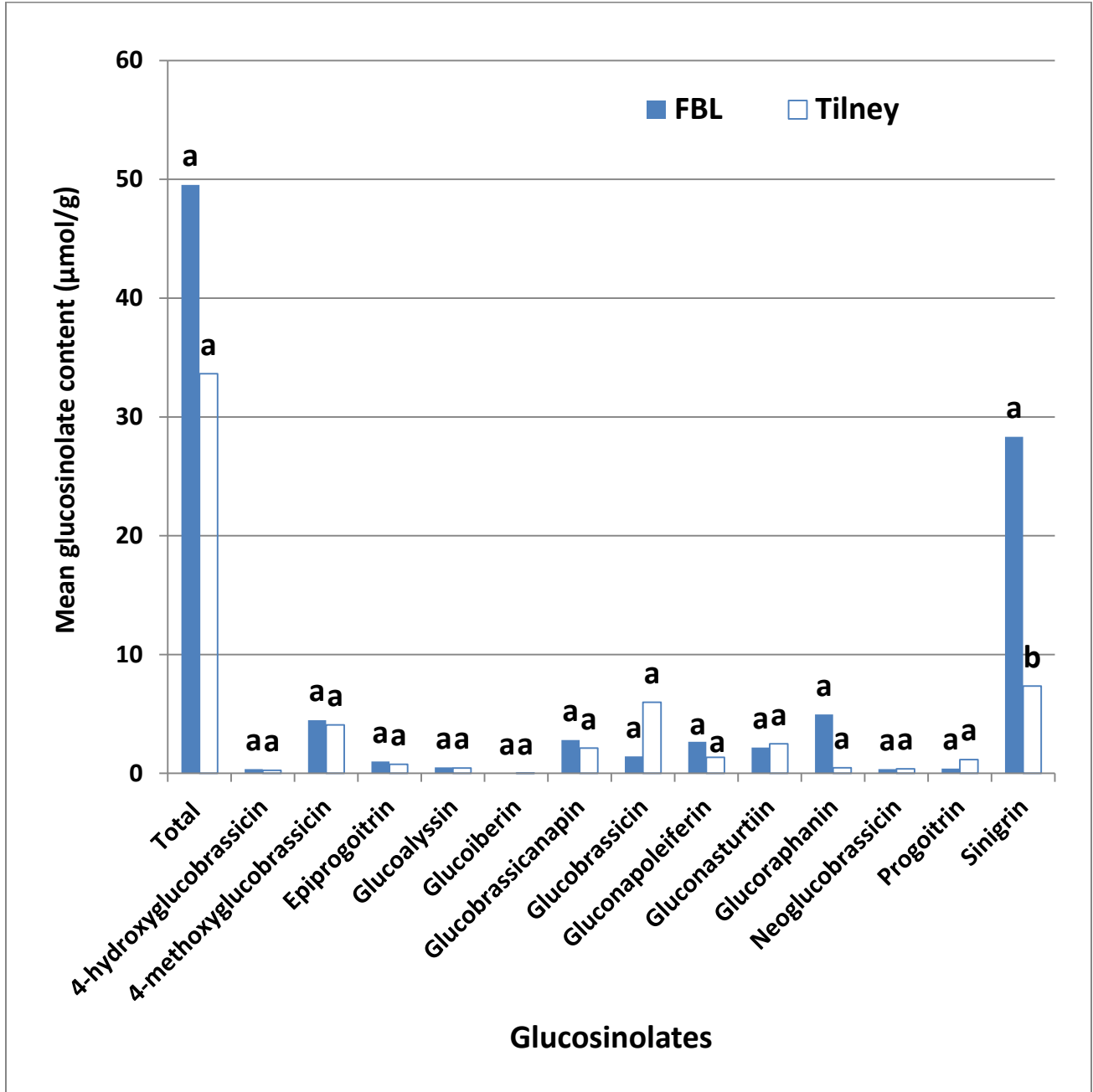
Fig. 2.1. Total and individual glucosinolate content in the freeze-dried tissues (µmol/g) of Florida Broadleaf (FBL) and Tilney mustard cultivars in the greenhouse trial.
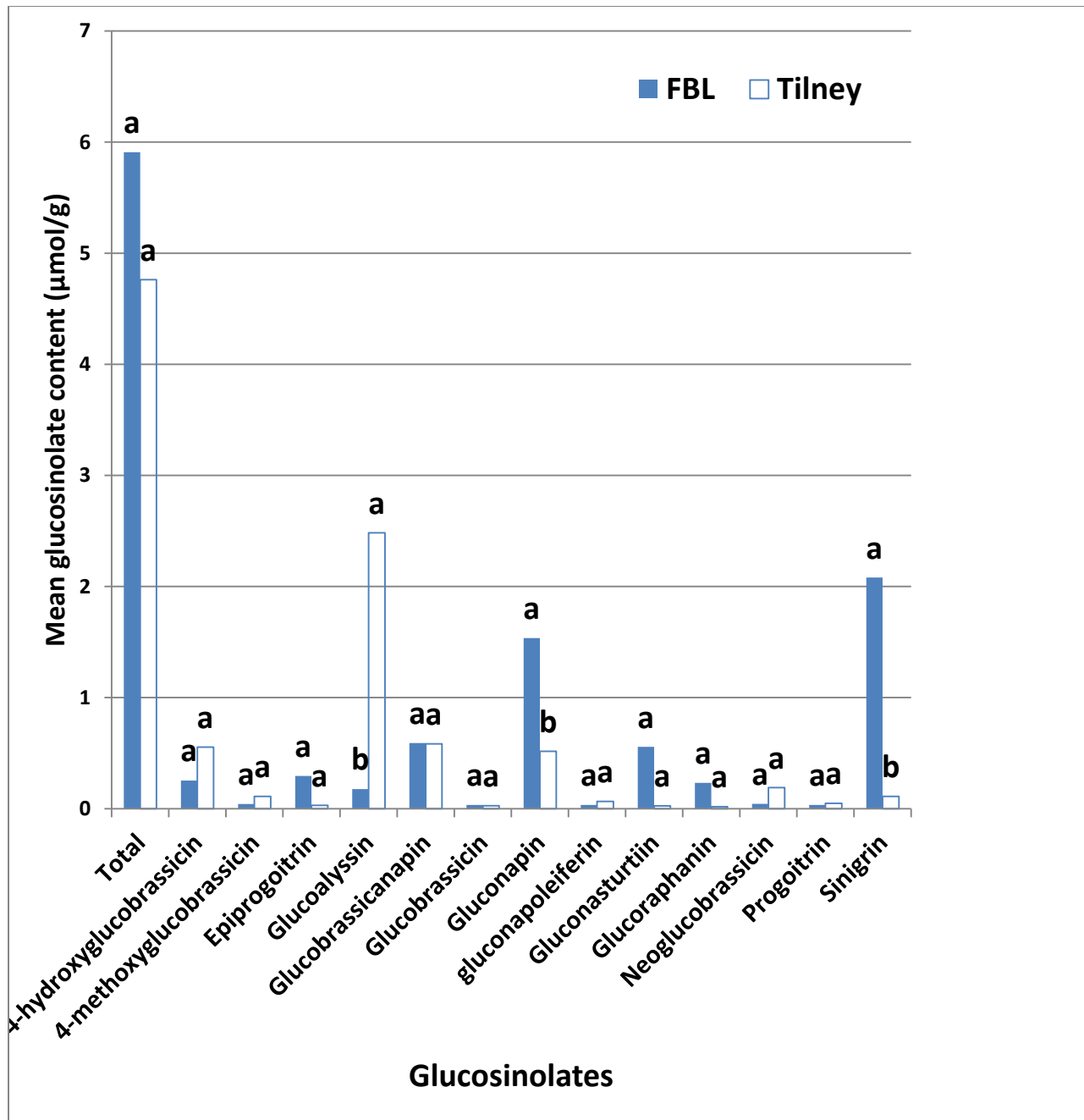
Fig. 2.2. Total and individual glucosinolate content in the freeze-dried tissues (µmol/g)) of Florida Broadleaf (FBL) and Tilney mustard cultivars averaged from four field trials.
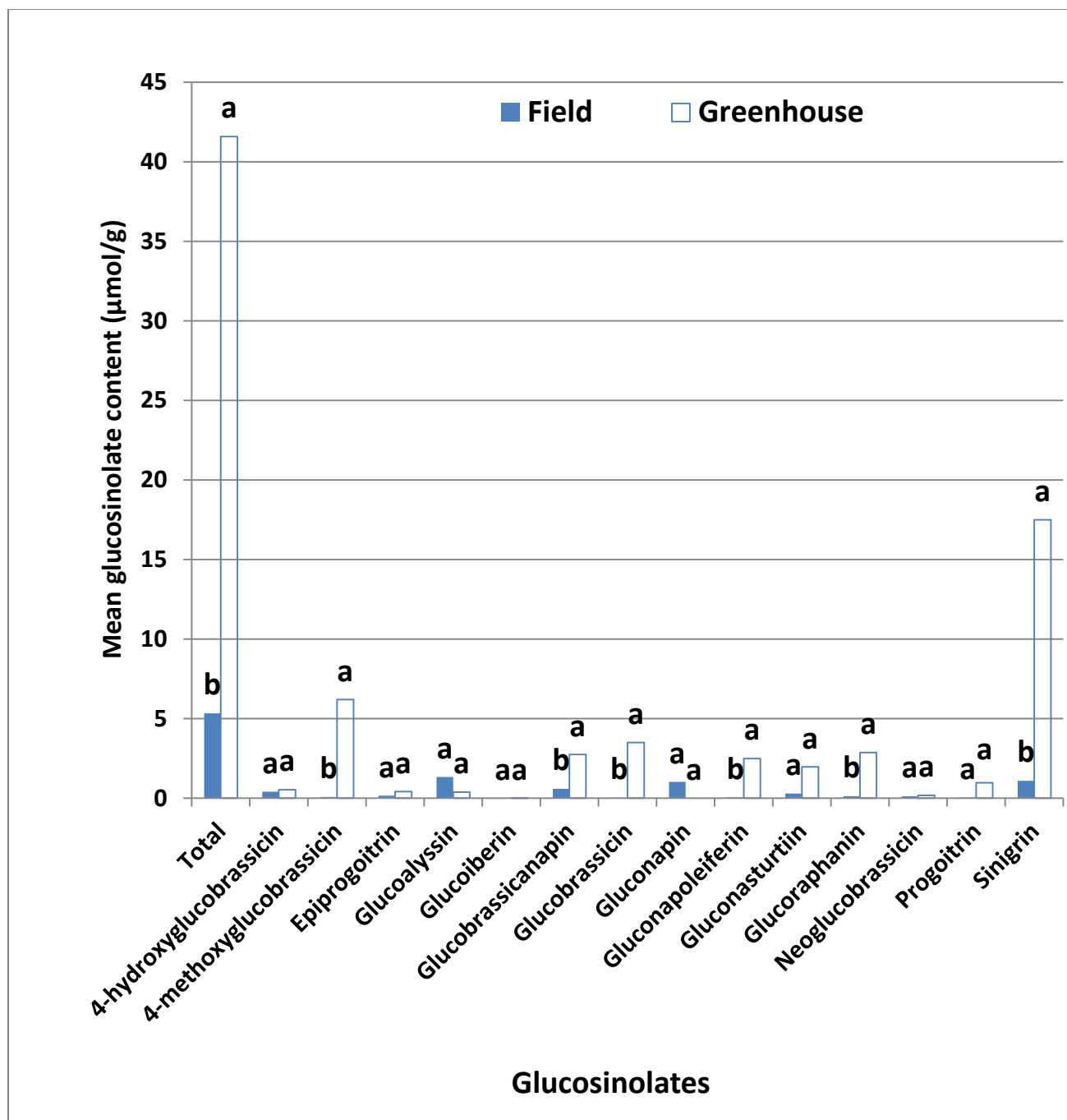
Fig. 2.3. Total and individual glucosinolate content in the freeze-dried tissues (μmol/g) of

Florida Broadleaf (FBL) and Tilney mustard cultivars averaged from field and greenhouse trials.
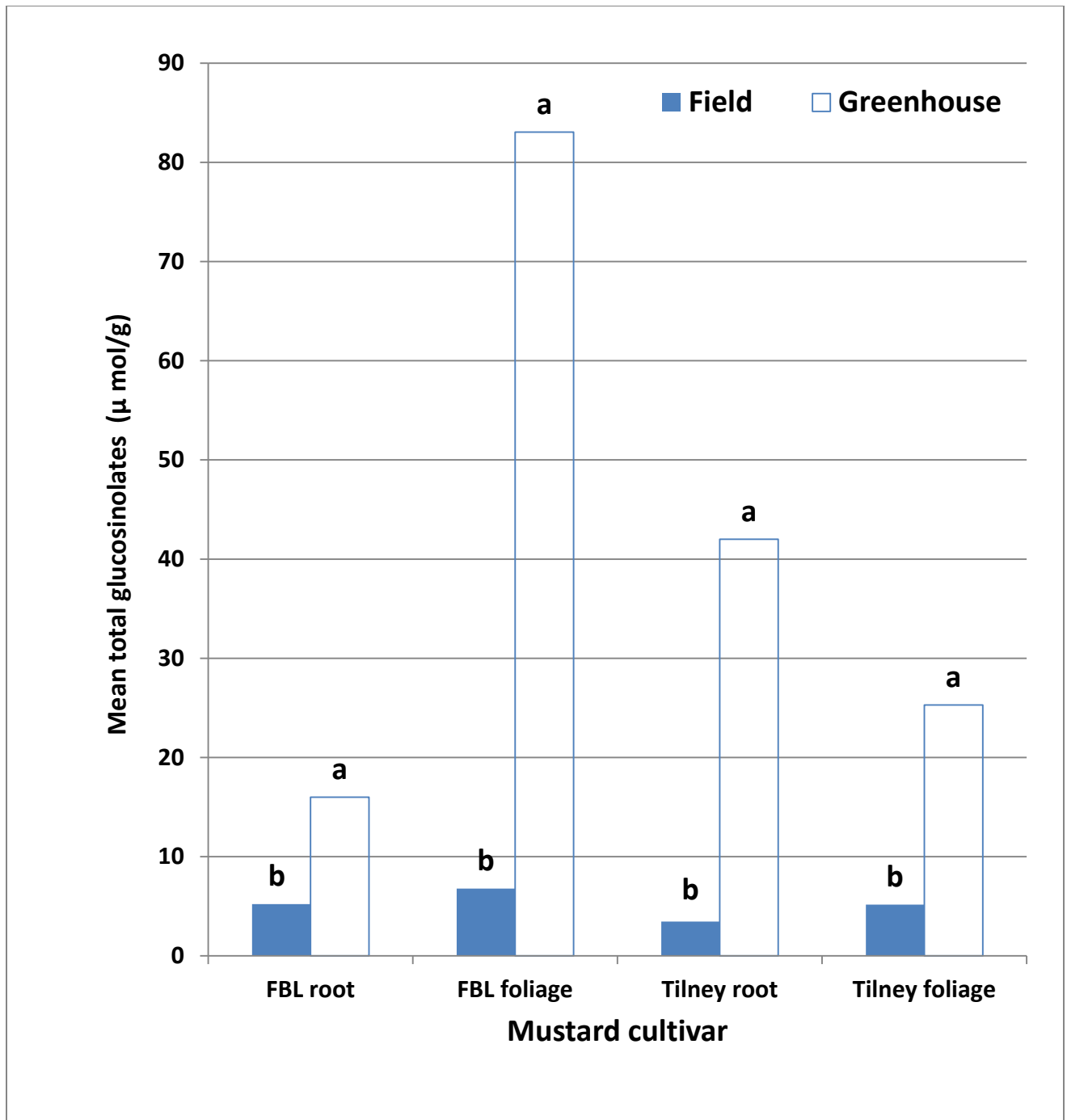
Fig. 2.4. Total glucosinolate content in the freeze-dried tissues of roots and foliage (µmol/g) of Florida Broadleaf (FBL) and Tilney mustard cultivars in the field (average of four trials) and greenhouse trials.

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CHAPTER 3

EFFECTS OF MUSTARD TREATMENTS ON *PHYTOPHTHORA CAPSICI* AND *FUSARIUM* SPP.

Inhibitory effects of 'Florida Broadleaf' (FBL) and 'Tilney' mustards were tested on *P. capsici* and *Fusarium* spp. in the laboratory, greenhouse, and field experiments. In the laboratory experiments, colony growth, sporangial production, zoospore germination, and oospore germination of *P. capsici* and colony growth of *Fusarium* species were tested in presence of mustard plant extracts. In the greenhouse trial, *P. capsici* crown infection on 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin were assessed in presence of mustard extracts. In the field trials, *P. capsici* and *Fusarium* spp. infection on 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin were determined after incorporation of mustard plants into the soil.

Materials and methods

Laboratory experiments

***Phytophthora capsici* and *Fusarium* cultures**

Phytophthora capsici isolates PC-1, PC-2, PC-3, and PC-4 were collected from infected pumpkin fruits from commercial fields in Illinois. Also, *Fusarium oxysporum* (F-2 and F-3) and *Fusarium solani* (F-1) were isolated from the soil samples collected from pumpkin fields in Illinois.

Mustard extract

Extracts of FBL, Tilney, and a 50:50 combination of FBL+Tilney were tested for inhibitory effect on *P. capsici*, *F. oxysporum*, and *F. solani* isolates in the laboratory. Fifty grams of plant tissues were ground in sterile-distilled water (SDW) using a Waring commercial laboratory blender (Waring Products Division, Conair Corp., McConnellsburg, PA) The extract was then passed through eight layers of cheese-cloth. The extracts were immediately filtered through 0.22 µm Millex syringe-driven filter unit (Millipore Corp., Billerica, MA) into sterilized beakers. The filtered mustard extracts were assessed for their inhibitory effect on colony growth, sporangial production, zoospore germination, and oospore germination of *P. capsici* and colony growth of *F. oxysporum* and *F. solani*.

Colony growth of *P. capsici*, *F. oxysporum*, and *F. solani*

Ten milliliters of mustard extract from each of FBL, Tilney, and FBL+Tilney mustards and sterile distilled water (SDW; control) were pipetted separately into 8 cm-diameter Petri plates. A 6-mm plug of *P. capsici* colony growing on lima bean agar (LBA) was cut and placed on LBA in another Petri plate. The plate with *P. capsici* plug was inverted onto the plate containing the mustard extract and both plates were taped together with parafilm. This procedure was repeated for the *P. capsici* isolates (PC-1, PC-2, PC-3, and PC-4), mustard extracts (FBL, Tilney, and FBL+Tilney) and control treatment (SDW). The same procedure was also carried out for *Fusarium* isolates (F-1, F-2, and F-3) with the mustard extracts (FBL, Tilney, and FBL+Tilney) and control treatment (SDW). All of the Petri plates were incubated at 24ºC under 12 hours light/12 hours darkness. Radius of the colonies were measured after 5 days. This

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experiment was designed as a factorial experiment in completely randomized design (CRD) with four replications. The experiment as a whole was repeated four times.

Production of *P. capsici* sporangia was also quantified. After five days of colony of growth in Petri plates, number of sporangia produced were counted directly under the light microscope. The counting was done at four different fields of view on each plate using a SZX12 microscope (Olympus Corp., Tokyo, Japan).

Germination of *P. capsici* zoospores

Zoospores of *P. capsici* isolates PC-1, PC-2, PC-3, and PC-4 were produced using the method by Babadoost and Islam (2002). Two hundred microliters of zoospore suspension in SDW (10^3 zoospores/ml) was added onto each lima bean agar (LBA) plate. Extracts of FBL, Tilney, FBL+Tilney, and SDW (control) were prepared as previously described. Ten milliliters of each of FBL, Tilney, FBL+Tilney and SDW was pipetted into different Petri plates. The Petri plate with zoospores was inverted over the Petri plate containing the mustard extract and both plates were taped together using parafilm. All of the Petri plates were incubated at 24ºC. Number of germinated zoospores were determined by using SZX12 microscope (Olympus Corp., Tokyo, Japan). This experiment was performed as a factorial experiment in CRD with four replications. The experiment as a whole was repeated four times.

Germination of *P. capsici* oospores

P. capsici oospores were tested for germination in presence of 0.22 µm-filtered extracts of FBL, Tilney, FBL+Tilney and SDW (control) treatments. *P. capsici* oospores were produced by pairing up isolates of PC-1, PC-2, and PC-3 (A1 mating type) with PC-4 (A2 mating type)

using the method described by Pavon et al. (2008). Two hundred microliters of oospore suspension in SDW (5×10^2 oospores/ml) was added into sterilized 1.5 ml microfuge tube containing 800 µl of 0.22 µm-filtered mustard extract. All of the microfuge tubes were incubated at 24ºC in darkness for 7 days. Number of oospores germinated in each mustard treatment was counted using a 'Hemacytometer' under BX41 light microscope (Olympus Corp., Tokyo, Japan). The experiment was performed as a CRD with four replications. The experiment as a whole was repeated four times.

Greenhouse trial

Greenhouse trial was designed to investigate the inhibitory effect of mustard treatments on *P. capsici* crown infection of cucurbits. A sterilized mix of soil:peat:perlite (1:1:1) was used throughout the greenhouse trial. Seeds of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin were sown in 48-cell flats. Ten-day-old and 20-day-old cucurbit seedlings were transplanted into 15.2 cm-diameter plastic pots containing the above-mentioned sterilized mix. Similarly, mustard plants were grown in the greenhouse in 15.2 cm-diameter pots. Thirty-five days after sowing seeds, mustard plants were uprooted and washed to remove adhering soil. Three mustard treatments (FBL, Tilney, and a 50:50 mixture of FBL+Tilney) and a control (without mustard) were included in the greenhouse trial. Extracts of FBL, Tilney, and FBL+Tilney mustards were prepared by grinding mustard tissues in SDW.

Zoospore suspension was prepared according to a method described by Babadoost and Islam (2002). Four isolates of *P. capsici* (PC-1, PC-2, PC-3, and PC-4) were included in the study. Zoospore suspensions from the four isolates were equally mixed and number of zoospores in the suspension mixture was adjusted to 2×10^5 spores/ml. Cucurbit seedlings were inoculated

by adding 10 ml zoospore suspension at the base of each cucurbit seedling. Immediately following the inoculation, 50 ml each of FBL, Tilney, FBL+Tilney and control (SDW) treatments were applied into the base of four different cucurbit seedlings. Seedlings were watered 3 times per day and development of *P. capsici* infection was assessed until 21 days after inoculating plants. The greenhouse trial was a factorial experiment in CRD with three replications. The greenhouse trial as a whole was repeated five times.

Field trials

Field trials included 2008-spring, 2008-fall, 2009-spring, and 2010-spring experiments. All field trials were conducted in two commercial fields in Tazewell County near Pekin, Illinois. Both fields had Phytophthora blight on pumpkins in 2007. One field was used for 2008-spring, 2009-spring, and 2010-spring trials, and a second field was used for 2008-fall trial.

The 2008-spring field trial was a split-split-split plot experiment performed in a randomized complete block design (RCBD) with three replications. The main plots included FBL, Tilney, and a 50:50 mixture of FBL+Tilney mustard treatments and a control (no mustard) treatment. In each main plot of FBL, Tilney, and FBL+Tilney, 806, 1345, and 1135 g of mustard seeds, respectively, was sown. Mustard plants were incorporated into the top 10-cm layer of the soil using shallow disking and field cultivator. Each main plot was divided into two sub-plots for two date treatments of sowing cucurbit seeds. Cucurbit seeds were sown either on the same day or four days after the incorporation of mustard plants into the soil. Three sub-sub plots for three cucurbit cultivars 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin were included. Two sub-sub-sub plots of standard fungicide-application and no fungicide-application were also included. Fungicide application included spray applications of famoxadone+

cymoxanil (Tanos 50WDG, at 700 g/ha)+copper hydroxide (Kocide-3000 46.1DF, at 1.68 kg/ha) alternated with dimethomorph (Forum 4.18 SC, at 350 ml/ha)+copper hydroxide (Kocide-3000 46.1DF, at 1.68 kg/ha).

For the 2008-spring trial, the field was cultivated and prepared on 5 April and mustard seeds were sown on 28 April. Mustard plants were incorporated into the soil on 13 June. Cucurbit seeds were sown on 13 June (the same day) and 17 June (after four days). The fungicide spray began on 9 July and applied at weekly intervals until a week before the plots were harvested. The plots were harvested on 28 September.

For the 2008-fall trial, field was cultivated and prepared on 17 September and mustard seeds were sown on 22 September. Mustard plants were left intact in the soil to overwinter. By early spring of 2009, all mustard plants were dead and almost completely disintegrated. The design of this field trial was similar to that of 2008-spring trial except that only one sowing date was included. This field trial was a split-split plot experiment in RCBD with three replications. The trial included four main plots (FBL, Tilney, FBL+Tilney, and control), three sub plots ('Eureka' cucumber, 'Magic Lantern' Pumpkin, and 'Dickinson' pumpkin), and two sub-sub plots (fungicide-application and no fungicide-application). Seeds of cucurbit crops were sown on 29 May, 2009. Fungicide spray application was similar to the 2008-spring trial and the spray application began on 20 June and continued at weekly intervals until a week before the plots were harvested. The plots were harvested on 3 September.

The 2009-spring and 2010-spring trials were conducted using the same design of 2008-fall trial. The design had four main plots (FBL, Tilney, FBL+Tilney, and control), three sub plots ('Eureka' cucumber, 'Magic Lantern' Pumpkin, and 'Dickinson' pumpkin), and two sub-sub plots (fungicide application and no fungicide application). In 2009-spring trial mustard seeds

were sown on June 9 and the plants were incorporated into the soil on 15 July. Seeds of cucurbit crops were planted on 16 July. Fungicide spray application began on 6 August and continued at weekly intervals until a week before the plots were harvested. The plots were harvested on 18 October. In 2010-spring trial mustard seeds were sown on 26 April and the plants were incorporated into the soil on 1 July. Seeds of cucurbit crops were planted on 2 July and fungicide spray application began on 23 July and continued at weekly intervals until a week before the plots were harvested. The plots were harvested on 5 October.

There were a total of 144 plots in the 2008-spring trial and a total of 72 plots each in the 2008-fall, 2009-spring, and 2010-spring field trials. Twelve cucurbit plants were maintained in each plot (10.67 m× 9.14 m). Data were taken from these four field trials in biweekly intervals, starting from four weeks after planting cucurbit seeds. Infection by *P. capsici* and *Fusarium* spp. were visually assessed by observing seedling death, vine lesions, and fruit infection of cucurbit plants. In each plot, twenty vines and all the fruits were examined for the infection.

Inoculum density of *P. capsici* and *Fusarium* spp. in soil

Soil samples were collected from top 15-cm of the soil using a soil core sampler (with a diameter of 1.8 cm), a week before mustard planting and two weeks after mustard incorporation into the soil. Dilution plating method was used to determine the number of germinating oospores of *P. capsici* and for colony forming units (cfu) of *Fusarium* spp. PARPH culture medium (French-Monar et al., 2007) was used to assess germination of *P. capsici* oospores. Similarly, Nash-Snyder media (Leslie et al., 2006) was used to determine the cfu of *Fusarium* spp. One gram of wet soil sample was added into 9 ml of SDW in a test tube. The test tube was shaken to suspend soil in SDW and 1 ml of the suspension was transferred into the second test tube

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containing 9 ml SDW. This serial dilution procedure produced 10^0, 10^{-1}, 10^{-2}, and 10^{-3} dilutions. Three replications, each with 500 μl soil suspension, from each of the dilution were added onto PARPH medium to determine the germinated oospores of *P. capsici* and onto Nash-Snyder medium to determine cfu of *Fusarium*. The plates were incubated at 24°C for 24 hours in darkness for germination of *P. capsici* oospores and at 24°C for 12 hr light/ 12 hr darkness for growth of *Fusarium* spp. Germinating oospores of   *P. capsici* and cfu of *Fusarium* spp. were counted using light microscopy. In addition to the PARPH plating, *P. capsici* oospores were also processed using sucrose-centrifugation method described by Pavon et al. (2008) to determine total number of oospores in soil samples.

Data analysis

Laboratory, greenhouse, and field data were analyzed using GLM procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC). Fishers protected LSD was used to compare treatment means at *P*=0.05.

Results

Laboratory experiments

Colony growth of *P. capsici*, *F. oxysporum*, and *F. solani*

Colony growth of *P. capsici* was significantly reduced, when the cultures were exposed to the extracts of FBL or FBL+Tilney, in comparison to the control treatment (Table 3.1). Mean colony growth reduction in *P. capsici* isolates were 85.50, 14.44, and 67.18% in presence of FBL, Tilney, and FBL+Tilney mustard treatments, respectively compared to control treatment. Exposing *Fusarium* cultures to the mustard extracts did not significantly affect the growth of the

colonies (Table 3.2). Colony growth reduction in F-1 and F-3 isolates were 20.28 and 10.83% in presence of Tilney and FBL+Tilney mustard treatments, respectively compared to control treatment. Colony growth of none of the *Fusarium* isolates were suppressed in presence of FBL extract (Table 3.2).

Production of *P. capsici* sporangia

Mean number of sporangia produced in four isolates of *P. capsici* per viewing field of the microscope were 67.96, 161.56, 73.83, and 163.51 in FBL, Tilney, FBL+Tilney, and control treatments, respectively. The FBL and FBL+Tilney treatments significantly reduced the sporangial production of PC-1 isolate compared to Tilney and control treatments (Table 3.3). Also, FBL, Tilney, and FBL+Tilney treatments significantly reduced sporangial production in PC-3 isolate compared to the control treatment (Table 3.3). No mustard treatments significantly affected the sporangial production in PC-2 and PC-4 isolates.

Germination of *P. capsici* zoospores

Mean percentages of zoospore germination were 75.11, 70.27, 81.07, and 67.40% in FBL, Tilney, FBL+Tilney, and control treatments, respectively. Zoospore germination of *P. capsici* isolates was not significantly affected by any of the mustard treatments (Table 3.4).

Germination of *P. capsici* oospores

Mean percentages of oospore germination were 40.61, 52.99, 43.33, and 57.10% in FBL, Tilney, FBL+Tilney, and control treatments, respectively. The FBL and FBL+Tilney treatments

significantly reduced percentage of oospore germination compared to control and Tilney mustard treatments (Fig. 3.1).

Greenhouse trial

No crown infection was observed in 'Eureka' cucumber (Table 3.5, Fig. 3.2). All 'Magic Lantern' pumpkin plants exhibited crown infection. The rates of crown infection in 'Dickinson' pumpkin ranged from 25.23% (FBL+Tilney treatment) to 50.69% (control treatment) (Table 3.5). The FBL and FBL+Tilney treatments significantly reduced the *P. capsici* crown infection in 'Dickinson' pumpkin compared to control treatment (Table 3.5).

Field trials

Vine infection

There was no vine infection in "Eureka' cucumber (Fig. 3.4). The rates of infected vines of 'Magic Lantern' and 'Dickinson' pumpkin combined were 19.11, 25.91, 22.96, and 19.33% in FBL, Tilney, FBL+Tilney, and control treatments, respectively. In none of the field trials, mustard treatments significantly reduced the rates of vine infection by *P. capsici* (Table 3.6). Overall mean percentages of vine infection were 24.04, and 26.34 in sprayed and unsprayed plots, respectively (Fig. 3.3). Overall mean percentages of vine infection in 'Magic Lantern' pumpkin and 'Dickinson' pumpkin were 27.95 and 18.95 %, respectively (Fig. 3.4). No vine infection by *Fusarium* was detected in any of the experimental crops.

Fruit infection

The rates of fruit infection by *P. capsici* were 29.48, 23.20, 21.71, and 26.32% in FBL, Tilney, FBL+Tilney, and control treatments, respectively. Percentages of fruit infection in 2009-spring treatment were the lowest among the four field trials (Table 3.7). The overall rate of fruit infection in sprayed plots (23.20%) was lower than that of unsprayed plots (29.11%) (Fig. 3.5). But the difference was not statistically significant. Overall rate of fruit infection in 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin were 25.74, 23.77, and 20.32%, respectively (Fig. 3.6), which were not significantly different from each other. No Fusarium infection on any fruit was observed.

Oospore density of *P. capsici* and cfu of *Fusarium* spp.

P. capsici oospores in the soil samples collected from fields failed to germinate on the PARPH culture medium. Mean number of oospores in one gram of soil prior to incorporation of mustards were 1.83, 1, 1 and 1.92 in the plots used later for seeding FBL, Tilney, FBL+Tilney, and control treatments, respectively. Mean number of oospores in one gram of soil after incorporation of mustards were 1.5, 1, 0.42, and 1.5 in FBL, Tilney, FBL+Tilney, and control plots, respectively. Mean number of oospores after incorporation of FBL+Tilney was significantly lower than that of the same plot area prior to incorporation of mustard plants (Fig. 3.7). Mean *Fusarium* cfu counts in one hundredth of a gram of soil (10^{-2} g) prior to the incorporation of mustards were 11.83, 11.78, 13.14, and 7.22 in FBL, Tilney, FBL+Tilney, and control treatments, respectively (Fig. 3.8). Mean *Fusarium* cfu counts after incorporation of mustards were 9.72, 11.22, 8.42, and 13.33 in FBL, Tilney, FBL+Tilney, and control treatments,

respectively. *Fusarium* cfu count was significantly lower in FBL+Tilney treated plots than that of the same plot area before the incorporation of mustard plants (Fig 3.8).

Discussion

In the laboratory study, extracts of *B. juncea* ('Florida Broadleaf') significantly reduced the colony growth of *P. capsici* isolates compared to that of *S. alba* ('Tilney'). Larkin and Griffin (2007) also reported that *B. juncea* completely inhibited the colony growth of *Phytophthora erythroseptica* while *S. alba* inhibited nearly 26% growth of *P. erythroseptica*. These findings indicate that some of the glucosinolates present in *B. juncea* and *S. alba* can reduce colony growth of *Phytophthora* spp. Considering the high quantity of sinigrin in 'Florida Broadleaf' mustard, hydrolysis products from this glucosinolate might have more suppressive effect on hyphal growth of *Phytophthora* spp. Sinigrin was also reported to inhibit the growth of soil-borne pathogen *Pythium irregulare* (Manici et al., 2000).

In the laboratory experiment, growth of *P. capsici* colony was suppressed as long as the culture plates were exposed to mustard extracts. When the culture plates were separated from the plates containing mustard extract, the colonies started growing. Fan et al. (2008) reported that extracts of *B. oleracea* var. *caulorapa* (Kohlrabi) suppressed colony growth of *P. capsici*, but the suppression was not persistent. The result of our study and result of Fan et al. (2008) indicated that the effect of glucosinolates on growth of *P. capsici* is temporary and lasts as long as the fumigation effect is present. Further studies are needed to determine the suppressive effects of individual glucosinolates in Brassica species on colony growth of *P. capsici*. Also, other mustard cultivars should be utilized to test their inhibitory effect on colony growth of *P. capsici*.

Mustard extracts were not effective in suppressing the colony growth of *F. oxysporum* and *F. solani* used in the laboratory experiments. The result of this study does not agree with the report by Larkin and Griffin (2007) that *B. juncea* substantially suppressed the colony growth of *F. oxysporum*. The difference between our result and the report by Larkin and Griffin could be due to difference in mustard cultivars used. Smits et al. (1993) reported that *F. oxysporum* can degrade glucosinolates (e.g., sinigrin) and prevent accumulation of toxic hydrolysis products. This might be another reason why the growth of *F. oxysporum* colonies were not significantly affected by mustards in our studies. Also, in our studies, except Tilney extract none of the other extracts affected the colony growth of F-1 isolate of *F. solani*. The reason for this result is unknown. Thus, we can conclude from the existing results that both FBL and Tilney were not effective in suppressing *Fusarium* growth effectively. More research is needed to clarify the effects of mustard on colony growth of *Fusarium* spp.

The results of our study showed that production of *P. capsici* sporangia was significantly suppressed in two isolates by FBL extract and in one isolate by Tilney extract. These findings indicate that there are interactions between glucosinolates and *P. capsici* isolates. Further studies are needed to determine the effectiveness of each glucosinolate on different isolates of *P. capsici*.

Tilney extract did not significantly affect germination of *P. capsici* oospores. The FBL extract reduced germination of *P. capsici* oospores by only 29% in comparison with control. Yet, 40.61% of *P. capsici* oospores germinated in presence of FBL extract. Also, the result of our study showed that germination of *P. capsici* zoospores was not significantly affected by any of the mustard extracts. Thus, the use of mustards as cover crops for management of *P. capsici* in the field should aim at suppressing hyphal growth and sporangial production rather than oospore and zoospore germination.

None of the cucumber seedlings in the greenhouse study and none of the cucumber vine in the field were infected by *P. capsici*. This finding does not agree with the report by Tian and Babadoost (2004) that all of the cucumber seedlings were infected by *P. capsici*. The cucumber cultivar used in the study of Tian and Babadoost was different from the cultivar ('Eureka') that we used in our study. 'Eureka' cucumber is likely to be carrying resistant gene(s) against vine infection by *P. capsici*, which is not resistant to fruit infection by *P. capsici*. The FBL extract reduced seedling infection of 'Dickinson' pumpkin by 38% compared to control treatment. But, FBL treatment did not significantly affect the *P. capsici* infection on 'Magic Lantern' pumpkin seedlings in the greenhouse study. Also, none of the mustard treatments significantly reduced the vine infection of either 'Magic Lantern' pumpkin or 'Dickinson' pumpkin in the field trials. So, using either FBL or Tilney, or a combination of these mustard cultivars as cover crops is not an effective method for management of vine infection of pumpkins. In addition, fruit infection of either "Eureka' cucumber, 'Magic Lantern' pumpkin or 'Dickinson' pumpkin was not significantly affected by mustard treatments in the field trials. Thus, effective control of *P. capsici* using mustard as a cover crop alone appears to be unlikely. However, utilizing mustard as a cover crop in an integrated approach (for example, in combination with fungicide application and crop rotation) for managing *P. capsici* in cucurbits may be a viable approach.

Sowing cucurbit seeds on the same day of mustard incorporation into the soil did not reduce their germination compared to sowing cucurbit seeds four days after mustard incorporation. Thus, cucurbit seeds can be sown any time after mustard incorporation in to the soil without significant effect on either seed germination or plant vigor.

The overall conclusion of the laboratory, greenhouse, and field studies during the three-year period showed that using mustard cultivar alone for management of *P. capsici* in cucurbits is not adequate. However, we tested only two cultivars of mustard. Testing of other mustard cultivars may have different results on the effectiveness of mustards for control of *P. capsici* in cucurbits.

TABLES

Table 3.1. Effects of mustard plant extracts on growth of *Phytophthora capsici* colony.

P. capsici isolate	Colony radius (cm)				Reduction in colony radius (%)[v]		
	FBL[w]	Tilney[w]	FBL+Tilney[x]	Control (SDW)[y]	FBL	Tilney	FBL+Tilney
PC-1	0.2111 g[z]	3.4028 abc	0.4111 g	3.5833 ab	94.11	5.04	88.53
PC-2	0.6056 g	3.8611 a	1.8972 def	3.8000 a	84.06	0.00	50.07
PC-3	0.7611 g	2.3440 cde	1.0889 fg	3.4278 abc	77.80	31.62	68.23
PC-4	0.4667 g	2.6389 bcd	1.2750 efg	3.3444 abc	86.05	21.09	61.88

[v] Percent reduction in colony radius for each mustard treatment relative to the colony radius for control treatment (sterile-distilled water).

[w] Mustard cultivars: 'Florida Broadleaf' (FBL) and 'Tilney'.

[x] A combination of 1:1 of 'Florida Broadleaf' and 'Tilney' mustards.

[y] SDW=sterile-distilled water.

[z] Values within the rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according to Fisher's protected LSD.

Table 3.2. Effects of mustard plant extracts on growth of *Fusarium* colony.

Fusarium isolate	Colony radius (cm)				Reduction in colony radius (%)[v]		
	FBL[w]	Tilney[w]	FBL+Tilney[x]	Control (SDW)[y]	FBL	Tilney	FBL+Tilney
F-1	4.0000 a[z]	3.1889 c	4.0000 a	4.0000 a	0.00	20.28	0.00
F-2	4.0000 a	4.0000 a	4.0000 a	4.0000 a	0.00	0.00	0.00
F-3	4.0000 a	4.0000 a	3.5667 b	4.0000 a	0.00	0.00	10.83

[v] Percent reduction in colony radius for each mustard treatment relative to the colony radius for control treatment (sterile-distilled water).

[w] Mustard cultivars: 'Florida Broadleaf' (FBL) and 'Tilney'.

[x] A combination of 1:1 of 'Florida Broadleaf' and 'Tilney' mustards.

[y] SDW=sterile-distilled water.

[z] Values within the rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according to Fisher's protected LSD.

Table 3.3. Effects of mustard plant extracts on sporangia production of *Phytophthora capsici*.

P. capsici isolate	FBL[w]	Tilney[w]	FBL+Tilney[x]	Control (SDW)[y]	FBL	Tilney	FBL+Tilney
	Sporangia production (number)				Reduction in sporangia production (%)[v]		
PC-1	52.33 c[z]	398.33 a	107.92 c	257.86 b	79.71	0.00	58.15
PC-2	95.06 c	65.89 c	64.11 c	55.58 c	0.00	0.00	0.00
PC-3	75.06 c	77.27 c	59.47 c	270.53 b	72.25	71.44	78.01
PC-4	49.39 c	104.75 c	63.83 c	70.08 c	29.52	0.00	8.92

[v] Percent reduction in sporangia production for each mustard treatment relative to the sporangia production for control treatment (sterile-distilled water).

[w] Mustard cultivars: 'Florida Broadleaf' (FBL) and 'Tilney'.

[x] A combination of 1:1 of 'Florida Broadleaf' and 'Tilney' mustards.

[y] SDW=sterile-distilled water.

[z] Values within the rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according to Fisher's protected LSD.

Table 3.4. Effects of mustard plant extracts on zoospore germination of *Phytophthora capsici*.

P. capsici isolate	Zoospore germination (%)				Reduction in zoospore germination (%)[v]		
	FBL[w]	Tilney[w]	FBL+Tilney[x]	Control (SDW)[y]	FBL	Tilney	FBL+Tilney
PC-1	87.88 abc[z]	74.92 abcd	93.56 ab	66.71 abcd	0.00	0.00	0.00
PC-2	48.22 d	42.56 d	52.08 cd	59.28 bcd	18.66	28.21	12.15
PC-3	75.56 abcd	64.22 abcd	79.17 abcd	68.67 abcd	0.00	6.48	0.00
PC-4	88.78 abc	99.39 a	99.50 a	74.97 abcd	0.00	0.00	0.00

[v] Percent reduction in zoospore germination for each mustard treatment relative to the zoospore germination for control treatment (sterile-distilled water).

[w] Mustard cultivars: 'Florida Broadleaf' (FBL) and 'Tilney'.

[x] A combination of 1:1 of 'Florida Broadleaf' and 'Tilney' mustards.

[y] SDW=sterile-distilled water.

[z] Values within the rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according to Fisher's protected LSD.

Table 3.5. Crown infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin and 'Dickinson' pumpkin by *Phytophthora capsici* in presence of 'Florida Broadleaf' (FBL), 'Tilney', and FBL+Tilney mustard extracts in the greenhouse trial.

Mustard treatment	Crown infection (%)		
	Eureka cucumber	Magic Lantern pumpkin	Dickinson pumpkin
FBL	0 a[y]	100 a	31.59 bc
Tilney	0 a	100 a	41.20 ab
FBL+Tilney	0 a	100 a	25.23 c
Control (SDW)[z]	0 a	100 a	50.69 a
LSD	NS	NS	13.63

[y] Values within each column followed by a letter in common are not significantly different (*P*=0.05) from each other according to Fisher's protected LSD.
[z] Sterile distilled water.

Table 3.6. Vine infection of 'Magic Lantern' and 'Dickinson' pumpkins in the field with 'Florida Broadleaf' (FBL), 'Tilney', and FBL+Tilney mustard treatments.

Trial	Vine infection (%)[y]			
	FBL	Tilney	FBL+Tilney	Control
2008-spring	27.48 abcd[z]	40.73 a	31.70 ab	39.93 a
2008-fall	12.58 cde	19.72 bcde	20.87 bcde	8.67 e
2009-spring	30.37 abc	29.44 abc	28.10 abcd	14.99 bcde
2010-spring	6.02 e	13.78 bcde	11.15 de	13.74 bcde

[y] Included cucurbits were 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin.
[z] Values within rows and columns followed by a letter in common are not significantly different (*P*=0.05) from each other according to Fisher's protected LSD.

Table 3.7. Fruit infection of cucurbits in the field with 'Florida Broadleaf' (FBL), 'Tilney', and FBL+Tilney mustard treatments.

Trial	Mustard treatment	Fruit infection (%)[x]	
		Sprayed[y]	Unsprayed
2008-spring	FBL	39.33 abcd[z]	31.05 bcdef
	Tilney	15.10 def	17.99 def
	FBL+Tilney	28.73 bcdef	27.83 bcdef
	Control	24.63 cdef	20.62 cdef
2008-fall	FBL	34.88 abcde	30.67 bcdef
	Tilney	16.70 def	59.22 a
	FBL+Tilney	13.33 ef	24.44 cdef
	Control	45.37 abc	51.22 abc
2009-spring	FBL	12.16 ef	8.69 f
	Tilney	5.94 f	8.87 f
	FBL+Tilney	6.22 f	22.89 cdef
	Control	6.16 f	5.90 f
2010-spring	FBL	21.39 cdef	21.87 cdef
	Tilney	21.53 cdef	30.85 bcdef
	FBL+Tilney	22.48 cdef	20.00 cdef
	Control	29.61 bcdef	15.77 def

[x] Included cucurbits were 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin.

[y] Combination of Tanos (50WDG, at 700g/ha)+Kocide-3000 (46.1 DF, at 1.68 Kg/ha) fungicides were sprayed, alternated weekly with sprays of Forum (4.18 SC, at 350 ml/ha)+Kocide-3000 (46.1 DF, at 1.68 Kg/ha) fungicides .

[z] Values within rows and columns followed by a letter in common are not significantly different (P=0.05) from each other according   Fisher's protected LSD.
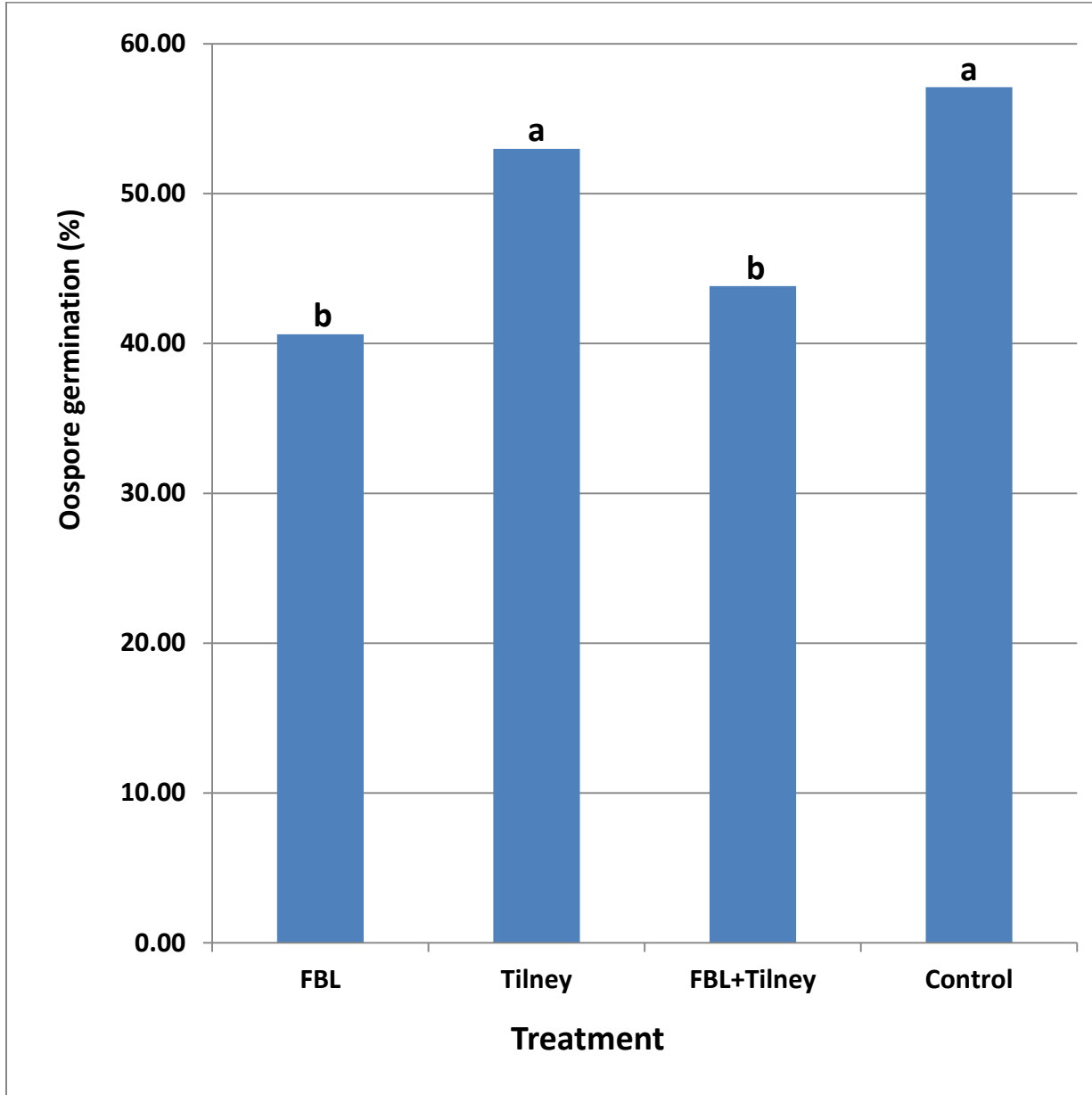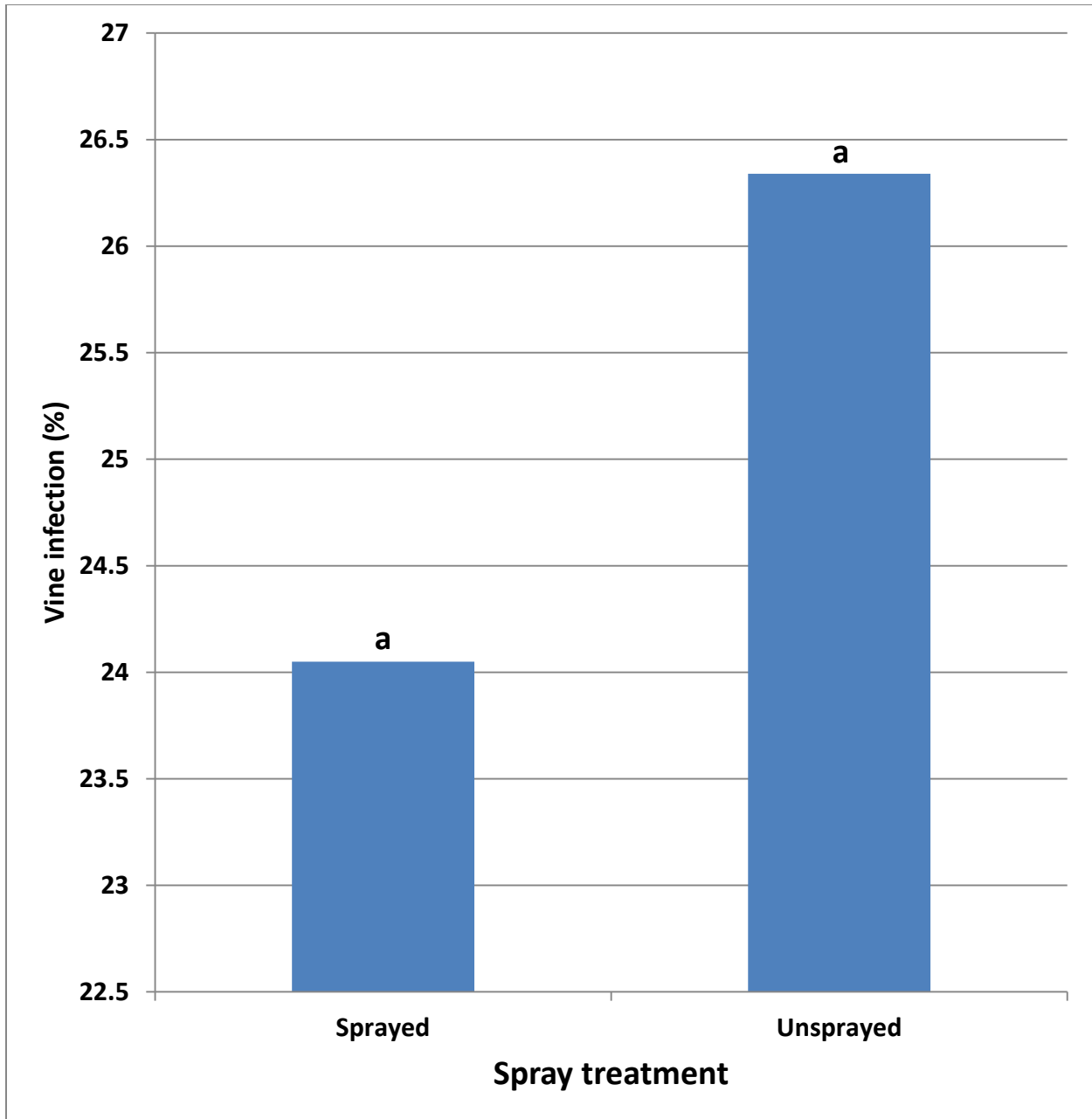
Fig. 3.1. Germination of *Phytophthora capsici* oospores exposed to Florida Broadleaf (FBL), Tilney, and FBL+Tilney mustard extracts in the laboratory experiments.
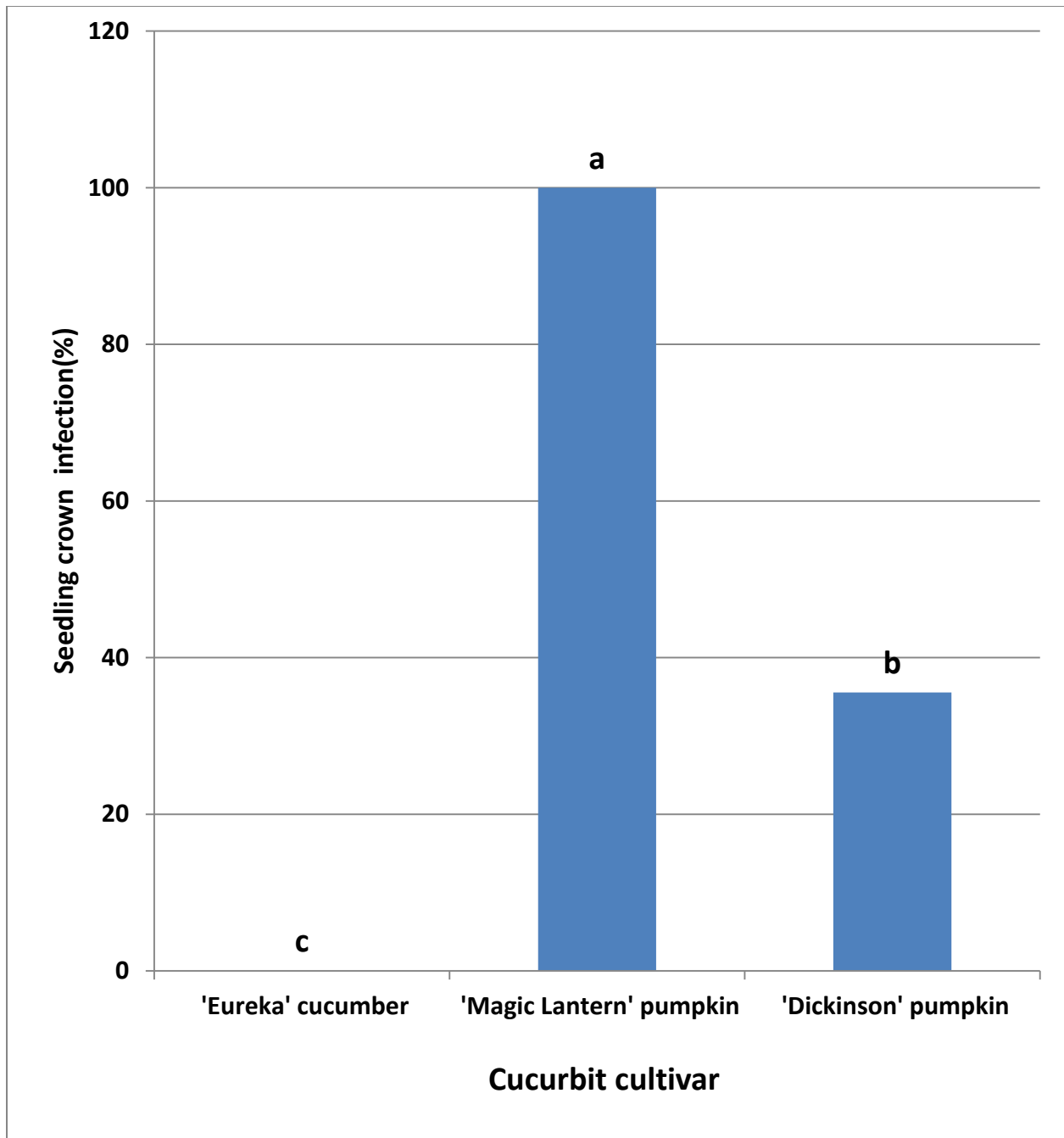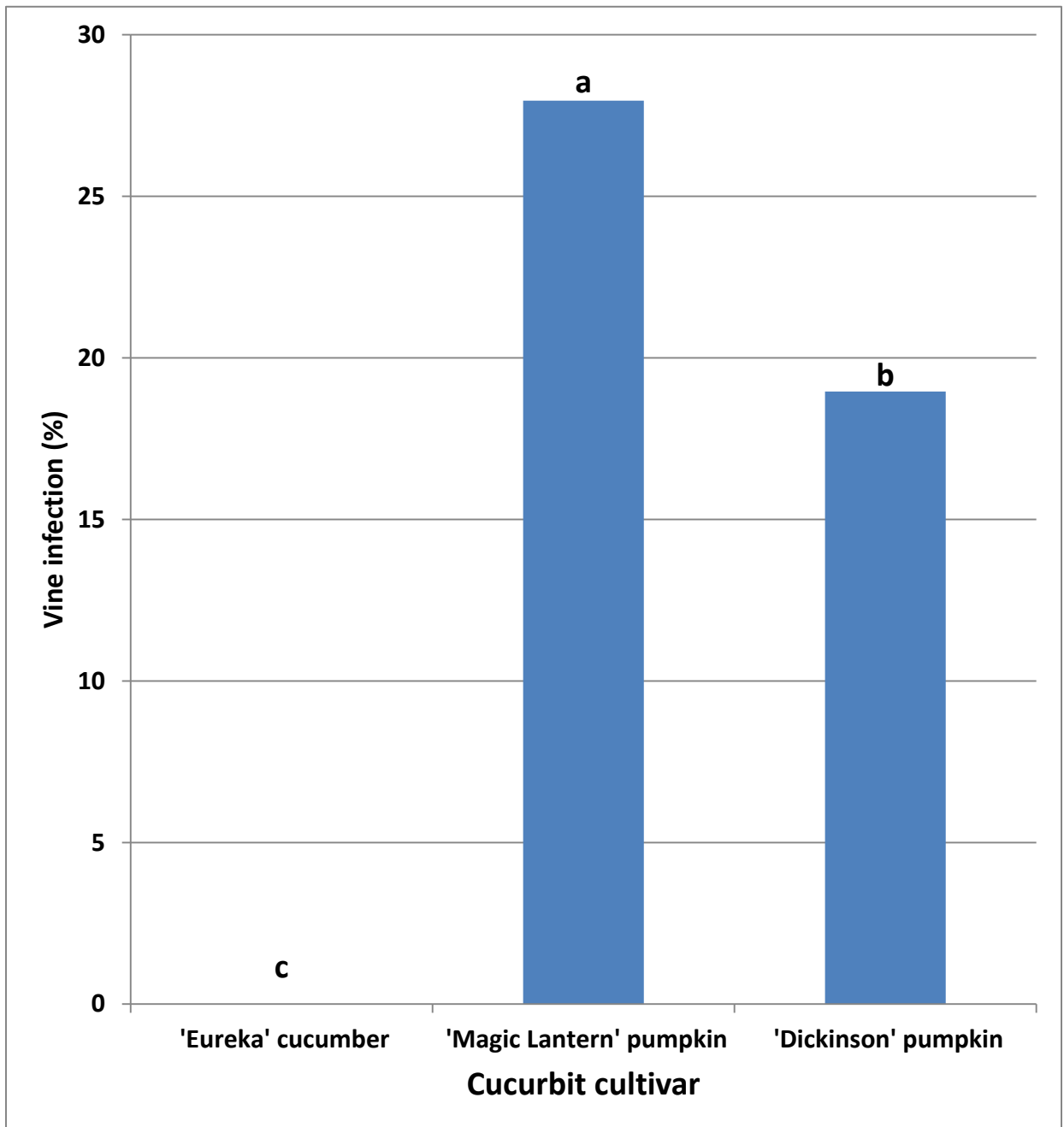
Fig. 3.2. Seedling infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin infected by *Phytophthora capsici* in the greenhouse trial.
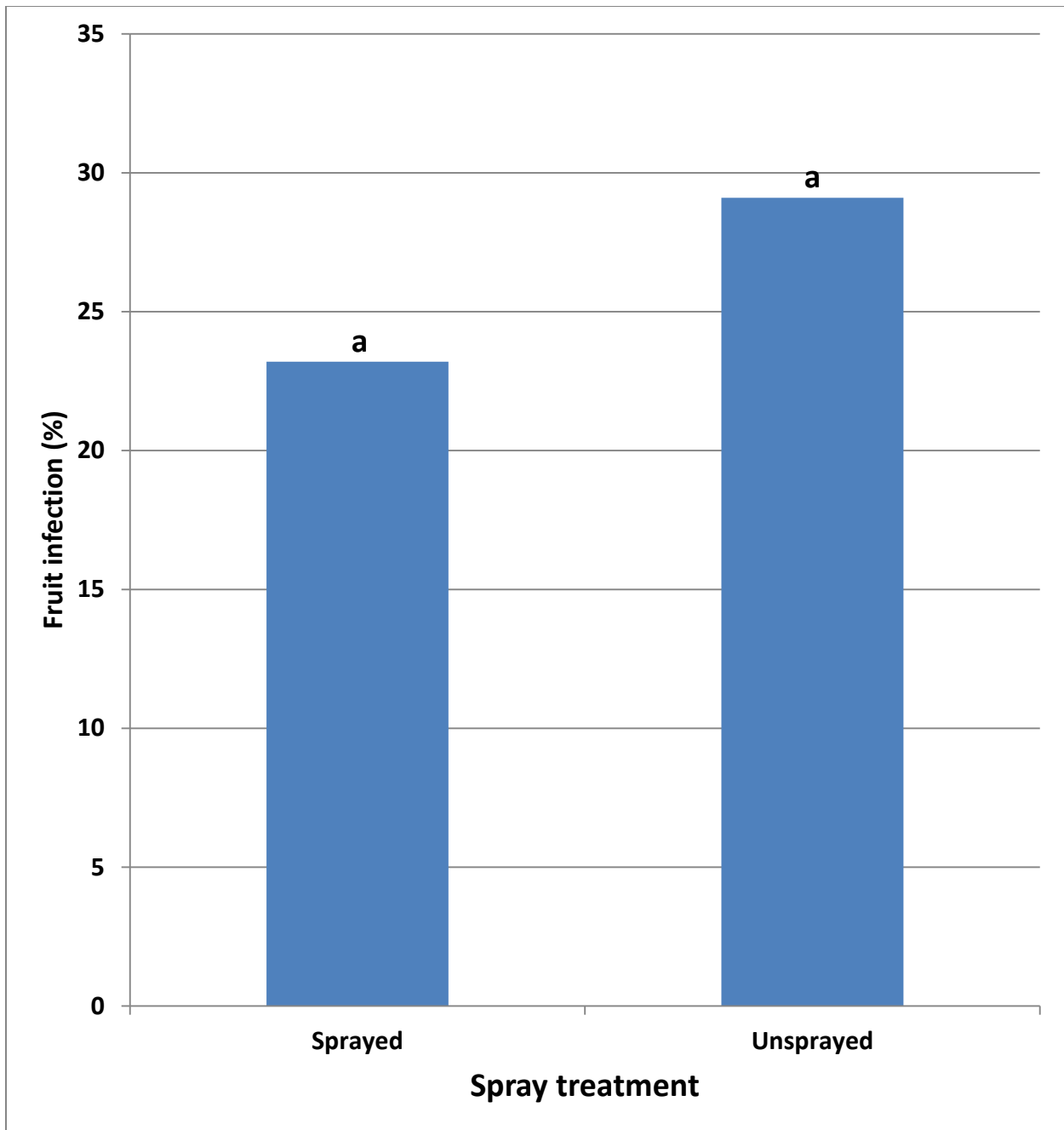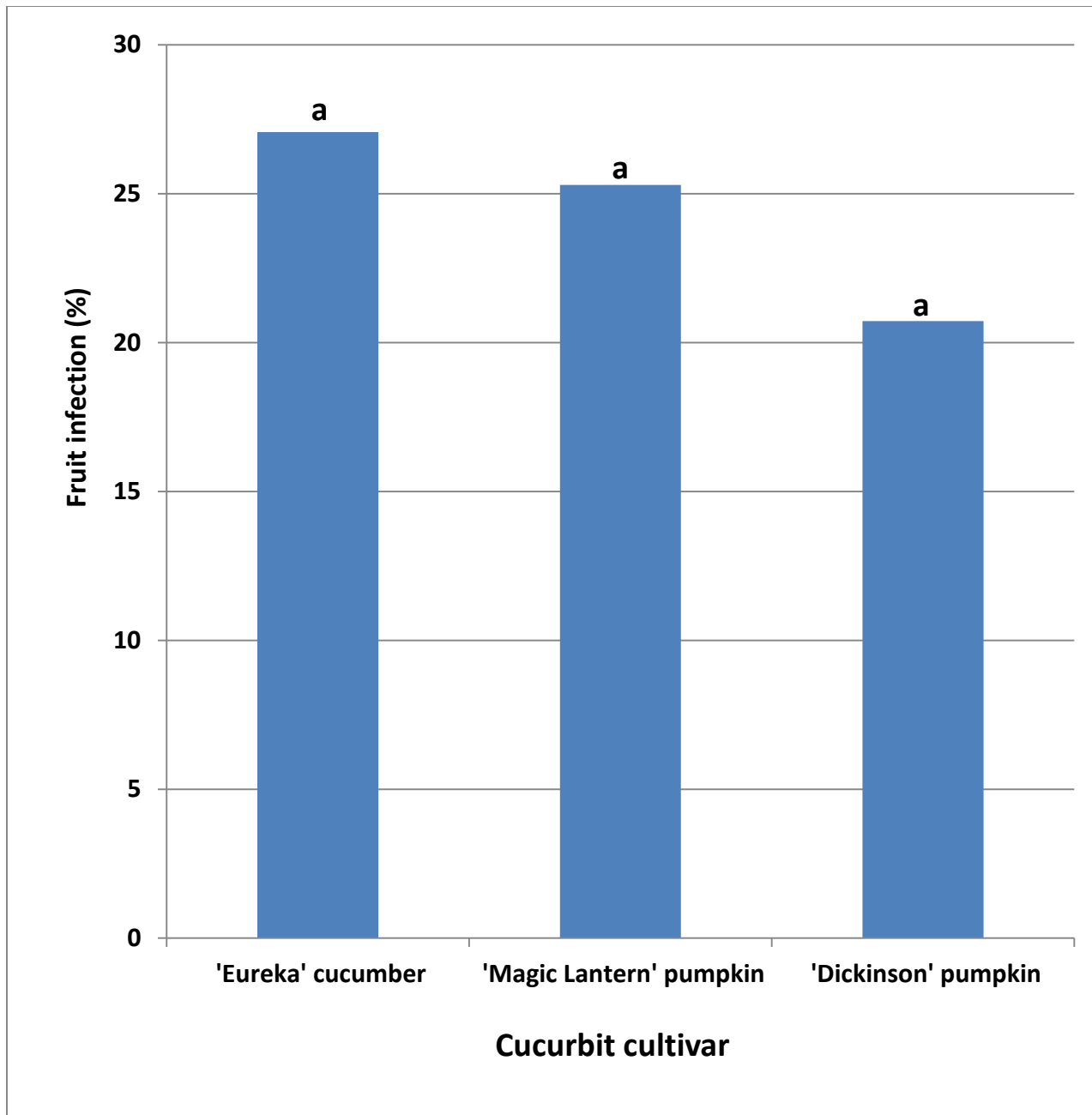
Fig. 3.3. Vine infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin by *Phytophthora capsici* in the sprayed and unsprayed field plots during 2008-2010.

Fig. 3.4. Vine infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin by *Phytophthora capsici* in the field plots during 2008-2010.

Fig. 3.5. Fruit infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin by *Phytophthora capsici* in the sprayed and unsprayed field plots during 2008-2010.

Fig. 3.6. Fruit infection of 'Eureka' cucumber, 'Magic Lantern' pumpkin, and 'Dickinson' pumpkin by *Phytophthora capsici* in the field plots during 2008-2010.
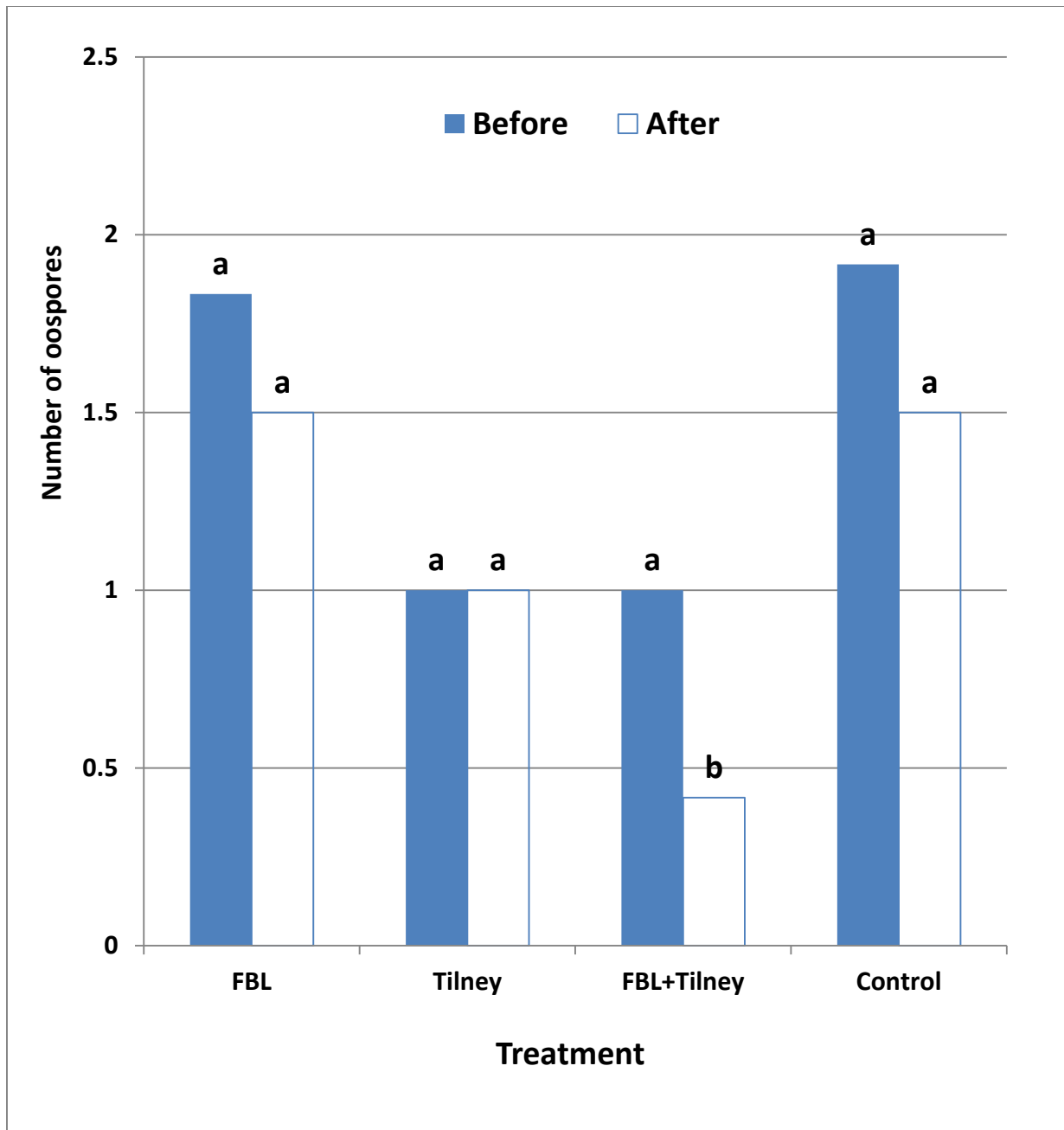
Fig. 3.7. Density of *Phytophthora capsici* oospores in one gram soil before and after incorporation of Florida Broadleaf (FBL), Tilney, and FBL+Tilney mustard and control treatments during 2008-2010 field trials.
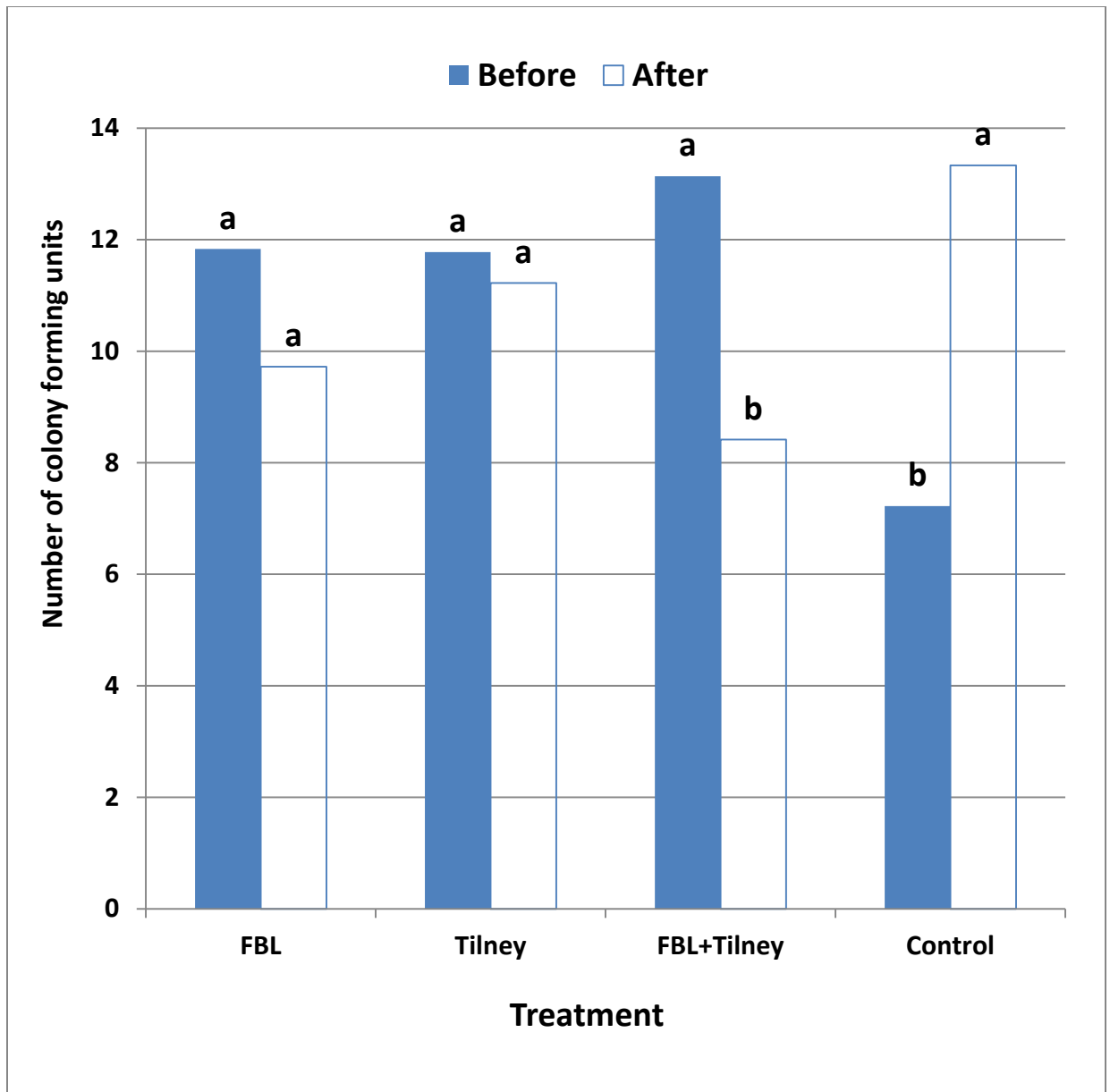
Fig. 3.8. Colony forming units (cfu) of *Fusarium* spp. in one hundredth of a gram of soil (10^{-2} g) before and after incorporation of Florida Broadleaf (FBL), Tilney, and FBL+Tilney mustard and control treatments during 2008-2010 field trials.

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