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Plant extracted essential oils as a contact fungicide seed treatment for organic corn

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Plant extracted essential oils as a contact fungicide seed treatment for organic corn

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To Lucille and Andrew

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Chapter 2.

Abstract

Planting date for organic corn (*Zea mays* L.) is delayed to avoid the cold and wet soils of spring. Conventional farmers can use fungicide seed treatments to protect the emerging seedling but few organic fungicides are available. The objective of this research was to determine whether essential oils can be used as a contact fungicide seed treatment for organic corn. Eighteen plant essential oils were screened for their fungicide properties against three common corn pathogens *Penicillium*, *Fusarium*, and *Pythium*. Five oils completely controlled all three pathogens *in vitro*. These oils were cinnamon, clove, oregano, savory, and thyme. The minimum inhibitory concentration (MIC) for all pathogens was 800 μL L^{-1} and there was no phytotoxicity seen in the germination test at rates up to 16 000 μL L^{-1} (MIC × 20). Field emergence of inbred and hybrid seeds treated with the essential oil were significantly lower than seed treated with the commercial fungicides Maxim XL, which is a conventional fungicide, and Natural 2, which is an organic fungicide, but not different from an organic fungicide Yield Shield or an untreated control.

Chapter 1. General Introduction

Introduction

Synthetic seed treatments are used to protect the corn seed from harmful pathogens during field emergence. This protection allows conventional farmers to plant earlier in the growing season, when the soil moisture and temperature are less favorable for seed germination. However, organic farmers must delay planting until later in the season to avoid field emergence problems associated with cold and wet soils since seed applied fungicides are unavailable. This delay in planting usually shortens the planting period considered optimum for maximizing grain yields. The use of natural seed treatment could benefit organic farmers by allowing earlier planting times and improving plant stand, which could also result in higher yields. There are some organic seed treatments comprised of beneficial bacteria or fungi currently available in the market. These organisms out compete with or antagonize the harmful pathogens on and around the seed, providing protection to the growing seedling. However, these types of treatments are specific to certain seedborne pathogens which are crop-species specific (International Seed Trade Federation, 2000; McSpadden Gardener and Fravel, 2002).

All effective seed treatments have several characteristics in common. The seed treatments should work consistently without adversely affecting the growing seedling. They should be harmless to humans or wildlife, break down easily and completely without harmful residues, and should not interfere with rhizobium in legumes. Most importantly, the seed treatment should be affordable (Agarwal and Sinclair, 1996). In order for a seed treatment to be effective and commercially viable, it needs to have these factors.

Seed pathogens have various methods of infection. Some pathogens are soilborne, such as *Pythium*, and are present in the soil. These pathogens attack the seed after planting and cause wilting and seedling damping off (McGee, 1994). Damping off symptoms are brown water-soaked areas in the stem of the plants. In most cases, seedling damping off leads to the death of the growing seedling. *Pythium* inhabits the soil and only infects the seed when the specific environmental conditions of cold soil, less that 10°C, and excessive moisture are in place (Munkvold, 1996). Control of *Pythium* is very important in Iowa where the springs are usually cool and wet. *Pythium* can greatly reduce plant stand of untreated corn and lower crop yields (Pedersen et al., 1986; Rao et al., 1978).

Seed pathogens can be seedborne, seed transmitted, or both. Seedborne pathogens are found on the surface or inside the seed (McGee, 1994). The environmental conditions that promote seed germination usually are favorable for pathogen growth and seed infection. Seed transmitted implies that the pathogen is transmitted from the seed to the growing plant. *Aspergillus* sp., *Fusarium* sp., *Alternaria* sp. and *Penicillium* sp. are common seed pathogens in corn. According to McGee (1994), *Fusarium* and *Penicillium* are seedborne, which implies the pathogen is on or in the seed, and seed transmitted, which implies the pathogen infects the plant through the seed, whereas *Aspergillus* and *Alternaria* are seedborne, but not seed transmitted.

Fusarium sp. and *Penicillium* sp. are common corn seedborne pathogens in Iowa. Seed infection with *Fusarium* sp. can originate from a variety of sources, but the most common are field residue and infected seeds (McGee, 1994). Fusarium stains consist of a pink colored mass of spores on the seed and white mycelium. The symptoms of this fungus are seedling rot and damping off where the seed embryo dies before germination. *Penicillium*

behaves much in the same way as *Fusarium,* but the spores of this fungus are transported by air and are found on plant residue in the field. The symptoms of *Penicillium* include seed rot and damping off. Seed infected with *Penicillium* are covered by white mycelium and a mass of bluish-green spores.

Some plant-produced essential oils have antifungal activity (Burt, 2004). Plant essential oils are extracted from plant materials such as bark, seeds, and leaves. Many plant essential oils with good antifungal activity have certain ingredients in common (Pawar and Thaker, 2006). Essential oils with good antifungal activity usually have phenolic or aromatic compounds in their chemical composition (Burt, 2004).The antifungal mode of action of these essential oils is not completely understood, but it is thought that due to their lipophilic and hydrophobic tendencies they can cross cell membranes and enter the cytoplasm (Burt, 2004; Pawar and Thaker, 2006). Moreover, the essential oils are believed to degrade the membrane and to allow cell leakage and loss of proton motive force (Burt, 2004). With the loss of membrane integrity a proton gradient cannot be sustained and proton motive force is lost. Proton motive force is the proton gradient plus the membrane potential (Hopkins, 1995). Lambert et al., 2001 determined the permeability of the pathogen cell membrane to essential oils mixed with a nuclear stain. The nucleus of the cell was stained when the stain was mixed with the essential oils, but was not when the stain was applied alone. They concluded that the essential oils penetrated the cell membranes, which was probably part of their mode of action.

Plant essential oils can inhibit growth of many fungal species. Some plant essential oils have antifungal properties and, when applied to the seed, eliminate the pathogens on and around the seed. Velluti et al., (2004) showed that essential oils of oregano, clove, cinnamon,

lemongrass, and palmarosa oils reduced the infection levels of *Fusarium verticillioides* in maize seeds. The essential oils eliminated the fungus and reduced the amount of fumonisins produced. Only five of the thirty-seven oils tested by Velluti et al., (2004) showed good control of *Fusarium verticillioides*. The essential oils were characterized using Gas Chromatography – Mass Spectrometry (GC-MS). Results revealed that the oils did not have many compounds in common.

Pawar and Thaker (2006) screened seventy-five essential oils for their antifungal properties against *Aspergillus niger*. They reported that only five of the seventy-five oils controlled pathogen growth. Essential oils extracted from *Cinnamomum zeylanicum* (bark and leaf), *Cinnamomum cassia*, *Syzygium aromaticum*, and *Cymbopogon citratus* had good antifungal activity, while cinnamon bark oil had the best control rate.

The oils of rosewood, bay, sassafras, and cinnamon inhibited the growth of *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp. *in vitro* (Simic et al., 2004). Cinnamon essential oil was the most effective at controlling the pathogen growth, while bay oil was the least. GC-MS characterization showed that the essential oils had different composition. The authors concluded that the antifungal activity of the oils was based on the interactions among the different compounds, rather than a few compounds providing the antifungal properties to all oils.

Large amounts of phenolic compounds, such as thymol and carvacrol, are found in most essential oils with good antifungal activity. *Penicillium* sp. growth *in vitro* was inhibited by the essential oils of oregano, thyme, dictamus, and marjoram oils (Daferera et al., 2000). The GC-MS characterization of the essential oils showed that essential oils of thyme, oregano, and dictamus oils had large amounts of the compound thymol. In contrast,

essential oils of marjoram contained a wide variety of aromatic compounds without a predominant element in the mixture. The authors also tested the inhibitory activity of a sample of pure thymol and concluded that pure thymol had similar pathogen inhibitory properties to the essential oils. Chatterjee (1990) showed that cassia, clove star-anise, geranium, and basil prevented the infection of maize seed *in vitro* by *Aspergillus flavus*, *Aspergillus Glaucus*, *Aspergillus niger*, and *Aspergillus sydowi*. The seeds were artificially inoculated with the pathogens and treated with the essential oils at rates from 0 to 50 μL/g of grain. The author defined "the effective level" of the essential oils to the rate capable of controlling the pathogens. Even though the five essential oils controlled the artificially inoculated fungi, the essential oils were unable to control other fungi already present on the seed. Montes-Belmont and Carvajal (1998) evaluated the fungicide properties of *Cinnamomum zeylanicum*, *Mentha piperita*, *Ocimum basilicum*, *Origanum vulgare*, *Teloxys ambrosioides*, *Syzygium aromaticum*, and *Thymus vulgaris*. These plant essential oils controlled *Aspergillus flavus* growth on artificially infected maize seeds germinated in the laboratory. Corn seedlings treated with these essential oils did not exhibit phytotoxicity. *Melaleuca alternifolia* essential oils are also high in phenolic compounds (Bishop and Thornton, 1997). The most abundant phenolic monoterpenes were thymol and carvacrol. A mixture of thymol and carvacrol had the same inhibitory effect against *Staphylococcus aureus* and *Pseudomonas aeruginosa* as the whole oregano essential oil (Lambert et al., 2001). Sivropoulou et al. (1996) also reported high levels of thymol and carvacrol in oregano essential oil. Whole oregano oil, and thymol and carvacrol were tested against different gram negative and gram positive bacteria. It was shown that when controlling the

bacteria there was no difference in control between the oil as a whole and the individual compounds.

The chemical composition of essential oils varies with plant genotype, growing conditions, and extraction method (Hammer et al., 1999). Therefore, essential oils composition should be assessed using GC-MS. Once the chemical make-up of the essential oil is determined, it can be referenced and compared to other essential oils of known antifungal activity.

The findings reported in the literature suggest that plant essential oils could be used as an alternative to synthetic fungicides, but further research needs to be done. Most of the research conducted thus far was done in the laboratory. There is little information in the literature about the effectiveness of these essential oils under field conditions or their effect on *Pythium sp*. growth.

The objectives of this research were to determine if plant-extracted essential oils show antimicrobial activity against selected corn pathogens; to determine the minimum amount of essential oil needed to control pathogen growth, or minimum inhibitory concentration MIC; to determine whether the essential oils are phytotoxic to corn seeds ; to analyze the essential oils by Gas Chromatography – Mass Spectrometry; to determine whether the essential oils can control seed corn pathogens in the laboratory, and finally to evaluate the field performance of the seeds treated with the essential oils

Thesis Organization

This thesis contains two manuscripts which were written for publication in scientific journals. Chapter 2 includes the initial screening of the eighteen essential oils against the

three selected corn pathogens *Fusarium* sp., *Penicillium* sp., and *Pythium* sp. *in vitro*. From the initial screening, five oils: cinnamon, clove, oregano, savory, and thyme were selected for further study. The minimum inhibitory concentration of the selected oils was then determined. Essential oils were then applied to seeds to test for phytotoxicity. The constituent compounds of the oils were then determined using GC-MS. In Chapter 3 the essential oils were applied to seeds which were inoculated with one of the three pathogens, seeds were then put in cold test germination. Seeds are then treated and planted in either a conventional or organic field. Emergence data was taken at both V2 and V5 growth stages. References are cited separately for each manuscript and a general conclusion follows the manuscripts.

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Chapter 2.In vitro testing for antifungal properties of essential oils

Introduction

Synthetic seed treatments are applied on seed corn (*Zea mays* L.) to protect the emerging seedling in the field. These seed treatments allow conventional farmers to plant earlier in the spring, when soils are wet and cold, and field conditions are less favorable for seedling growth. Organic farmers, however, must delay planting to avoid seedling emergence problems associated with cold and wet soils. This necessary delay jeopardizes the organic producer's chances of planting their crop within the dates recommended for maximizing corn yields. Organic farmers could benefit from natural seed treatments that would lengthen planting time, improve stand establishment, and possibly increase yields. There are some organic seed treatments available in the market based on beneficial bacteria or fungi. These beneficial organisms have an antagonistic mode of action, and interact and suppress the pathogens present on the seed or immediately around it. These types of treatments are specific for a certain seedborne pathogens (International Seed Trade Federation, 2000; McSpadden Gardener and Fravel, 2002).

All effective seed treatments have several characteristics in common. Seed treatments must provide protection to the seed from soil and seedborne pathogens without adversely affecting seedling growth. They also must be harmless to humans or wildlife, break down easily and completely without harmful residues, and should not interfere with rhizobium in legumes. Most importantly, seed treatments should be affordable (Agarwal and Sinclair, 1996).

Seed pathogens have various methods of infection. Soilborne pathogens, such as *Pythium*, are present in the soil. These pathogens attack the seed after planting and cause seedling wilting or damping off (McGee, 1994). The symptoms are brown water-soaked tissue in portions of the stem which can be lethal to the germinated seedling. *Pythium* usually infects the seed when the soil is cold, less than 10°C, and moisture is excessive (Munkvold, 1996). These pathogens are always in the soil and usually are harmless until the specific environmental conditions are in place. Alternatively, seedborne pathogens are found on or in the seed (McGee, 1994). The environmental conditions favorable for seed germination are also ideal for pathogen growth and consequent seedling infection. For example, *Aspergillus, Fusarium, Alternaria* and *Penicillium* are seedborne fungi. *Fusarium* produces a pink colored mass and white mycelium on the seed, causing seedling rot. *Penicillium* also causes rot and damping off. The fungus produces a white mycelium and a bluish-green colored mass of spores on the seed.

The essential oils of some aromatic plants have antifungal properties. When these essential oils are applied to the seed, they eliminate the soilborne and seedborne pathogens. Velluti et al. (2004) showed that the essential oils from oregano, clove, cinnamon, lemon grass, and palmarosa oils reduced the levels of *Fusarium verticillioides* infection in maize seeds. These essential oils also reduced the amount of fumonisins produced by the fungus. Only five of the thirty-seven oils tested by Velluti et al. (2004) showed good control of *Fusarium verticillioides*. The Gas Chromatography – Mass Spectrometry (GC-MS) characterization of the oils yielded very few compounds in common among the effective essential oils.

The essential oils of rosewood, bay, sassafras, and cinnamon inhibited the growth of *Aspergillus sp.*, *Fusarium sp.*, and *Penicillium sp. in vitro* (Simic, et al., 2004). From these oils, cinnamon oil was the most effective at controlling pathogen growth, while bay oil was the least. Gas chromatography-mass spectrometry characterization of the oils showed that there were no similarities in the composition of the oils. The authors concluded that the antifungal activity of the oils depended on the interaction of all the constituent compounds, rather than a few common compounds providing antifungal properties to all essential oils.

Research shows that aromatic compounds isolated from plant essential oils also have antifungal activity. *Penicillium* growth was inhibited by oregano, thyme, dictamus, and marjoram oils (Daferera *et al*, 2000). The chemical composition of the essential oils was characterized using GC-MS. Thyme, oregano, and dictamus oils showed large amounts of the compound thymol. Marjoram, in contrast, contained a wide variety of aromatic compounds without one being predominant. The authors also tested whether pure thymol would inhibit *Penicillium* growth. They concluded that pure thymol had a similar inhibiting effect on pathogen growth than the essential oils containing thymol. Chatterjee (1990) showed that cassia, clove star-anise, geranium, and basil prevented *in vitro* infection of maize seeds by *Aspergillus flavus*, *Aspergillus Glaucus*, *Aspergillus niger*, and *Aspergillus sydowi*. The seeds were inoculated with the pathogens and treated with amounts of the essential oils ranging from 0 and 50 μL/g of grain. The authors defined "the effective level of the oil" as the concentration of the oils capable of controlling the pathogens. Even though the five oils controlled the artificially inoculated fungi, they were unable to control other fungi already present on the seed. These studies suggest that plant essential oils could be used as an alternative to synthetic fungicides, but there is little information about the effectiveness of the

essential oils on *Pythium sp*. growth, the minimum concentration of the oil capable to inhibit corn pathogens, or the potential phytotoxic effect of the essential oils to corn seeds.

The objectives of this study are to evaluate the efficacy of the essential oils at controlling two seedborne and one soilborne pathogen in corn, to determine the minimum inhibitory concentration of the oil capable of inhibiting pathogen growth *in vitro*, and assess possible phytotoxic effect of the oils to corn seed.

Material and Methods

Identification, isolation, culture, and purification of the seed pathogens

The corn pathogens selected for this study were Fusarium seed rot, Penicillium seedling blight, and Pythium root rot (Figure 1). *Penicillium* sp. and *Fusarium* sp. were isolated from a corn seed sample sent for testing to the Iowa State University Seed Testing Laboratory. To isolate the pathogens, the seed sample was cultured on selective media. PDA was used for the isolation and purification *Penicillium* sp. and Nash and Snyder selective media was used for *Fusarium* sp. The media were incubated in a growth chamber at 25°C for 14 d (*Fusarium* sp) and 3 d (*Penicillium* sp.). *Penicillium* sp. and *Fusarium* sp. mycelium growth were excised from the seed surface or the PDA media. Distinctive colonies from the pathogens were re-isolated and plated into fresh media for purification. The pathogenicity of these cultures was not tested as mycelia were isolated from infected symptomatic seeds, and therefore, considered pathogenic. *Pythium* sp. cultures were obtained from the Seed Health Testing Lab at the Iowa State University Seed Testing Laboratory.

After purification, *Fusarium* sp. and *Pythium* sp. were stored as 4.3mm cores of media containing mycelium. *Penicillium* sp. was stored as a spore solution. The *Fusarium* sp.

cores and the *Penicillium* sp. spores were stored inside vials containing a 15% glycerol solution and frozen at -80°C (Mao *et al*., 1997). The *Pythium* sp. cores were placed inside vials containing sterile de-ionized water and stored at 15°C.

Plant essential oils

Eighteen essential oils were chosen for these experiments: anise (*Pimpinella anisum*), basil (*Ocimum basilicum*), bay (*Laurus nobilis*), caraway (*Carum carvi*), cinnamon (*Cinnamonum zeylanicum*), clove (*Eugenia caryophyllata*), coriander (*Coriandrum sativum*), lavender (*Lavendula officinalis*), lemongrass (*Cymbopogon citratus*), nutmeg (*Myristica fragrans*), oregano (*Origanum minutiflorum*), pepper (*Piper nigrum*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), savory (*Satureia montana*), tea tree (*Melaleuca alternifolia*), and thyme (*Thymus vulgaris*). Most oils were obtained from Amrita (Fairfield, Iowa) except for cinnamon which was obtained from Aromaland (Santa Fe, New Mexico).

Evaluation of the antifungal properties of the oils in-vitro

Pathogen culture

The pathogens were cultured prior to using in the experiments. The frozen cores were thawed and plated in the center of the Petri dish containing PDA. *Fusarium sp*. 4.3mm-cores were cultured for 14 d, while *Penicillium sp*. spores and *Pythium sp*. cores were cultured for three days to allow mycelium to completely fill the Petri dish. One hundred microliters of *Penicillium sp*. spore solution were plated on a Petri dish with PDA media and spread in a

thin layer over the entire Petri dish, using a glass rod. All Pathogens were cultured at 25°C and 50 % relative humidity.

Media preparation and pathogen incubation

Essential oils were mixed with PDA growth media at a concentration of l000 μL L^{-1}. Growth media was prepared by adding 39 grams of PDA to one liter of distilled water. The PDA and water were heated on a hot plate, constantly mixing with a magnetic stirrer. The mixture was allowed to boil for 60 s and subdivided into 60ml flasks. The flasks were sterilized by autoclaving at 121°C for 15 min. Twenty μL of the essential oils were mixed into the 60 ml of PDA inside a laminar-flow hood (Bioguard, Baker Co. Inc., Sanford, ME) using an autoclaved magnetic stirrer. The mixture was subdivided into three Petri dishes, pouring 20 ml of PDA per plate. Media was allowed to cool and solidify for 24 h. The essential oils were not autoclaved to avoid denaturing or volatilizing the aromatic compounds in the oils. Additional control Petri dishes containing PDA and essential oils were prepared and incubated to evaluate possible pathogen contamination in the essential oils.

A different pathogen was plated onto each of the three plates. A small core of a pathogen culture was placed on each plate under the laminar-flow hood. The *Pythium sp*. and *Fusarium sp*. cores were taken in a circular pattern equidistant from the center of the plate so mycelia in each core were of the same age. The *Penicillium sp*. cores were taken randomly from the individual colonies growing throughout the plate. Plates were sealed with parafilm and inverted so that water would not condense on the agar surface. The inoculated plates were randomized within rows on trays, and placed in single plate-high rows and 17 plates in each tray. A total of four trays were used per replication. The trays were placed inside modified food service carts (Lincoln Foodservice Products, Fort Wayne, IN) and incubated in

the dark inside a constant 25°C germination chamber. The door of the cart was left ajar, to prevent accumulation of high concentrations of volatile compounds from the essential oil inside the cart. Three types of control plates were included in each replication: three PDA media plates each with and without essential oil to verify media and essential oils sterility, and four PDA media plates inoculated with the pathogen to determine their viability and growth. There were also *Pythium* sp. inoculated control plates in opposite corners of each of the trays to determine whether essential oil volatility influenced pathogen growth anywhere within the cart.

Measurement of fungal growth

Fungal growth measurements were taken every 24 h for 7 d. The mycelium diameter was measured using a digital caliper (Fischer Scientific, Pittsburgh, PA) and recorded in millimeters. Two measurements from each plate were taken at a 90° angle from each other. The two measurements were averaged to determine the diameter of the fungal colony. Two diameter measurements were taken to account for the uneven circular pattern of the pathogen colony. The entire experiment was replicated three times. Data were analyzed using PROC ANOVA in SAS (SAS Institute, Cary, NC) and means were separated. Even though mean separations were conducted, the essential oils were chosen based solely on their ability to completely inhibit growth of all three pathogens over the time of measurement.

Minimum Inhibitory Concentration (MIC)

Five essential oils were selected from the previous experiment based on their ability to completely suppress pathogen growth *in vitro*. These oils were: cinnamon, clove, oregano, savory and thyme. Minimum inhibitory concentration (MIC) was determined by diluting the

essential oils in PDA media to concentrations of 100, 200, 400, and 800 μL L^{-1}. Sixty ml of PDA were mixed with 6, 12, 24, and 48 μL of essential oil to obtain the desired concentrations. *Pythium* sp., *Penicillium* sp., and *Fusarium* sp. inoculum cores were cultured in PDA to obtain sufficient fungal mycelium for the MIC experiment. *Pythium* sp. and *Penicillium* sp. completely covered the Petri dish within 3 d while *Fusarium* sp. did in 13 d. Mycelium cores were extracted in a circular or random pattern around the center so that the mycelium is all of the same age. Pathogen culture and fungal growth measurements were conducted as previously described. The MIC experiments were replicated three times. Data were analyzed using PROC ANOVA in SAS and means were separated. Even though mean separations were conducted, the essential oils were chosen based solely on their ability to completely inhibit growth of all three pathogens over the time of measurement.

Phytotoxicity of the essential oils

The standard germination test was used to determine phytotoxicity of the oils. Seeds of inbred LH 332 and hybrid LH 334 X LH 391 were obtained from Holden's Foundation Seeds LLC (Williamsburg, Iowa). The essential oils were applied to the LH 332 seeds at four different rates. The rates were: 800 μL L^{-1} (MIC), 1 600 μL L^{-1} (MIC × 2), 8 000 μL L^{-1} (MIC × 10), and 16 000 μL L^{-1} (MIC × 20) mixed in a soybean oil carrier A soybean oil-treated seed was also included in the experiment to evaluate possible phytotoxicity by the soybean oil carrier, as well as an untreated control. Four replications of fifty seeds were planted in two rows on top of a 30cm x 60cm rolled towel standard germination test (Figure 2). The towels were rolled and placed upright in pails and covered with a clear plastic bag

(Figure 3). Towels within replications were randomized within the pails. The seeds were evaluated after seven days according to AOSA rules (AOSA, 2006).

Gas Chromatography – Mass Spectrometry

The eighteen essential oils were characterized using GC-MS. The Iowa State University Chemical Instrumentation Facility supplied the equipment and personnel to conduct the GC-MS analysis. The goals of the analysis were to identify the major compounds in the essential oils and their amounts. A Micromass GCT (Waters Corporation, Beverly MA) was used to conduct the analysis, in conjunction with an Agilent 7683 Autosampler and an Agilent 6890 gas chromatograph, as well as a Micromass time of flight mass spectrometer. A VF-5MS capillary column (length 30m, inside diameter 0.25mm, film thickness 0.25 microns, and 5% phenyl 95% methyl silicone) was used. The data were processed in a Compaq Deskpro PC with Windows XP. The software used was MassLynx 4.0 (Waters Corporation, Beverly MA). Chemical libraries were used to identify the compounds found by the GC-MS. The libraries used were the National Institute of Standards and Technology (NIST), 2002, as well as the Wiley Registry of Mass Spectral Data, 7th Edition (John Wiley and Sons, Inc.).

The data collected were analyzed based on the retention time. Knowing the relative retention time, the retention index can be calculated. The retention index is used to standardized results across laboratory technicians. This retention index is compared to a numerical Kovat's index used to classify chemical compounds. The peaks and the area under the peaks on the mass spectrometer graph were analyzed, the individual spectra were compared to known spectra, and the compounds were identified based of the Kovat's number

and the individual mass spectra. Mass spectra and Kovat's numbers were compared to know spectra in the literature such as those found in Adams (2004).

Results and Discussion

Evaluation of the antifungal properties of the oils in-vitro

Based on the information provided in the literature, eighteen plant essential oils were selected for these experiments to determine their potential antifungal activity against one soilborne pathogen, *Pythium* sp., and two seedborne pathogens, *Penicillium* sp. and *Fusarium* sp.

Fusarium sp. mycelium grew very slowly, but some mycelium growth was observed within the first 24 h of incubation. *Pythium* sp. and *Penicillium* sp., on the other hand, grew more aggressively and completely covered the Petri dish surface in a few days. However, the initial mycelium growth was observed after 48 to 72 h.

Five essential oils completely suppressed pathogen growth in all pathogens within the 168 h period of the experiments (Tables 1-3). These oils were cinnamon, clove, oregano, savory and thyme. Although other essential oils could significantly suppress growth of some of the pathogen, only five could completely suppress growth of all three pathogens. Fourteen oils were able to significantly suppress *Pythium sp*. mycelium growth, (Table 2), while nine oils were able to suppress *Penicillium* sp. growth (Table 3). Daferera *et al*., (2000) also found a significant reduction in the radial growth of *Penicillium digitatum* when oregano, thyme, dictamus, and marjoram essential oils were mixed into the PDA media at concentrations up to 250µg/ml.

The eighteen essential oils chosen for these experiments also had varying degrees of antifungal activity. Burt (2004) also reported that the essential oils have inherent amount of activity, and their composition depends on the methods of extraction and distillation, and the part of the plant from where the oils were extracted. Essential oils of consistent composition and antifungal activity are difficult to obtain because oil composition changes from plant to plant and year to year (Burt, 2004). This variation can be problematic when using the essential oils as seed treatments. However, some of the variation can be managed if the precise composition of the essential oils is known. GC-MS Gas Chromatography Mass Spectrometry analysis can be used as a tool to estimate and possibly remediate these differences in essential oils composition. Once the constituent compounds of the oils are known, their antifungal activity can be compared to other results obtained from oils of slightly different composition.

The essential oils capable of controlling all three pathogens during the entire length of this experiment were selected for assessing the minimum inhibitory concentration of the essential oils. These five oils were cinnamon, clove, oregano, savory and thyme.

Minimum Inhibitory Concentration

Minimum inhibitory concentration or MIC is the lowest concentration at which the essential oils control the pathogen (Mann and Markham, 1998). Tables 4-6 show the MIC for each pathogen. Oregano essential oil controlled *Pythium* sp. growth at a concentration of 100 $\mu L\ L^{-1}$ (Table 4), while thyme oil controlled the same pathogen at a concentration of 400 $\mu L\ L^{-1}$. *Fusarium* sp. growth was more difficult to control compared to the other two pathogens (Table 5). Oregano essential oils suppressed *Fusarium* sp. growth at a rate of 200

μL L^{-1}. Most of the other oils suppressed the pathogen at concentrations of 400 or 800 μL L^{-1}. Minimum inhibitory concentration for *Penicillium* sp. was usually between those of *Fusarium* sp. and *Pythium* sp. Table 7 shows the MIC for each of the essential oils and pathogen combinations. Values of MIC ranged between 100 and 800 μL L^{-1}. The highest MIC of 800 μL L^{-1} was selected for the purpose of standardization of future experiments. Other researchers also found that MIC varied greatly with the antifungal activity of the essential oil and its constituents. The minimum inhibitory concentrations were found using an agar diffusion method (Daferera *et al*, 2003; Hammer *et al* 1999). Daferera *et al*., (2003) showed that oregano, thyme, dictamus, and marjoram oils inhibited the growth of *Fusarium* sp. at concentrations of 150, 200, 250, and 300μg/ml, respectively. Fifty-two essential oils were screened to determine their MIC against 10 species of bacteria (Hammer *et al*, 1999). The authors found that lemongrass, oregano, and bay oils could inhibit all the bacteria species at a concentration of 2.0% (v/v) or greater.

There are many citations in the literature reporting the MIC of these essential oils for a large number of bacterial pathogens but, to our knowledge, this is the first time that MIC is reported for fungal pathogens specific to corn seed.

Phytotoxicity of the oils to corn seeds

Cinnamon, clove, oregano, savory, and thyme essential oils were applied to inbred corn seeds. These essential oils were applied at four concentrations: 800, 1600, 8000 and 16 000 μL L^{-1}. The maximum rate was arbitrarily set at 20 times the MIC. Seeds were germinated in the lab using the standard germination test. Only inbred seed was used in these experiments because they are less vigorous than hybrid seed and, consequently, are

more likely to show phytotoxicity symptoms. The mean germination percentage for the different essential oils and concentrations ranged from 89.0% to 97.5% (Table 8). Means were not significantly different ($P< 0.05$); therefore, the essential oils did not have phytotoxic effects.

Other researchers also reported lack of phytotoxicity effects in corn from several essential oils. Corn seeds treated with a 10% solution (10^5 μL L^{-1}) of cinnamon, thyme, clove, peppermint, or basil showed no reduction in germination after 6-8 d and after 20 d (Montes-Belmont and Carvajal, 1998).

Chemical analysis of the essential oils

The chemical composition of the essential oils was characterized using GC-MS. Table 9 shows the most important compounds of five essential oils with good antifungal activity; these essential oils are cinnamon, clove, oregano, savory and thyme. A compound was considered important when its concentration was in excess of 8% of the essential oil. Cinnamon oil had only one major compound which was trans-cinnamaldehyde, whereas clove oil had three major compounds that combined made up about 97% of the essential oil. The major compounds in clove were eugenol (60.6%), trans-caryophyllene (26.9%), and eugenol acetate (9.33%). Damien-Dorman *et al.* (2000) reported a similar composition for clove essential oil. The authors found very high concentration of eugenol (91.2%) in clove oil, with smaller concentrations of eugenyl acetate and beta caryophyllene 2.9 and 4.1%, respectively. In our case, the concentration of eugenol was lower than previously reported in the literature, but the concentration of the other compounds was higher.

The chemical composition of oregano oil also had three important compounds para-cymene (13.6%), gamma-terpinene (11.4%), and carvacrol (57.3%). Other researchers also reported these compounds were the most abundant in oregano essential oil (Daferera *et al.*, 2000; Sivropoulou *et al.*, 1996). However, these authors reported high levels of thymol (63.3% and 31.8% respectively) which was not present or was in a very small proportion in the oregano essential oils used in these experiments. The predominant compounds present in savory were the same three present in oregano, but in different proportion. The major compounds and amounts in savory essential oil were: para-cymene (12.4%), gamma-terpinene (37.6%), and carvacrol (33.0%).

The three important compounds in thyme essential oil were para-cymene (8.94%), linalool (41.4%), and lavandulol (10.2%). Cosentino *et al.* showed that the antifungal activity of *thymus* essential oil was due to the effect of phenolic compounds, such as carvacrol and thymol present in the oil.

Most major compounds in the essential oils had some chemical similarities. For example, most compounds had either 10 or 15 carbons and consisted of benzene rings with different functional groups (Table 9). These similarities seem to indicate some relationship between these compounds and the antifungal properties of the essential oils and possibly a compound in low concentration. Future research into essential oils seed treatments should involve finding a reliable delivery system for the oils, as well as determining the proper application rates for the essential oil.

Conclusions

Five essential oils were effective at controlling three common corn pathogens *in vitro*. These essential oils were cinnamon, clove, oregano, savory, and thyme. Concentrations as low as 100 μL L^{-1} of oregano essential oil were capable of inhibiting *Pythium* sp. growth in agar media, while 400 μL L^{-1} of thyme were needed to obtain the same results. *Fusarium* sp. growth was more difficult to inhibit compared to the other pathogens and 800 μL L^{-1} of all essential oils were capable of inhibiting growth of all pathogens.

These essential oils applied to the seed were innocuous to the corn seedlings even at concentrations as high as 16 000 μL L^{-1}. The chemical composition of the essential oils was determined using GC-MS. The essential oils showed some similarities in chemical composition having, in general, high levels of phenolic compounds with 10 to 15 carbons and consisting of benzene rings. Future research should include synthesizing and testing the constituent compounds individually to assess their antifungal properties. Chemically synthesizing these compounds would limit their use in organic farming system, but could provide new opportunities for the seed treatment industry. The effectiveness of these constituent compounds in pure form should also be compared to that of the essential oils. It is possible that the chemical compounds of the essential oils are only effective when working together in a synergistic manner.

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Table 1. Mean *Fusarium* mycelium radial growth in agar media with a concentration of 1000 μL L^{-1} of eighteen essential oils.

	Mycelium radial growth over time						
Essential oil	24 h	48 h	72 h	96 h	120 h	144 h	168 h
	---------- mm ----------						
Control	8.9 ab	29.4 a	31.8 a	43.1 a	47.6 a	72.3 a	80.9 a
Anise	9.7 a	10.5 b	13.3 b	14.3 bc	14.9 bcd	16.3 d	18.0 de
Pepper	7.9 abc	11.7 b	15.6 b	20.4 b	28.1 b	49.1 b	61.7 b
Basil	6.0 bcd	7.4 b	7.9 b	8.5bc	9.3cd	11.3 de	16.0 de
Lemongrass	5.8 bcd	6.4 b	7.8 b	10.2 bc	10.4 cd	10.7 de	10.9 de
Lavender	5.7 bcd	6.3 b	7.1 b	7.7 c	8.0 cd	8.9 de	23.9 cd
Coriander	5.4 cd	8.4 b	9.6 b	11.2 bc	13.5 bcd	18.3 cd	27.5 cd
Peppermint	5.3 cd	7.3 b	8.0 b	8.9 bc	9.8 cd	10.2 de	10.8 de
Bay	5.3 cd	6.5 b	7.2 b	8.0 c	8.9 cd	9.7 de	10.7 de
Nutmeg	5.2 cd	7.1 b	9.1 b	11.5 bc	13.8 bcd	16.9 d	20.8 de
Sage	4.8 cd	7.2 b	8.2 b	9.1 bc	10.0 cd	12.5 de	18.1 de
Rosemary	4.7 cd	7.0 b	8.8 b	14.2 bc	18.5 bc	29.4 bc	41.3 bc
Caraway	4.3 d[†]	11.3 b	11.7 b	14.1 bc	14.1 bcd	14.2 de	14.1 de
tea tree	4.3 d	4.3 b	7.1 b	7.2 c	7.9 cd	7.7 de	8.0 de
Cinnamon	4.3 d	4.3 b	4.3 b	4.3 c	4.3 d	4.3 e	4.3 e
Clove	4.3 d	4.3 b	4.3 b	4.3 c	4.3 d	4.3 e	4.3 e
Oregano	4.3 d	4.3 b	4.3 b	4.3 c	4.3 d	4.3 e	4.3 e
Savory	4.3 d	4.3 b	4.3 b	4.3 c	4.3 d	4.3 e	4.3 e
Thyme	4.3 d	4.3 b	4.3 b	4.3 c	4.3 d	4.3 e	4.3 e

Means within a column followed by a different letter are significantly different ($P<0.05$)
[†] Minimum growth is considered 4.3 mm because of the size of the pathogen core

Table 2. Mean *Pythium* mycelium radial growth in agar media with a concentration of 1000 μL L^{-1} of eighteen essential oils.

	Mycelium radial growth over time						
Essential Oil	24 h	48 h	72 h	96 h	120 h	144 h	168 h
	---------- mm ----------						
Control	51.6 a	79.7 a	83.1 a‡	83.1 a	83.1 a	83.1 a	83.1 a
Pepper	16.2 b	40.6 b	56.9 b	63.4 b	63.4 b	63.4 b	63.4 a
Coriander	4.3 c†	4.3 c	16.1 c	22.7 c	24.0 c	24.0 c	24.0 bc
Nutmeg	4.3 c	4.3 c	4.3 c	10.5 cd	12.7 cd	21.5 cd	29.2 b
Rosemary	4.3 c	4.3 c	4.3 c	5.2 cd	6.1 cd	6.9 cd	8.4 bc
Anise	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Basil	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Bay	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Caraway	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Lavender	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Lemongrass	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
tea tree	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Peppermint	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Sage	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Cinnamon	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Clove	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Oregano	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Savory	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c
Thyme	4.3 c	4.3 c	4.3 c	4.3 d	4.3 d	4.3 d	4.3 c

Means within a column followed by a different letter are significantly different ($P<0.05$)

† Minimum growth is considered 4.3mm because of the size of the pathogen core

‡ Maximum growth is considered 83.1 mm because of the size of the plate

Table 3. Mean *Penicillium* mycelium radial growth in agar media with a concentration of 1000 μL L^{-1} of eighteen essential oils.

	Mycelium radial growth over time						
Essential Oil	24 h	48 h	72 h	96 h	120 h	144 h	168 h
	---------- mm ----------						
Control	8.1 a	14.1 a	33.9 a	39.0 a	41.6 a	44.6 a	46.6 ab
Pepper	5.3 b	12.0 b	28.8 a	34.0 ab	38.1 ab	40.6 a	43.0 abc
Rosemary	5.0 bc	9.6 c	14.6 b	22.7 bc	35.9 abc	38.9 a	42.2 abc
Sage	4.3 c[†]	5.6 d	12.1 b	22.8 bc	36.5 abc	39.3 a	56.1 a
Nutmeg	4.3 c	4.7 d	10.0 b	19.2 bcd	26.0 abc	44.6 a	49.7 ab
Coriander	4.3 c	4.3 d	4.3 b	17.0 cd	21.3 bcd	31.2 ab	36.9 bcde
Lavender	4.3 c	4.3 d	4.3 b	10.8 cd	15.5 cd	25.7 ab	30.3 bcd
tea tree	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	10.6 b	18.0 cde
Basil	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	7.2 b	7.6 de
Anise	4.3 c	4.3 d	4.3 b	4.8 d	4.3 d	5.4 b	5.5 de
Bay	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Caraway	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Lemongrass	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Peppermint	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Cinnamon	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Clove	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Oregano	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Savory	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e
Thyme	4.3 c	4.3 d	4.3 b	4.3 d	4.3 d	4.3 b	4.3 e

Means within a column followed by a different letter are significantly different ($P<0.05$)
[†] Minimum growth is considered 4.3 mm because of the size of the pathogen core

Table 4. *Pythium* radial growth in agar media with four concentrations of essential oil..

Essential Oil Concentration	Mycelium radial growth over time						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
	---------- mm ----------						
100 µL L^{-1}							
control§	46.2 a	83.1 a‡	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a
Cinnamon	4.3 b†	13.9 b	24.0 b	24.0 b	24.0 b	24.0 b	24.0 b
Clove	4.3 b	4.3 b	24.0 b	35.2 b	43.7 ab	43.7 ab	43.7 ab
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	13.3 b	26.6 b	31.5 b	39.0 ab	41.0 ab
Thyme	7.2 b	16.0 b	31.9 b	35.4 b	38.4 ab	40.7 ab	41.1 ab
200 µL L^{-1}							
Control	46.2 a	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Thyme	4.3 b	7.2 b	11.0 b	15.1 b	18.3 b	20.2 b	20.0 b
400 µL L^{-1}							
Control	46.2 a	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Thyme	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
800 µL L^{-1}							
Control	46.2 a	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a	83.1 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Thyme	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b

Means within a column followed by a different letter are significantly different ($P<0.05$)
† Minimum growth in mm given by the size of the pathogen core
‡Maximum growth in mm given by the size of the Petri dish
§The mean for control samples is repeated for each concentration of the essential oil to facilitate comparisons among means

Table 5. *Fusarium* radial growth in agar media with four concentrations of essential oil.

Essential oil Concentration	Mycelium radial growth over time						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
	---mm---						
100 µL L^{-1}							
control§	7.6 a	17.6 a	37.9 a	59.4 a	78.3 a	82.2 a	83.1 a‡
Cinnamon	8.2 a	9.5 a	10.1 b	13.3 b	18.6 b	24.7 b	31.5 b
Clove	9.3 a	13.5 a	14.7 b	17.7 b	21.8 b	25.9 b	30.2 b
Oregano	7.6 a	8.8 a	11.6 b	17.4 b	15.7 b	16.5 b	16.7 b
savory	6.1 a	7.8 a	8.1 b	8.7 b	9.3 b	11.1 b	14.0 b
Thyme	5.5 a	7.8 a	10.9 b	16.8 b	24.8 b	32.2 b	42.5 b
200 µL L^{-1}							
Control	7.6 a	17.6 a	37.9 a	59.4 a	78.3 a	82.2 a	83.1 a
Cinnamon	4.7 ab	8.6 ab	8.7 b	8.7 bc	8.7 bc	11.7 b	13.9 bc
Clove	5.4 ab	15.2 ab	16.5 b	20.5 b	20.3 b	19.9 b	20.3 bc
Oregano	4.3 b†	4.3 b	4.3 b	4.3 c	4.3 c	4.3 b	4.3 c
savory	5.3 ab	5.7 ab	5.7 b	5.7 c	5.6 bc	6.2 b	5.7 bc
Thyme	7.5 ab	8.1 ab	8.5 b	10.2 bc	13.7 bc	18.9 b	25.5 b
400 µL L^{-1}							
Control	7.6 a	17.6 a	37.9 a	59.4 a	78.3 a	82.2 a	83.1 a
Cinnamon	4.3 b	4.3 b	6.6 b	7.3 b	7.2 bc	8.0 bc	8.1 bc
Clove	4.3 b	6.8 b	7.7 b	7.8 b	7.9 bc	7.9 bc	7.9 bc
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 c	4.3 c	4.3 c
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 c	4.3 c	4.3 c
Thyme	5.0 b	7.1 b	7.6 b	10.5 b	12.2 b	14.6 b	18.3 b
800 µL L^{-1}							
Control	7.6 a	17.6 a	37.9 a	59.4 a	78.3 a	82.2 a	83.1 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Thyme	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b

Means within a column and essential oil concentration followed by a different letter are significantly different ($P<0.05$)

† Minimum growth in mm given by the size of the pathogen core

‡ Maximum growth in mm given by the size of the Petri dish

§The mean for control samples is repeated for each concentration of the essential oil to facilitate comparisons among means

Table 6. *Penicillium* radial growth in agar media with four concentrations of essential oil.

Essential oil Concentration	Mycelium radial growth over time						
	24 h	48 h	72 h	96 h	120 h	144 h	168 h
	----------mm----------						
100 µL L^{-1}							
control‡	6.0 a	22.3 a	39.9 a	49.9 a	61.9 a	65.4 a	67.9 a
Cinnamon	4.3 b†	6.8 b	9.4 b	11.0 b	15.4 b	16.6 b	25.1 ab
Clove	4.3 b	12.3 ab	21.9 ab	24.7 ab	26.5 ab	27.4 b	35.5 ab
Oregano	4.3 b	4.3 b	6.0 b	7.5 b	8.5 b	9.4 b	10.5 b
savory	4.3 b	9.5 ab	19.6 ab	22.6 ab	28.1 ab	28.4 ab	29.7 ab
Thyme	4.3 b	12.5 ab	20.9 ab	24.4 ab	30.3 ab	33.0 ab	37.7 ab
200 µL L^{-1}							
Control	6.0 a	22.3 a	39.9 a	49.9 a	61.9 a	65.4 a	67.9 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 c	4.3 c	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 c	4.3 c	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 c	4.3 c	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 c	4.3 c	4.3 b
Thyme	4.3 b	12.8 ab	24.2 ab	27.2 ab	32.2 b	33.3 b	34.4 c
400 µL L^{-1}							
Control	6.0 a	22.3 a	39.9 a	49.9 a	61.9 a	65.4 a	67.9 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Thyme	4.3 b	7.1 b	11.1 b	19.9 b	22.2 b	24.0 b	24.0 b
800 µL L^{-1}							
Control	6.0 a	22.3 a	39.9 a	49.9 a	61.9 a	65.4 a	67.9 a
Cinnamon	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Clove	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Oregano	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
savory	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b
Thyme	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b	4.3 b

Means within a column and essential oils concentration followed by a different letter are significantly different ($P<0.05$)

† Minimum growth in mm given by the size of the pathogen core

§The mean for control samples is repeated for each concentration of the essential oil to facilitate comparisons among means

Table 7. Minimum amount ($\mu L\ L^{-1}$) of essential oils of cinnamon, clove, oregano, savory and thyme necessary to inhibit mycelium growth in *Pythium* sp., *Penicillium* sp. and *Fusarium* sp. over 168h.

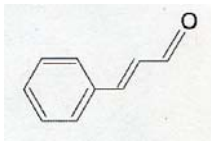
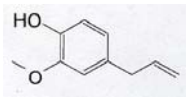
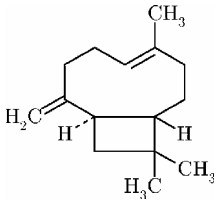
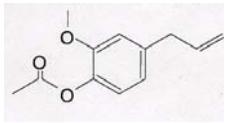
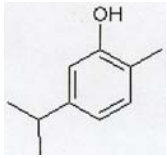
	Essential Oils				
Pathogen	Cinnamon	Clove	Oregano	Savory	Thyme
	---------- $\mu L\ L^{-1}$ ----------				
Pythium	200	200	100	200	400
Penicillium	200	200	200	200	800
Fusarium	800	800	200	400	800

Table 8. Mean germination percentage of corn seeds treated with five essential oils at four concentrations (800, 1 600, 800, and 16 000 μL L^{-1}) for phytotoxicity experiment. Means include untreated control and soybean oil used as a carrier in these experiments.

Treatment / Concentration (μL L^{-1})	Percent Germination*
Untreated Control	97.0
Soybean Oil	96.0
Cinnamon / 800	93.0
Cinnamon / 1 600	97.5
Cinnamon / 8 000	95.0
Cinnamon / 16 000	92.5
Clove / 800	89.0
Clove / 1 600	94.5
Clove / 8 000	96.5
Clove / 16 000	96.5
Oregano / 800	96.0
Oregano / 1 600	94.5
Oregano / 8 000	95.5
Oregano/ 16 000	93.5
Savory / 800	96.0
Savory / 1600	94.0
Savory / 8 000	96.5
Savory / 16 000	95.0
Thyme / 800	96.5
Thyme / 1 600	97.0
Thyme / 8 000	96.0
Thyme / 16 000	96.0

*Means were not significantly different ($P < 0.05$)

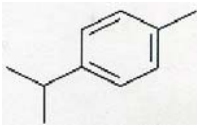
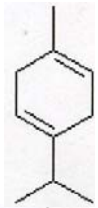
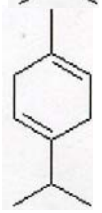
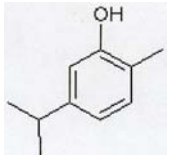
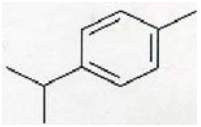
Table 9. Gas Chromatography-Mass Spectrometry of five selected oils with main constituents

Oil	Component Name	Molecular structure	Chemical Formula	MW[a]	Chemical Name	% area[b]
Cinnamon (*Cinnamonum zeylanicum)*	(E) cinnamaldehyde		C_9H_8O	132.16192	(E)-3-phenyl-2-propenal	74.0
Clove (*Eugenia caryophyllata*)	Eugenol		$C_{10}H_{12}O_2$	164.20408	2-Methoxy-4-(2-propenyl)phenol	60.6
	(E) caryophyllene		$C_{15}H_{24}$	204.35556	4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene	26.9
	Eugenol acetate		$C_{12}H_{14}O_3$	286.92936	2-Methoxy-4-(2-propen-1-yl)-phenyl acetate	9.33
Oregano (*Origanum minutiflorum*)	Carvacrol		$C_{10}H_{14}O$	150.22056	2-methyl-5-(1-methylethyl)phenol	57.3

[a] molecular weight

[b] area under the peak expressed as a percentage of total areas of all peaks

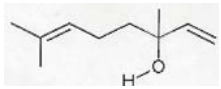
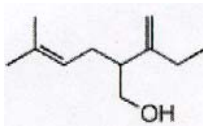
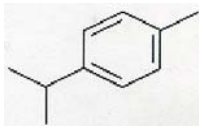
Table 9 (Cont.)

Oil	Component Name	Molecular structure	Chemical Formula	MW[a]	Chemical Name	% area[b]
Savory (*Satureia montana*)	para-cymene		$C_{10}H_{14}$	134.22116	1-methyl-4-(1-methylethyl)benzene	13.6
	γ-terpinene		$C_{10}H_{16}$	136.23704	1-Isopropyl-4-methyl-1,4-cyclohexadiene	11.4
	γ-terpinene		$C_{10}H_{16}$	136.23704	1-Isopropyl-4-methyl-1,4-cyclohexadiene	37.6
	Carvacrol		$C_{10}H_{14}O$	150.22056	2-methyl-5-(1-methylethyl)phenol	33.0
	para-cymene		$C_{10}H_{14}$	134.22116	1-methyl-4-(1-methylethyl)benzene	12.4

[a] molecular weight

[b] area under the peak expressed as a percentage of total areas of all peaks

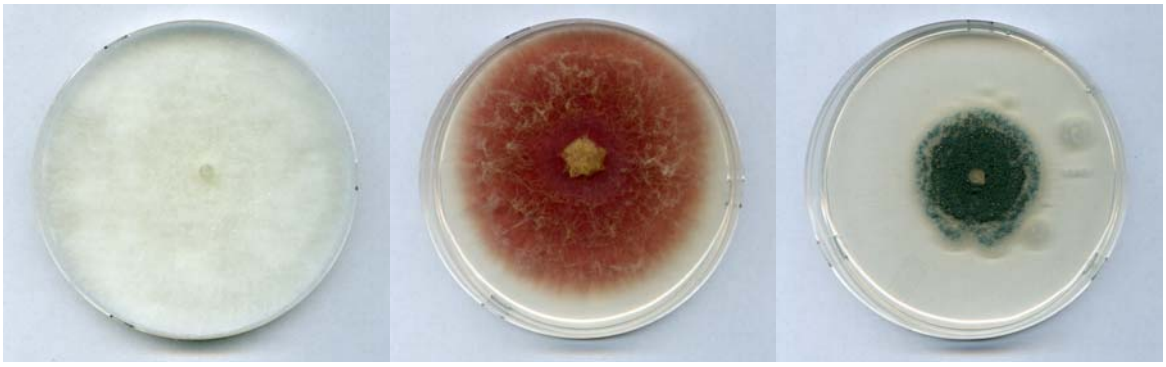
Table 9 (Cont.)

Oil	Component Name	Molecular structure	Chemical Formula	MW[a]	Chemical Name	% area[b]
Thyme (*Thymus vulgaris*)	Linalool		$C_{10}H_{18}O$	154.25232	3,7-Dimethyl-1,6-octadien-3-ol	41.4
	lavandulol		$C_{10}H_{18}O$	154.25232	2-Isopropenyl-5-methyl-4-hexen-1-ol	10.2
	para-cymene		$C_{10}H_{14}$	134.22116	1-methyl-4-(1-methylethyl)benzene	8.94

[a] molecular weight

[b] area under the peak expressed as a percentage of total areas of all peaks

Figure 1. Pythium, Fusarium, and Penicillium mycelium and spores growing on potato dextrose agar media inside Petri dishes.



Pythium Fusarium Penicillium

Initial pathogens cores were 4.3mm in diameter. The pathogens were allowed to grow until the surface of the Petri dishes were completely covered (83.1mm in diameter).

Figure 2. Standard germination test where fifty corn seeds were planted in two rows on a wet 30cm x 60cm rolled towel. Towels were incubated at 25°C for seven days with 12 hours of light.

Figure 3. Rolled paper towels standard germination tests placed upright in a pail. Towels were randomized within pails.

Chapter 3. In vivo testing of plant essential oils for use as contact fungicide seed treatments

Introduction

Seed treatments are widely used in the U.S. seed industry to control fungal pathogens in germinating corn seedlings. These seed treatments enable corn seedlings to withstand pathogen pressure until either the plant is large enough to withstand the pathogen or the environmental conditions around the seed are no longer conducive to pathogen growth. Fungicide seed treatments protect the germinating seedlings from the detrimental effects of seed and soil fungi, especially in the cold (less than 10°C) and wet (excess moisture) soils of the Midwest. Until recently, Captan was the most widely used seed treatment in corn seed, but in recent years, treatments such as Maxim XL, active ingredient (a.i.) Metalaxyl (Syngenta Crop Protection, Greensboro, NC), Allegiance, a.i. Metalaxyl (Bayer CropScience, Research Triangle Park, NC), Dynasty, a.i. Azoxystrobin (Syngenta Crop Protection, Greensboro, NC), and Trilex, a.i. Trifloxystrobin (Bayer CropScience, Research Triangle Park, NC) have gained popularity.

Conventional grain producers, those who use non-organic practices, have a wide range of seed treatments options to protect their seeds when planting under stressful field conditions. For a certified organic producer, seed treatment options are limited, as synthetic chemicals are excluded from an organic farming system. A few options available to producers are, planting later in the spring when soil conditions are more favorable or using an organically approved seed-applied fungicide. Planting later, however, reduces the length of the growing season as well as the final grain yield (Kaspar et al., 1987; Swan et al., 1987).

Alternatively, organic farmers can use an organically approved seed treatment, but there are few fungicide seed treatments currently approved for organically grown corn. Some of these organic treatments are Yield Shield (Bayer CropScience, Research Triangle Park, NC) and Natural 2 (AgriCoat LLC, Soledad, CA) which protect the seed based on beneficial microbes and their antagonistic interaction with pathogens.

Plant essential oils are effectively used in the food industry as antimicrobials. They control pathogen growth and prevent food spoilage. Plant essential oils also were used against a number of seed pathogens for their antifungal activity (Montes-Belmont and Carvajal, 1998; Pawar and Thaker, 2006; Daferera et al., 2000). However, very little is known about the use of essential oils as contact seed treatment for corn. Some promising research has shown that plant essential oils are effective against seed borne and soil borne pathogens *in vitro* (Chatterjee, 1990; Velluti et al., 2004), but little research has been done to determine their effectiveness *in vivo*. The effectiveness of a seed treatment *in vivo* can be affected by the environment in which corn seeds are planted, as well as the amount of pathogen inoculum that the seed carries. This environmental pressure can overwhelm the seed treatment and reduce it effectiveness. It is important that the seed treatment provides good initial pathogen control in the field to allow the germinating seedling to emerge.

Use of essential oils to control seed pathogen growth *in vitro* has been well established (Velluti et al., 2004; Montes-Belmont and Carvajal, 1998; Pawar and Thaker, 2006; Daferera et al., 2000). *Fusarium verticillioides*, *Fusarium proliferatum*, and *Fusarium graminearum* growth was reduced *in vitro* by lemongrass, cinnamon, clove, palmarosa, and oregano oils (Velluti et al., 2004). Montes-Belmont and Carvajal (1998) found that when essential oils were applied to corn seeds and seeds were inoculated with an *Aspergillus flavus*

spore solution, fungal growth was suppressed. The seeds were immersed in pure essential oil and sprayed with a spore solution. The oils of clove, cinnamon, oregano, epazote, thyme, basil, and peppermint reduced the contamination in 5-d seedlings by 100%. *Cinnamomum zeylanicum* (bark), *Cinnamomum zeylanicum* (leaf), *Cinnamomum cassia*, *Syzygium aromaticum*, and *Cymbopogon citratus* controlled *Aspergillus niger* in the laboratory (Pawar and Thaker, 2006). Seventy-five oils were initially screened for antifungal activity against *Aspergillus niger*. Five essential oils were selected from the preliminary screening for their antifungal activity. The pathogen *Penicillium digitatum* was controlled by oregano, thyme, dictamus, and marjoram oils at a concentration between 250 and 400 µg/ml (Daferera et al., 2000).

The objectives of this study were to evaluate the antifungal efficacy in the lab and the field of five essential oils (cinnamon, clove, oregano, savory, and thyme). The goal was to assess the ability of the essential oils to be used as seed treatments in corn seeds.

Material and Methods

Laboratory performance of seeds treated with essential oils

Pathogen inoculation of healthy seeds

Untreated seeds were plated on potato dextrose agar (PDA) growth media to assess initial seedborne pathogen infection. Seeds from the inbred LH 332 and the hybrid LH 334 X LH 391 were sterilized using a 1% Clorox solution (The Clorox Company, Oakland, CA). Seeds were submersed in the solution for 1 min rinsed in deionized water for 1 min, and air dried at room temperature (approx. 23 °C) on top of paper towels.

The disinfected corn seeds were artificially inoculated with spores of *Fusarium* sp. and *Penicillium* sp. harvested from Petri dished with PDA media inoculated with the pathogens. *Penicillium* sp. spores were produced after 4 d. and *Fusarium* sp. macroconidia were produced after 14 d of incubation time. The spores were collected by rinsing the fungal colonies with sterile water. Spore and macroconidia were counted using a hemocytometer (American Optical, Buffalo NY). The *Penicillium* sp. spore solution was corrected to a concentration of 10^6 spores per ml, and the *Fusarium* sp. macroconidia solution to 10^4 macroconidia per ml. Seeds were submersed in the spore solution for five minutes and air dried at room temperature (approx. 23 °C) on top of paper towels.

Seed treatment application

Infected seeds were divided into 100-seed samples and placed in re-sealable plastic bags. The essential oil treatments were added to the seeds inside the bag and each bag was shaken for 60 s. The oils were applied at two rates, the previously determined minimum inhibitory concentration rate (MIC) of 800µL L^{-1} and a rate of 16 000µL L^{-1} (20 × MIC). The five essential oils used in these experiments were cinnamon, clove, oregano, savory, and thyme, the same oils previously determined to have good antifungal activity. Also, the commercial seed treatments Maxim XL, Natural 2, and Yield shield were applied at the label-recommended rates and used as controls. Seeds from four different seed treatments were planted in each tray, trays were only large enough to hold four treatments, and treatments were replicated four times in an incomplete block design so that all treatments could occur with every other treatment and that all treatments could be compared. Samples were randomized within a tray, and trays within a cart.

Cold test

The tray method cold test was used in these experiments (AOSA, 2002). Seeds were placed on one sheet of Kimpak™ with 1000 ml of chilled (10°C) water. Four different treatments were randomly planted in each tray. The trays were covered with a mixture of 1 soil: 4 sand. The soil was artificially inoculated with *Pythium* sp. to increase the seed's exposure to this soilborne pathogen.

Pythium sp. inoculum was grown on PDA media until the entire plate was completely covered with mycelia. The Pythium-infected media was cut into 1 cm^2 pieces. The pieces of PDA and mycelia were mixed with the sand-soil mixture and used as a substrate for the cold test. To avoid cross contamination with other seedborne pathogens, seeds used in this experiment were surface sterilized prior to applying the essential oils seed treatment and planting in the cold test.

The cold tests were placed in a constant 10°C growth chamber for seven days in the dark and moved to constant 25°C growth chamber with continuous light for seven days. The experiments were replicated four times, replications were planted over 7d. Both cold test germination percentages and pathogen infection data were collected at the end of the test period.

Field performance of seeds treated with essential oils

Field trials

To evaluate the effectiveness of the essential oils as seed treatments in corn, field emergence trials were conducted at six sites in Iowa; three sites were under organic farming systems and three were under conventional farming systems. For this experiment, organic

farming systems sites are defined as farms under organic cultural practices of soil fertility and weed control management, but not necessarily certified as organic farms. The organic sites were Ames, Cresco, and Crawfordsville. The Ames and Cresco organic sites were certified organic while the Crawfordsville location is under organic practices but not certified. Conventional sites were Ames, Nashua, and Crawfordsville. At the Ames and Crawfordsville sites, the organic and conventional fields were less than 1 km apart. Sites were chosen so that the organic and conventional sites were in close proximity. The distance between the conventional field at Nashua and the corresponding organic field at Cresco was about 80 km.

Seed treatment application

Cinnamon, clove, oregano, savory, and thyme essential oils were selected for their efficacy *in vitro* and their lack of phytotoxicity, and used as seed treatments for the *in vivo* experiments. Seeds from inbred LH 332 and hybrid LH 334 X LH 391 were treated with the essential oils using a laboratory batch treater. The inbred and hybrid genotypes were representative for genotypes that would be used in organic and conventional farming systems. Seeds samples from each site were weighed and treated.

The five essential oils were applied at two rates, the MIC (800 μL L^{-1}) and 20×MIC (16 000 μL L^{-1}) using a micropipette. Soybean oil was used as a carrier of the essential oils. The controls in the organic sites were untreated seed and seed treated with the soybean oil used as a carrier. Two commercially available organic fungicides, Natural 2 and Yield Shield, were also used as controls at all the sites. Seed treated with a synthetic seed treatment, Maxim XL, at the label rate 0.016 mg/seed was planted as an additional control in the conventional sites. This synthetic fungicide is not labeled for organic use and was not

applied to seeds planted in the organic sites. All of these treatments were replicated in the field three times.

Field plot design

The field plot design was a split plot in which the inbred and hybrid were randomized within a block. Plots were planted at a plant population rate of 68,600 plants / hectare. Seeds were planted in rows spaced 76 cm in the conventional sites and 96 cm in the organic sites. Each plot consisted of two 5.5m-long rows. Plots were planted during the third week of April through the third week of May (Table 10). The first plantings were at the Crawfordsville farm in southeastern Iowa and the last at the Natvig farm by Cresco in northeast Iowa.

The conventional sites were in a corn-soybean rotation and used conventional cultural practices. Synthetic fertilizers, such as anhydrous ammonia and urea, and pre- or post-emergence herbicide were applied in the fall or spring at all conventional sites.

Soil fertility at the organic sites was managed with non-synthetic fertilizers, such as manure or a cover crop (oats) plowed under the soil. Weed control was achieved primarily by rotary hoeing and cultivation to reduce weed pressure.

Soils at the Ames conventional and organic sites were classified as a Clarion loam soil; soils were a Kalona silty clay loam at the Crawfordsville sites; a Floyd loam soil at the Nashua conventional site; and three soil types: a Clyde silty clay loam, Floyd loam, and Oran loam at the Cresco organic site.

Soil moisture was adequate for germination at planting for all sites. All sites were planted using a John Deere Max Emerge Planter with ALMACO cone units.

Field emergence data were collected at growth stage V2 and growth stage V5, 7 and 21 days after planting respectively. Total emerged seedlings in each row were counted and divided by the total number of seeds planted in each row to obtain the emergence percentage.

Statistical Analysis

Data from the laboratory seed infection experiment were analyzed as an incomplete block design. This design was chosen for the laboratory experiments because only four treatments could be planted on each tray. Repeated treatments in each block allowed the independent comparison of each treatment. The whole experiment was replicated four times and the data was analyzed using Proc Mixed (Appendix 12).

Data from the organic and conventional sites were analyzed as a randomized complete block design with three replications (Appendix 10,11). Since the synthetic commercial seed treatment (Maxim XL) could not be used in the organic sites, data were analyzed first excluding seed samples treated with Maxim XL at every site. Data from the conventional sites were reanalyzed to include the Maxim XL-treated seed samples. Data were analyzed using Proc Mixed in SAS (SAS Institute, Cary, NC). Emergence means separation was conducted using the Tukey means separation method.

Results and Discussion

Evaluation of antifungal properties of the oils in the lab

Our goal was to evaluate the efficacy of the essential oils seed treatment at protecting corn seed from soilborne and seedborne pathogens in the lab and the field. When all laboratory data were analyzed together, the main effects, pathogen, genotype, and treatment, as well as the interactions among these factors were significant ($P < 0.05$, Appendix 12). The

varieties used in these experiments were selected for their contrasting seed vigor. The higher seed vigor of the hybrid is partially due to heterozygocity and hybrid vigor, while the nearly homozygous inbred has low seed vigor.

Table 11 shows the mean cold test germination percentage for inbred and hybrid corn seed treated with a commercial or essential oil seed treatment. The germination percentage of inbred seeds inoculated with *Fusarium* sp. and treated with thyme 800 μL L^{-1} was not significantly different ($P < 0.05$) from the commercial seed treatments Natural 2, Yield Shield, and the untreated control. The cold test germination of inbred seed treated with Natural 2 was consistently higher than most other treatments for seeds inoculated with *Fusarium* sp. and *Penicillium* sp., and significantly higher than all other treatments for seeds tests in soil inoculated with *Pythium* sp. The lowest germination percentage for Fusarium-inoculated inbred seed was 78.2%, and was recorded for seed treated with clove at a rate of 800 μL L^{-1}. There were no significant cold test germination differences between seeds treated with the essential oils at a concentration of 800 μL L^{-1} and 16 000 μL L^{-1}.

The germination percentage of Penicillium-inoculated inbred seeds treated with Maxim XL and Natural 2 was significantly higher than seeds treated with the essential oils, untreated and soybean oil controls, and seeds treated with the commercially available organic seed treatment, Yield Shield (Table 11). The inbred seeds treated with the essential oils had very low germination percentages. All of the essential oils treatments except for two were not significantly different from the untreated control. Both savory 800 μL L^{-1} and thyme 16 000 μL L^{-1} were significantly lower than the untreated control possibly indicating a negative effect from these oils. The lowest germination percentage was recorded for seeds treated with savory 800 μL L^{-1} (29.9%), which was not significantly different from the other

essential oils. There were no significant differences in the cold test germination percentage of seeds treated with a rate of 800 or 16 000 μL L^{-1}, similarly to results obtained for Fusarium-inoculated seed. The cold test germination percentage of seeds treated with Yield Shield was half of the other two commercial treatments.

Maxim XL and Natural 2 provided the highest level of protection when treated inbred seeds were planted in Pythium-inoculated soil. Inbred seeds treated with Yield Shield germinated significantly lower ($P < 0.05$) than Maxim XL and Natural 2, but significantly higher than the untreated control. Four essential oil treatments were not significantly different than Yield Shield. These oils were thyme 800 μL L^{-1}, thyme 16 000 μL L^{-1}, clove 800 μL L^{-1}, and oregano 800 μL L^{-1}. The lowest mean germination percentage again was recorded for seeds treated with savory 16 000 μL L^{-1}.

The stressful test conditions of the cold test reduced the germination percentage of the inbred seed. In these experiments, the untreated control ranged from 90.4% for *Fusarium* sp. to 32.3% for *Pythium* sp. When commercial fungicides were applied to the seed, germination percentages improved to 86.1 % and 92.2 % for *Fusarium* sp. and *Penicillium* sp.

The hybrid seed was vigorous and germinated better than the inbred seed under the stressful conditions of the cold test. The cold test germination percentage of Penicillium- and Pythium-inoculated, untreated controls were not significantly different from the seed treated with Natural 2 and Yield Shield. Hybrid seeds had higher cold test germination percentages than the inbred, probably due to their higher seed vigor. This higher seed vigor of the hybrid also affected seeds inoculated with *Fusarium* sp. All seed treatments used in this experiment effectively controlled *Fusarium* sp. on hybrids seed (Table 11). The mean cold test

germination ranged from 90.2% for seeds treated with soybean oil to 97.5% for Yield Shield treatment.

Maxim XL, Natural 2, and soybean oil effectively controlled *Penicillium* sp. in hybrid seed (P <0.05) (Table 11). In addition two essential oils, clove 16 000 μL L^{-1}, and cinnamon 800 μL L^{-1}, were not significantly different from these treatments. The lowest mean cold test germination percentage was recorded for hybrid seeds treated with the essential oil thyme 16 000 μL L^{-1}. For all other essential oil treatments, there were no significant differences between the high (16 000 μL L^{-1}) and low (800 μL L^{-1}) rates.

Maxim XL and Natural 2 were also the best treatments for protecting the seed against *Pythium* sp. Oregano 800 μL L^{-1}, Yield Shield, and thyme 800 μL L^{-1} were not significantly different from Natural 2. All remaining essential oil treatments were not significantly different (P < 0.05) from the untreated control. The lowest mean cold test percentage was obtained in seeds treated with thyme 16 000 μL L^{-1}.

There were no significant differences between the two rates of the essential oils (800 μL L^{-1} and 16 000 μL L^{-1}). The failure of the essential oils to control these pathogens, in the cold test, seems to indicate that the effective rates of the essential oils *in vitro* differ from those *in vivo*.

Plant essential oils have been used in previous studies to control seed corn storage pathogens *in vivo* (McGee and Misra, 1988). Healthy corn seeds were infected with a spore solution of *Aspergillus* sp. and treated with different essential oils (Montes-Belmont and Carvajal, 1998). The essential oils were applied at rates from 1 to 10% of the essential oils to determine the optimal rate of treatment, and their individual constituent compounds of the oils were also used as treatments. Kernel infection rate was greatly reduced by the

compounds linalool, eugenol, and the oils of cinnamon, peppermint, clove, thyme, and basil. The authors concluded that oils and their individual constituent compounds can be used to control these pathogens in storage.

The essential oils of *Thymus vulgaris*, *Origanum vulgare*, *Origanum dictamus*, and *Origanum majorana* inhibited *Penicillium digitatum* radial growth inside Petri dishes in the lab (Daferera et al., 2000). These essential oils controlled the germination and growth of conidia when infected seeds were treated at concentrations of 250 µg/mL. *Penicillium digitatum* is a seed storage pathogen and seed infection usually occurs post harvest. Corn storage pathogens can deteriorate corn stored to be used as grain or seed. When the intended use is for seed, the pathogens can infect the seed before and during germination. The research done on using plant essential oils to control storage pathogens can be helpful to determine whether plant essential oils can be used as seed treatments.

Field performance of seeds treated with essential oils

The same varieties and treatments also were planted in the field under organic and conventional cropping systems. However, the commercial seed treatment Maxim XL is not approved for use in organic farming systems, thus we could not collect emergence data for Maxim XL at every site. Seeds treated with Maxim XL were planted only at the conventional sites.

Table 12 shows the mean emergence percentage at each growth stage for inbred and hybrid seed treated with commercial and essential oils across all sites and farming systems. Natural 2 protected inbred seed significantly better than the other treatments. The beneficial effects of this commercial seed treatment were observed at both growth stages V2 and V5.

The V2 emergence of inbred seed treated with Natural 2 was 73.5%, while the emergence of inbred seed treated with essential oils, ranged from 29.6% to 36.9%. When data from the conventional farming system were re-analyzed to include results from seed treated with Maxim XL, the emergence percentage of seeds treated with Maxim XL was significantly higher than all other treatments (data not shown). The emergence percentages of inbred seed treated with the essential oils were not significantly different from the untreated and soybean oil controls ($P< 0.05$). The V5 emergence percentage of inbred seeds treated with Natural 2 was highest, while all other treatments were lower and not significantly different from each other. The mean emergence percentage ranged from 71.3% for Natural 2-treated inbred seed to 30.3% for soybean oil-treated control.

regardless of the seed treatment (Table 12). At V5, the emergence of Natural 2-treated hybrid seed was significantly higher than oregano 800 $\mu L\ L^{-1}$, but not different from all other treatments ($P< 0.05$). The hybrid seeds were more vigorous than inbred seeds and their field emergence percentage was higher. The field emergence of the untreated control hybrid seed was not different from the essential oils treated seed.

Overall, there were no significant differences between the higher concentration 16 000 $\mu L\ L^{-1}$ and the lower concentration of 800 $\mu L\ L^{-1}$ of the essential oils. This lack of response could be associated with a concentration of the oils below the necessary threshold for seed protection under the stressful field conditions or with a premature volatilization of the aromatic compounds in the oils. The seeds must be treated less than 24 hours before planting. The time between treating and planting was sometimes longer than 24 hours since sites were sometimes a long distance apart and weather may have delayed planting. Further research should be conducted to resolve some of these issues. The emergence percentage of

inbreds seed was significantly different ($P < 0.05$, Appendix 7, 8) than hybrid seed, regardless of seed treatment. The emergence percentage of inbreds was about 30 percent lower than that of the hybrid seed.

Previous research addressed the effectiveness *in vivo* of the essential oils on corn seeds in the laboratory (Chatterjee, 1990). To our knowledge, this is the first time that essential oils were used as seed treatments in the field. Preliminary research showed that most of the essential oils applied at the minimum inhibitory concentration, controlled corn seed pathogen *in vitro*, but there was little information available in the literature for a comparison *in vivo.* The results from our study showed that these five essential oils applied at high doses do not have phytotoxic effect on corn seed and provided some protection against pathogens in the lab and the field. However, none of the essential oils were as effective as the synthetic seed treatment Maxim XL or the organic seed treatment Natural 2.

Conclusions

The five essential oils, cinnamon, clove, oregano, savory, and thyme, effectively protected infected corn seedlings in the cold test. The effectiveness of the essential oil treatments varied for each pathogen. *Fusarium* sp. was easily controlled by most essential oils, while *Penicillium* sp. was the most resistant.

The essential oils used in this experiment lacked phytotoxic effect even at the highest rate tested (16 000 μL L^{-1}). The most effective essential oils were thyme, clove, and oregano, and both rates (800 μL L^{-1} and 16 000 μL L^{-1}) of the essential oil provided similar fungicidal action. These results indicate that higher rates could be used to control the fungi without detrimental effects to the seeds.

The essential oils were not as effective as the commercial products for controlling these pathogens. One possible explanation is that the rate necessary to control the pathogen *in vitro* is much lower than the rates necessary to control the pathogen *in vivo* in the laboratory or in the field. The lower rates necessary to control the pathogen *in vitro* could be the result of higher concentration of volatile compounds inside the Petri dish that dissipate when the seeds are treated and tested *in vivo*. Inside the enclosed environment of the Petri dish, the pathogen is affected not only by the essential oils in the media, but also by the volatile compound released by the oils into the air.

The results of the field experiments yielded similar results to those of the laboratory. Seedling emergence of essential oils treated seeds was not significantly different from that of the untreated control. Future research should explore the possibility of using higher rates of essential oils. Increasing the application rates could better suppress the pathogens on and around the germinating corn seedling to a level comparable to commercially available fungicides.

This research advances our knowledge on the use of plant essential oils as corn seed treatments for use in organic production. Further research must be done to determine the effective rate of application *in vivo*. Organic farmers need an alternative to synthetic pesticides, and essential oils show some promise for the control of seed pathogens in corn which will be used as grain as well as seed.

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Table 10. Field experiments' planting dates by site.

Site	Planting Date	Soil Type
Ames (Conventional)	4/26/2006	Clarion Loam
Ames (Organic)	5/11/2006	Clarion Loam
Crawfordsville (Conventional)	4/20/2006 Hybrid - 4/21/2006 Inbred	Kalona Silty Clay Loam
Crawfordsville (Organic)	4/21/2006	Kalona Silty Clay Loam
Nashua (Conventional)	4/24/2006	Floyd Loam
Cresco (Organic)	5/23/2006	Floyd Loam, Clyde Silty Clay Loam, Oran Loam

Table 11. Cold test germination percentage of inbred and hybrid corn seeds infected with *Fusarium* sp., *Penicillium* sp., and *Pythium* sp., treated with commercial and essential oil seed treatments.

		Genotype					
		Inbred			Hybrid		
Treatment		Fusarium	Penicillium	Pythium	Fusarium	Penicillium	Pythium
		%					
Commercial							
	Maxim XL	86.1 bcde*	92.2 a	85.9 b	91.5 a	98.8 a	97.6 a
	Natural 2	95.0 a	92.9 a	96.5 a	95.5 a	94.7 ab	94.7 ab
	Yield Shield	92.2 ab	43.6 b	40.2 c	97.5 a	87.2 bcde	89.4 bc
Control							
	Soybean Oil	78.2 f	43.5 b	39.1 cd	90.2 a	92.7 abc	85.4 c
	Untreated Control	90.4 abc	38.6 bc	32.3 defg	93.1 a	88.1 bcd	86.7 c
Essential Oils							
	Cinnamon 800 µL L^{-1}	82.8 cdef	33.7 cd	31.1 efg	95.6 a	87.2 bcde	85.8 c
	Clove 800 µL L^{-1}	77.8 f	35.3 cd	35.4 cdef	93.0 a	86.2 cdef	82.2 c
	Oregano 800 µL L^{-1}	81.6 def	34.7 cd	35.1 cdef	91.4 a	86.8 cdef	88.2 bc
	Savory 800 µL L^{-1}	83.4 cdef	29.9 d	29.4 fg	95.3 a	81.8 def	82.4 c
	Thyme 800 µL L^{-1}	88.4 abcd	33.0 cd	42.6 c	96.3 a	82.9 def	87.1 bc
	Cinnamon 16 000 µL L^{-1}	78.2 f	32.6 cd	31.7 defg	94.9 a	80.2 ef	85.5 c
	Clove 16 000 µL L^{-1}	78.4 ef	33.7 cd	30.9 efg	93.5 a	87.4 bcde	85.6 c
	Oregano 16 000 µL L^{-1}	79.3 ef	31.3 cd	29.6 fg	92.3 a	85.5 cdef	84.5 c
	Savory 16 000 µL L^{-1}	79.2 ef	35.2 cd	24.8 g	90.9 a	86.3 cdef	83.7 c
	Thyme 16 000 µL L^{-1}	79.6 ef	30.6 d	38.2 cde	91.7 a	79.2 f	82.1 c

*Means within a column followed by the same letter are not significantly different ($P < 0.05$)

Table 12. Emergence percentages for inbreds and hybrids at different growth stages across all sites and farming systems.

Treatment		Genotype			
		Inbred		Hybrid	
Commercial		V2	V5	V2	V5
	Natural 2	73.5 a*†	71.3 a	82.0 a	81.4 a
	Yield Shield	46.6 b	45.4 b	76.3 a	74.1 ab
Control					
	Soybean Oil	29.9 c	30.3 b	70.7 a	68.7 ab
	Untreated	40.2 bc	39.7 b	72.1 a	69.4 ab
Essential Oils					
	Cinnamon 800 µL L^{-1}	29.7 c	33.2 b	76.1 a	73.6 ab
	Clove 800 µL L^{-1}	34.9 bc	35.8 b	75.7 a	71.6 ab
	Oregano 800 µL L^{-1}	32.7 c	31.8 b	73.6 a	67.1 b
	Savory 800 µL L^{-1}	32.8 bc	34.1 b	73.3 a	70.7 ab
	Thyme 800 µL L^{-1}	33.9 bc	33.8 b	75.4 a	73.6 ab
	Cinnamon 16 000 µL L^{-1}	36.6 bc	36.1 b	73.5 a	71.6 ab
	Clove 16 000 µL L^{-1}	31.3 c	29.4 b	74.8 a	71.2 ab
	Oregano 16 000 µL L^{-1}	32.5 c	36.9 b	72.4 a	68.8 ab
	Savory 16 000 µL L^{-1}	31.3 c	32.6 b	73.6 a	70.8 ab
	Thyme 16 000 µL L^{-1}	29.6 c	32.3 b	73.3 a	70.1 ab

* Means within a column followed by the same letter are not significantly different ($P < 0.05$)

† Maxim XL was included in the conventional sites. For the inbred V2, V5, and the hybrid V2, Maxim XL was not significantly different from the means followed by a's ($P < 0.05$). For V5 conventional site Maxim XL was only significantly greater than soybean oil, oregano 16 000 µL L^{-1}, and savory 800 µL L^{-1}.

Chapter 4. General Conclusions

Eighteen plant essential oils were screened to determine their antifungal properties and to assess their possible use as a fungicide seed treatment for organic seed corn. Five essential oils (cinnamon, clove, oregano, savory, and thyme) completely inhibited growth of all three corn pathogens *in vitro* (*Fusarium* sp., *Penicillium* sp., and *Pythium* sp.). The minimum inhibitory concentration (MIC) of the essential oils ranged from 100 to 800 μL L^{-1}. Therefore, a base concentration of 800 μL L^{-1} of essential oil was arbitrarily set for the rest of the experiments.

The essential oils used in these experiments did not produce phytotoxicity symptoms for the growing seedlings. Inbred seeds treated with these five essential oils at concentrations up to 16 000 μL L^{-1} showed no symptoms of phytotoxicity as evaluated by using the standard germination test. Higher concentrations of the essential oils should be used in future experiments to determine the rate of the essential oil at which phytotoxicity symptoms may occur. This information would be useful to determine the highest rate of application to use as seed treatment in corn seeds.

The five oils were also characterized by Gas Chromatography – Mass Spectrometry (GC-MS). The major constituent compounds of each essential oil were determined by comparing the results of the analysis to known spectra. The most abundant compounds of each oil were identified and compared to the predominant compounds in the other four oils and in the literature. The compounds present in the five essential oils were 10- and 15-carbon compounds with benzene groups. Eugenol, carvacrol, terpinene, and linalool were the predominant aromatic compounds in most oils. The antifungal properties of the oils seem

to be associated with these aromatic compounds. Future research should compare the efficacy of the pure forms of these constituent compounds to that of the whole essential oil to prove if the constituent compounds are related to the antifungal activity of the essential oil as a whole.

The standard germination test was used to evaluate the germination of artificially infected inbred and hybrid corn seeds treated with two rates (800 and 16 000 μL L^{-1}) of the five essential oils. There were no significant differences in germination percentage between the two rates of the essential oils. The germination percentage of seeds treated with the essential oil was significantly lower than the commercial seed treatments Natural 2, and Maxim XL. However, some essential oil seed treatments were not significantly different from the commercial organic seed treatment Yield Shield. The germination percentages of hybrid seeds inoculated with *Fusarium* sp. spores were not significantly different for all seed treatments. Because Maxim XL has restricted use for organic farming, the essential oils could provide farmers an alternative to Yield Shield.

Inbred and hybrid seeds were treated with the essential oils and planted in the field under conventional and organic cropping systems. The emergence of inbred seeds treated with the essential oil was significantly lower than seed treated with Natural 2, at both plant growth stages, V2 and V5. However, the emergence percentage of hybrid seeds treated with the essential oils was not significantly different from Natural 2 at developmental stages V2 and V5, except for hybrid seeds treated with 800 μL L^{-1} of oregano at growth stage V5. The synthetic seed treatment, Maxim XL, could not be used on seed planted at the organic sites, thus data including Maxim XL were reanalyzed separately for the conventional sites. Seeds treated with Maxim XL had the highest emergence percentage compared to all other

treatments. Results from this study indicate that the concentrations of the essential oils used in these experiments are insufficient to protect the seed in the field. Higher concentrations of the essential oils should be used before a definite conclusion can be reached about the effectiveness of the essential oils as seed treatments. To our knowledge, these experiments are the first attempt to use plant essential oils as seed treatments to improve corn field emergence and promote early planting in organic and organic farming systems.

Appendices

Appendix 1. Emergence percentage for Inbreds at each site at growth stage V2.

	Site					
Treatment	Crawfordsville Conventional	Ames Conventional	Nashua Conventional	Crawfordsville Organic	Ames Organic	Cresco Organic
Maxim XL	61.6	92.7	72.8	†	†	†
Natural 2	70.8	90.1	70.6	54.4	62.3	79.8
Yield Shield	80.5	15.1	29.4	18.9	70.6	60.5
Soybean Oil	69.5	10.9	13.2	8.3	44.3	38.2
Untreated Control	69.5	15.6	28.1	10.5	51.7	63.1
Cinnamon 800 µL L^{-1}	72.7	9.4	14.5	9.6	42.1	54.4
Clove 800 µL L^{-1}	69.5	11.5	11.4	6.1	71.1	47.8
Oregano 800 µL L^{-1}	64.1	7.8	13.6	14.9	59.6	30.7
Savory 800 µL L^{-1}	69.3	12.0	13.2	14.5	59.2	36.4
Thyme 800 µL L^{-1}	66.1	8.9	15.8	10.5	57.0	44.3
Cinnamon 16 000 µL L^{-1}	71.9	7.8	19.3	17.5	54.8	48.2
Clove 16 000 µL L^{-1}	56.3	3.6	12.7	9.2	47.8	46.9
Oregano 16 000 µL L^{-1}	88.3	16.1	11.4	12.3	50.4	50.0
Savory 16 000 µL L^{-1}	72.7	13.5	11.4	5.3	44.7	51.3
Thyme 16 000 µL L^{-1}	77.3	10.4	11.8	10.5	42.1	60.5

† Maxim XL is a synthetic fungicide, so it could not be used in certified organic fields.

Appendix 2. Emergence percentage means for Hybrids at each site at growth stage V2.

	Site					
Treatment	Crawfordsville Conventional	Ames Conventional	Nashua Conventional	Crawfordsville Organic	Ames Organic	Cresco Organic
Maxim XL	80.7	94.8	78.1	†	†	†
Natural 2	85.4	92.7	75.0	81.1	93.9	60.5
Yield Shield	93.8	65.1	56.6	70.2	88.2	70.6
Soybean Oil	92.2	59.9	41.2	54.4	93.4	71.1
Untreated Control	89.6	59.4	62.3	60.1	87.3	57.0
Cinnamon 800 µL L^{-1}	92.2	58.3	65.4	61.0	89.5	75.4
Clove 800 µL L^{-1}	90.6	72.4	57.0	57.0	90.8	61.8
Oregano 800 µL L^{-1}	77.6	64.6	53.1	56.1	86.0	65.4
Savory 800 µL L^{-1}	94.8	60.9	52.2	62.7	92.5	61.4
Thyme 800 µL L^{-1}	93.2	67.2	61.8	65.8	87.7	65.8
Cinnamon 16 000 µL L^{-1}	94.3	69.3	54.4	56.6	92.5	62.7
Clove 16 000 µL L^{-1}	90.1	62.5	57.9	66.2	87.3	63.2
Oregano 16 000 µL L^{-1}	91.7	64.1	47.4	61.4	80.7	67.5
Savory 16 000 µL L^{-1}	95.3	65.6	61.4	57.5	89.0	56.1
Thyme 16 000 µL L^{-1}	93.8	65.1	58.8	63.6	88.6	52.6

† Maxim XL is a synthetic fungicide, so it could not be used in certified organic fields.

Appendix 3. Emergence percentage for Inbreds at each site at growth stage V5.

	Site					
Treatment	Crawfordsville Conventional	Ames Conventional	Nashua Conventional	Crawfordsville Organic	Ames Organic	Cresco Organic
Maxim XL	88.0	91.7	92.7	†	†	†
Natural 2	93.8	88.5	84.4	32.5	62.3	79.4
Yield Shield	73.4	15.1	44.8	18.0	71.9	56.6
Soybean Oil	64.1	9.9	18.2	5.7	40.4	41.2
Untreated Control	79.7	15.6	36.5	9.2	51.3	49.1
Cinnamon 800 µL L^{-1}	55.2	9.9	21.4	8.3	37.3	46.1
Clove 800 µL L^{-1}	53.6	12.0	15.6	5.7	71.9	50.4
Oregano 800 µL L^{-1}	65.5	8.3	18.2	13.6	59.2	31.1
Savory 800 µL L^{-1}	59.9	10.9	20.3	12.3	59.6	33.8
Thyme 800 µL L^{-1}	68.8	8.3	22.4	8.8	56.1	39.0
Cinnamon 16 000 µL L^{-1}	56.8	7.8	26.0	30.7	53.5	44.7
Clove 16 000 µL L^{-1}	62.5	4.2	19.8	9.2	48.2	43.9
Oregano 16 000 µL L^{-1}	59.9	16.1	16.1	11.0	46.1	45.6
Savory 16 000 µL L^{-1}	66.7	13.0	17.7	3.9	44.7	41.7
Thyme 16 000 µL L^{-1}	63.0	10.4	15.1	8.8	41.7	38.6

† Maxim XL is a synthetic fungicide, so it could not be used in certified organic fields.

Appendix 4. Emergence percentage means for Hybrids at each site at growth stage V5.

	Site					
Treatment	Crawfordsville Conventional	Ames Conventional	Nashua Conventional	Crawfordsville Organic	Ames Organic	Cresco Organic
Maxim XL	93.2	96.4	93.8	†	†	†
Natural 2	64.6	94.8	90.6	79.4	96.1	66.7
Yield Shield	91.1	65.6	72.9	67.5	88.2	72.4
Soybean Oil	88.0	59.4	57.3	53.5	93.4	72.8
Untreated Control	91.7	59.4	75.5	59.2	86.8	60.0
Cinnamon 800 µL L^{-1}	91.1	58.3	82.3	62.3	89.0	73.7
Clove 800 µL L^{-1}	92.2	72.9	73.4	61.4	91.2	62.7
Oregano 800 µL L^{-1}	95.3	64.6	72.9	54.4	87.3	67.1
Savory 800 µL L^{-1}	93.2	61.5	64.6	64.9	90.4	65.4
Thyme 800 µL L^{-1}	88.0	67.2	75.7	64.9	97.7	68.9
Cinnamon 16 000 µL L^{-1}	91.7	70.3	68.8	56.1	92.1	62.3
Clove 16 000 µL L^{-1}	92.7	62.0	72.9	64.9	87.3	68.9
Oregano 16 000 µL L^{-1}	89.6	64.1	64.6	68.4	80.3	67.5
Savory 16 000 µL L^{-1}	89.1	65.6	76.6	56.1	89.9	64.0
Thyme 16 000 µL L^{-1}	89.6	67.7	74.0	62.7	90.4	55.3

† Maxim XL is a synthetic fungicide, so it could not be used in certified organic fields

Appendix 5.
ANOVA for Phytotoxicity

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	21	311.09090	14.81385	1.30	0.2058
error	66	750.00000	11.36363		
total	87	1061.09090			

Appendix 6.
ANOVA for stand establishment (V2) at the organic sites

Source	DF	Type III SS	Mean Square	F Value	Pr > F
genotype	1	69112.00000	69112.00000	10.13	0.0861
treatment	14	7439.95588	531.42542	3.31	0.0007
treatment*genotype	14	3173.25843	226.66131	1.41	0.1794
location	2	47843.00000	23921.00000	3.51	0.2219
location*genotype	2	13643.00000	6821.33605	42.43	<.0001
location*treatment*variety	56	9003.05222	160.76879	1.09	0.3309
residual	180	26554.00000	147.51975		

Appendix 7.
ANOVA for stand establishment (V2) at the conventional sites

Source	DF	Type III SS	Mean Square	F Value	Pr > F
genotype	1	73265.00000	73265.00000	19.53	0.0476
treatment	14	26726.00000	1909.00097	4.35	<.0001
treatment*genotype	14	8130.17295	580.72664	1.32	0.2230
location	2	76516.00000	38258.00000	10.17	0.0895
location*genotype	2	7524.89963	3762.44981	8.57	0.0006
location*treatment*genotype	56	24742.00000	441.81329	3.80	<.0001
residual	170	19745.00000	116.14858		

Appendix 8.
ANOVA for stand establishment (V5) at the conventional sites

Source	DF	Type III SS	Mean Square	F Value	Pr > F
genotype	1	94571.00000	94571.00000	23.04	0.0408
treatment	14	41309.00000	2950.62288	9.67	<.0001
treatment*genotype	14	17588.00000	1256.31277	4.12	<.0001
location	2	54802.00000	27401.00000	6.67	0.1303
location*genotype	2	8210.34071	4105.17035	13.45	<.0001
location*treatment*genotype	56	17092.00000	305.20936	3.12	<.0001
residual	180	17586.00000	97.70146		

Appendix 9.
ANOVA for stand establishment (V5) at the organic sites

Source	DF	Type III SS	Mean Square	F Value	Pr > F
genotype	1	84680.00000	84680.00000	17.43	0.0529
treatment	14	6561.41633	468.67259	3.47	0.0004
treatment*genotype	14	2678.60623	191.32901	1.42	0.1767
location	2	49116.00000	24558.00000	5.05	0.1652

location*genotype	2	9717.07961	4858.53980	35.95	<.0001
location*treatment*genotype	56	7569.14948	135.16338	1.00	0.4933
residual	180	24441.00000	135.78537		

Appendix 10.

ANOVA for V2 data at all sites

Source	DF	Type III SS	Mean Square	F Value	Pr > F
farming system	1	454.21587	454.21587	0.01	0.9392
genotype	1	144171.00000	144171.00000	27.21	0.0064
farming system*genotype	1	344.84083	344.84083	0.07	0.8112
treatment	13	21356.00000	1642.80729	7.57	<.0001
farming system*treatment	13	1943.27771	149.48290	0.69	0.7706
treatment*genotype	13	7272.32917	559.40993	2.58	0.0039
farming system*treatment*genotype	13	1160.66583	89.28198	0.41	0.9630
location	2	13534.00000	6767.19442	0.11	0.9005
farming system*location	2	122437.00000	61219.00000	11.54	0.0218
farming system*location*genotype	4	21263.00000	5315.75998	24.50	<.0001
farming system*location*treatment*genotype	104	22731.00000	218.57054	1.64	0.0005
residual	345	46091.00000	133.59789		

Appendix 11.

ANOVA for V5 data at all sites

Source	DF	Type III SS	Mean Square	F Value	Pr > F
farming system	1	234.28156	234.28156	0.00	0.9539
genotype	1	182396.00000	182396.00000	40.36	0.0031
farming system*genotype	1	586.33099	586.33099	0.13	0.7369
treatment	13	22259.00000	1712.20945	9.43	<.0001
farming system*treatment	13	3520.01723	270.77056	1.49	0.1329
treatment*genotype	13	10566.00000	812.74541	4.47	<.0001
farming system*treatment*genotype	13	4271.62897	328.58684	1.81	0.0508
location	2	2052.39076	1026.19538	0.02	0.9810
farming system*location	2	105890.00000	52945.00000	11.71	0.0213
farming system*location*genotype	4	18084.00000	4521.03132	24.93	<.0001
farming system*location*treatment*genotype	104	18954.00000	182.25302	1.54	0.0021
residual	345	41948.00000	118.49830		

Appendix 12.

ANOVA for Pathogen Infection Experiment

Source	DF	Type III SS	Mean Square	F Value	Pr > F
pathogen	2	45920.00000	22960.00000	275.78	<.0001
genotype	1	99301.00000	99301.00000	1192.71	<.0001
pathogen*genotype	2	23956.00000	11978.00000	143.87	<.0001
treatment	14	20943.00000	1495.89910	60.33	<.0001
treatment*pathogen	28	8022.79074	286.52824	11.41	<.0001
treatment*genotype	14	10058.00000	718.42412	28.70	<.0001
treatment*pathogen*genotype	28	3123.83933	111.56569	4.51	<.0001
tray(pathogen*genotype)	84	6061.46334	72.16027	2.62	<.0001
residual	186	5121.78665	27.53648		

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