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Investigating mycelial-crop residue mat application to reduce early-colonizing weeds in row-crop agriculture

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Abstract

Herbicide use within conventional agriculture has contributed to greatly increased crop yields since its widespread adoption, but environmental concerns regarding overuse and reliance on selective herbicides continue to mount. Using five fungal species and two crop residues in a factorial design, I created a novel slurry to control weeds through inhibition by the mycelial mat formed after application to soil. I monitored weed stem counts and the strength of the mycelial mat under the treatments. Additionally, as a proxy for crop yield, I measured the wet and dry mass of crop plant grown under application treatments. Weed prevalence was significantly reduced when compared to a bare soil control, but not when compared to a substrate only control without fungal inoculum. Similar strength values were recorded between treatments and control, suggesting poor colonization of the substrate under greenhouse and field conditions. No significant weed reduction was achieved in field trials.

Keywords

Mycelia, inoculum, slurry, agriculture, early competing weeds, crop residue, biotechnology, saprotrophic fungi, herbicide

Summary for Lay Audience

Greater agricultural yields are required to continue to meet the global food demands of the increasing human population. One tool employed by modern agriculture to help meet demand is the use of herbicides to control weeds which, left uncontrolled, compete with crop plants for energy and resources. Herbicide use has contributed to greater realized yields, but concerns exist regarding negative environmental impacts and over-reliance causing decreased effectiveness of these herbicide regimens. In this study, I tested a novel approach to control weeds without traditional herbicides. I formulated liquid slurries with different combinations of fungal cultures and waste from Ontario agricultural crops to be applied on crop fields. To determine effectiveness, I measured the number of weeds present in treated plots, plots covered with crop waste only, and uncovered plots. I also measured the strength (penetration resistance) generated by the slurry once applied, as well as crop plant weights to detect any negative or positive effects on plant growth. I found the treatments greatly reduced weed counts compared to uncovered plots but provided similar weed reduction compared to plots covered with just crop waste. The slurry did not generate a fungal mat of significant strength compared to bare soil, nor were there any significant changes to weights of the crops grown. Although this trial did not achieve weed control using fungal growth, alternative combinations of fungi and crop wastes may yield greater power to control weed growth, and different applications may yield other economic and environmental benefits, such as reduction of crop residues and winter soil erosion, and improvement of soil organic matter.

Co-Authorship Statement

This work was conceptualized, undertaken, and drafted with input from Dr. R. Greg Thorn and our industrial sponsors Dietmar Walch, Murray Good and Terry Good of Natures Balance, Inc., and will be revised for publication with their co-authorship.

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List of Abbreviations

AMF = Arbuscular mycorrhizal fungi

BcF = Biocontrol fungi

C:N = Carbon : Nitrogen

EPSP = 5-enolpyruvylshikimate-3-phosphate synthase

LFH = Laminar flow hood

MAS = Macroaggregate structure

MEA = Malt extract agar

SOM = Soil organic matter

1 Introduction

1.1 Weed control

1.1.1 Weed pressure in agriculture

Globally and within Ontario, weeds are responsible for the greatest production losses in row-crop agriculture, with estimates of average yield losses greater than 30% (Soltani et al., 2016; Oerke, 2005), and even higher farm-gate losses after factoring in costs of tillage and the purchase and application of herbicides used to control weeds. Improper control of weed pressure can result in crop yield losses exceeding 80% (Ontario Ministry of Agriculture, Food, and Rural Affairs, 2017). Currently, different regimens are used to control weed pressure including herbicide application, conventional and alternative tilling, and diversified cropping (Sharma et al., 2021). Modern methodology has shown reduction in weed pressure on a global scale, but challenges exist in utilization and adoption. Here, I focus primarily on pertinent environmental concerns with modern weed control, though concerns regarding economic and human health should also be acknowledged (Weersink et al., 1992; Kanissery et al., 2019; Sharma et al., 2021).

1.1.2 Traditional weed control

Within conventional agriculture, a dual-faceted approach towards weed management is predominantly observed (MacLaren et al., 2020; Chauhan, 2020). Combined use of tillage (mechanical disturbance of the soil) and herbicide application currently employed by many farmers leads to significant reduction in weed prevalence, and in turn greater crop yield (Chauhan, 2020). While current practices have led to dramatic reduction in weed pressure, concern is mounting against the environmental risks and hazards of systemic, broad-spectrum herbicides (Fernandez-Cornejo et al., 2014; Kanissery et al., 2019; Camargo et al., 2019). The

environmental costs of traditional weed control focused on tillage, herbicide-resistant crop varieties and whole-field herbicide application include alteration of soil structure, runoff patterns and natural soil diversity, significant greenhouse gas emissions, nitrogen remobilization, herbicide drift, increased soil erosion, and soil fertility loss, as well as horizontal transfer of herbicide-resistance genes into weed populations (Pollegioni et al., 2011; Kanissery et al., 2019; Heap, 2019).

1.1.2.1 Costs of tillage

Conventional agriculture has increased yield dramatically over the past 100 years to meet the ever-increasing food demand imposed by humanity. Tillage refers to a broad scope of mechanical intervention designed to disturb the soil structure to prepare the field for cropping as well as to displace and control weeds (Busari et al., 2015; Šarauskis et al., 2018). Combined with herbicide use, tillage has allowed far greater crop yields to be realized, but the negative environmental impacts of the practice have been a topic of recent discussion (Uri et al., 1999; Van Oost et al., 2000; Žurovec et al., 2017). A Canadian study showed labile forms of organic carbon and nitrogen found in agricultural soils increase through reduction of tillage (Malhi et al., 2008). Additionally, frequent tillage can be responsible for deterioration of macroaggregate structure (MAS) (i.e., soil structures larger than 250 μm) in cultivated soils (Zheng et al., 2018). Reduction of MAS and crop cover residue through tilling contribute to increased rates of soil erosion due to increased impact displacement of soil through rainfall (Williams et al., 2009; Busari et al., 2015). Conversely, fungal exudates of polysaccharides and glycoproteins in soils contribute to the formation of MAS, adding beneficial resistance to natural soil erosion and physical displacement (Caesar-TonThat & Cochran, 2000).

1.1.2.2 Costs of herbicide application

Currently, herbicide regimens are widely adopted in commercial agriculture. In the U.S. it is estimated over 95% of cultivated soybean, sugar beet, cotton, and maize are treated with some form of herbicide (Wang et al., 2018). Modern herbicides are effective in the reduction of weed pressure but use of repeated herbicide protocols can lead to accumulation of weed populations resistant to the specific site of action targeted by the herbicide (Funke et al., 2006; Wang et al., 2018). Glyphosate (N-phosphonomethyl-glycine), the most globally prevalent herbicide, acts to control weeds by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSP) (Funke et al., 2006; Pollegioni et al., 2011). EPSP activity drives regulation of the production of certain amino acids such as tryptophan and phenylalanine (Funke et al., 2006). Failure to synthesize these amino acids restricts protein synthesis and other biosynthetic pathways, causing plant death. To date, 54 weed species have shown emerging resistance to EPSP inhibition (Heap, 2019). In Canada, species with resistant populations include Giant Ragweed (*Ambrosia trifida*), Common Ragweed (*Ambrosia artemisiifolia*), Tall Waterhemp (*Amaranthus tuberculatus*), and Birdsrape Mustard (*Brassica rapa*) (Heap, 2019). In total, over 500 unique instances of herbicide-resistant weed populations have been documented worldwide (Heap, 2019.

In addition to concerns regarding herbicide resistance, significant losses have been observed due to herbicide drift (Sharma et al., 2021; Egan et al., 2014b; Pingali, 2012). Herbicide drift refers to the physical movement of herbicide, during or shortly after application, to unintended sites. This movement can occur unintentionally due to vapor drift, surface or subsurface water flow, and as airborne particles deposited during rainfall (Egan et al., 2014a). A recent meta-analysis documented susceptibility in neighbouring cotton fields to 2,4-dichlorophenoxyacetic acid (2,4-

D), a prevalent broadleaf herbicide (Egan et al., 2014b). Herbicide drift damage can extend into neighbouring non-target terrestrial plants and neighbouring arthropod communities and has been suggested to act as a stressor inducing epigenetic changes, DNA mutations, and other genetic alterations that could confer herbicide resistance (Egan et al., 2014a; Vieira et al., 2019). According to MacLaren (2020), a focus on more sustainable methods and applications for controlling weed pressure is crucial to meeting global food demand while conserving ecosystem integrity, diversity, and health.

1.1.3 Fungi in weed control

Fungal-based herbicides, or "mycoherbicides" exist, with 12 products currently marketed in the United States and Canada (Triolet et al., 2020). Though application of mycoherbicides have increased over the past three decades, utilization in large-scale agriculture is limited (Harding & Raizada, 2015). Acting as a primary constraint on market capture is the tendency of mycoherbicides to be highly targeted or specific to a single prevalent weed species. This contrasts with other methods of weed control which may be more desirable as they are effective against a broad range of weed species. Prominent genera utilized within mycoherbicidal applications include *Colletotrichum*, *Phytophthora*, and *Sclerotinium* (Harding & Raizada, 2015; Triolet et al., 2020). The standard method of action of marketed mycoherbicides utilizes phytopathogenic compounds produced by the fungus to reduce or inhibit plant growth (Triolet et al., 2020).

1.2 Roles of fungi

1.2.1 Fungi as decomposers

Throughout the fungal kingdom a wide variety of lifestyles can be observed. Fungi can act pathogenically, antagonising living plants, animals and humans. Fungi can also serve as a beneficial or commensal partner in obligate symbioses. For example, fungi exhibiting mycorrhizal lifestyles intimately associate with roots of living plants. This association can provide protection to the host plant from root pests, as well as allowing greater access to water and nutrients that were previously unreachable (Smith & Read, 2008; Treseder & Lennon, 2015). Lifestyles exhibited by fungi are highly varied and can contain numerous stages of different ploidy. One of the most prevalent lifestyles are the saprotrophic fungi or the "decomposers".

With a holistic view towards soil as a dynamic heterogenous living substrate, it can be said fungi act as "biological regulators" due to their influence on biogeochemical cycling and soil health. Saprotrophic Agaricomycetes (mushrooms) are specialized decomposers of high carbon: nitrogen (C:N) plant materials (Worrall et al. 1997). Saprotrophic fungi inhabit soils globally with a great degree of success due to their ability to produce and exude various extracellular enzymes (Frąc et al., 2018). Biological processes such as directed hyphal growth, translocation of mycelial cytoplasm, and the ability to re-assimilate nitrogen from lysed hyphae, help enable these specialized fungi to grow in constraining, nitrogen-poor substrates (Miller & Jastrow, 1990). Fungi can decompose organic matter through the degradation of cellulose and hemicelluloses in plant cell walls (Treseder & Lennon, 2015; Finlay & Thorn, 2019). Fungal decomposition of soil organic material generally proceeds through a well-defined pathway. Involving numerous fungal species, decomposition proceeds initially by degrading labile compounds prior to degrading more recalcitrant structural materials (Deacon, 2005; Finlay & Thorn, 2019; Frąc et al., 2018). Through the ability to decompose organic matter, saprotrophic fungi facilitate and mediate nutrient cycling in soils. Because of their ability to bridge the

separation between inorganic and organic nutrients in soils, fungi play a necessary role in facilitating and mediating nutrient cycling. This process is exemplified through the ability of certain specialized saprotrophic fungi to degrade compounds such as lignin, lignocellulose, and chitin, recalcitrant compounds integral to the maintenance of cellular structure in living plants and fungi (Lindahl et al., 1999; Miller & Jastrow, 1990).

Though historically saprotrophic fungi have been considered the sole decomposing fungal lifestyle, there is increasing recent evidence that mycorrhizal fungi can facilitate or hamper decomposition in soils (Lindahl & Tunlid, 2015; Shah et al., 2016). Two distinct forms of mycorrhizal fungi exist: ectomycorrhizal and endomycorrhizal, or arbuscular mycorrhizal fungi (AMF). Both are obligate symbionts with suitable plant species, however AMF penetrate the cell walls of symbiotic root tissue for nutrient exchange, while ectomycorrhizal fungi remain exterior to the root cells and form an intricate association matrix, the Hartig net (Smith & Read, 2008). Some species of ectomycorrhizal fungi have been observed decomposing soil organic matter (SOM) through oxidation (Shah et al., 2016). The mechanisms used are currently being investigated, as many ancestral genes coding for lignocellulose-degrading enzymes have been lost (Shah et al., 2016). Similarly, AMF have lost the ability to produce many ancestral enzymes responsible for decomposition, but their hyphae have been observed colonizing litter in soil (Went & Stark, 1968; Bunn et al., 2019). It is hypothesized these fungi may indirectly influence decomposition of SOM, though the mechanisms of influence remain unclear (Bunn et al., 2019).

1.2.2 Fungi in agriculture

The edibility of certain mushrooms has been widely known and documented historically. More recently, certain fungal species, which are primarily saprotrophic, have been domesticated and are cultivated on a large scale globally. *Agaricus bisporus*, which can be harvested as button

mushrooms, cremini, and portabella types, accounted for 98% of Canadian mushroom production in 2017 (Government of Canada, 2019). With over 20 billion pounds of *A. bisporus* produced globally in 2017, cultivation of edible mushrooms represents a significant agricultural market (Siwulski et al., 2020). Beyond cropping utility, fungi are commonly utilized in plant crop agriculture to control plant pathogens and improve soil health. Continued research surrounding the effects of saprobic fungal soil community members on plant biomass productivity, nutrient cycling, and soil aggregation suggest increasing incorporation of saprobic fungi into agricultural practices (Peterson et al., 1984; Savary et al., 2019).

1.2.2.1 Fungi as biological control

Global trends suggest a shift from chemical-based weed and pest control to an increasing prevalence of biocontrol agents. Fungi can act as biocontrol agents and assume different mechanisms of action dependent on the species of fungus used, the pest to be controlled, and other immediate environmental factors. The simplest of these mechanisms are direct effects produced by the crop plant due to the addition of biocontrol fungi (BcF). The presence of BcF can induce defensive responses in the crop plant and raise its resistance to the pathogen (Savita & Sharma, 2019). Conversely, the presence of specific BcF can also enhance crop growth and offset antagonistic pathogenic effects (Thambugala et al., 2020). Acting indirectly through the mechanism of antibiosis, BcF can produce and exude secondary metabolite compounds or antibiotics to inhibit the growth of targeted pests (Sood et al., 2020). Finally, BcF can exert bidirectional parasitic or predatory pressure on the antagonizing pest. In the role of a predator, BcF can penetrate the dermis or cuticle of target pest species and exert control directly with no need for ingestion (Savita & Sharma, 2019). Addition of BcF can also provide a surrogate species to be parasitized in lieu of the crop plant, allowing the pest to derive nutrition directly

from the fungus and thus mitigating effect on the crop species (Thambugala et al., 2020; Sood et al., 2020).

1.2.2.2 Fungi as soil additives

Environmentally beneficial roles of saprobic fungi are being further recognized and understood in terms of their importance in nutrient cycling and natural soil diversity (Miller and Jastrow, 1990). The transfer of litter-derived carbon to soil can be facilitated through inherent fungal processes, providing benefits to soil health (Frey et al., 2001). The abundance of fungi in soils is directly correlated with increased MAS formation (Miller & Jastrow, 2000; Lucas et al., 2014; Totsche et al., 2018). AMF have been observed to produce glomalin external to their hyphae (Miller & Jastrow, 2000). Glomalin acts as an adhesive compound, both producing MAS and assisting to maintain soil structure during disruptive events (i.e., physical disturbance, moisture changes) (Rillig et al., 2003). MAS are viewed as beneficial to soil health as they contribute to improved porosity, decreased erosion, and higher proportions of sequestered carbon (Miller & Jastrow, 2000). MAS are more readily decomposed when compared to microaggregates (<250 μm), thus able to provide increased organic matter sources for plant roots and fungi (Totsche et al., 2018).

1.3 Project overview

This project investigated a novel method of weed control for row-crop agriculture that, if successful, has the potential to reduce or eliminate the need for chemical weed control and may have side benefits of increasing soil health and reducing nutrient losses and greenhouse gas emissions. The application seeks to take advantage of profuse mycelial growth and other inherent fungal processes to create a barrier impenetrable to germinating weeds. The resistance will be generated by the propensity of the chosen fungi to decompose the provided plant waste, and in

doing so, bind the substrate with mycelial growth, a mechanism that has been exploited by a number of commercial applications listed below. Sufficient mycelial coverage and homogeneity of the application is facilitated through generation of plant waste slurry, mixed with blended inoculum of selected fungi prior to deployment. Numerous species of bacteria, as well as fungi, are known to be opportunistic decomposers of SOM. To avoid significant bacterial interference, plant waste of a high C:N ratio was chosen to form the substrate, and the experimental fungi selected were fast-growing and efficient decomposers of such recalcitrant material. Theoretically, the mycelial mat generated would prove dense enough and of sufficient tensile strength to halt any undesirable germinating seeds below from penetrating it.

Application of bio-based composite materials, driven primarily by a desire to offer viable alternatives to petroleum-based products, have greatly increased in prevalence and visibility in recent years. The first patent covering the use of fungal materials in industrial application was granted in 2011 and since then, 25 additional patents have been granted (Cerimi et al., 2019). Ecovative Design LLC., a bio-tech company specializing in developing mycelium-based products for applications such as textiles, electrical circuit boards, and composites, owns 45% of all current fungal industrial patents (Cerimi et al., 2019). The significant tensile and flexural strength of mycelial materials have led to successful industrial applications as polystyrene replacements, as well as substitutes for foam-like, wood-like and cork-like materials (Appels et al., 2019). The extent to which fungal-based materials can replace current manufacturing techniques is still emerging, but the potential benefit and diversity of possible applications are vast.

1.4 Research question

Can a mat of fungal mycelium formed by an application to soil of an inoculated slurry of waste crop biomass function as an effective deterrent of early colonizing weeds in row-crop agriculture?

1.5 Objectives

Objective 1: Develop a mix of ground cellulosic crop residue with fungal inoculum capable of being applied as a slurry to the soil surface and then forming a mycelial mat.

Predictions:

1. A tangible fungal-crop residue mat will be formed by some or all inoculum-residue combinations.
2. Differences in colour, visual growth and quantifiable physical traits will be observed in specific inoculum-residue combinations.

Objective 2: Quantify changes in early colonizing weed prevalence within treatment plots by measuring direct stem counts at multiple time points

Predictions:

1. Weed prevalence will be significantly lower in treatment plots as compared to control plots.
2. Weed reduction as a percentage will vary based on the specific inoculum-residue combinations used in the plot.

Objective 3: Quantify changes in crop species yield within treatment plots by measuring fresh- and dry-weight biomass (roots and shoots) at harvest as proxies for yield.

Predictions:

1. Crop biomass will be consistent between treatment and control plots, showing no reduction in growth due to fungal mat treatment.

Objective 4: Investigate the strength generated by fungal mats to resist seedling penetration in greenhouse trials. A ¼' soil penetrometer will be used to create holes in the mat to facilitate crop seed planting, returning a shear strength value of the mat at near onset and at termination of the trial.

Predictions:

1. Documentable strength values will be returned in treatment plots, displaying shear strength above and beyond the baseline values recorded in the no application control plots.
2. Documentable strength will vary based on specific inoculum-residue combinations.

2 Materials and Methods

2.1 Study species

2.1.1 Crop residue

For this experiment, two crop residues were used, *Zea mays* (corn or maize) and *Triticum aestivum* (wheat) due to their prominent utilization within Canadian agriculture, and in turn the associated prevalence of residue remaining after harvest. The two species accounted for

13,563,300 and 35,183,000 tonnes of harvested crop weight, respectively, during the 2020-2021 growing season (AAFC, 2020). During the same season, yield by weight of all crop species in Canada was estimated at 99,577,300 tonnes (AAFC, 2020), showing a capture percentage of total Canadian crop yield for *Z. mays* and *T. aestivum* of 48.95% by weight (AAFC, 2020).

2.1.2 Fungal species

Five fungal species were used as treatments for this experiment: *Pleurotus ostreatus*, *Irpex lacteus*, *Hypsizygus ulmarius*, *Picipes rhizophilus*, and *Fomitopsis betulina*. All are saprotrophic, which was part of the selection criteria to achieve fungal-driven decomposition of the crop residue for mat generation.

2.1.2.1 *Pleurotus ostreatus*

The oyster mushroom, *P. ostreatus*, is a commonly cultivated edible fungus (Beltran-Garcia et al., 1997). Widespread in many temperate forests globally, including southwestern Ontario, the oyster mushroom is a white-rot fungus acting as a primary necrotrophic decomposer of various hardwood trees (Stamets & Chilton, 1983; Pavlík & Pavlík, 2013). Due to the presence of associated white-rot peroxidases, *P. ostreatus* is commonly explored in applications for bio- and mycoremediation (Rhodes, 2014; Pavlík & Pavlík, 2013). While *P. ostreatus* is primarily saprotrophic, *Pleurotus* species supplement their high-carbon woody diet with consumption of both nematodes and bacteria, a notable distinguishing trait compared to the other four species used here (Barron & Thorn, 1987; Dijksterhuis et al., 1994; Feldman et al., 2020).

2.1.2.2 *Irpex lacteus*

Like *P. ostreatus*, *I. lacteus* is a white-rot fungus acting primarily as a necrotrophic decomposer of hardwood (Novotný et al., 2009). One of the most common wood-rotting fungi, *I. lacteus*

produces polypore (pored) or hydnoid (toothed) fruiting bodies, unlike *P. ostreatus* which produces agaricoid (gilled) fruiting bodies (Phillips, 2010). Inedible and generally undesirable for culinary purposes, *I. lacteus* is considered a viable option for biotechnical applications due to its ability to withstand soilborne and aquatic pollution (Novotný et al., 2009; Yao et al., 2017).

2.1.2.3 *Hypsizygus ulmarius*

This species is commonly known as the elm oyster, with fruiting bodies similar in agaricoid form to the true oyster mushroom (*P. ostreatus*). The two can be differentiated visually as the gills of *H. ulmarius* are not decurrent, meaning the gills do not extend down the stem, unlike *P. ostreatus* (Stamets, 2005; Greeshma et al., 2016). Although *H. ulmarius* is found fruiting primarily on wounded elm trees (*Ulmus* sp.) in temperate forests, it is unclear if it is strictly saprotrophic, or possesses parasitic lifestyle tendencies as well (Hofstetter et al., 2014).

Hypsizygus ulmarius has a moderate ability to degrade lignin, an ability more commonly associated with white-rot fungi, although it is classified as a brown-rot fungus due to observable decay patterns (Redhead & Ginns, 1985; Hori et al., 2013). Additionally, *H. ulmarius* has been trialed for development in mycoremediation applications due to its enzymatic ability to decolourize various industrial dyes (Ravikumar et al., 2013).

2.1.2.4 *Picipes* (= *Polyporus*) *rhizophilus*

A polyporoid species, *P. rhizophilus* exists saprotrophically as a specialized decomposer of various genera of grasses (Vlasenko & Turmunkh, 2020). Not commonly cultivated or regarded as an edible species. *P. rhizophilus* is red listed as vulnerable in various European countries by the International Union for Conservation of Nature (IUCN 2021). It is believed reduction of

specific habitats due to human impact and livestock grazing contribute to its rarity (Vlasenko & Turmunkh, 2020).

2.1.2.5 *Fomitopsis betulina*

The birch polypore, *F. betulina* (formerly *Piptoporus betulinus*), causes a brown rot of trees exclusively within the genus *Betula.* Unlike *H. ulmarius, F. betulina* does not possess lignin-degrading enzymes or ability (Camlibel, 2020). A parasitic necrotroph, *F. betulina* is prominent on weakened trees, eventually leading to death (Camlibel, 2020). *F. betulina* is not commonly regarded as a desired edible species, however recent pharmacological studies have shown promising results in antibacterial, antiviral, and neuroprotective properties attributed to specific compounds produced by the fungus (Pleszczyńska et al., 2017).

2.2 Fungal material

2.2.1 Genetics and agar culture

All fungal cultures utilized in the project were sourced from Canadian colleagues or collected by the author or supervising professor, Dr. R.G. Thorn. Candidate species were then isolated in pure culture (if necessary) and identified by macro- and micromorphology with a retained voucher specimen. Following species confirmation, hyphal growth of all candidate species on agar media (MEA, 12.5 g/L Bacto malt extract, 15 g/L Bacto agar; Nobles 1948) and in liquid broth media (12.5 g/L Bacto malt extract) was observed to better inform candidate selection, with profuse or rhizomorphic hyphal growth noted as desirable traits and poor growth in liquid broth a criterion for exclusion. Prior to commencement of formal testing, five trial candidate species were selected. Cultures of *Irpex lacteus, Hypsizygus ulmarius, Pleurotus ostreatus, Fomitopsis betulina,* and *Picipes rhizophilus* met all desired criteria and were selected for further testing.

The selected fungal candidates are all saprotrophic, non-pathogenic basidiomycete species (mushroom fungi), and were maintained in 100 x 15 mm polystyrene Petri dishes on MEA at room temperature.

2.2.2 Liquid broth

On 13 January 2021, thirty 1 L thick-walled glass beakers were filled with 500 mL distilled water and 6.25 g of malt extract. Each beaker was homogenized using a magnetic platform and stir bar until clear. The beakers were then covered with a double layer of aluminum foil and autoclaved for 20 minutes at 121 ^{o}C and 15 psi. Once cooled, the covered broth was transferred to the laminar flow hood (LFH) for inoculation. Three beakers per experimental species were inoculated from agar culture. To standardize inoculation, a 7 mm punch was used to take five disks of agar from the active growing edge of agar plate containing experimental cultures. The five agar disks were transferred directly to a single beaker of broth, briefly removing and then replacing the sterile aluminum foil. This process was repeated for 12 of the 15 beakers. The three remaining beakers were to be included in control slurries, and as such were inoculated with sterile agar disks using the same procedure but no fungal cultures. Post-inoculation, broth cultures were placed on a New Brunswick G10 gyratory shaker platform and rotationally incubated at 100 rpm and room temperature (approximately 21 ^{o}C) for a period of seven days (Figure 2.1).

Figure 2.1. Liquid broth culture of *Irpex lacteus*. Shown in 12.5 g/L malt extract broth after incubation period of seven days.

2.3 Slurry preparation

2.3.1 Liquid culture preparation

Immediately prior to experimental slurry formation, incubated liquid cultures were pulse blended to disperse aggregate mycelial growth. An immersion hand blender was soaked in fresh 15% bleach (0.75% sodium hypochlorite) to sterilize for a period of 15 minutes, then rinsed with 70% ethanol within the LFH. Liquid cultures were transferred to the LFH and pulsed for 3 seconds with the hand blender. After each liquid culture, the blender was rinsed in ethanol and any excess ethanol was shaken off. Once blended, the cultures were re-covered with sterile aluminum foil and transported to the University of Western Ontario greenhouse preparatory room.

2.3.2 Slurry preparation

Directly after pulse blending the liquid broth cultures, slurries to be used in greenhouse pot trials were formulated. For each greenhouse trial, six slurries were created. Five slurries were experimental treatments, each containing one species of fungus. The remaining slurry formed a substrate control, containing the liquid broth with no fungal inoculum. Slurries were prepared in 20 L pails with lids. Prior to use, all buckets and lids were wiped with 70% ethanol. Ground, non-sterile agricultural substrate (*Z. mays* or *T. aestivum* in alternating trials) was loosely packed into 1 L beakers to measure out 3 L per pail. Three liquid cultures (1.5 L) of a single species of experimental fungus were then added to the same pail, and the pail was labeled. Finally, 3.5 L of tap water was measured using a 1 L graduated cylinder and added to each pail. The lid was then closed firmly, and the pail agitated by vigorous shaking for 30 seconds and set aside. This process was repeated until six slurries were completed.

2.3.3 Slurry maturation

After formulation, the slurries were matured in the greenhouse potting room at the University of Western Ontario for a period of 5 days (Figure 2.2). Each day, the slurries were vigorously shaken for a period of 30 seconds to disperse the fungal inoculum within and to stimulate aerobic growth (Kim et al., 2010). Slurries remained sealed through the duration of the incubation period.

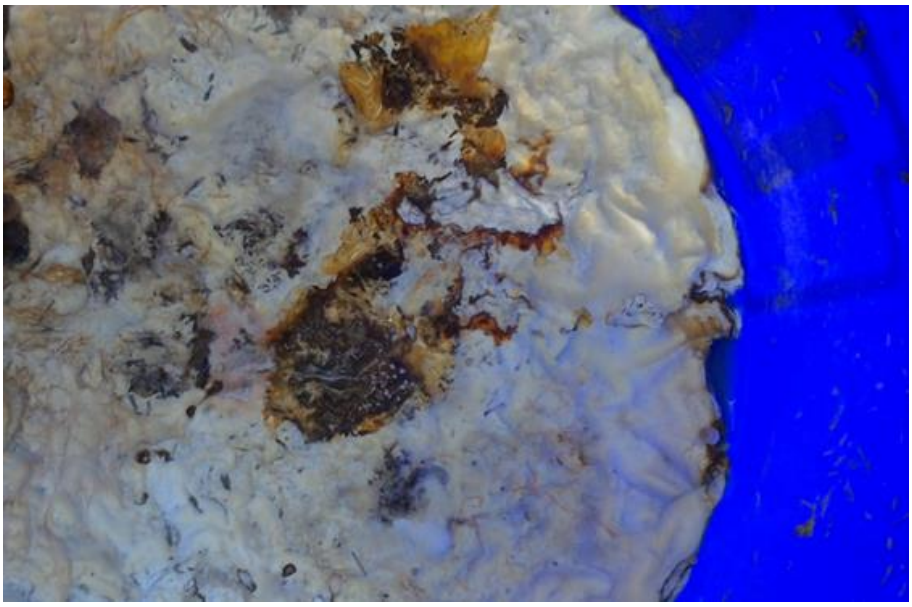

Figure 2.2. Top-down view of over-matured slurry. This *Irpex lacteus* slurry was matured for much longer than five days, resulting in a visible mat of mycelium forming on the surface.

2.4 Greenhouse Trials

2.4.1 Preparatory methodology

Soil used was a 50:50 mixture of field soil from the Environmental Sciences Western Field Station and general-purpose Pro-Mix HP growth medium with mycorrhizae (Premier Tech Home and Garden, Rivière-du-Loup, Quebec, Canada). Soil was combined volumetrically and mixed by hand to ensure homogenization. Prior to potting, Ag. Distributors Inc. All Purpose Plant Food slow-release fertilizer (10-10-10) was added to the soil mixture at a rate of 30 mL per 20 L of soil. Thirty-five 47 cm x 12.7 cm plastic window box planters were used per greenhouse trial. Five planters were used for each of the five fungal species, with the remaining ten planters used as two control treatments. Five planters were used as a substrate control containing the

prescribed amount of slurry without experimental fungal inoculum, and five were used as a no application control to which no slurry was added.

The planters were filled with the soil mixture to a depth of 15 cm. Approximately 54 (1 cm^3) of *Chenopodium album* seeds were added to each planter, and softly raked into the soil with a small hand rake. Fungal slurry (1 L per planter) was added to the soil surface of each planter using a thick-walled 1 L beaker. This rate of slurry application was calculated to be 16.75 L/m^2 (approximately 16.75 mm depth before soaking in). The slurry was poured evenly over the surface of the planter and allowed to settle undisturbed. Each planter was labelled via small plastic stakes with the substrate and fungus combination it contained.

2.4.2 Greenhouse trial overview

Planters were transferred to a greenhouse bench, elevated off the floor on large mesh racks and surrounded by wire frame cages to protect against mice (Figure 2.3). The wire frame cages were of thin gauge, as not to impact light intensity or restrict air flow. Watering took place each day for a duration of five seconds per planter using a shower head hose attachment. The five second duration was measured volumetrically and determined to provide about 750 mL of water.

Figure 2.3. View of eight replicate planters in protective cage. The closest replicates show visible soil surface, indicative of no application control treatments to which no slurry was added. *Zea mays*, the crop utilized in the study, is also visible.

On day five, initial fungal mat strength tests were conducted. These tests were conducted using a 6 mm soil penetrometer (Humboldt Manufacturing, Elgin, IL, USA) and recorded in kg/cm^2. In each planter, five equidistant central sites were tested as per manufacturer instructions (Figure 2.4). The sites utilized for strength testing created five 12 mm depressions in the surface. These sites were used to facilitate crop seed (*Z. mays*) planting without incurring additional disturbance to the substrate.

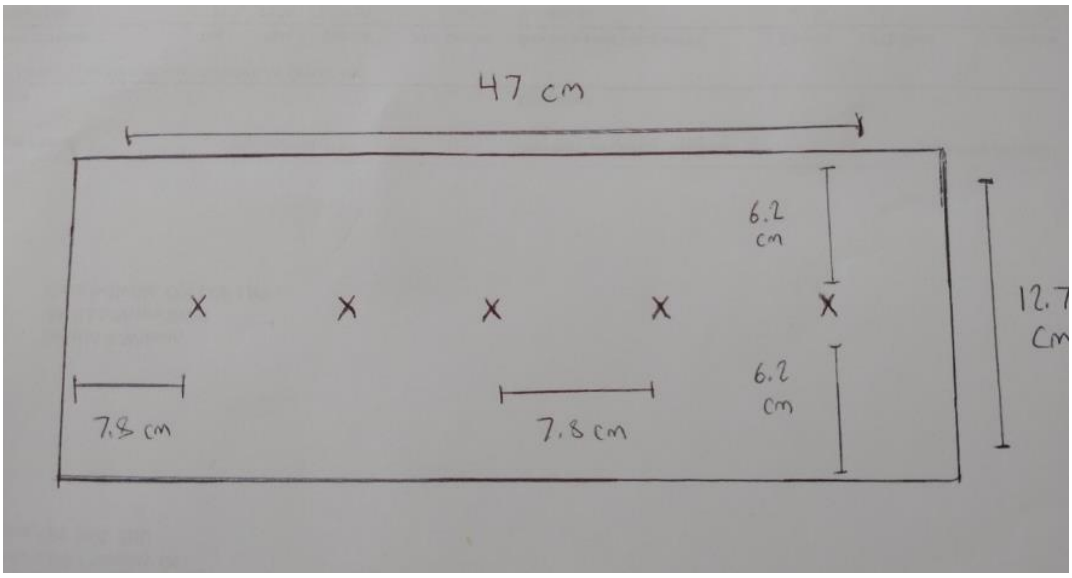

Figure 2.4. Diagram of strength test sites in one planter. The "x" symbols on the diagram indicate strength test/crop planting sites

Three days prior to strength testing (day two, one day post slurry application), corn seeds were incubated in a shallow dish lined with moist paper towel. For all greenhouse trial replicates, the selected crop seed was 2019 season field *Zea mays*. Approximately 200 seeds were placed within the dish, and then covered with another layer of moist paper towel. The dish was then covered with a glass panel to retain humidity. The seeds were allowed to incubate at 21 ^{o}C until they were sown on day 5. When sown, seeds with visible signs of germination were selected to ensure viability of the individual plant. Watering did not occur on day five until after completion of the strength testing and planting of crop seeds. Watering helped cover the crop seeds with soil and substrate to mitigate possible desiccation without further disruption to the surface.

2.4.3 Trial progression and data collection

On day 12 (one week after crop planting) counts of weed prevalence and crop plants were conducted. For every planter, five viable crop seeds were sown, so crop seed counts were conducted solely to detect potential inhibitory effects posed by the specific treatment. As the field soil contained seed of various weed species, weed seedling counts were divided into three categories, distinguished visually: *C. album* (controlled), monocot seedlings (uncontrolled), and other dicot seedlings (uncontrolled). *Chenopodium album* seedlings were easily distinguished by their slender oval cotyledons, tall slender stem, and ovoid first true leaves. Monocot seedlings were distinguishable by their parallel venation and slender leaves, whereas dicots possess broad leaves and branching venation. Seedling counts were recorded on a per-planter basis. Identical counts were conducted on days 19, 26, 33, and 40, following the same structure and procedure outlined above (Table 2.1).

Strength testing was repeated on day 40 for all planters, in accordance with the protocol outlined on day 5. Four sites were sampled equidistantly between corn plants, as well as one additional site equidistant to the outermost corn plant and the edge of the planter, yielding an equal number of strength values as the initial test. Crop plants were harvested from the planters taking care to gather all plant tissue (above and below ground). Any adherent soil was washed away from the plant roots. Harvested plant tissue from a single treatment was aggregated by treatment into one container to be weighed. The wet mass of each treatment (5 planters) was weighed in grams. The containers were then dried for a 72-h period using a hot air convection drier at 70 ^{o}C. After the 72-h drying period, the dry mass of each treatment was recorded.

Table 2.1. Itinerary table describing the activities undertaken during the greenhouse trials, and their corresponding day.

Day	1	5	12	19	26	33	40
Activity	- Sow weed seed and apply slurry	- Sow crop seed - Initial strength test	- Crop seedling count + weed stem count	Crop seedling count + weed stem count	Crop seedling count + weed stem count	Crop seedling count + weed stem count	- Crop seedling count + weed stem count - Final strength test - Crop biomass testing

2.5 Field trial

2.5.1 Logistics and preparation

A field trial was conducted in the spring of 2021 as the primary method of in situ testing, with the goal of providing a real-world scenario simulation and scalability information. The trial was conducted on an active agricultural field owned by the partner organization Natures Balance, located at 157366 7th Line, Meaford, ON. Fourteen plots measuring 10 ft (3.05 m) x 15 ft (4.57 m) (13.9 m^2) were arranged in a 7x2 grid formation, with one plot of each treatment. Six plots were replicates using *Z. mays* as the slurry substrate (i.e., five fungal species and substrate control) and 6 plots were replicates using *T. aestivum* as the slurry substrate. The two remaining plots had no slurry applied (see Fig 2.5).

F. bet Straw	P.rhi Corn
P. ost Corn	None Straw
Irpex Straw	None None
F.bet Corn	Hyps Straw
P.rhi Straw	P.ost Straw
Irpex Corn	None Corn
None None	Hyps Corn

Figure 2.5. Layout of species-substrate plots in field trial. Each square represents a 10 x 15 ft replicate plot. Upper text in each box indicates fungal species (F. bet = *Fomitopsis betulina*, Hyps = *Hypsizygus ulmarius*, P. ost = *Pleurotus ostreatus*, P. rhi = *Picipes rhizophilus*); bottom text indicates substrate used.

Liquid fungal cultures were grown in 10 L Pyrex bottles using identical methodology as greenhouse slurry liquid cultures. Pyrex bottles were filled to 5 L for safe autoclave sterilization. Due to limited availability of large vessels, one liquid culture was divided in half, with 2.5 L being utilized in the *T. aestivum* slurry, and 2.5 L utilized in the *Z. mays* slurry. Slurries were formulated on site in 125 L pails five days prior to application. Eighty L of slurry was formulated for each plot, about one third the rate of application used in greenhouse trials (5.74 L/m^2), based on both logistical constraints as well as costs as a potential product.

Each slurry contained 75 L of water, 35 L of ground substrate, and 2.5 L of liquid fungal culture. Each barrel was covered with a lid and mixed by hand using a large wooden stir rod daily. The stir rod was wiped clean with 70% ethanol between mixings. The slurries were matured for 5 days at 21 ^{o}C in a climate-controlled environment, then applied to field plots by hand on 1 May 2021.

2.5.2 Data collection

Beginning seven days after slurry application, weed stem counts were conducted on days 12, 19, 26 and 33. Weeds were counted using the same categories as in the greenhouse trials (section 2.3.3). Strength testing was not conducted due to the sparsity of the mat present.

2.6 Statistical analysis

Means, standard deviations, and standard errors were calculated with summarySE in the package Rmisc (Hope, 2013). The effects of substrate type and fungal species used were treated as main effects ($\alpha = 0.05$) in an analysis using two-way analyses of variance (ANOVA). Type II sum of squares were utilized as sample sizes of groups were equal. Prior to ANOVA, Q-Q plot of standardised residuals was used to discern there were no concerning deviations from normality. Additionally, Levene's test determined significant heterogeneity of variance ($p<0.05$), however as sample sizes are equal ANOVA is robust to violations of homogeneity of variance. In instances where ANOVA returned significant main effect(s), Tukey's multiple comparison test was utilized to determine inter-level significance. All statistics were run in RStudio (version 1.2.5033) using R version 4.0.0 and the packages MASS, Rmisc, and tidyr (Hope, 2013;

Venables & Ripley, 2002; Hope, 2013; Horikoshi & Tang, 2019; Wickham et al., 2019; Wickham & Henry, 2019; RStudio team, 2019; R core team, 2019).

3 Results

3.1 Greenhouse trials

Between January and June 2020, eight replicate greenhouse trials were conducted. Some mycelial growth was observed in greenhouse trials, but the profuse mat cover that was predicted failed to form in any treatment.

3.1.1 Weed stem counts

3.1.1.1 *Chenopodium* stem counts in greenhouse trials

Reduction in stem counts was observed for both species and substrate treatments (Table 3.1; Figure 3.1). No statistically significant interactive effect was observed between species and substrate treatments (Table 3.1). No application control replicates, where no fungal inoculum or slurry of substrate was applied, had the highest *C. album* counts, compared to other treatments (Figure 3.1). Within the substrate control treatments, the wheat (*T. aestivum*) substrate had 23% higher weed stem counts compared to corn (*Z. mays*) substrate. In general, replicates treated with *P. ostreatus*, *I. lacteus*, *H. ulmarius*, *P. rhizophilus*, *F. betulina*, and the substrate controls all had lower *C. album* counts compared to the no application control.

Weed stem counts in treatments of *Picipes rhizophilus* combined with the wheat (*T. aestivum*) substrate averaged 59% of those in no application controls. The treatment of *I. lacteus* combined

with corn (*Z. mays*) substrate had the lowest average weed stem counts, representing an 82% reduction compared to averaged no application control counts, however this was not significantly different from all other substrate-inoculum combinations. The effect of the substrate control treatments (i.e., substrate with no fungal inoculum) with the wheat (*T. aestivum*) substrate on inhibiting weed stem growth was statistically comparable to all other treatment combinations (Figure 3.1).

Table 3.1. Summary statistics (p and F-values) of two-way ANOVAs for weed stem counts fungal mat strength tests, and crop weights measured on *Zea mays* plants in greenhouse trials. Plants were grown under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays* and *Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina*, *Hypsizygus ulmarius*, *Irpex lacteus*, *Pleurotus ostreatus*, and *Picipes rhizophilus*). Bold values are significant ($\alpha = 0.05$).

	Species		Substrate		Species × Substrate	
Greenhouse Trials	p-value	F-value	p-value	F-value	p-value	F-value
Chenopodium count	**<0.0001**	**92.21**	**<0.0001**	**21.67**	0.09	1.84
Monocot count	**0.01**	**2.65**	0.08	3.02	0.91	0.34
Dicot count	0.65	0.70	0.50	0.45	0.70	0.64
Strength	0.89	0.38	0.49	0.48	0.73	0.60
Crop mass (wet)	0.17	1.59	0.89	0.02	0.82	0.48
Crop mass (dry)	0.13	1.78	0.83	0.04	0.82	0.47

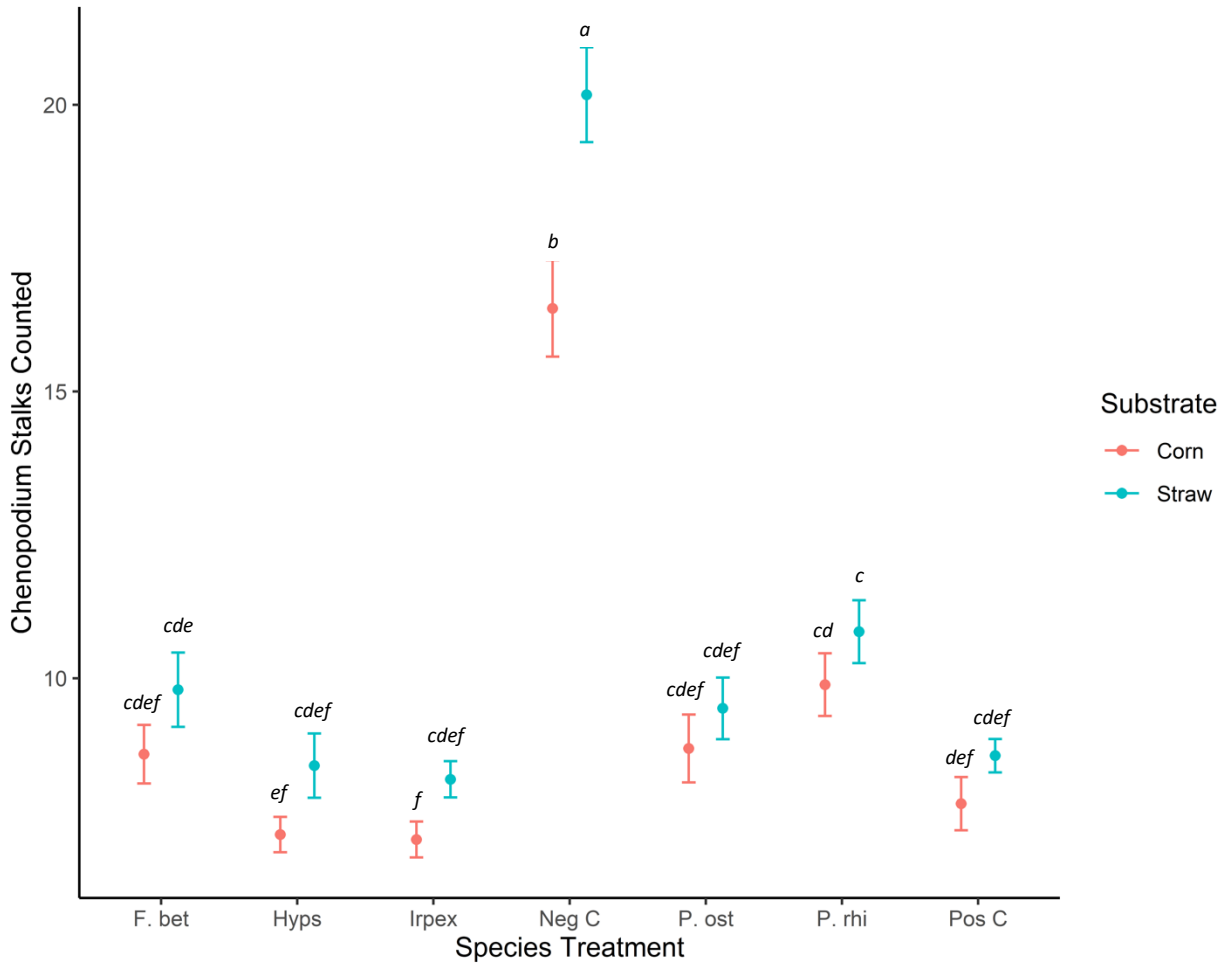

Figure 3.1. Mean stem counts (±1 SE) of *Chenopodium album* measured in greenhouse trials. Trials were conducted under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays and Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina* "F. bet", *Hypsizygus ulmarius* "Hyps", *Irpex lacteus* "Irpex", *Pleurotus ostreatus* "P. ost", and *Picipes rhizophilus* "P. rhi"). Two control treatments were used, substrate control (no inoculum "Pos C") and no application control (no substrate or inoculum, "Neg C"). Though no substrate was added, the colour difference in no application control distinguishes replicates grown in conjunction with each substrate during greenhouse trials. Lettering indicates significance as determined by Tukey's multiple comparison test.

3.1.1.2 Monocot stem counts in greenhouse trials

Variation in monocot stem counts was observed across species treatment levels (Table 3.1; Figure 3.2). There was no statistically significant effect observed between substrate treatments on monocot weed stem numbers (Table 3.1). Additionally, no statistically significant interactive effect was observed between species and substrate treatments on monocot weed stem numbers (Table 3.1). *Pleurotus ostreatus* combined with wheat (*T. aestivum*) substrate had 64% fewer monocot stems than the substrate control treatment (no fungal inoculum added) combined with corn (*Z. mays*) substrate (Figure 3.2). It should also be noted that the average monocot stems counted per replicate was low, with a total average of 1.1 stems counted per replicate. Monocot seeds were not controlled in the experiment, with their main source stemming presumably from wild seeds present in the field site soil utilized.

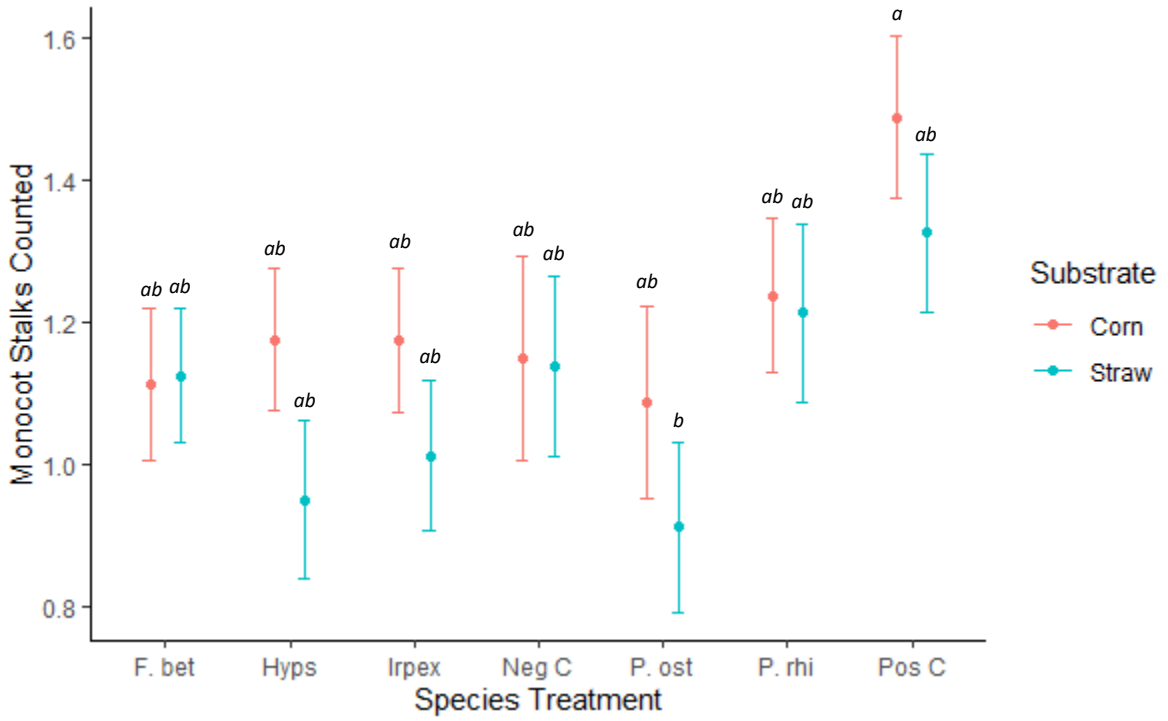

Figure 3.2. Mean stem counts (±1 SE) of monocot species measured in greenhouse trials. Trials were conducted under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays and Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina* "F. bet", *Hypsizygus ulmarius* "Hyps", *Irpex lacteus* "Irpex", *Pleurotus ostreatus* "P. ost", and *Picipes rhizophilus* "P. rhi"). Two control treatments were used, substrate control (no inoculum "Pos C") and no application control (no substrate or inoculum, "Neg C"). Though no substrate was added, the colour difference in no application control distinguishes replicates grown in conjunction with each substrate during greenhouse trials. Lettering indicates significance as determined by Tukey's multiple comparison test.

3.1.1.3 Dicot stem counts in greenhouse trials

There were no significant main effects observed in replicate dicot stem counts for species or substrate treatments (Table 3.1). No statistically significant interactive effect was observed for dicot stem counts in the greenhouse trials (Table 3.1). Dicot seeds (excluding *C. album*) were not controlled, and low total counts averaging 0.6 stems per replicate were observed (Figure 3.3).

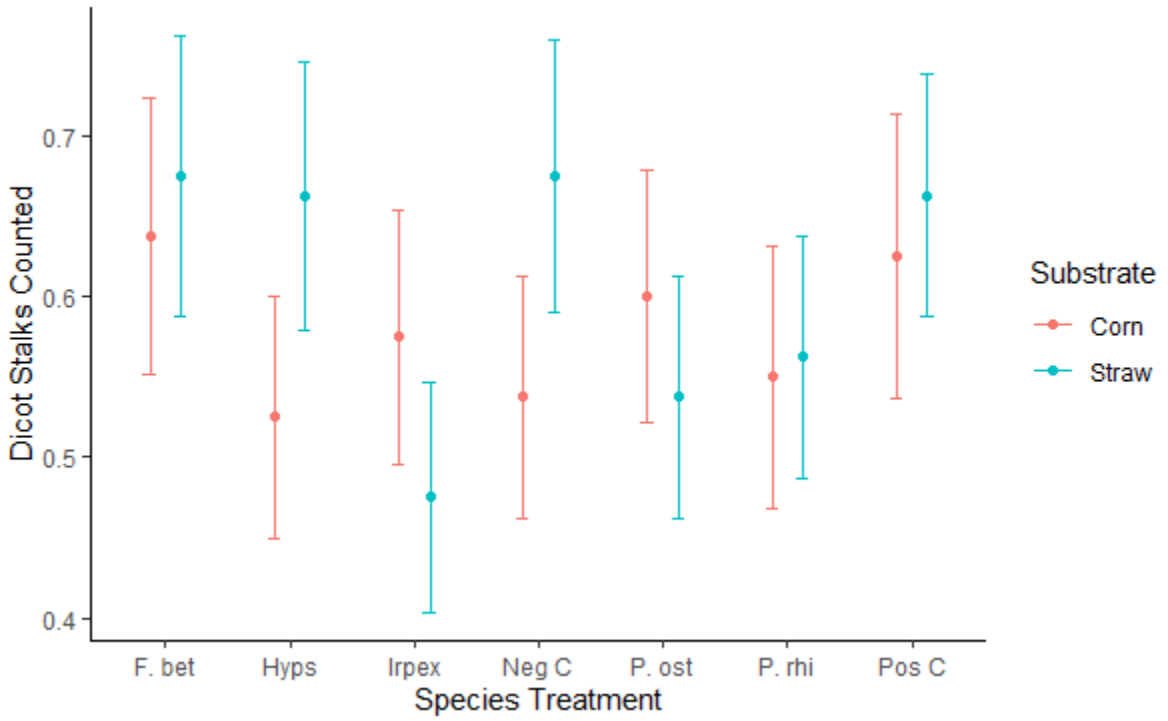

Figure 3.3. Mean stem counts (±1 SE) of dicot species measured in greenhouse trials. Trials were conducted under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays and Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina* "F. bet", *Hypsizygus ulmarius* "Hyps", *Irpex lacteus* "Irpex", *Pleurotus ostreatus* "P. ost", and *Picipes rhizophilus* "P. rhi"). Two control treatments were used, substrate control (no inoculum "Pos C") and no application control (no substrate or inoculum, "Neg C"). Though no substrate was added, the colour difference in no application control distinguishes replicates grown in conjunction with each substrate during greenhouse trials.

3.1.2 Mat strength

Some mycelial growth was observed in the greenhouse trials, but the profuse mat cover that was predicted failed to form in any treatment. There were no significant main effect differences in the greenhouse mat strength measurements for species, substrate treatments or their interaction (Table 3.1; Figure 3.4).

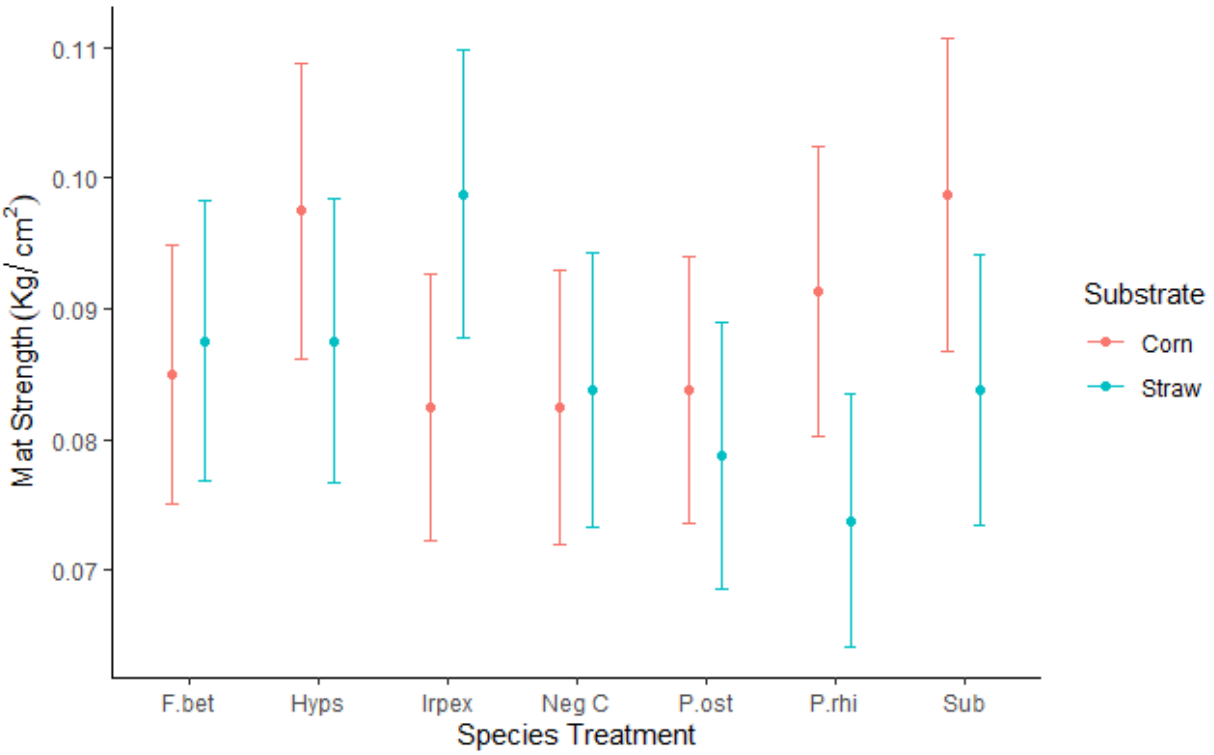

Figure 3.4. Mean strength values (±1 SE) of fungal mat measured in greenhouse trials. Trials were conducted under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays and Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina* "F. bet", *Hypsizygus ulmarius* "Hyps", *Irpex lacteus* "Irpex", *Pleurotus ostreatus* "P. ost", and *Picipes rhizophilus* "P. rhi"). Two control treatments were used, substrate control (no inoculum "Sub") and no application control (no substrate or inoculum, "Neg C"). Though no substrate was added, the colour difference in no application control distinguishes replicates grown in conjunction with each substrate during greenhouse trials.

3.1.3 Crop yield

3.1.3.1 Crop wet mass

There were no significant treatment differences in measured wet mass (above and belowground biomass) of *Zea mays* grown as a proxy crop in the greenhouse trials (Table 3.1; Figure 3.5), nor was a significant interaction effect observed.

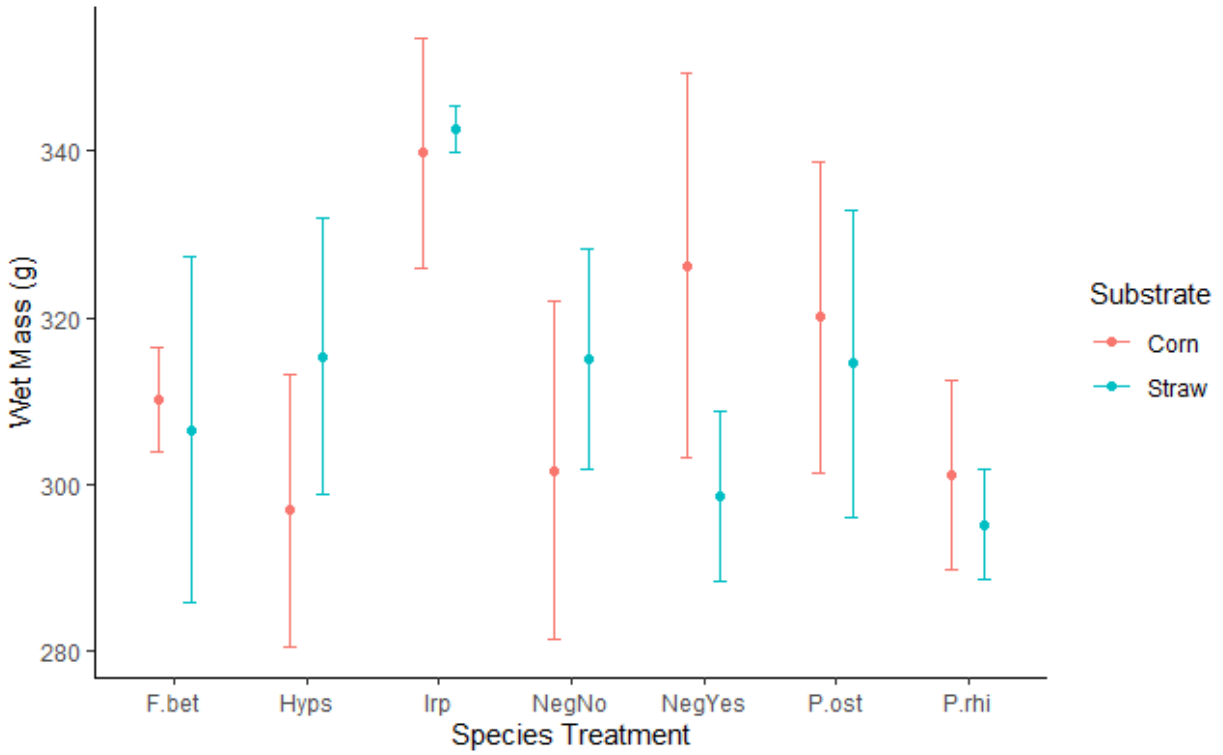

Figure 3.5. Mean crop wet mass values (±1 SE) of *Zea mays* harvested in greenhouse trials. Trials were conducted under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays and Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina* "F. bet", *Hypsizygus ulmarius* "Hyps", *Irpex lacteus* "Irpex", *Pleurotus ostreatus* "P. ost", and *Picipes rhizophilus* "P. rhi"). Two control treatments were used, substrate control (no inoculum "NegYes") and no application control (no substrate or inoculum, "NegNo"). Though no substrate was added, the colour difference in no application control distinguishes replicates grown in conjunction with each substrate during greenhouse trials.

3.1.3.2 Crop dry mass

There were no significant differences in measured dry mass (above and belowground biomass) of *Zea mays* grown in greenhouse trials (Table 3.1; Figure 3.6). No significant interaction effect

was observed on dry mass of *Zea mays* grown in greenhouse trials (Table 3.1). Dry weight mass harvested from *I. lacteus* treatment in combination with either substrate was consistently at the higher range of all treatments, but not statistically significant ($\alpha = 0.05$).

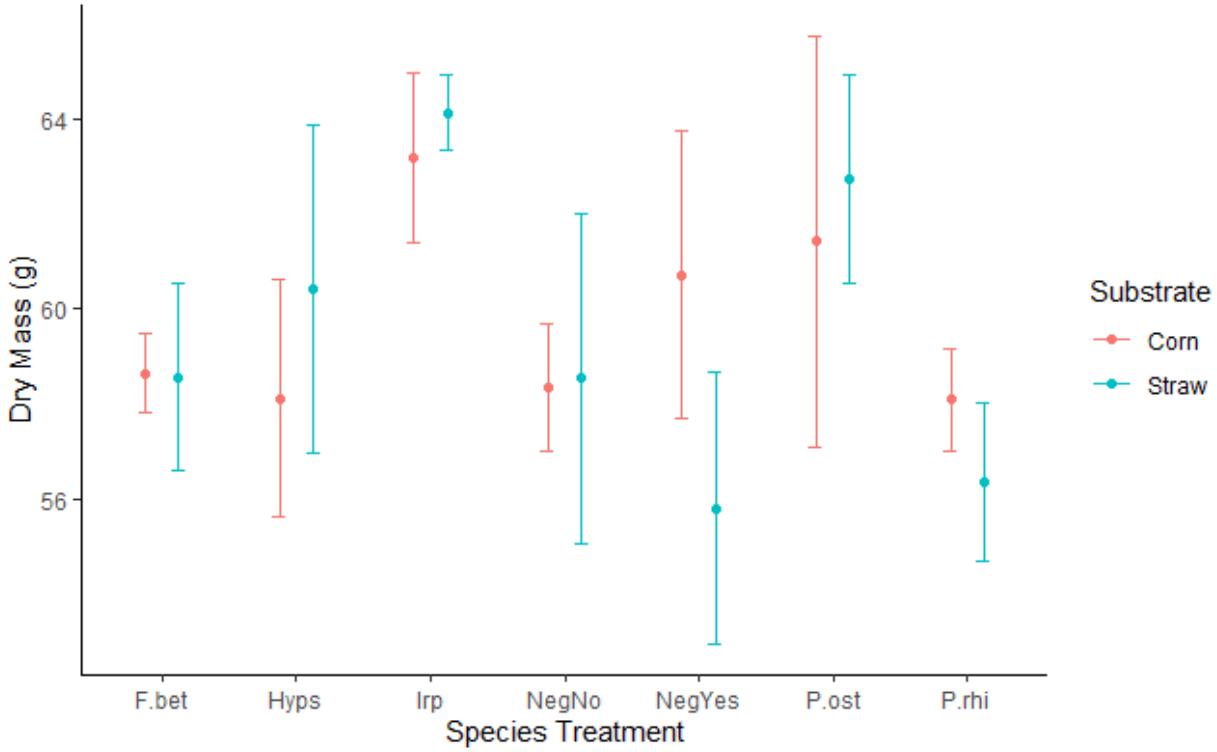

Figure 3.6. Mean crop dry weight values (±1 SE) of *Zea mays* harvested in greenhouse trials. Trials were conducted under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays and Triticum aestivum*), and five fungal inocula (*Fomitopsis betulina* "F. bet", *Hypsizygus ulmarius* "Hyps", *Irpex lacteus* "Irpex", *Pleurotus ostreatus* "P. ost", and *Picipes rhizophilus* "P. rhi"). Two control treatments were used, substrate control (no inoculum "NegYes") and no application control (no substrate or inoculum, "NegNo"). Though no substrate was added, the colour difference in no application control distinguishes replicates grown in conjunction with each substrate during greenhouse trials.

3.2 Field trial

Beginning in May 2021, a field trial was conducted on an active agricultural plot owned by the industrial partner Natures Balance. Substrate application was reduced to scale feasibly and the profuse mat cover that was predicted failed to form in any treatment. Mean values from the trial were recorded and reported (Table 3.2). However, as a single replicate was used for each pairwise combination, additional statistical analysis was not undertaken.

Table 3.2. Mean values for weed stem counts in field setting. Plants were grown under fungal slurry applications comprised of factorial combinations of two substrates (*Zea mays* and *Triticum aestivum*; values from both combined here), and five fungal inocula (*Fomitopsis betulina, Hypsizygus ulmarius, Irpex lacteus*, *Pleurotus ostreatus*, and *Picipes rhizophilus*), or substrate only controls (values for corn and wheat combined), or no application controls (n=2).

Field Trial		
	Monocot	Dicot
Fomitopsis betulina	53.1	29.4
Hypsizygus ulmarius	56.8	29.4
Irpex lacteus	55.4	29.6
Pleurotus ostreatus	57.0	27.5
Picipes rhizophilus	60.4	30.5
No application control	63.9	28.8
Substrate only control	59.4	29.6

4 Discussion

4.1 Slurry maturation

There was no significant increase in penetration resistance in the greenhouse trials in the treatment pots, suggesting limited colonization of the crop residue by the fungal treatments. Mean mycelial mat strength values observed closely resembled the values for the no application control, which represented the strength of the soil surface without any applications added (Figure 3.4). An example of the dense and pliable mat that I hoped for on soil was produced by *Irpex lacteus* colonizing wheat (*T. aestivum*), harvested from the surface of a slurry container four weeks post-inoculation (Fig. 4.1). In this experiment, all slurries were matured for a period of five day before being applied to the soil surface. This maturation time was chosen to permit a higher number of repetitions through limiting the duration of each repetition to 40 days. This timeline was based on literature results, where the growth rates of filamentous fungi within liquid broth media displayed a rapid proliferation period between 42-60 hours post-inoculation (Meletiadis et al., 2001). However, the previously noted study utilized broth media rich in labile nutrients (e.g., Sabouraud dextrose broth) which may produce a more rapid period of fungal proliferation compared to the crop residue substrate used in this experiment. I believe modification of the slurry maturation duration before application could lead to greater mat formation on the soil surface and increased efficacy in weed reduction. Extending the slurry maturation period beyond five days would provide additional time for the fungal inoculum to colonize the substrate. Superior colonization during application could provide increased tolerance to periodic drought conditions, which are known to inhibit fungal growth (Weinberg et al., 2008). Figure 4.1 displays a well-formed mycelial mat, after a maturation of 4 weeks in

slurry. As the desired outcome is for the mat to form after application to the soil surface, a maturation period of longer than 5 days, but shorter than 4 weeks should be investigated in future studies.

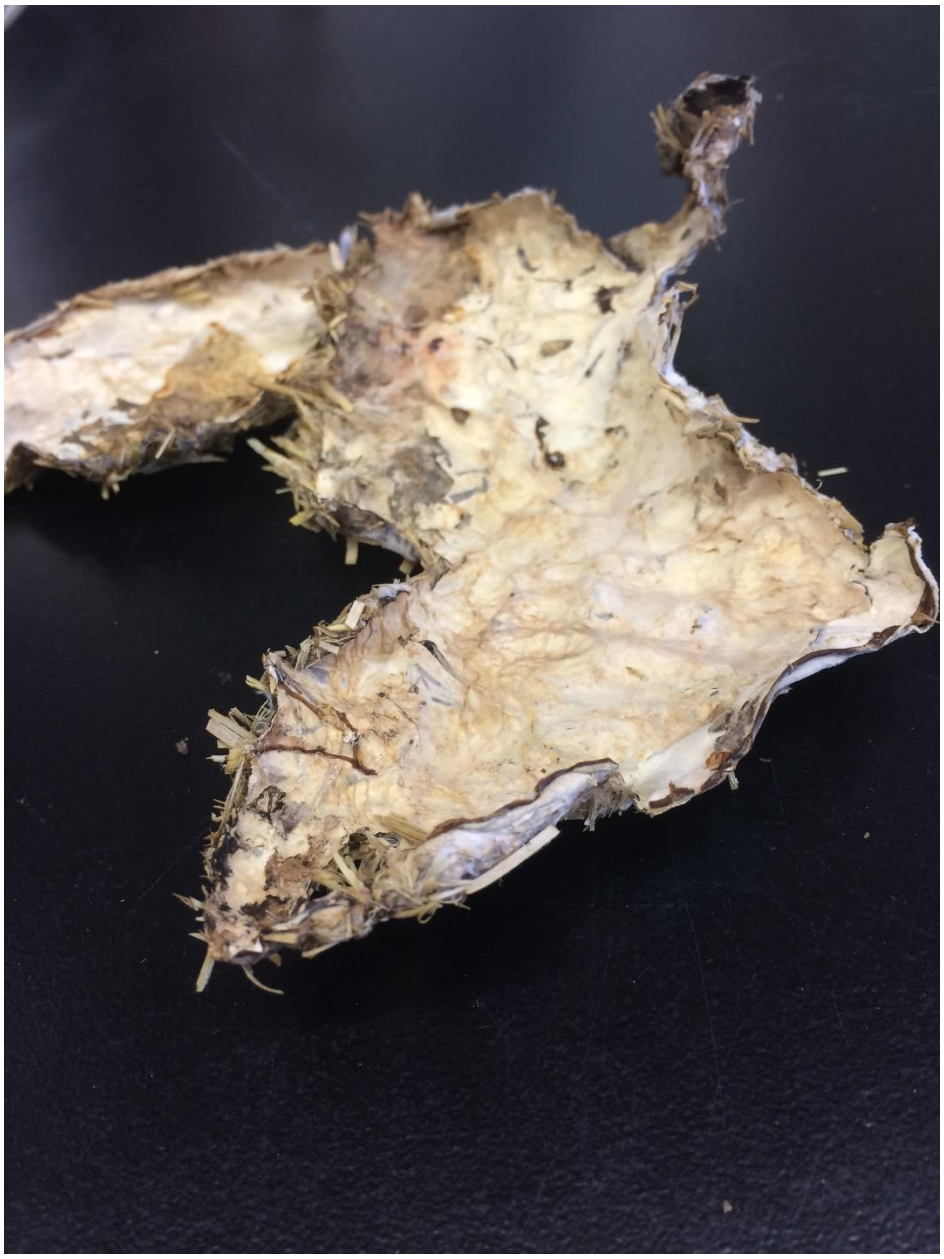

Figure 4.1. Dried mat of *Irpex lacteus* colonizing wheat (*Triticum aestivum*). The mat was matured for a four-week period under standard slurry growth conditions.

The ability to form a profuse mat, as seen within slurry, yet not on the soil surface, suggests that periodic reductions in environmental moisture may inhibit complete colonization of the residue by the fungal mycelium. Moisture fluctuations may represent a significant barrier to proliferation of fungal mycelium within residue once applied to the soil surface. The presence or absence of moisture is a well-known limiting factor in fungal proliferation: harvested grains are best hermetically stored in a reduced moisture content environment to prevent and inhibit detrimental fungal growth (Weinberg et al., 2008). In fact, relative humidity is a stronger predictor of fungal abundance in natural systems when compared to ambient temperature, lending insight into how critical maintaining moisture may prove in successfully forming a soil surface mat (Talley et al., 2002). Although a test could be accomplished in a greenhouse trial by applying a plastic film over the freshly applied slurry on the soil for one to two weeks to see if a better mycelial mat developed, reliance on additional plastic film would make the application impractical in the field.

4.2. Weed reduction and crop yield in mulching practices

Greenhouse trial crop mass did not show significant inter-treatment variation when measured wet (immediately post-harvest), or after drying for a period of 72 hours. The watering regimen utilized during the greenhouse trials was standardized, but no application control treatments likely experienced greater evaporation from the wet soil surface due to their lack of substrate cover, which in turn could have influenced water assimilation and harvested crop weight. The phenomenon of increased soil moisture after mulch application is well documented in the literature (Kader et al., 2017; Qu et al., 2019; Wang et al., 2021). Traditional mulch regimens can influence crop yield by not only reducing weed biomass, but also by increasing moisture content in the covered soil (Petrikovszki et al. 2020).

No significant reductions in weed counts were observed in the field trial. Although a literature review suggests that a fungal-crop residue combination is a novel approach, other weed reduction applications involving plant residue mulch have shown similar results. For example, Duppong et al. (2004) investigated crop biomass and weed reduction in cultivation of two species of medicinal herb, catnip (*Nepeta cataria*) and St. John's wort (*Hypericum perforatum*) under natural mulch treatments. All treatments, as well as the substrate control, showed significant weed biomass reduction when compared to the no treatment no application control (Duppong et al., 2004). In a meta-analysis of 74 studies investigating the effects of mulching on crop yield, mulching practices significantly increased crop yield, up to 60% when compared with no-mulching (Qin et al., 2015).

4.3. Future directions

I found that the traits measured (mycelial mat strength, crop wet or dry mass, and weed counts) were not greatly influenced by treatments of single fungal-residue combinations. This experiment had a narrow focus on a novel idea, the use of a fungal-inoculated slurry of agricultural waste to control weed emergence; because of the novelty of the application, limited guiding literature was available. In future trials, inclusion of additional untrialed fungal species, alternative crop residues, or longer slurry maturation could generate a more effective mycelial mat. This experiment was limited to single fungal-residue combinations, whereas an expanded experiment could be conducted with permutations of multiple fungal inoculants combined with multiple distinct crop residues.

Modulation of crop residue size after processing or additional compound inclusion prior to trialing could also be investigated in an attempt to increase associated mycelial strength or

cellular accessibility of the residues. To illustrate this concept, Appels et al. (2020) treated mycelial films with submersion in 0-32% aqueous glycerol to investigate tensile strength, mycelial density, and strain after drying. The authors found significant increases in tolerated strain and mycelial density produced were observed when submerged in greater than 1% glycerol (Appels et al., 2019). Inclusion of additional compounds into the experimental slurries may provide increased mycelial strength in future studies.

One additional strategy, centered around concerns regarding periods of reduced moisture availability inhibiting growth of the mat, is to apply the slurry to soils in late fall, allowing the mat to colonize during winter months in preparation for the following crop season. This has the advantage of limiting drought and desiccating conditions more commonly experienced during summer months. Also, it is possible that after application, the slurry would be covered by snowfall. This would provide an increased level of protection against varied temperatures, wind, and evaporation compared to if the application were left uncovered. For this approach, fungal species able to survive and thrive in cold conditions would need to be selected. In conventionally tilled crops, such a winter mulch might have benefits in reducing winter soil erosion, whereas in no-till crops, a winter mycelial mulch might prove effective in better decomposition of previous crop residues and release of these nutrients into the field soil.

Overall, in greenhouse trials neither the modulation of fungal treatment, nor the substrate used decreased *Chenopodium album* stem numbers significantly. Limited impacts were also observed in other measures such as monocot stem counts, dicot stem counts, mat strength, and crop mass. Rather, most significant variations between the treatments occurred between the no application control and all other treatments. As it pertains to the qualities monitored in this study, very little

beneficial change was observed between fungal treatment applications and the substrate control, which can be viewed as a traditional mulch comprised primarily of lignocellulosic materials. As the demand for environmentally conscious agricultural techniques continues to grow, potential roles of fungi need to continue to be increasingly explored.

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