Evaluating King Stropharia mushroom as an intercrop in asparagus and tomato production; the potential for utilizing mushroom residue as a dietary supplement for poultry

by

Olalekan Ayodele Sipasi

B.Tech., Federal University of Technology, Akure, 2007
M.S., University of Ibadan, 2014

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Horticulture and Natural Resources
College of Agriculture

KANSAS STATE UNIVERSITY
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Abstract

Stropharia rugosoannulata (L.), the King Stropharia or Wine Cap mushroom, is a wood-loving fungus that grows well on organic mulches. Some vegetables can be successfully grown using organic mulches like woodchips or straw. As a result, farmers may be able to enhance their income by intercropping Stropharia mushrooms with their vegetable crops. However, no study has been identified that assesses this strategy's potential or effect on vegetable yields. Two studies were carried out in the Kansas State University Willow Lake Student Farm, Manhattan, KS to evaluate King Stropharia mushroom as an intercrop in vegetables (Asparagus and Tomato) production, as well as a review paper on the potential of utilizing Spent Mushroom Substrate SMS as a dietary supplement in poultry production.

The first study looked at intercropping King Stropharia mushrooms and asparagus by applying woodchips at three depths (4, 8, and 12 in), utilized a randomized complete block design (RCBD) with four replications and included bareground controls. Across both 2022 and 2023 asparagus harvest began earlier with decreasing depths of mulch, and therefore lasted longer. Inoculated plots also produced more marketable spears than uninoculated plots. And, 12 in woodchip mulch significantly inhibited asparagus emergence and is therefore not recommended. Stropharia-asparagus intercrop shows great promise. In fact, in 2023, mushrooms were observed in uninoculated plots demonstrating the gregarious growth of Stropharia in woodchip mulch on asparagus plots.

The Second study evaluated King Stropharia mushrooms intercropped with tomato using woodchips, wheat straw, and soy straw (all at 6 in depth). The experiment also utilized a randomized complete block design (RCBD) with four replications and included bareground controls. The tomato study also included a positive mulch control using black plastic. In the fall

of 2021, black plastic, inoculated wheat straw, and uninoculated soy straw and woodchip mulch provided earlier harvest than unmulched plots, while the soy treatments and bareground and black plastic controls increased total fruit numbers. In 2022, overall tomato production decreased, resulting in no marketable fruit. These results suggest that a Stropharia-tomato intercrop may not be feasible without additional work. The relative success in asparagus plots supports a need for further research to evaluate other Stropharia-vegetable [or fruit] intercrop candidates and/or strategies to improve compatibility.

The third chapter was a review paper on the potential of utilizing spent mushroom substrate as dietary supplement in poultry industry. Mushrooms produced enormous residue especially from the growth substrate that end up in landfill and causing several environmental hazards. Research has shown that for every 1kg of culinary mushrooms produced there are 3.25 – 5 kg of SMS, which is able to as dietary supplement into poultry feed directly or indirectly through being fed to mealworms, that are then utilized as protein source for poultry feed. Even as this is a viable solution, it is worthy of note that the inclusion ratio of SMS in poultry and mealworm feed needs to be more studied.

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Dedication

This work is dedicated to my unborn child

Chapter 1 - Introduction

Agricultural mulches are used to enhance the soil environment to promote plant growth (Vandenberg and Tiessen 1972, Bot and Benites, 2005, Chakraborty et al. 2008). Plastic mulch films, which are used to manipulate soil temperature, increase soil moisture, and block weeds from competing with the cash crop, have been standard in vegetable production since the 1950s (Hill et al., 1982; Shogren, 2000; Schonbeck, 1998; Schonbeck and Evanylo, 1998). In 2012, 89.2 thousand t of plastic mulch was use in North America was anticipated to be 89.2 thousand t in 2012, and is expected to reach 106 thousand t by 2017 (MarketsandMarkets, 2012), it is estimated that the global market for polyethylene mulch films is expected to grow at a rate of 6.5% per year (beginning in 2021) to $15.7 billion USD by 2026 (Markets and Markets., 2012). In commercial tomato production, black polyethyene plastics are usually use for increased yields, rather than bare soil. This is because plastic mulches are simple to apply, store, and transport, their use has significantly increased (Brault et al., 2002; Steinmetz et al., 2016). However, black plastic pose several challenges aside from the high cost, there are also challenges around long-distance transportation of used black plastics to limited processing areas, high cost of recycled resin in comparison to open-market virgin resin, contamination brought on by dirt and mixing several kinds of plastic (resin) (Garthe and Kowal, 1993), in order to avert these menace, many growers decide to use nearby landfills for the disposal of this garbage (Olsen and Gounder, 2001; Shogren, 2000). Nonetheless, some producers burn their plastic garbage, which has a negative impact on both the environment and public health (Shogren and Hochmuth, 2004). Hence, the choice of organic mulches which as beneficial after use effect of retiling into the field for its benefits.

Organic mulches in vegetable production long predates plastic films because they have the potential to improve soil organic matter content, inhibit weed growth, and reduce or stop erosion (Bot and Benites 2005; Nalayini 2007; Kader et al. 2019, Rathore et al. 1998). Crop residue mulching utilizes organic wastes generated from a previous crop left in place, such as straw, maize stalks, or stubble of leafy organic material (Erenstein, 2002; Bot and Benites 2005). Mulching with crop residues has shown promise in the production of yam (*Dioscorea rotundata* Poir; Olasantan 1999), wheat (*Triticum aestivum* L.; Chakraborty et al. 2008), and groundnut (peanut, *Arachis hypogaea* L; Ghosh et al. 2006; Opara-Nadi and Lal 1987). Organic mulches may also be imported from offsite; those mulches include (though, are not limited to) woody materials like woodchips, bark, or sawdust; straw from grain crops; and compost, which is becoming more readily available in areas where municipalities have green waste recycling programs (Merfield 2002). Organic mulches may present other challenges or conditions that are less ideal. Some organic mulches cool the soil relative to bare ground, and contrary to black plastic, which warms the soil (Caldiz et al., 2001; Sinkevičienė., 2009). Though this has proved beneficial for some crops in areas with exceptional heat during the growing season (Sinkevičienė., 2009). While weed control is a primary function of mulch, if the mulch material contains weed seeds or other viable propagules, the mulch may contribute to increased weed pressure (Merfield 2002). Organic mulches eventually break down and need to be replaced, a problem shared with plastic films. However, broken down organic mulches may be safely tilled into the soil, adding organic matter, potentially building soil quality (Bajoriene., 2013; Saroa and Lal (2004)

Crop residues, such as leafy stubble, grain straw, maize (*Zea mays* L.) stalks, and other organic material, have long been used to preserve soil moisture, soil temperature, reduce erosion, increase soil organic matter, and to suppress weed growth/development (Erenstein, 2002; Bot and Benites 2005; Nalayini, 2007; Kader et al., 2019, Rathore et al., 1998). Other types of organic mulch include woody materials like woodchips, bark, or sawdust, and compost, among others (Merfield, 2002). Organic mulches are regularly used in the production of brambles (*Rubus* spp.), tree fruits, and grapes (*Vitis vinifera* L.; citation); and, have also been applied successfully to perennial vegetable crops like yam (*Dioscorea rotundata* Poir; Olasantan 1999) and rhubarb (*Rhuem rhabararum*; citation). Although a primary function of mulch is to suppress weeds, organic mulches may also increase weed pressure if the mulch material includes weed seeds or other viable propagules (Merfield, 2002). By first composting the mulch, this problem might be reduced; but, before applying the compost to the ground, it must be heated to a temperature high enough to destroy any weed seeds (Munn, 1992)

Many ecosystems rely on fungi's capacity to decompose organic materials (Dai et al., 2021). As a result of this decomposition process, the soil environment consistently receives useful amounts of carbon, nitrogen, hydrogen, and other minerals (Dai et al., 2021). Some of these terrestrial fungi are cultivated for human consumption. Over the past 20 years, the global market for edible mushrooms has grown significantly, with yearly production being projected to be in excess of $14 billion USD (Organic Centre Wales, 2006). Currently, 11,898,399 t of culinary and medicinal mushrooms were produced in 2019; China, Japan, India, Iran, the United States of America, Canada, the European Union, Russia, and Australia accounted for 98% of the total output (FAOSTAT, 2021).

King Stropharia (*Stropharia rugoso-annulata* Farlow ex. Murrill) is a wood-loving, terrestrial mushroom with high nutritional and medicinal values (Wu et al., 2012, Liu et al., 2019), which was recommended by the Food and Agricultural Organization for expanded cultivation in developing countries (De Oliveira et. al, 2020). Also known as the wine cap mushroom, King Stropharia is one of the most recently domesticated, non-mycorrhizal, commercial mushrooms. In 1960s' Germany, King Stropharia was originally domesticated for use by small-scale commercial growers (Szudyga 1978). The first success of wine cap mushrooms was recorded in wooden vegetable frames with amended cereal straw as substrate; however, the yield was inconsistent (Szudyga 1978). By 1989, the commercial production of King Stropharia in Europe had reached approximately 1300 $tons \cdot year^{-1}$ (Domondon and Poppe 2000).

Production of King Stropharia is relatively easy and has been shown to be successful on wheat (*Triticum aestivum* L.) or flax (*Linum* spp.) straw (Szudyga, 1978); uncased mixed wood chip/soil substrate and wheat straw, cased with the wood chip/soil mixture (Bruhn, 2010); mulberry sawdust (Li et al., 2012); and, a mixture of rice husk, corncobs, and sawdust as substrate (Gong et al., 2018). Additionally, wood chips from basswood (*Tilia* spp.), poplar/cottonwood (*Populus* spp.), and alder (*Alnus* spp.) supported higher Stropharia yields than wheat straw, especially when the wood chips were obtained in the winter (Domondon and Poppe, 2000; Stamets, 2000). Sawdust, farmyard manure, leaves, and hay suppress King Stropharia production (Szudyga 1978).

Like other wood-loving mushrooms, most commercial production utilizes logs or bagged sawdust as a growing medium (Miller and Sayner 2023). However, the demand for sawdust to produce heating and [meat] smoking pellets, animal bedding, pulp, mulch, and sheet lumberer is increasing pressure on mushroom farmers. Shortages of high-quality oak (*Quercus* spp.) sawdust have recently become more common. As a result, mushroom farmers have started looking for alternative substrates (Royse and Sanchez 2007).. *Auricularia Spp.* mushrooms have proven capable of being intercropped with sugar cane in China, where the carbon dioxide produced encourages the sugar cane's growth (Oei 1991). Amomgst vegetables that has been successfully intercropped in organic and plastic mulches are Asparagus and Tomato’s.

Asparagus (*Asparagus officinalis L.*) is primarily grown for food, and only the very young, spear-shaped shoots are consumed. Asparagus, and other species related to it, are purported to have therapeutic benefits (Pegiou et. al., 2019). Asparagus has been farmed for more than 2,000 years (Pegiou et al., 2019; Li et al., 2021), and at least 60 countries grow asparagus on over 1.6million ha (FAOSTAT, 2021). Fresh asparagus accounts for nearly three-quarters of the global market, with canned and frozen asparagus accounting for the remainder . While China produces over 84% of the global asparagus supply (7.8 million tonnes; Statistica, 2019), North America (Mexico and United States) cultivates 31,800 ha yearly (Drost et al., 2023).

Asparagus production is greatly influenced by environmental factors. Temperature is the main determinant of development and growth, spear yield, and asparagus quality in the majority of asparagus-producing regions (Alam et al., 1998; Srivastava et al., 2018; Hoberg et al., 2003; Heißner et al., 2006). Asparagus spears harvested in the early spring contain more protein, carotenoids, and flavonoids than those gathered in other times of the year (Shou 2007).In most temperate regions, spears are harvested from April to June, but in southern China, plastic-covered tunnels provide a harvest season from March to June in the spring and from September to October in the fall (Chen et al., 2022). Due to cool spring temperatures, even inside plastic tunnels, early asparagus output is constrained and marked by late emergence and poor growth

(Herner, 1993). As a result, new growing methods may prolong harvest periods and reduce production risk in early spring.

Mulches provide a simple and affordable method for preserving soil warmth and prolonging the growing season for many vegetable crops. The output of various horticulture crops can be efficiently increased using this technique, and the quality of the nutrients can be improved (Deng et al. 2022, Dyduch et al. 2014, Ramadhan 2021, Zhang et al. 2021, Wang et al. 2018). Plastic mulches are a staple of vegetable production enabling earlier planting dates in tomato (Singh and Kamal., 2012), hot pepper (Iqbal et al., 2009), watermelon (Parmar et al., 2013), among others (Mahadeen., 2014)." One of the ways mulching benefits crops is by limiting emergence of weeds that would compete with the crop. In asparagus, the newly emerging shoot is the harvestable unit, making plastic mulch films untenable. However, organic mulches, like straw and woodchips, also have the capacity to increase soil temperatures in the early spring (Choi and Chung.,1997; Ramakrishna., 2006). An alternative that may be used to lengthen the harvesting season in early spring in China and speed up the harvesting of green asparagus growing beneath plastic tunnels is black plastic mulch, a recently developed mulching technique. Additionally, the black plastic mulch approach encouraged early spear emergence with improved marketable spear quality as compared to the organic mulch and non-mulching treatment. The diameter, marketable length, and look of the spear were improved by the black plastic mulch. These consequences may be related to how it raises soil and surface temperatures considerably in the spring (Chen et al., 2022).

This study also looked at the intercropping of tomato with organic mulches in mushroom inoculation, with black plastic being the negative control and bare ground the positive control.

Indigenous to the Andean region of South America, the domesticated tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetable crops worldwide (Kumar et al., 2020). Tomato production increased more than 54% between 2000 and 2014, when approximately 170 million tons of fresh and processed tomatoes were produced globally in 2014 (FAO, 2017), on 5 million hectares (FAO, 2017). China is the world's top tomato producer, with the United States, India, the European Union, and Turkey producing significant tomato crops—roughly 70% of the world's tomato supply (Geetha and Rani., 2020). In the United States, roughly 35 billion pounds (16 million tons) of tomatoes were produced in 2015 (Guan et al., 2018); From 2010 to 2016, 3.3 billion pounds of tomatoes were cultivated in fields in the US yearly, which has experienced a downward trend due to opened boarder importation policies from Mexico. (Baskins et al., 2019). Florida and California produce around two-thirds of the nation's fresh tomatoes, while California produces nearly 95% of the processed tomatoes Around 197 million t tomatoes were produced worldwide in 2019 (FAO., 2021), with 75% going toward the fresh market and 25% going toward processing. Third-largest tomato grower in the world is the USA. The top two producers in the USA, accounting for two thirds of the country's total producing area, are California and Florida (Costa., 2018).

Tomatoes are a nutrient-dense food that may be eaten raw or cooked and can contribute significantly to a balanced diet, hence the importance of its cultivation (Bhowmik et al., 2012). Consumers' desire to purchase fresh produce that is safe, environmentally friendly, and cultivated responsibly is expanding in addition to their desire to purchase inexpensive produce. In the United States, customers are even prepared to pay more for food that is grown locally, organically, or with a reduced carbon footprint (Onozaka and McFadden., 2011; Paul and Rana., 2012). Additionally, according to research, from 2002 to 2007, the market share of fresh food farmed locally grew from 4 billion US dollars to 5 billion US dollars (Porjes., 2007; Giovannucci et al., 2010). As the market share grows, the food waste around the world has also being on the increase. According to estimates by the FAO (FAO, 2019), almost 1.3 billion tonnes of the world's annual food production are either lost or wasted. Over the decades, there has been an evolution in the space of animal feeds and feedstuff in relation to plant residue inclusion in animal feed. Crop residues are undoubtedly rich sources of plant nutrients with the potential to increase the agricultural ecosystem sustainability and effective use of marginalized resources. Crop residues should be used and/or disposed of properly; otherwise, it encourages the reckless practice of on-field residue burning and pollution, which results in an irreversible loss of nutrients, soil fertility, and the environment (Neeraj et al, 2023). India for example produces a significant amount of crop wastes, which are used in agriculture, industry, and energy. (Hiloidhari et al, 2014) claims India produces more than 686 million tonnes (Mt) of crop residue every year, of which 368 Mt comes from cereal crops. Among cereal crops, rice and wheat are the dominating crops, contributing about 154 and 131 Mt, respectively, in the total crop residue production. In India, crop residue is mainly used as cattle feed and cooking fuel, whereas in countries like China, Indonesia, Thailand, Bangladesh and Sri Lanka, it is utilized as feedstock in bioenergy generation, organic fertilizers, and paper industry s captured by (Kumar et al, 2023). It is interesting to note that, 3.25 kg of SMS is created for every 1 kg of edible fungi harvested (Zhao et. al., 2012), and in China alone, there were 8.9 million t of edible mushrooms produced in 2015 (Liu 2019), this will produce some 29 million t SMS. In as much as this is conventionally referred to as farm-generated waste, it has created enormous potential in the agricultural industry. The unique and favorable characteristics of mushrooms, particularly their bioactive components, have piqued the interest of researchers and agriculturists alike (Bonanno et al., 2019). These components hold great potential for being utilized as natural feed additives for livestocks, with the aim of enhancing their performance and overall well-being. Mushrooms boast an array of bioactive compounds, such as polysaccharides, polyphenols, and antioxidants, which have been found to have various positive effects on human and animal health. Studies have also shown that these same compounds can also have significant benefits for livestock. Incorporating mushroom-derived additives into livestock feed can lead to improved digestion, enhanced immune function, and increased resistance to stress and diseases (Bonanno et al., 2019). Additionally, these natural additives have the potential to promote weight gain, improve feed efficiency, and even enhance the quality of animal products such as meat and milk. The appeal of using mushrooms as feed additives lies not only in their potential to boost livestock performance but also in their eco-friendliness. Being a natural product, mushroom-derived additives align with the growing demand for sustainable and environmentally friendly farming practices (Bonanno et al, 2019). Mushrooms have long enjoyed popularity due to their health benefits and medicinal and tonic attributes. These remarkable fungi contain a rich array of bioactive compounds that have been harnessed for their therapeutic properties in traditional medicine across various cultures. From boosting immune function and reducing inflammation to providing antioxidants and essential nutrients, mushrooms offer a natural and holistic approach to enhancing overall well-being (Bederska-Łojewska et al 2017). Moreover, ongoing research continues to uncover new and exciting potential uses for mushrooms in modern medicine, making them a captivating subject for scientific exploration and a sought-after ingredient in the culinary world (Teng et al, 2016).

Therefore, the present study will evaluate: 1) the depth of wood-chip mulch impact asparagus production, and how; 2) the effect of intercropped wine-cap mushroom impact asparagus production; 3) the effect of wine cap fruiting bodies production when intercropped with established asparagus using various depths of commercially available, mixed-species, woodchip mulch. 4) the effect of how wheat- and soy-straw mulch perform compared to black plastic mulch and bare ground for tomato production in northeastern Kansas; 5) attempts to determine whether wine-cap mushroom will successfully grow as an intercrop with tomato; and 6) whether or not mushroom inoculation affect the yield and quality of tomato positively or negatively; and 7) a review paper on the inclusion of mushrooms and its residue in poultry feed.

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Chapter 2 - Effect of intercropping asparagus with King Stropharia mushroom in woodchip mulch at three depths

Abstract

Intercropping mushrooms with field-grown vegetables, may provide farmers increased revenues without sacrificing bed space or investing in indoor production facilities. The King Stropharia mushroom (*Stropharia rugosoannulata*), also called wine cap, can colonize organic material, like woodchips or straw mulch, making it a good candidate for intercropping with vegetables that can be grown on organic mulches. However, no research has been identified that evaluates the impact of this strategy on the yields of vegetable. This project seeks to answer three questions: 1) will wine-cap mushroom grow as an intercrop with asparagus 2) will intercropping mushroom impact the yields of asparagus and spear diameter, and 3) will woodchip mulch affect the yield of asparagus and spear diameter at four depths. The experiment utilized a randomized complete block design (RCBD) with two treatments: intercropping – inoculated and non-inoculated and mulch depth – 0 in (control), 4 in, 8 in, and 12 in. Wine cap mushrooms successfully established in the inoculated plots. The depth of woodchip mulch had no effect on the timing or overall yield and marketability of wine cap; though, the shallower 4 in mulch produced larger fruiting bodies, on average, compared to deeper mulch treatments. During the first spring harvest, wine-cap mushrooms were observed on the edges of non-inoculated plots, signaling how gregarious the mushrooms produced with asparagus in woodchip mulch. However, this contaminated our non-inoculated plots; therefore, we were not able to evaluate the effects of mushroom on asparagus growth. Woodchip mulch did not have an impact on the date of first harvest or harvest duration, except at a depth of 12 in, where the start date was delayed, reducing the overall length of the

production season. Relative to bare ground, we saw no increase in the yield of asparagus (by number or weight) with woodchip mulch except in 12 in of woodchips—which decreased the number and weight of harvested asparagus. In 2023, 8 in, non-inoculated woodchips produced spears over 80 % larger, on average, than the 4 in and 12 in, inoculated plots on average. While the experiment did provide proof of concept for intercropping wine-cap mushrooms with asparagus, further research is needed to determine whether intercropped mushrooms have a direct impact on asparagus production.

Introduction

Asparagus (*Asparagus officinalis L.*) is a perennial crop grown for its young, tender shoots which has been farmed for over 2,000 years as a food crop (Pegiou et al., 2019; Li et al., 2021), but is also purported to have therapeutic benefits (Pegiou et. al., 2019). Asparagus is produced on over 1.6 million ha across at least 60 countries and had an estimated global farm-gate value over $13 billion in 2022 (FAOSTAT, 2024). Fresh asparagus accounts for nearly three-quarters of the global market, with canned and frozen asparagus accounting for the remainder (Chitrakar et al., 2019, Global Asparagus Market 2017). In 2022, China led global asparagus production accounting for over 87% (7.68 million t) of the global asparagus supply, followed by Peru, Mexico, and Germany, which all produced more than 100,000 t (377,246 t, 304,822 t, and 110,300 t, respectively; FAOSTAT, 2024). In the same year, the United States ranked tenth in global asparagus production, growing over 24,000 t on about 6,000 ha for a total value of $74.4 million (FAOSTAT, 2024).

The growth, development, yield, and quality of asparagus spears are greatly influenced by temperature (Alam et al., 1998; Srivastava et al., 2018; Hoberg et al., 2003; Heißner et al., 2006). Asparagus spears harvested in early spring contain more protein, carotenoids, and flavonoids than those gathered during other times of the year (Shou, 2007). In the Northern Hemisphere, spears are typically harvested from April to June, but in southern China, plastic-covered tunnels allow harvest to begin in March and repeat in September and October (Chen et al., 2022). Those plastic tunnels help to offset the cool, spring temperatures that limit early asparagus output by delaying emergence and slowing early growth (Chen et al., 2022). As a result, new growing methods may prolong harvest periods and reduce production risk in early spring.

Asparagus is generally considered a poor competitor with weeds as its canopy development is delayed by early-spring harvests and its fine-textured foliage does little to shade out growing weeds. Tillage and/or post-emergent, selective herbicides are generally preferred to reduce weed competition in asparagus (Folwell et al., 1997). However, tillage is not possible in seeded asparagus, except between rows, because of the shallow crowns of the asparagus. Further, the fine texture of asparagus ferns and complete removal of dead ferns in the fall contribute to a lack of soil cover during winter and early spring. As a result, soil erosion has been problematic for producers in areas with high precipitation in the off-season (Warman, 1990). Improperly timed tillage can exacerbate the erosion potential, resulting in shallow crowns that are more vulnerable during subsequent tillage events. While mulch has long been used to limit weed competition in vegetable production, plastic mulches, prevalent in conventional vegetable production, find limited use in asparagus. One study in China reported earlier harvest, increased spear diameter and length, and overall quality improvement when asparagus was mulched with black plastic (Chen et al., 2022). However, a plastic mulch thin enough to allow spear penetration would need to be replaced each year, a process which may damage dormant asparagus crowns and adds a significant cost to growers for purchase, installation, removal, and disposal. Crop residues and other organic materials, such as grain straw, maize (*Zea mays* L.) stalks, wood debris like woodchips, bark, and sawdust, and compost, among others, have a much longer history of use as mulch, with the added benefit of building soil organic matter as they naturally decompose (Erenstein, 2002; Bot and Benites, 2005; Nalayini, 2007; Kader et al., 2019, Rathore et al., 1998). Organic mulches are regularly used in the production of brambles (*Rubus* spp.; Tommila et al., 2022), perennial fruit crops (Hammermeister, 2016), including grapes (*Vitis vinifera* L.; Cataldo et al., 2020); and, have also been applied successfully to perennial vegetable crops like yam (*Dioscorea rotundata* Poir; Olasantan, 1999) and rhubarb (*Rhuem rhabarbarum*; Cojocaru et al., 2016). Although a primary function of mulch is to suppress weeds to improve production, thereby increasing the income of the grower, organic mulches delay spring asparagus without eliminating the need for hand weeding (Kaiser and Ernst, 2020); they may provide refugia for overwintering insects and pathogens (Bohnenblust and Tooker, 2010 ; Douglas and Tooker, 2012 ; Hammond and Stinner, 1987b ; Mischler et al., 2010 ; Thorbek and Bilde, 2004) and increase weed pressure if the mulch material includes weed seeds or other viable propagules (Merfield, 2002).

Intercropping is growing two or more different crops concurrently on a single plot of land, based on the idea that increasing ecological diversity will help offset damage caused by insect pests, diseases, and weeds (Theunissen, 1994; Theunissen and Schelling, 1996; Theunissen, 1997; Baumann et al., 2000). Intercropping in vegetable systems has been documented to increase productivity (referred to as the land-equivalent ratio, or LER), quality, and/or profitability per unit area of numerous vegetable crops grown in field, greenhouse, and hydroponic systems (Varghese, 2000; Yildirim and Guvenc, 2004; Guvenc and Yildirim, 2005; Yildirim and Guvenc, 2005; Guvenc and Yildirim, 2006; Singh et al., 2012; Blair et al., 2016; Kuepper and Dodson (Duncan), 2016; Maucieri et al., 2017). In asparagus, intercropping low-growing legumes between rows may help with both the lack of ground cover and weed control in mature stands (Warman, 1990). In addition to improving soil conditions—especially nitrogen content—an intercropped legumes and asparagus may also limit nutrient loss, erosion, and weed competition (Shafer and Garrison, 1986).

Many ecosystems rely on fungi's capacity to decompose organic materials (Dai et al., 2021). As a result of this decomposition process, the soil environment consistently receives useful amounts of carbon, nitrogen, and other minerals (Dai et al., 2021). Some of these terrestrial fungi produce organs that are edible, nutritious, and, thus, cultivated for human consumption. Over the past 20 years, the global market for edible mushrooms has more than doubled from $19.6 billion in 2002 to $46.5 billion in 2022 (FAOSTAT, 2024). In 2022, 48.3 million t of culinary and medicinal mushrooms were produced globally (FAOSTAT, 2024). China alone produces 94% of the worlds cultivated mushrooms, with no other country producing more than 1% of the supply (FAOSTAT, 2024) The United States of America ranked third in mushroom production in 2022, behind Japan, contributing 318,600 t, worth just over $1 billion (FAOSTAT, 2024; NASS, 2024).

The wine cap (*Stropharia rugoso-annulata* Farlow ex. Murrill) is a wood-loving, terrestrial mushroom with high nutritional and medicinal values (Wu et al., 2012, Liu et al., 2019), which the Food and Agricultural Organization (FAO) of the United Nations recommends for expanded cultivation in developing countries (Ben-Hur de Oliveira, 2020). Also known as King Stropharia, the wine cap is one of the most recently domesticated, non-mycorrhizal, commercial mushrooms. Wine cap was originally domesticated in 1960s' Germany for use by small-scale commercial growers (Szudyga 1978). The first successful crop of wine cap mushrooms was recorded in 'wooden vegetable frames' with amended cereal straw as substrate; however, the yield was inconsistent (Szudyga 1978). By 1989, the commercial production of wine caps in Europe had reached approximately 1300 $t{\cdot}y^{-1}$ (Domondon and Poppe 2000). Now, production of wine caps is relatively easy and has been shown to be successful on wheat (*Triticum aestivum* L.) and flax (*Linum* spp.) straw (Szudyga, 1978); uncased mixed wood chip/soil substrate and wheat straw, cased with the wood chip/soil mixture (Bruhn, 2010); mulberry sawdust (Li et al., 2012); and, a mixture of rice husk, corncobs, and sawdust as substrate (Gong et al., 2018). Additionally, woodchips from basswood (*Tilia* spp.), poplar/cottonwood (*Populus* spp.), and alder (*Alnus* spp.) supported higher yields of wine caps than wheat straw, especially when the woodchips were obtained in the winter (Domondon and Poppe, 2000; Stamets, 2000). Sawdust, farmyard manure, leaves, and hay suppressed wine-cap production (Szudyga 1978). Like other wood-loving mushrooms, most commercial production utilizes logs or bagged sawdust as a growing medium (Nikšić et al., 2022; Royse, 1997,). However, the demand for sawdust to produce heating and [meat] smoking pellets, animal bedding, pulp, mulch, and sheet lumber is increasing pressure on mushroom producers (AGMRC, 2018). Shortages of high-quality oak (*Quercus* spp.) sawdust have recently become more frequent. As a result, mushroom growers have started looking for alternative substrates (Royse and Sanchez 2007).

Intercropping mushrooms with crops and vegetables. Auricularia spp. mushrooms have proven capable of being intercropped with sugar cane in China, where the carbon dioxide produced during fungal respiration encourages the sugar cane's growth (Oei 1991). Although, a Sustainable Agriculture Research and Education (SARE) project report from Tennessee indicated that wine cap did not successfully produce fruiting bodies when intercropped with beet, cabbage, sweet potato, and tomato, likely due to high heat (Wells, 2020). However, despite anecdotal claims of success (Kalenius, 2022), no peer-reviewed reports have been identified that evaluate the potential to intercrop wine-cap mushrooms with vegetable crops in an organic mulch applied to the crop. Further, no studies have been identified that have evaluated the impact of intercropping mushrooms on the vegetable crop they are grown with.

The present study will evaluate the following questions:

1) Does the depth of wood-chip mulch impact asparagus production, and how?
2) Does intercropped wine-cap mushroom impact asparagus production?

3) Can wine cap produce fruiting bodies when intercropped with established asparagus using various depths of commercially available, mixed-species, woodchip mulch?

Materials and methods

The study was conducted at Kansas State University (KSU) Willow Lake Student Farm (WLSF), located at latitude 39.2498 °N, longitude 96.5726 °S and 1,180 ft above mean sea level near Manhattan, Kansas. The soil is Eudora silt loam is coarse-silty, mixed, mesic Fluventic Hapludolls, that is rarely flooded (Wagner et al., 1992; Natural Resources Conservation Service [NRCS], 2021). WLSF falls in a Köppen-Geiger Dfa climate region, categorized as having a humid continental climate with hot summers and year-round precipitation (Köppen.earth, 2024 based on Beck et al., 2023) The site has an annual mean temperature of 53.7 °F, receiving an average 35.8 in of precipitation (National Center for Environmental Information [NCEI], 2024). The site averages 185 frost-free days, and is classified as prime farmland (NRCS, 2021). During the growing season (usually from 15 April through 20 October), the daily low temperatures range from 40.2 °F to 67.5 °F (mean T_{low} = 57.1 °F) and the daily high temperatures range from 65.7 °F to 90.0 °F (mean T_{high} 80.9 °F; NCEI, 2024).

Experimental design: The experiment utilized a randomized, complete block design with four replicate blocks. Treatments included four levels of mulch depth: 0 in/no-mulch (control), 4 in, 8 in, and 12 in; and, two levels of inoculation: inoculated with *S. rugosoannulata* and non-inoculated. Because the no-mulch control lacked suitable growing media for inoculation, there were a total of seven treatment combinations: (1) bare ground/no-mulch control; (2) 4-in inoculated woodchip mulch; (3) 4-in non-inoculated woodchip mulch; (4) 8-in inoculated woodchip mulch; (5) 8-in non-inoculated woodchip mulch; (6) 12-in inoculated woodchip mulch; and, (7) 12-in non-inoculated woodchip mulch The experiment was installed in two rows 100 ft long in the center of five rows of asparagus established two years prior (in 2019). Blocks comprised one half of each row; therefore, each row contained 14 total plots—seven per block. Plots measured 4 ft wide and 7 ft long and were mulched edge-to-edge (except no-mulch/control plots). Along each boundary between an inoculated plot and an non-inoculated plot, a mylar-lined insulation sheet (R-3.7 Reflectix® Reflective Insulation, Reflectix, Inc., Markleville, Indiana, USA) was installed between the plots as mulch was installed as a barrier against cross-contamination of the non-inoculated plots.

Site preparation. Asparagus cv. Purple Passion had been established in the experimental plots 2 y prior to the experiment by digging a trench 8 in deep and laying asparagus crowns in the base of the trench, with crowns faced up, then backfilled with native soil and watered in. On 22 December 2020, each row was rototilled (Model 749, BCS Group, Abbiategrasso (MI), Italy) to a depth of 4 in to incorporate the prior year's ferns and break up the roots and rhizomes of grassy weeds. On 7 May 2021, prior to installing the mulch treatments, each row was rototilled to a depth of 4 in to minimize weed competition and to incorporate a granular, pre-plant fertilizer (Suståne 8-2-4, Suståne Natural Fertilizer, Inc, Cannon Falls, Minnesota, USA) was surface applied at a rate of 80 $lb \cdot N \cdot ac^{-1}$, 20 $lb \cdot P_2O_5 \cdot ac^{-1}$, 40 $lb \cdot K_2O \cdot ac^{-1}$.

Preparing wine-cap spawn. Maple (*Acer* spp.) wood shavings and sawdust (collectively "media") was acquired from a local cabinet maker (Homestead Cabinets, Wamego, Kansas, USA) and hydrated to 75% moisture content by weight. Eighteen autoclavable bags, with 50-micron filters (Size 14A, Unicorn Bags, Plano, Texas, USA), were filled with 6 lb of hydrated media, then autoclaved at 250 °F and 15 psi for 2 h. After cooling for 24 h, each bag was inoculated with 1/2 cup (35 - 40 g) of wine-cap grain spawn (Field and Forest Products, Peshtigo, Wisconsin, USA). Inoculated bags were sealed then incubated at 80 °F for 23 days in complete darkness, until the outer surface of the sawdust was observed to be greater than 90 % colonized by mycelium.

Two yd^3 of a commercial, mixed-species, wood-chip mulch (Hummel Tree Service, Manhattan, Kansas, USA), acquired in early spring 2021, was disinfected by boiling in two batches by loading 1 yd^3 into a 240 gal stock tank, adding water until the chips began to float, and covering the stock tank with multiple layers of burlap, then bringing the water to a boil and holding that temperature (>210 °F) for 2 h. The woodchips were spread on a new tarp in a high tunnel and the process was repeated. The pasteurized woodchips were allowed to cool under ambient conditions for 24 h, then inoculated with approximately 108 lb sawdust spawn. The spawn was mixed into a with a spade that had been pasteurized with the woodchips, and the mixed woodchips were mounded to incubate. The pile was covered with a plastic tarp to reduce contamination, and the temperature was monitored using an analog compost thermometer. When the temperature of the pile exceeded 120 °F two days after inoculation, the tarp was removed. White mycelium was observed over most of the pile within 21 d. However, a gray green mold was also observed, so eight samples were randomly collected from throughout the pile and sent out for PCR testing (Fungal Ecology Lab, Manhattan, Kansas, USA) to confirm both the presence of *S. rugosoannulata* and to identify the contaminant(s). Seven of the eight samples returned as *S. rugosoannulata,* while one was identified as *Fusarium solani* [(Mart.) Sacc. (1881)], a relatively ubiquitous terrestrial fungi with the potential for pathogenicity in solanaceous crops.

Installation of mulch treatments. A wooden frame 4 ft wide, 7 ft long, and 11.5 in tall was constructed of 2 in by 12 in construction lumber; and, mulch treatments were installed on 11 May 2021. Inoculated mulch treatments were installed by layering woodchips into the frame in

2-in layers. This was accomplished by constructing a series of screeds of 2 in by 2-, 4-, 6-, 8-, and 10-in dimensional lumber allowing for layers to be set at 10-, 8-, 6-, 4-, and 2-in depths, respectively; a flat board was used to screed mulch at 12-in depth. Inoculated plots received 4 gal of spawn spread evenly between every 2-in layer (no inoculum was spread on the soil surface or top of the finished bed. Therefore, 4-in, inoculated plots received 4-gal inoculum at 2-in depth; 8-in, inoculated plots received 4 gal of inoculum each at 2 in, 4 in, and 6 in, for a total of 12 gal of inoculum; and, 12-in, inoculated plots received 4 gal of inoculum each at 2 in, 4in, 6in, 8in, and 10 in, for a total of 20 gal of inoculum. Non-inoculated mulch treatments were installed by filling the frame to near its final depth and using the screed to identify and fill in any voids. After each layer, and during bulk filling of non-inoculated plots, water was sprayed onto the beds to both hydrate the mulch to facilitate inoculum transfer, and to achieve relatively consistent compaction across all mulch treatments.

Plot Maintenance. The plots were hand watered twice each day, in the afternoon and evening, when summer temperatures exceeded 90 °F. Weeds were managed by hand and the time it took to hand-weed each plot was measured with a stopwatch on 06/05/2021, 08/21/2021, and 5/13/2022. To ensure consistent measurement of weeding times, the stopwatch was started immediately before a plot was weeded using a machete (to remove top growth) or hand-pulling. When all top growth was removed, the stopwatch was stopped and the time recorded. At the close of each season, all dead ferns and visible weeds were completely removed.

Asparagus harvest. Asparagus spears were harvested every 1-3 d from 23 April to 19 May 2022 and from 13 April to 25 May 2023; there were single extended harvest intervals of 7 and 4 d in 2022, and of 5 d in 2023, though no non-marketable spears resulted. All spears that had reached 6 in height, or taller, were collected during each harvest. Spears were weighed (Valor 7000, Ohaus Corporation, Parsippany, New Jersey, USA), counted, and sorted into groups of marketable and non-marketable spears. Asparagus that were non-marketable were further categorized according to the primary cause of non-marketability, then the number and weight in each category were recorded. Marketable spears were sorted by USDA standards (USDA, 2006), then counted and weighed.

Mushroom harvest. To minimize edge effects, mushrooms were harvested from the center 2 ft, across the width of the bed, and the center 5 ft along the length of each plot. Mushrooms were hand harvested, carefully cut with a just below the mulch surface serrated harvest knife, placed into a harvest crate, and evaluated immediately to avoid moisture loss. During the first mushroom harvest, four mushrooms (each presenting with a different colored/shaded cap), were reserved for identification. As all mushrooms were generally the same shape and within the range of colors expected of wine-cap mushrooms, a spore print test was carried out to confirm the identification (Fig. 2.1). All spore prints supported a positive identification of *S. rugosoannulata*, and no further tests were conducted during subsequent harvests as no other fruiting bodies differed visibly from the normal range of phenotypic expression of the species. Total and marketable number and weight were recorded, and marketable mushrooms were again counted, weighed, and recorded by USDA grade and size (USDA, 2022). Non-marketable mushrooms were categorized by the primary cause of non-marketability (open veil, immature, pest damage, misshapen and other damages), counted, weighed, and recorded.

Procedure for PCR on extended woodchip mulch.

To confirm the identity of substrate colonizing fungi, we PCR-amplified the Internal Transcribed Spacer (ITS) of the ribosomal RNA gene with the forward ITSiF (Gardes and Bruns, 1993) and reverse ITS4 (White et al., 1989) primers in 50 µl PCR reactions. The volumes and final concentrations of reagents were as follows: 2.5 µl forward and reverse primer (0.5 µM), 5 µL 10-2 diluted template DNA, 25 µL of 2X Phire Plant Direct PCR Master Mix, and 17.5 µL molecular grade water. The PCR reactions included an initial denaturing step for 30 s (98°C) and were followed by 35 cycles of 10 s of denaturing (98°C); 30 s of annealing (54°C); 1 min of extension (72°C); and concluding with a 9 min final extension (72°C). The PCR reactions included sterile molecular grade RNA- and DNA-free water as a negative control and Saccharomyces cerevisiae as a positive control. The amplicons were purified and Sanger-sequenced at the Eurofins Genomics (https://eurofinsgenomics.com/en/home/) in both directions. Forward and reverse reads were pairwise aligned and BLASTed to NCBI GenBank to retrieve the closes matches.

Statistical analysis. A one-way ANOVA model was generated with PROC GENMOD in SAS 9.4 (SAS Institute Inc., Cary, NC). The three levels of the mulch effect and the two levels of the inoculation effect were combined to make six different treatments as the fixed effect. The effect combination was processed further using the CONTRAST procedure to compare the control (bare ground) to other treatment effects. No random effects were specified except for the two-year models—to consider the effect of growing season (year); however, growing season data were ultimately analyzed and presented separately. Comparisons were carried out using two-sided tests, with $\alpha = 0.05$. Finally, Tukey's adjustment was applied to pairwise comparisons.

Results

Environmental conditions. The environmental condition (rainfall, maximum air temperature, average air temperature, and minimum air temperature) varied across the growing seasons, as seen in Fig. 2.2.

Asparagus

First harvest date differed by mulch depth in 2022 and 2023 (P = 0.0019 and 0.0110 respectively; Table 21). In 2022, asparagus grown without mulch emerged earlier than 8-in, inoculated and 12-in, mulched plots, and 4-in, mulched plots emerged earlier than 12-in, inoculated plots (Table 2.1). In 2023, asparagus in 4-in, inoculated plots emerged earlier than in plots with 12 in of mulch (Table 2.1).

The average length of harvest was impacted by mulch treatment in both years (P = 0.0002 and 0.0030 in 2022 and 2023, respectively; Table 2.1). In both years, plots with 12 in of inoculated mulch were harvested for fewer days than bare-ground plots; and in 2023, 12-in, inoculated plots were harvested for fewer days than in 8-in, mulched plots and 4-in, inoculated plots. In 2023, 4 in of inoculated woodchip mulch delivered a longer harvest period than 12-in, mulched plots and bare ground produced longer than 12-in, inoculated plots (Table 2.1).

In 2022 and 2023, the total number of spears per plot differed by mulch treatment (P = 0.0071 and 0.0192 respectively; Table 2.1). In 2022, 4-in, inoculated plots produced more spears than plots mulched with 12 in of woodchips; and all plots produced more spears than in the 12-in, inoculated treatment except 8-in, inoculated and 12 in non-inoculated plots (Table 2.1). While in 2023, 4 and 8-in, inoculated plots produced more spears than 12-in, inoculated plots.

Differences in total weight were also present between mulch treatments in both years (P = 0.0153 and 0.0018 in 2022 and 2023 respectively; Table 2.1). In 2022, bare ground and 4 in mulched plots produced greater yield by weight than 12-in, inoculated plots. In 2023, all treatments produced greater yields than 12-in, inoculated plots except the 12 in non-inoculated plots (Table 2.1).

In 2022, treatments had no effect on marketability of the asparagus spears (Table 2.2). While a treatment effect was detected for percent marketable spears by number in 2022 ($P = 0.0190$), pairwise comparisons among all treatment detected no differences. In 2023, percent marketable by number and by weight and the average weight per marketable spear were impacted by mulch treatment ($P = 0.0091$; Table 2.2). The 4 in non-inoculated plots produced a lower percent of marketable spears, by number, than bare ground, 8-in, inoculated and 12-in, mulched plots (Table 2.2). For percentage marketable by weight in 2023, non-inoculated 4 in plots also produced a lower marketable yield than the 8-in, inoculated plots (Table 2.2). The average weight per marketable spear grown in 8 in non-inoculated plots was greater than those grown in 4-in, inoculated and 12-in, inoculated plots ($P = 0.0134$; Table 2.2).

Treatments had no effect on the size distribution (diameter) of marketable spears except for the percent of small-sized spears (5/16 in $\geq$ x < 1/2 in) in 2023 ($P = 0.0065$; Table 2.3). Spears harvested from 4-in, inoculated and 12-in, inoculated plots produced a greater proportion of small spears (by weight) than bare-ground and 4 in non-inoculated plots; and, 8-in, inoculated plots produced a greater proportion of small spears (by weight) than 12 in non-inoculated plots (Table 2.3).

Asparagus grades are primarily determined by spear diameter (Grade 1 is medium and larger with greater color consistency than Grade 2, which comprise small and very small spears) and did not differ due to mulch treatment in 2022 (Table 2.4). However, in 2023, 12-in, inoculated plots produced a greater proportion of Grade 2 spears (by weight) relative to bare-ground and 4-

in, inoculated plots, while bare ground produced a greater proportion of Grade 1 spears (by weight) than 12-in, inoculated plots (*P* = 0.0204 in both years; Table 2.4).

Mushrooms

In 2023, wine-cap mushroom production was insufficient to perform statistical analyses; therefore, the yield of mushrooms is presented for 2022 only. Mulch depth did not produce differences for any measured yield parameter, except the average weight per marketable cap (*P* = 0.0132; Table 2.5). Further, marketable mushroom quality only differed in the percent of small-to-medium Grade 1 mushrooms (*P* = 0.0097; Table 2.6). Mushrooms grown in 4-in woodchip mulch were larger than caps grown in deeper mulch treatments (Table 2.5). The proportion of small-to-medium, Grade 1 mushrooms was smaller in 4-in treatments than in 8 and 12 in of mulch (Table 2.6). Mulch depth only produced a difference in the primary cause of non-marketability for fruiting bodies misshapen enough to be deemed non-marketable (*P* = 0.0411; Table 2.7). In that case, the 8-in, mulch treatment produced a greater proportion of misshapen fruiting bodies than those that emerged from mulch 12 in deep (Table 2.7).

One important note, in the fall of 2022 wine-cap mushrooms were observed on the edges of some non-inoculated plots. No fruiting bodies were found within the center of the beds where asparagus or mushroom data were collected. However, as a result, we opted not to analyze the asparagus data for differences due to inoculation alone.

Discussion

Asparagus Results. Some publications recommend woodchip or straw mulch. Woodchip mulch did not have a significant impact on the date of first harvest or harvest duration for the 4 in or 8 in treatments. However, mulching with woodchips to a depth of 12 in had a negative impact on the start date and overall length of the growing season. The additional distance new spears had to traverse from crown to daylight likely caused the delayed harvest. As our overall yields in 12-in mulch were also less than in shallower mulch treatments, the energy stored in the roots and crowns was likely insufficient for the effort. The depth that crowns are initially buried inversely affects the yield (total weight, spears/plot, spears/plant, weight/spear and weight/plant) of asparagus (Lindgren, 1990); but, depth becomes insignificant after more than four years when the crowns come together to settle at the same depth (Lindgren, 1990; Yeager and Scott, 1939; Young, 1940). Our plantings were only two years old when the mulch treatments were applied, and, therefore, likely impacted by the overall depth after mulching.

Mulching with woodchips has been shown to successfully increase the yield and quality of lettuce (Jenni et al. 2002), tomato (Horimoto et al., 2022), and pumpkin (Splawski et al., 2016), among others. In Turkey, asparagus covered with black plastic mulch produced larger spears and a greater overall yield (by weight) than without mulch, with no increase in the number of spears (Jakše and Maršić, 2015). We saw no increase in the number or weight of asparagus spears grown in woodchip mulch, relative to bare ground. Instead, 12 in of woodchip mulch decreased both the overall number and weight of harvested asparagus spears. In 2023, 8-in, non-inoculated woodchips produced spears over 80 % larger, on average, than the 4-in and 12-in, inoculated plots (Table 2). Given that spears of lower average weight occurred in both shallower and deeper mulches, this result was likely due to random error. Size distribution was not consistently affected by mulch depth, except in 2023, when 12-in, inoculated plots produced a greater proportion of small spears relative to bare ground, and 4-in and 8-in, non-inoculated plots. Jakše and Maršić (2015) found that asparagus grown without mulch (bare-ground control) produced larger spears. Asparagus grades are based, in part, on spear diameter such that spears less than 0.5 in diameter do not qualify for Grade 1 designation. This alone would explain the greater proportion of Grade 2 spears in 12-in, inoculated plots in 2023.

Mushroom results. Mushroom productivity was primarily limited to the first spring following mulch inoculation (in 2022). In 2023, too few mushrooms were observed in the research plots to be able to analyze statistically. Bruhn et al. (2010) experienced a similar reduction in production and recommended trialing fall substrate additions or rotating Stropharia production into other fields/plots.

Woodchip mulch depth had no effect on the timing or overall yield and marketability of wine caps in the present study. The shallower 4-in mulch produced larger fruiting bodies on average, compared to the deeper mulch treatments. Lower spawn rates have been shown to increase the size of oyster (*Pleurotus cornucopiae*) mushrooms (Royse, 2002; Royse et al., 2004). Here, the 8-in and 12-in treatments received 1.50 and 1.67 times more initial inoculum than the 4 in treatments. One theory that may account for larger fruiting bodies in an otherwise identical population holds that greater resource-availability favors the larger mycelial growth necessary to produce [larger] fruiting bodies (Bässler et al., 2014). Further, the smaller surface area:volume ratio of a larger mushrooms may protect the fruiting body from drying (Bässler et al., 2016). And, while we did not measure moisture content of the mulch or the soil beneath it, the relatively high surface area:volume ratio of the shallower mulch would tend toward faster desiccation.

However, given that wine caps tend to be larger than most agaricus types found in U.S. markets, the relatively small caps in deeper mulches may not be a detriment.

While deeper mulches tended to produce a greater proportion of Grade 1 mushrooms (P = 0.1799), the only difference in the size and grade of wine caps in this study were a smaller proportion of small-to-medium Grade 1 mushrooms in 4 in woodchip mulch relative to deeper mulches. We found no references correlating the size of the fruiting body with an increased occurrence of defects affecting marketability. Except for the proportion of misshapen fruiting bodies, which was greater in 8-in mulch than 12 in, the primary causes of non-marketability were not affected by mulch depth. High temperature (Jodon et al., 1981) fluctuations in relative humidity and elevated CO_2 concentrations (Aminuzzaman et al., 2022), and excess sunlight (Stamets, 2000) have been implicated as potential causes of fruiting body deformation. CO_2 concentrations remain relatively stable from day-to-day in the open atmosphere and wine cap is touted as being relatively tolerant of full-sun conditions (Mudge and Gabriel, 2014). The greatest incidence of misshapen caps occurred on 4 June 2022 when the minimum, average, and maximum temperatures at the study site were 13.6 °C, 18.9 °C, and 26.4 °C, respectively—well within the range of temperatures tolerable to wine cap (Bruhn et al., 2010)—and 3 – 4 days after a substantial rain event (>50 mm). The average relative humidity on that date was close to the seasonal average. The most anomalous environmental condition on that date was that it was the first day with a low temperature above 10 °C after the rain event, which saw temperatures as low as 7.5 °C, on 2 June 2022. However, we have found no report of warming morning temperatures causing deformation of developing mushrooms.

Future research on intercropping wine-cap mushrooms with vegetable crops, should provide ample space between plots to reduce interplot contamination, as mylar insulation was insufficient to prevent *S. rugosoannulata* mycelium from encroaching on non-inoculated plots. Further, using different tools for the inoculated and the non-inoculated plots, or having a sterilization protocol before using tools in a new plot, especially before use in non-inoculated plots after use in inoculated plots, will help avoid contamination of the non-inoculated plots.

Conclusion

Mulching asparagus with woodchip mulch can be an effective method to reduce weed competition in asparagus, reducing the labor required for weed management. The yield of asparagus was relatively unaffected by mulching with woodchips until the depth of mulched reach 12 in, where harvests were delayed, and the overall yield and quality of asparagus were diminished. Due to the aggressive nature of wine-cap mushrooms in the woodchip mulch used in this study, we were unable to determine directly whether the presence of inoculum in the mulch had an impact on asparagus growth. But, in general, the presence of *S. rugosoannulata* did not appear to negatively impact asparagus production relative to bare ground. The depth of woodchip mulch produced only minor effects on wine-cap production, such that 4-in mulch produced somewhat larger, but perhaps lower quality mushrooms than the deeper treatments. These results suggest that wine cap intercropped with asparagus, and other vegetable crops, warrants further consideration both by researchers and growers.

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Table 2.1 – Effect of mulch depth and inoculation with Stropharia rugosoannulata on yield of intercropped asparagus in 2022 and 2023[i].

Mulch Depth (in)	Inoc.[ii]	First Harvest Date		Average length of harvest period		Total Number of spears (#/plot)		Total Weight of spears (g/plot)	
		2022	2023	2022	2023	2022	2023	2022	2023
Bare ground	**No**	114.00 c	111.75 ab	23.50 abc	32.25 ab	14.00 ab	13.00 ab	285.65 a	467.55 a
4	**No**	116.50 bc	121.75 ab	21.50 abcd	21.50 abcd	14.75 ab	15.00 ab	317.65 a	361.35 a
4	**Yes**	117.75 bc	109.00 b	20.25 abcd	34.50 a	16.50 a	19.00 a	281.05 a	359.05 a
8	**No**	120.50 abc	122.00 ab	18.00 bcd	22.50 abc	13.50 ab	14.25 ab	262.40 ab	446.00 a
8	**Yes**	123.00 ab	113.25 ab	14.50 cd	30.75 abc	12.75 abc	19.25 a	254.00 ab	489.35 a
12	**No**	124.75 ab	125.00 a	10.75 cd	16.75 bcd	6.00 bc	7.75 ab	104.40 ab	234.90 ab
12	**Yes**	128.31 a	125.68 a	6.50 d	11.00 d	3.50 c	5.00 b	30.30 b	58.05 b
	***P* value**	**0.0019**	**0.0110**	**0.0002**	**0.0030**	**0.0071**	**0.0192**	**0.0153**	**0.0018**

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

Table 2.2 – Effect of mulch depth and inoculation with *Stropharia rugosoannulata* on marketability of intercropped asparagus in Manhattan, KS in 2022 and 2023[i].

Mulch depth (in)	Inoc.[ii]	Percent Marketable				Average Weight per Marketable Spear (g)	
		(#/#)		(w/w)			
		2022	2023	2022	2023	2022	2023
Bare ground	**No**	91.00	87.00 a	92.00	93.85	20.94	37.62 ab
4	**No**	90.00	54.00 b	90.00	65.62	18.75	30.84 ab
4	**Yes**	100.00	80.00 ab	100.00	83.92	16.23	23.15 b
8	**No**	92.00	70.00 ab	93.00	86.82	24.93	42.69 a
8	**Yes**	100.00	94.00 a	100.00	98.71	22.71	28.71 ab
12	**No**	89.00	92.00 a	90.00	91.05	17.24	30.25 ab
12	**Yes**	100.00	93.00 a	100.00	97.35	17.58	23.34 b
	***P* value**	**0.0190**	**0.0091**	NS	NS	NS	**0.0134**

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

Table 2.3 – Effect of woodchip mulch depth and inoculation with Stropharia rugosoannulata on the size of marketable spears of intercropped asparagus in Manhattan, KS in 2022 and 2023[i].

Mulch depth		% Very Small (w/w) [iii]		% Small (w/w)		% Medium (w/w)		% Large (w/w)		% Very Large (w/w)	
(in)	Inoc.[ii]	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
Bare ground	**No**	2.62	0.99	15.68	1.15 c	27.08	12.99	18.33	31.47	36.19	53.39
4	**No**	3.60	2.26	28.82	0.02 c	6.62	12.29	25.22	46.38	35.70	39.06
4	**Yes**	8.74	2.90	7.47	22.54 ab	32.01	11.98	25.57	21.36	26.39	41.26
8	**No**	1.57	0.41	5.11	3.53 bc	19.35	07.19	26.07	41.36	47.90	47.51
8	**Yes**	1.99	2.50	15.20	7.93 abc	8.34	20.30	44.34	36.31	30.02	32.98
12	**No**	0.18	3.20	6.51	5.92 abc	54.63	1.15	5.06	30.12	33.62	59.61
12	**Yes**	0.00	3.72	29.18	26.34 a	50.63	18.58	20.19	47.20	0.00	4.15
***P* value**		NS	NS	NS	**0.0065**	NS	NS	NS	NS	NS	NS

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

iii. USDA grading Standard of asparagus spears

Table 2.4 - Effect of woodchip mulch depth and inoculation with *Stropharia rugosoannulata* on the grade size weights of intercropped asparagus spears in Manhattan, KS in 2022 and 2023[i].

		Percent Grade 1 (w/w)[iii]		Percent Grade 2 (w/w)	
Mulch depth	**Inoc.[ii]**	**2022**	**2023**	**2022**	**2023**
Bareground	**No**	81.60	97.86 a	18.31	2.14 b
4'	**No**	67.54	97.73 ab	32.43	2.28 ab
4'	**Yes**	83.98	74.60 ab	16.22	25.44 b
8'	**No**	93.32	96.06 ab	6.70	3.94 ab
8'	**Yes**	82.70	89.60 ab	17.20	10.41 ab
12'	**No**	93.31	90.88 ab	6.70	9.12 ab
12'	**Yes**	73.77	69.94 b	25.95	30.06 a
	***P* value**	NS	**0.0204**	NS	**0.0204**

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

iii. By weight of USDA grade of asparagus spears.

Table 2.5 - Effect of mulch depth on spring harvest of *Stropharia rugosoannulata* yield when intercropped with asparagus in Manhattan, KS in 2022. [i]

Mulch Depth (in) [ii]	First Harvest Date (Julian date)	Harvest period (days)	Total Number (#/plot)[iii]	Total Weight (g/plot)	Marketable Yield		
					Percent (#/#)	Percent (w/w) [iv]	Avg. Weight (g)
4	121.75	34.75	53.00	1488.75	75.34	75.68	29.92 a
8	118.13	27.50	55.50	1372.73	62.82	59.38	22.29 b
12	129.50	27.00	56.25	1275.25	87.78	86.64	24.35 b
***P* value**	NS	NS	NS	NS	NS	NS	**0.0132**

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different ($P > 0.05$) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA) at three deferent heights

iii. Percentage by number of mushrooms

iv. Percentage by weight of mushroom

Table 2.6 - Effect of mulch depth on the size distribution and quality grade of *Stropharia rugosoannulata* grown on woodchips in Manhattan, KS in 2022 and 2023[i].

Mulch Depth (in)[iii]	Grade 1[ii] Small – Medium (% w/w)[iv]	Grade 1 Large (% w/w)	Grade 1 Total (% w/w)	Grade 2 Small – Medium (% w/w)	Grade 2 Large (% w/w)	Grade 2 Total (% w/w)
4	11.93 b	28.20	40.14	10.78	49.11	59.89
8	28.70 a	31.12	59.83	3.65	36.56	40.21
12	24.58 a	32.34	56.92	9.47	33.59	43.06
***P*-value**	**0.0097**	NS	NS	NS	NS	NS

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Mushroom grade according to USDA grading standard

a. **U.S. No. 1** consists of fresh mushrooms of similar varietal characteristics which are mature, at least fairly well shaped, well trimmed, free from open veils, disease, spots, insect injury, and decay, and from damage by any cause.
b. **U.S. No. 2**. The requirements for this grade are the same as for U.S. No. 1 except for a greater tolerance for open veils and a larger tolerance for defects.

c.

iii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA) at three deferent heights

iv. Percentage by weight of mushroom

Table 2.7 - Effect of mulch depth on the causes of non-marketability by percent of *Stropharia rugosoannulata* grown on woodchips in Manhattan, KS in 2022 and 2023[i].

Mulch Depth (in) [ii]	% Open Veil (w/w) [iii]	% Immature (w/w)	% Pest damage by (w/w)	% Misshapen by (w/w)	% Other damage (w/w)
4	16.29	25.84	26.53	31.33 ab	0.00
8	12.63	0.10	29.99	62.41 a	0.00
12	34.51	0.92	12.82	21.12 b	5.62
***P* value**	NS	NS	NS	**0.0411**	NS

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA) at three deferent heights

iii. Percentage by weight of mushroom

Figure 2.1 - Spore print to confirm *Stropharia rugosoannulata* expansion on woodchips in Manhattan, KS in 2022[i].

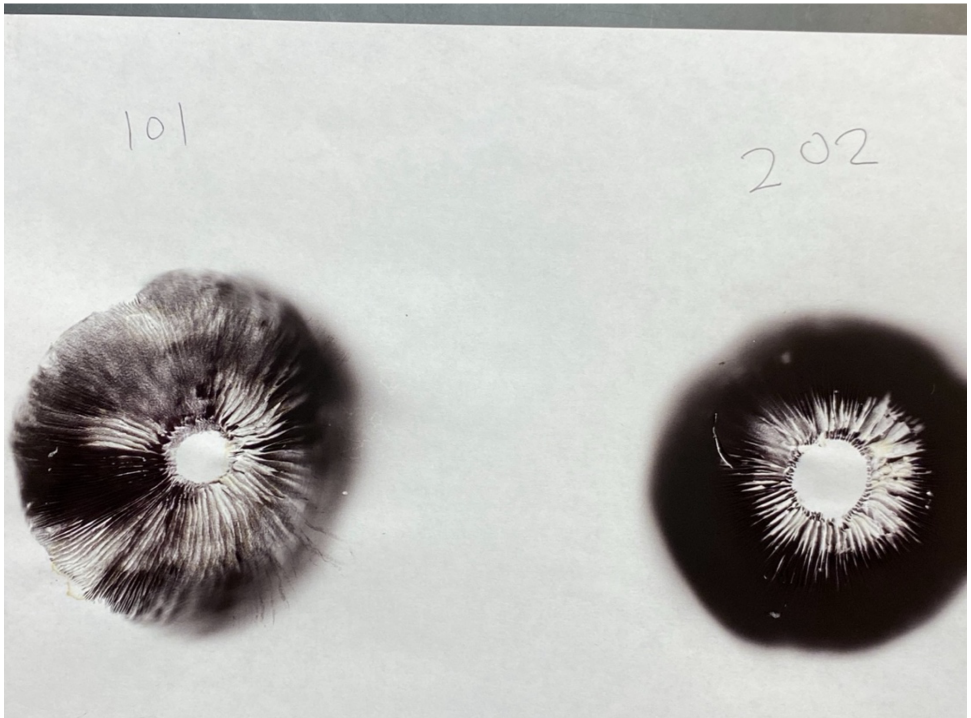
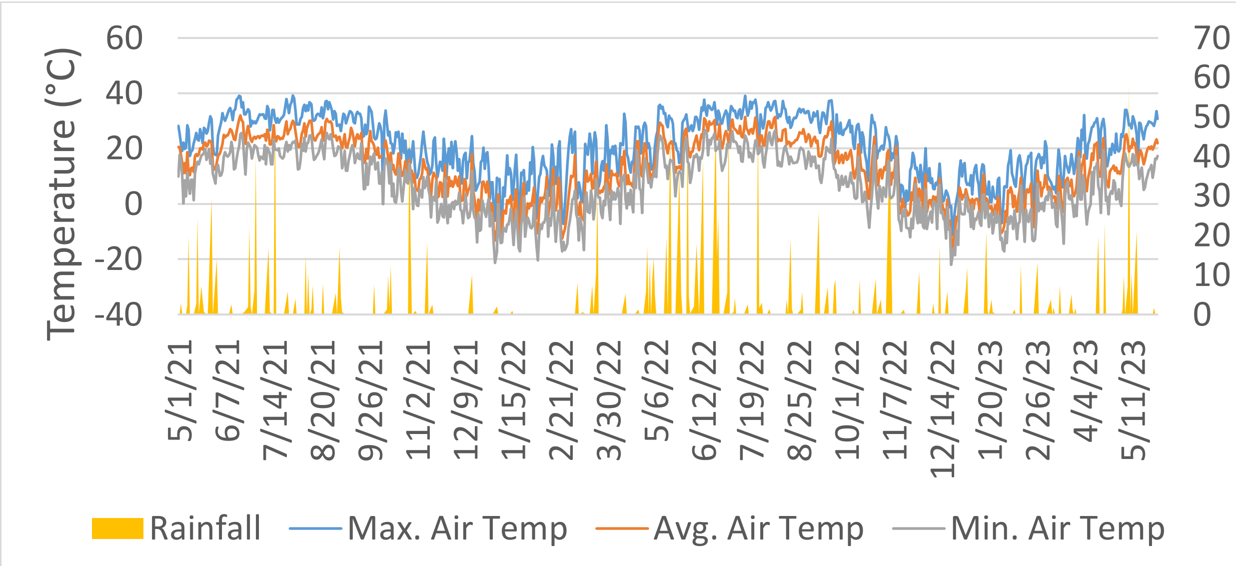


Figure 2.2 - The environmental conditions (rainfall, maximum air temperature, average air temperature, and minimum air temperature) varied across the growing seasons



Chapter 3 - Effect of growing tomatoes on four different types of mulches in Manhattan, KS

Abstract

Intercropping mushrooms with field-grown vegetables, may provide farmers increased revenues without sacrificing bed space or investing in indoor production facilities. The King Stropharia mushroom (*Stropharia rugosoannulata*), also called wine cap, can colonize organic material, like woodchips or straw mulch, making it a good candidate for intercropping with vegetables that can be grown on organic mulches. However, no peer-reviewed research has been identified that evaluates the impact of this strategy on the yields of vegetable. This project evaluated whether: 1) organic mulches affect tomato yield and quality in northeastern Kansas; 2) wine-cap mushroom will grow successfully when intercropped with tomato; and, 3) mushroom inoculation will affect the yield and quality of tomato. We established experimental plots at Kansas State University's Willow Lake Student Farm in the spring of 2021 to evaluate the effect of intercropping wine cap mushroom with tomato (*Solanum lycopersicum* L. cv. Galahad) across the 2021 and 2022 growing seasons. The experiment utilized a randomized, complete block design (RCBD) with four replications. There were two factors: mulch type and mushroom inoculation. The treatments were: inoculated woodchips, non-inoculated woodchips, inoculated wheat straw, non-inoculated wheat straw, inoculated soybean straw, non-inoculated soybean straw, black plastic (non-inoculated positive [mulch] control), and bare ground (non-inoculated negative [mulch] control). Soy straw performed comparably with black plastic mulch in virtually every metric and woodchips tended to reduce tomato yields. Soy straw required substantially less time to weed than other organic mulch treatments. And while installing and planting into straw

mulch was more labor-intensive than installing plastic mulch, straw does not require the capital investment or learning curve of using a plastic-mulch layer. A few wine caps emerged from woodchips in a buffer plot planted with tomato in fall 2021; however, no fruiting bodies were observed in experimental plots in either year of this study. This suggests that the mushrooms may grow in woodchips when intercropped tomato plants, but further research would be needed to determine whether the incompatibility we observed was due to interactions between the intercropped species or cultivars, or was a function of the local climate and/or soil conditions. Despite the failure to produce a mushroom crop, there appeared to be a slight protective effect of mulch inoculation on tomato fruit marketability during the second year—when heavy pathogen loads decimated the crop.

Introduction

Indigenous to the Andean region of South America, the domesticated tomato (*Solanum lycopersicum* L.) is one of the most widely consumed vegetable crops worldwide (Kumar et al., 2020). In 2022, tomato was cultivated on 12.1 million ac, including over 260,000 ac in the United States (FAOSTAT, 2024). Global tomato production was estimated to be over $130 billion in 2022 (FAOSTAT, 2024), with the United States reporting a 2023 tomato harvest worth $2.0 billion (USDA NASS, 2023). Together with China, the top producer of tomato globally, the United States, India, the European Union, and Turkey, produce roughly 70% of the world's tomato supply (Geetha and Rani., 2020). California and Florida produce around two-thirds of the nation's fresh tomatoes; and, California produces nearly 95% of the processed tomatoes (Guan et al., 2018).

Tomatoes are a nutrient-dense food that may be eaten raw or cooked and can contribute significantly to a balanced diet (Kumar et al., 2012). Delgadillo-Díaz et al. (2019) linked tomato consumption to a decreased risk of cancer, inflammatory processes, and chronic non-communicable diseases, like coronary heart disease, hypertension, diabetes, and obesity.

Agricultural mulches are used to enhance the soil environment to promote plant growth and development (Vandenberg and Tiessen 1972, Bot and Benites, 2005, Chakraborty et al. 2008). Plastic mulch films have been used since the 1950s to improve vegetable crop production by modifying soil temperature, conserving soil moisture, and blocking weeds from competing with the cash crop (Hill et al., 1982; Shogren, 2000; Schonbeck, 1998; Schonbeck and Evanylo, 1998). Plastic mulch films have been shown to improve yields in potato (Yalan et al., 2024), raspberry (McIntosh et al., 2023), rice (Gao et al, 2023), maize (Cheruiyot, 2023), and okra (Chauhan, 2024), and tomato (Castellano-Hinojosa, 2024). Plastic mulch films are relatively low-cost, and simple to apply, store, and transport, leading to a steady increase in their use over time (Brault, 2002; Steinmetz, 2016). However, plastic mulches are generally used for a single season, and then must be removed from the field and disposed of. Recycling of these films is complicated by adhesion of soil and chemical residues (Kaiser et al., 2017) and is geographically limited (Gómez and Escobar, 2022; Garthe and Kowal, 1993). As a result, many growers transport spent mulch films to nearby landfills (Olsen and Gounder, 2001; Shogren, 2000), others may burn the plastic, producing a negative impact on both the environment and public health (Shogren and Hochmuth, 2004).

Organic mulches include (though, are not limited to) woody materials like woodchips, bark, or sawdust; straw from grain crops; and compost, which is becoming more readily available in areas with green-waste recycling programs (Merfield 2002). The use of organic mulches in vegetable production long pre-dates plastic mulch films, primarily for their potential to inhibit weed growth and reduce or stop erosion, while improving soil organic matter content (Bot and Benites 2005; Nalayini 2007; Kader et al. 2019, Rathore et al. 1998). Organic mulches eventually break down and need to be replaced, a problem shared with plastic films. However, decomposed organic mulches may be safely tilled into the soil, adding organic matter and potentially building soil quality (Bajoriene, 2013; Saroa and Lal 2004). However, some organic mulches cool the soil relative to bare ground (Caldiz et al., 2001; Sinkevičienė, 2009); though, in areas with exceptional heat during the growing season, cooling the soil with mulch has been shown to benefit potato (Sinkevičienė, 2009), strawberry (Orde et al, 2021), and lettuce (Gheshm and Brown, 2020). Further, if a mulch material contains weed seeds or other viable propagules, the mulch may contribute to increased weed pressure in defiance of one of its primary purposes (Merfield, 2002).

Growing two or more different crops simultaneously on a single plot of land is known as intercropping. This practice is based on the idea that enhancing ecological diversity may improve natural disease, insect, pests, and weed suppression (Theunissen, 1994; Theunissen and Schelling, 1996; Theunissen, 1997; Baumann et al., 2000). Vegetable intercropping systems have been documented to increase vegetable productivity per unit area and profitability in both field and greenhouse settings (Varghese, 2000; Yildirim and Guvenc, 2004; Guvenc and Yildirim, 2005, 2006; Yildirim and Guvenc, 2005). Total yield of tomato intercropped with lettuce produced a greater yield in both crops, while there were no difference in the dry matter content and plant height parameters of the intercropped tomato compared to each crop grown in monoculture (Reynafarje et al., 2014). Other studies have shown overall benefits when intercropping tomato with bell pepper (Sandhu et al., 2021), potato-onion ((Li et al., 2020), snow peas (Kong et al., 2020).

Fungi plays an important role in the ecology of every biome on Earth (Kauserud et al., 2012), and make up this planet's most varied kingdom (Orwin, 2011; Tedersoo et al., 2014). Fungi provide: (a) ecological services vital to the survival of terrestrial vegetation (Humphrey, 2010); (b) bioremediation and decomposition of wastes and toxins (Asplund et al., 2018); and, (c) a source of food for animals, including humans (Amara., 2023). In 2023, the United States produced just over 300 thousand t of culinary and medicinal mushrooms (CMM) worth over $1 billion (USDA-NASS 2023). However, the majority of mushroom production is in China, where over 45 million t of CMM were produced in 2022, comprising 94% of global production that year (FAOSTAT., 2024).

The King Stropharia, or wine-cap mushroom (*Stropharia rugosoannulata* Farlow ex. Murrill), is one of the most recently cultivated non-mycorrhizal mushrooms to come into commercial use. In the 1960s, wine cap was domesticated in Germany for use by small-scale commercial farmers and homeowners (Szudyga, 1978). It has been proposed as a potential economic and subsistence target for underdeveloped nations by the Food and Agriculture Organization (Ben-Hur de Oliveira et al., 2019). In addition to its role in human health and nutrition, *S. rugosoannulata* has shown potential for the biodegradation of pharmaceuticals (Hu, 2020) and diuron (Bruhn, 2010) in contaminated water. Stropharia can also degrade 2,4,6-trinitrotoluene (TNT; Weiß, 2004), polycyclic aromatic hydrocarbons (Steffen, 2007), synthetic dyes (Jarosz-Wilkołazka, 2002), and other pharmaceutical compounds like ifosfamide, carbamazepine, venlafaxine, iopromide, diclofenac, and cyclophosphamide (Castellet-Rovira, 2018). Since wine cap is a both a primary and secondary decomposer, it can easily colonize organic substrates like woodchips and straw mulch in vegetable garden (Szudyga 1978). Because of its ease of production in organic materials also commonly used as mulches, the wine cap may be a good candidate for intercropping with mulched vegetable crops, like tomato. If growing wine cap mushrooms is compatible with vegetable production, farmers may benefit from the additional revenue stream made possible by the intercrop.

To evaluate the potential for intercropping wine cap mushrooms with tomato, the present study examines three questions: 1) how will wheat- and soy-straw mulch perform compared to black plastic mulch and bare ground for tomato production in northeastern Kansas; 2) will wine-cap mushroom successfully grow as an intercrop with tomato; and 3) will mushroom inoculation affect the yield and quality of tomato positively or negatively.

Materials and methods

The present study was conducted at Kansas State University's (KSU) Willow Lake Student Farm (WLSF) located in Manhattan, Kansas (39.2494° N, 96.5742° W) at an elevation of 1043 ft above mean sea level. The experimental site is situated 1,060 ft above mean sea level, and the soil is classified as a Eudora silt loam, a coarse-silty, mixed, mesic Fluventic Hapludolls, which is rarely flooded (Wagner et al., 1999). The National Resources Conservation Service (2021). WLSF falls in a Köppen-Geiger Dfa climate region, categorized as having a humid continental climate with hot summers and year-round precipitation (Köppen.earth, 2024 based on Beck et al., 2023) The site has an annual mean temperature of 53.7 °F, receiving an average 35.8 in of precipitation (National Center for Environmental Information [NCEI], 2024). The site averages 185 frost-free days, and is classified as prime farmland (NRCS, 2021). During the growing season (usually from 15 April through 20 October), the daily low temperatures range from 40.2 °F to 67.5 °F (mean T_{low} = 57.1 °F) and the daily high temperatures range from 65.7 °F to 90.0 °F (mean T_{high} 80.9 °F; NCEI, 2024).

Experimental design. The experiment utilized a randomized, complete block design (RCBD) with four replications. There were two factors, mulch type and mushroom inoculation. The mulch treatments included: commercially available, mixed-species woodchips (Hummel Tree Service, Manhattan, Kansas, USA), local wheat straw (Michael Ebert, Saint George, Kansas, USA), local soybean straw straw (Michael Ebert, Saint George, Kansas, USA), black plastic (Berry Global Inc., Evansville, Indiana, USA), as a positive mulch control, and bare ground, as a no mulch control. Two mushroom treatments were: inoculated with wine-cap mushroom spawn (Field and Forest Products Inc., Peshtigo, Wisconsin, USA) and non-inoculated. Because only the organic mulches would serve as viable substrates, each replicate block (row) contained the following treatments randomly assigned within the row: inoculated woodchips, non-inoculated woodchips, inoculated wheat straw, non-inoculated wheat straw, inoculated soybean straw, non-inoculated soybean straw, black plastic (not inoculated), and bare ground (not inoculated). Replicate blocks comprised complete rows, spaced 6 ft on-center, with eight experimental plots per row. Each plot measured 2 ft wide and 10 ft long. An additional 10 ft long buffer plot was installed at each end of every row and mulched with woodchips and planted with tomato.

Site Preparation and Planting. On 26 May 2021, experimental plots were prepared by twice rototilling (Model 749, BCS Group, Abbiategrasso (MI), Italy) four 100 ft rows to a depth of 6 in, aligned approximately southwest-to-northeast, in a 100 ft by 100 ft production block. . A single drip tape (10 mil, 12 in emitter spacing, 0.45 $gal \cdot min^{-1} \cdot 100$ ft; T-Tape [510-12-450], Rivulis, Gvat, Israel) was laid in the center of the row down the entire length. In the 2021 growing season, drip tape was laid on the soil surface. However, in 2022 growing season, the drip tape was moved to the top of the organic mulches to ensure a moist substrate for the mushroom spawn.

Mulch Application. To ensure consistent depth of organic mulches, a wooden frame 11.5 in tall, 2 ft wide, and 10 ft long was constructed and placed over the plot. Non-inoculated organic mulches were installed by filling the frame with the appropriate mulch to depth of approximately 6 in and spread by hand. A wooden screed 5.5 in tall was then placed inside the frame such that it hung from the top of the frame and its bottom surface was 6 in above the original soil level. The screed was worked up and down the length of the frame to lightly compress the mulch and create a level top surface. The mulches were wetted before carefully removing the frame. The sides of the mulch were allowed to collapse naturally to a stable angle. For inoculated plots, the same process was used as non-inoculated plots except that a series of screeds (9.5 in, 7.5 in, and 5.5 in) were employed to gradually bring the mulch level up to 2 in, 4, in, and 6 in, respectively. After the 2 and 4 in depths were filled, leveled, and wetted, 2 gal of wine-cap mushroom spawn was spread evenly across the top of the layer. To install black plastic mulch, furrows were hand dug 6 in deep and 8 in wide such that 24 in of level soil was left between the furrows. The mulch was pulled tight across the bed and the edges were placed in the furrows and covered with the previously excavated soil.

Fertility Management. A granular, pre-plant fertilizer (Suståne 8-2-4, Suståne Natural Fertilizer, Inc., Cannon Falls, Minnesota, USA) was surface applied at a rate of 80 $lb \cdot N \cdot ac^{-1}$, 20 $lb \cdot P_2O_5 \cdot ac^{-1}$, 40 $lb \cdot K_2O \cdot ac^{-1}$ on 26 May 2021, immediately prior to each mulch treatment application. A soluble fish emulsion fertilizer (Alaska Fish Fertilizer 5-1-1, Lilly Miller Brands, Altanta, GA, USA) was injected into irrigation water every 3 weeks from 17 June until 19 August at a rate of at a rate of 10 $lb \cdot N \cdot ac^{-1}$, 2 $lb \cdot P_2O_5 \cdot ac^{-1}$, 2 $lb \cdot K_2O \cdot ac^{-1}$, and on 20 May, 10 June, 14 July, and 5 and 26 August 2022 at a rate of 20 $lb \cdot N \cdot ac^{-1}$, 4 $lb \cdot P_2O_5 \cdot ac^{-1}$, 4 $lb \cdot K_2O \cdot ac^{-1}$.

Planting and maintenance. Tomato cv. Galahad (Johnny's Selected Seeds, Albion, Maine, USA), an early-season, hybrid, determinate cultivar, was started from seed in the greenhouse, and 6 – 7 weeks old seedings were transplanted to the field on 26 May 2021 and 3 May 2022. Plants were trellised using the Florida weave method (Kelbert et al., 1966). In 2021, the plants were minimally pruned to keep stems and fruit off the ground. However, in 2022, the plants were pruned weekly to maintain a more open canopy and remove dead or diseased foliage.

In 2021, the crops were irrigated for 45 minutes once per day throughout the growing season. In 2022, the irrigation regimen remained the same until we observed significant wilting in the plots on the 11th of August. From that point, we added an additional watering cycle in the hottest afternoons, 3-4 days per week, until the termination of the experiment on the 26 September 2022.

Weeding. The area between rows was maintained by periodic mowing and tilling down the center of the alley, and hand weeding along bed edges. The plots were weeded as needed; and, the time spent on weeding the research plots was recorded.

Harvest and fruit quality sampling. Tomato fruits were harvested from one to three times per week, depending on the stage of fruit development observed in the field. Tomatoes were harvested when fruits were a minimum of 75% red ripe. In 2021, harvest occurred from 3 August to 15 September; and in 2022, tomatoes were harvested from 16 July to 2 September. Fruits were weighed, counted, and sorted into groups of marketable and non-marketable fruit. Tomatoes that were non-marketable were categorized according to the primary cause of unmarketability, before number and weight were recorded. Marketable fruits were sorted by USDA standards for small, medium, large, extra-large, and jumbo sizes (USDA, 1991), then counted and weighed. A random sample of marketable fruit from each plot—one to three tomato fruit per plot per harvest (when available) for three harvests on 31 August and 3 and 10 September 2021 (four plots were harvested on 15 September to augment the 10 September harvest) was reserved for fruit quality tests. Sample fruits were kept at 22 ^{o}C in a paper bag until 100% of the fruit had reached the red-ripe stage. Fruit quality analyses were performed on 4, 8, and 15-16 September 2021 for each sample collection, respectively. In 2022, there were not enough marketable tomato fruit of sufficient size to perform quality analyses.

Juice Content. Ripe fruit was sliced in half; one half was weighed and placed in a tared drying tin and the fresh weight (FW) was recorded. The other half was reserved for other measurements.

Weighed samples were then oven dried for 48 hours at 80 ^{o}C, and the dry weight (DW) was recorded. Juice content was calculated according to Eq. 1.

$$[(FW - DW) / FW] \times 100 \qquad \text{Eq. 1}$$

Texture. An approach modified from Lana et al. (2007) was used to measure texture based on the stiffness of the axial pericarp. An L-500 g mechanical force gauge (Ametek, Hunter Spring Division, Hatfield, Pennsylvania, USA) was mounted on a manual drill press (Craftsman Model 9-25921, Sears, Roebuck, and Co., Chicago, IL) and fitted with a 3 mm probe. A stop was set to ensure that the probe stopped exactly 3 mm above the surface of the plate. Tomato halves reserved in a previous step were sliced once to create a 6 mm thick sample from the equatorial region of the fruit. A slice was positioned so that the force gauge's tip was directly over the pericarp, the gauge was reset to zero, and the probe was lowered 3 mm into the pericarp. The fruit was rotated to place fresh pericarp below the probe, and the process was repeated until four measurements were collected for each fruit sample. The four texture measurements (F), expressed in gram-force units (gf), were averaged and converted to Newtons according to Eq. 2.

$$F \times 9.807 \times 10^{-3}\ N \cdot gf^{-1} \qquad \text{Eq. 2}$$

Total soluble solids. The remaining halves of each tomato (reserved in a previous step), including the slices used for the texture measurements, was pulverized for 2 min in a blender (Oster 6832, Jarden Consumer Solutions, Boca Raton, FL). The tomato puree was centrifuged (Beckman TJ-6, Beckman Instruments, Inc., Palo Alto, CA) 10 min at 3,000 rpm and the liquid fraction (juice) was extracted with a pipette. Total soluble solids (TSS) were measured as ^{o}Brix from three to four drops of the juice using a digital refractometer (Palm Abbe digital refractometer, Misco, Cleveland, Ohio, USA). The remaining liquid extract was reserved for further testing.

pH and titratable acidity. pH was measured using 30 mL of the previously reserved tomato juice using a digital, benchtop pH meter (Beckman Φ 32 Beckman Instruments, Inc., Fullerton, California, USA). 4 mL of the extracted tomato juice was diluted to 50 mL with de-ionized (DI) water, agitated on a magnetic stir plate (Fisherbrand™ Isotemp™ Hotplate Stirrer, Fisher Scientific, Waltham, Massachussetts, USA), and titrated to pH 8.1 ± 0.1 using 0.2 N NaOH. To confirm the titration's endpoint. Percent titratable acidity (TA; citric acid equivalent) was determined utilizing the following formula:

$$TA\ (\%\ w/v) = (N \times V_1 \times Eq.wt.) / (V_2 \times 1000\ mg/g) \times 100 \qquad \text{Eq. [3]}$$

where N = normality of the titrant = 0.2; V_1 = volume of titrant (mL); Eq.wt. = mg/mEq of citric acid (CA) = 64 mg/mEq; and V_2 = volume of the sample (mL) = 4 mL (Sadler and Murphy, 2003).

Statistical Analysis

A one-way ANOVA model was generated with PROC GENMOD in SAS 9.4 (SAS Institute Inc., Cary, NC). The five levels of the mulch effect and the two levels of the inoculation effect were combined to make eight different treatments as the fixed effect. The effect combination is processed further using the CONTRAST procedure to compare positive control (black plastic) and negative control (bare ground) to other treatment effects. No random effects were specified except for the two-year models—to consider the effect of growing season (year); however, growing season data were ultimately analyzed and presented separately. Comparisons were carried out using two-sided tests, with $\alpha = 0.05$. Finally, Tukey's adjustment was applied to pairwise comparisons.

Results

Mulch treatment had an effect on the number of days to first tomato harvest (DTH) in 2021 and 2022 (P = 0.0045 and 0.0004, respectively; Table 3.1). Mulching with black plastic, inoculated wheat straw, and non-inoculated soy straw and woodchips resulted in earlier harvest than bare ground in 2021 (P = 0.0045; Table 3.1). However, in 2022, the first harvest from plants growing in non-inoculated woodchip mulch was later than all mulch treatments except bare ground, which produced its first harvest later than black plastic and non-inoculated soy straw (P = 0.0004; Table 3.1).

Mulch treatment had an effect on the total number of tomato fruit per plot in 2021 and 2022 (P = 0.0001 and 0.0004, respectively; Table 3.1). In 2021, tomato plants grown on soy straw, bare ground, and black plastic produced a greater number of fruit than those grown in woodchip mulch, while plants grown on wheat straw produced more fruit than those on non-inoculated woodchip mulch (P = 0.0001; Table 3.1). In 2022, overall fruit production decreased, and plants grown on all mulch treatments except woodchip mulch produced a greater number of fruit than those on bare ground, and those on black plastic and non-inoculated soy straw produced more fruit than those on non-inoculated woodchips (P = 0.0004; Table 3.1).

Mulch treatment had an effect on the total weight of tomato fruit per plot in 2021 and 2022 (P = 0.0020 and 0.0041, respectively; Table 3.1). In 2021, tomato plants grown on woodchip treatments produced lower total fruit weight than those grown on soy straw and bare ground (P = 0.0020; Table 3.1). In 2022, soy straw treatments produced greater fruit weight than plants grown on non-inoculated woodchips and bare ground, while tomato plants grown on black plastic also produced greater total weights than those grown on bare ground (P = 0.0041; Table 3.1).

In 2021, the percent (w/w) of total fruit deemed marketable and the average weight per marketable fruit did not differ by mulch treatment (P = 0.0968 and 0.2847, respectively; Table 3.1). In 2022, there were not enough marketable fruits across all treatments to perform statistical analyses.

Marketable fruit quality was evaluated only in 2021. Due to excessive fruit cracking and general decline of tomato plants across all treatments from bacterial wilt (*Ralstonia solanacearum* [Smith, 1896] Yabuuchi et al., 1995), there were not enough marketable fruits available for fruit quality analysis in 2022. Total soluble solids differed by mulch treatment (P = 0.0007; Table 3.2). Tomatoes grown on inoculated woodchip mulch had higher TSS than those grown on wheat straw, non-inoculated soy, and black plastic mulch (P = 0.0007; Table 3.2). Further, bare ground, inoculated soy straw, and non-inoculated woodchips treatments generated tomato fruit with greater TSS than those grown in inoculated wheat straw (P = 0.0007; Table 3.2). There were no differences in the pH or titratable acidity due to mulch treatment (Table 3.2). However, the ratio of TSS to TA in tomatoes from inoculated woodchip plots were higher than those grown in either wheat straw treatment (P = 0.0079; Table 3.2). Tomatoes grown on inoculated woodchips had lower juice content than all other treatments except non-inoculated woodchips (P = 0.0005; Table 3.2). Firmness of fruit grown in inoculated woodchip plots and non-inoculated wheat straw was significantly higher than those grown on inoculated wheat straw (P = 0.0410; Table 3.2).

Mulch treatment had effect on weeding time in 2021 (P-value: <.0001; Fig. 3.1); weeding times were not measured in 2022. Plots mulched with black plastic mulch took significantly less time to weed than all other mulch treatments except non-inoculated soy straw. The soy straw mulches took less time to weed than all other organic mulches. Bare ground required more time to remove weeds than all other treatments except non-inoculated woodchips (Fig. 3.1).

While we were able to measure the impact of mulch type and mushroom inoculation on tomato production, no mushrooms grew within our sampling frame during either year of this study. Therefore, no mushroom production results can be presented.

Discussion

Mulch materials of all sorts (organic and inorganic) have proven to increase crop yields, including tomatoes, relative to bare ground (Liu et al., 2002; Bhatt, 2004; Olfati, 2008). However, in the present study our results were mixed. Here, black plastic consistently produced harvests earlier than unmulched, bare ground production. This aligns with the majority of the literature evaluating the efficacy of black plastic in tomato production (Schonbeck and Evanylo, 1998; Grassbaugh et al., 2004; Andersen, 2012). However, the organic mulches were less consistent. In 2021, inoculated wheat straw, and non-inoculated soy straw and woodchips produced earlier yields than bare ground. But, in 2022, only the non-inoculated soy straw kept pace with black plastic to produce earlier yields than bare ground. Schonbeck and Evanylo (1998) found that increased temperatures for the first 30-40 days after transplant improved earliness and that retained soil moisture under organic mulches offset late-season water demand due to high temperature or drought. A study in India demonstrated soy straw increasing both soil temperature (+0.79 °C) and soil moisture (12.26 %) over bare ground (Chavan and Khodke, 2009). However, the same study reported that wheat straw increased soil temperatures and soil moisture more than soy straw (+1.15 °C and 17.81 % over bare ground, respectively; Chavan and Khodke, 2009). This conflict between the relative performance of wheat and soy straws in the present study and Chavan and Khodhe (2009), suggests that mulch effects on soil temperature and soil moisture are only part of the story.

In 2021, we saw no benefit from mulch treatments on the total number of fruits per plot. Indeed, woodchips, both inoculated and non-inoculated, reduced the number of fruits harvested by as much as 40 % compared to bare ground. However, in 2022, when significant disease pressure (primarily due to septoria leaf spot [*Septoria lycopersici* Speg. (1881)] and tomato pith necrosis

[*Pseudomonas corrugata* Roberts and Scarlett, 1981 emend. Sutra et al., 1997]) practically decimated the plots, the mulches appeared offer somewhat of a protective effect. Disease pressure reduced the number of tomatoes per plot by nearly 98 % in the bare-ground plots from 2021 to 2022. The woodchip mulch, while not differing statistically from bare ground, only saw less than a 90 % reduction in fruit number, with total numbers 100 – 250 % higher than in bare-ground plots. The overall weight of tomatoes harvested dropped in 2022 also represented similar decreases to the reduced numbers over the same interval. Moura et al. (2005) reported reductions in fruit number upon infection of tomato with *P. corrugata. S. lycopersici* is likewise known to reduce yields of tomato (Wyenandt et al., 2008). Splashing of rain and irrigation water on the soil is a vector for transmission of both pathogens. It is likely the mulches either served as a barrier to rainfall splashing on the soil, as well as reducing the velocity of water striking the soil, in the case of the organic mulches (Wyenandt et al., 2008).

We found no evidence that inoculation of the mulch with wine-cap spawn affected tomato fruit marketability, or the average weight of marketable fruit. As we have not identified other research that has evaluated mushroom-tomato intercropping, we consider this a first report that *S. rugosoannulata* does not negatively impact tomato production.

Total soluble solids was elevated in tomato fruits from plots covered by inoculated woodchips, while it was the lowest in inoculated wheat straw. However, those °Brix measurements coincided with the lowest and highest juice contents, respectively, among all treatments. The relationship between water content and the concentration of soluble solids in fruit is well-established (Cowan et al., 2014, Mitchell et al., 1991). Further, the number of fruits per plant has an inverse relationship with TSS concentrations (Walker et al., 1978)—which our data in woodchip plots seems to support. It is likely that wheat straw absorbed and/or held little water from either rainfall or irrigation allowing a large share to enter the soil matrix, relative to woodchips. Wine-cap mushrooms are best produced in woodchip mulch (Szudyga, 1978); and, mycelium was more visible in woodchip mulches compared to the straw mulches. Mycelium composites have a higher percentage of water absorption range (40 to 580 based on weight percentage) than standard products (polyurethane, 0.01 to 72), polystyrene, 0.03 to 9, phenolic formaldehyde resin, 1 to 15, plywood, 5 to 49), and wood, 5 to 190). This is the main limitation of mycelium composites for insulation purposes (Sukhtesaraie and Hosseini., 2016). The structure of the mycelium and the hydrophilic properties of lignocellulosic materials are responsible for this significant water absorption. If this made less moisture available for tomato fruits, TSS could be increased as juice content is reduced. Tomato TSS and juice content also may be influenced by cultivar, cultivation technique, fruit maturity, soil conditions, and other environmental factors (Helyes, 2006, Abd El-Salam, 2002).

The relative amount of sugars and organic acids in tomato fruits, represented crudely by the TSS:TA ratio, is a factor in the overall perception of sweetness and compatibility for processing. Elevated sugar concentrations, in concert with sufficient, but not overwhelming acid content, enhances sweetness, contributing to the overall flavor and usefulness of tomato fruit (Beckles, 2011). Beckles (2011) indicates that a “good-tasting table tomato” should have TSS ≥ 5, TA ≥ 0.4, corresponding to a TSS:TA ratio of 12.5, while Kadar et al. (1977) stated that good tomato flavor is realized with a ratio ≥ 10. The TSS:TA ratios measured in the present study ranged from 17.2 – 24.6, all substantially greater than those in either reference, despite the sub-standard values of both TSS and TA (Beckles, 2010). Given that mulch treatments and mushroom inoculation produced no detectable differences in the pH or TA of tomato fruit, the relative

TSS:TA advantage of tomatoes from inoculated woodchip mulch relative to those from wheat straw plots is primarily the result of increased TSS in the former.

Firmness of tomato fruits at harvest is strongly correlated with ripeness (Beaulieu and Gorny, 2001). In this study, all fruits were held until they reached full red-ripe stage for fruit quality measurements. As such, the ripeness stage should have had little influence on the measured firmness. Temperature plays an important role in fruit development and directly influences fruit firmness (Sams, 1999). This is likely due to increased density in fruit tissues under cooler conditions (Sams, 1999). Moreno et al. demonstrated a linear relationship between soil temperature and fruit firmness at harvest in Spain (2014). Hong et al. observed greater firmness in tomato fruit mulched with hairy vetch than those grown on black plastic (2000). However, in sub-Saharan Africa, Samaila et al. (2011) found firmness decreased from black plastic > rice straw mulch > bare ground. However, neither Hong nor Samaila reported soil temperatures. Sunscald, calcium content, and certain plant-growth regulators (ethephon) are also known to impact tomato firmness (Sams, 1999). Given that wheat straw treatments resulted in both the second-highest (non-inoculated wheat straw followed inoculated woodchips) and lowest (inoculated wheat straw) measures of fruit firmness, it is likely that the our results will offer no new insights into the matter.

All of the mulch treatments (except non-inoculated woodchips) reduced weeding times relative to bare ground, and black plastic resulted in the most significant time savings compared to all of the other mulch treatments except non-inoculated soy straw. While we did not survey weeds directly, the overall volume of weeds in the plots seemed to be strongly [negatively] correlated with the weeding times. Two primary considerations in the efficacy of a mulch as a weed barrier are 1) the amount of light the mulch blocks from reaching the soil surface, and 2) the difficulty of the weed seedling to emerge into the light—as a physical barrier (Coolong, 2012). Black polyethylene mulch is effective in both blocking light and providing a physical barrier, while the efficacy of organic mulches varies (Coolong, 2012; Lamont, 1993). Increasing the depth of an organic mulch will boost its efficacy in weed suppression by reducing light and extending the path the [resource-limited] weed seedling must traverse to access the light (Aldrich, 1984; Ozores-Hampton, 1998; Coolong, 2012). Here, wheat straw seemed the most easily displaced by wind and rodent activity—its straight, lightweight stems did not "lock" together like the kinked and branched stems of the soy straw—thereby allowing more light penetration and a shorter path for seedling emergence. Additionally, soybean residues have been reported to have both allelopathic effects on weeds (Massantini et al., 1977; Einhellig and Leather, 1988) and stimulatory effects on some crops (Narwhal, 2006), though no reports on soy straw stimulating tomato have been identified.

While it is unfortunate that wine cap mushrooms did not grow as an intercrop in the present study (though, a small cluster of these mushrooms emerged in a buffer plot in early-October 2021), a number of factors may be considered. First, tomato plants have a diversity of protective compounds with antimicrobial properties in root, leaf, and stem tissue (Kravchenko et al., 2003; Tam et al., 2021; Karačić, 2024). Second, although *S. rugosoannulata* is generally a good competitor, the widespread infection of tomato plants in the second year suggest that a high fungal and bacterial load was present in the soil [and likely mulch] environment. Third, frequent weeding and plant management resulted in regular disruption of the organic mulches, disrupting the mycelial networks' growth and development. Finally, as in any intercrop, success is rarely guaranteed and proof-of-concept trials, such as this, are essential to screen for both ecological and economic compatibility.

Conclusion

Soy straw outperformed wheat straw and woodchips, performing comparably with black plastic mulch in virtually every measure of tomato production undertaken in this study. While soy straw required substantially less time to weed than the other organic mulch treatments, installing and planting tomato starts into the straw was more labor-intensive than installing plastic mulch—but without the capital investment or learning curve of a plastic mulch layer. However, despite the vast acreage of soy production in the Midwest, soy straw is a relatively rare product. It's potential as a mulch for tomato, and presumably other crops, might open up a new market for Midwest farmers utilizing soy in their rotations. Woodchip mulch produced fewer tomato fruit and reduced overall yields than non-mulched plots in this study, indicating that it might not be a good choice for tomatoes in hot, humid climate of northeastern Kansas. Wine-cap mushrooms did not grow in our experimental plots—despite a few caps having emerged from woodchips in a buffer plot during the first fall after mulch inoculation. Those few mushrooms suggest that a tomato-wine cap intercrop may be possible. However, further research would be needed to determine whether the lack of productivity we saw was species-specific, cultivar-specific, or a function of the northeast Kansas climate or local soil conditions. Despite the failure to produce a mushroom crop, there appeared to be a slight protective effect of mulch inoculation on tomato marketability during the second year when a heavy pathogen load decimated the tomato crop. In this region, a viable strategy to intercrop wine-cap mushrooms with tomato would have to accommodate annual rotation of tomato plants into fresh soils to manage disease pressure.

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Table 3.1 - Effect of mulch type and inoculation with *Stropharia rugosoannulata* mushroom on yield of 'Galahad' tomato in Manhattan, KS in 2021 and 2022[i].

		Total Fruit						Marketable Fruit	
		Days-to-First Harvest		Number (#/plot)		Weight (kg/plot)		% of Total (w/w) [iii]	Weight per fruit (g)
Treatment		2021	2022	2021	2022	2021	2022	2021	2021
Non-inoculated		68.9	87.2	106.3	11.0	22.5	2.0	49.76 b	0.212
Inoculated		69.3	82.9	101.2	12.3	21.9	2.3	55.25 a	0.216
***P*-value**		0.3193	0.2048	0.6796	0.9220	0.7798	0.7841	0.0141	0.8839
Mulch Treatment	**Inoc** [ii]								
Bare ground	**No**	72.3 a	97.3 ab	121.3 a	2.8 c	24.8 a	0.6 c	53.6	201.1
Black Plastic	**No**	67.0 b	75.0 c	114.0 a	16.0 a	23.1 ab	2.8 ab	51.7	208.9
Wheat Straw	**No**	71.3 ab	79.5 bc	107.5 ab	13.8 ab	22.4 ab	2.4 abc	51.0	206.2
Wheat Straw	**Yes**	67.0 b	81.5 bc	107.3 ab	12.8 ab	23.7 ab	2.1 abc	51.3	214.1
Soy Straw	**No**	67.0 b	75.5 c	116.3 a	17.0 a	25.4 a	3.1 a	50.2	212.1
Soy Straw	**Yes**	70.5 ab	83.3 bc	118.3 a	14.3 ab	25.1 a	3.2 a	60.9	215.3
Woodchips	**No**	67.0 b	108.8 a	72.5 c	5.5 bc	16.6 b	1.1 bc	42.3	233.8
Woodchips	**Yes**	70.5 ab	84.0 bc	78.0 bc	9.8 abc	16.6 b	1.6 abc	53.5	219.7
***P*-value**		0.0045	0.0004	0.0001	0.0004	0.0020	0.0041	NS	NS

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different ($P > 0.05$) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

iii. By weight if marketable tomato fruits.

Table 3.2 - Effect of mulch type and inoculation with *Stropharia rugosoannulata* mushroom on fruit quality of 'Galahad' tomato in Manhattan, KS in 2021[i].

Treatment	Total Soluble Solids (°Brix)	pH	Titratable Acidity (% Citric-acid eq.)	Juice content (%)	Firmness (gF)	TSS:TA Ratio [iii]
Non-inoculated	4.362	4.42	0.222	95.0	271.71	20.25
Inoculated	4.411	4.44	0.218	94.8	264.52	20.74
***P*-value**	0.5107	0.4567	0.1855	0.3733	0.2014	0.1227

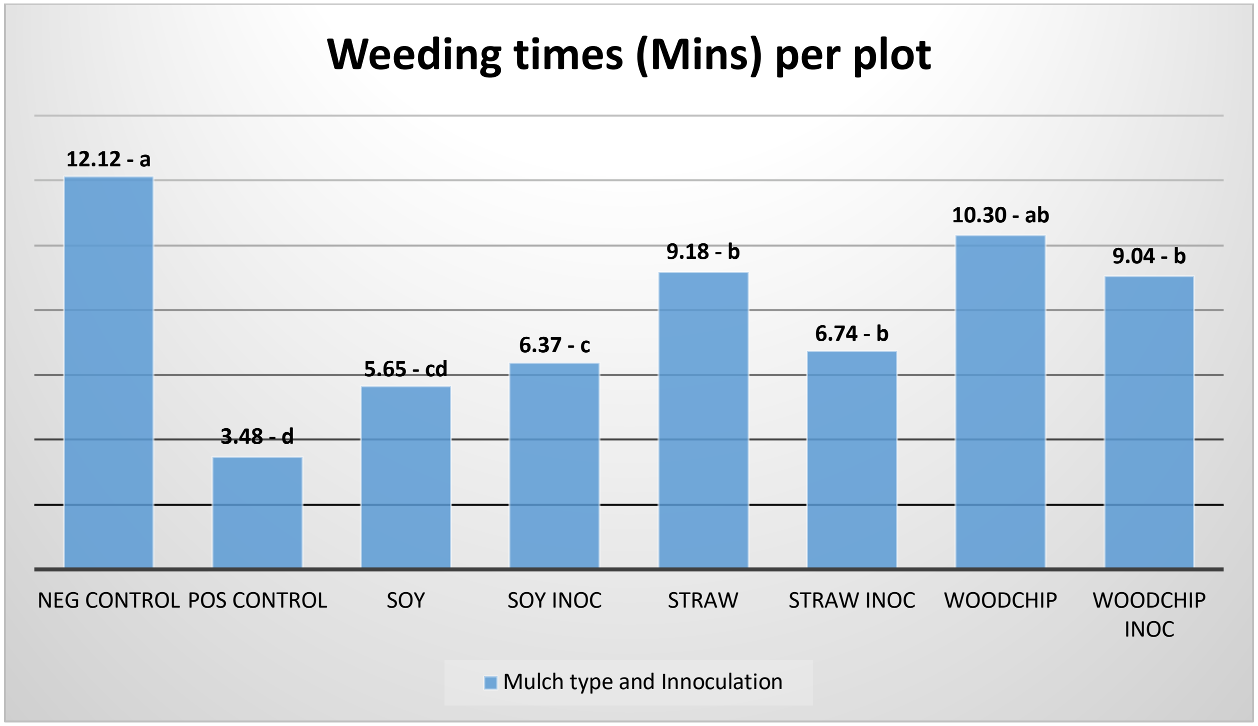
Mulch Treatment	Inoc [ii]	Total Soluble Solids (°Brix)		pH	Titratable Acidity (% Citric-acid eq.)	Juice content (%)		Firmness (gF)		TSS:TA Ratio [iii]	
Bareground	**No**	4.63	ab	4.40	0.213	95.0	a	266.16	ab	21.83	ab
Black Plastic	**No**	4.11	bc	4.44	0.199	95.1	a	252.93	ab	22.48	ab
Wheat Straw	**No**	4.24	bc	4.43	0.235	94.9	a	290.86	a	18.13	b
Wheat Straw	**Yes**	3.80	c	4.38	0.223	95.3	a	233.01	b	17.20	b
Soy Straw	**No**	4.27	bc	4.44	0.228	95.1	a	273.56	ab	19.46	ab
Soy Straw	**Yes**	4.54	ab	4.45	0.231	94.9	a	262.38	ab	20.40	ab
Woodchips	**No**	4.50	ab	4.39	0.232	94.8	ab	270.36	ab	19.90	ab
Woodchips	**Yes**	4.89	a	4.48	0.202	94.3	b	298.19	a	24.62	a
***P* value**		0.0007		NS	NS	0.0005		0.0410		0.0079	

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different ($P > 0.05$) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with S. rugosoannulata sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

iii. Total Soluble Solids to Titratable Acidity ratio of the tomato fruits

Figure 3.1 - Average number of minutes to hand weed a 20 sq.ft. tomato plot by mulch type and inoculation with wine-cap (*Stropharia rugosoannulata*) mushroom spawn during the 2021 growing season in Manhattan, KS. (*P*-value: <.0001)



Chapter 4 - Mushrooms and its residue inclusion in chicken feed

Abstract

Since the middle of the past century, there has been a steady increase in the demand for premium chicken meat worldwide. By 2025, the globe will produce 139.19 million tons of chicken meat, up from 7.56 million tons in 1961. The main factor influencing the quality of chicken meat and the producer's profitability is feed. However, there are growing concerns around the antibiotics and other synthetic feed additives used in promoting the growth of broiler chickens and increase egg production in the industry globally. Natural alternatives that could be used as antibiotic substitutes are becoming more popular as a result of the ban on the use of antibiotics in poultry. Hence, this review focused on the utilization of culinary and medicinal mushrooms (CMM) a source of energy and nutrients, with the potential to increase agricultural sustainability through the effective use of this marginalized resource such as culled mushroom and Spent Mushroom Substrate (SMS). The inclusion of several types of CMM has shown to improve the health of poultry due to their phenolic compounds, increased immune activities, and antioxidants. The inclusion of CMM has also shown to be effectively replace some of expensive feed stuff such as soybean meal which is expensive, providing the birds with comparable micro and macro nutrients for improved health and maximum profit for the poultry farmer. Beyond being an effective substitution for macro and micro-nutrients in poultry diet, CMM waste has shown to be an excellent source of feed for mealworm, which is an excellent source of protein in poultry industry, which in-turn help in reducing the agricultural pollution in the mushroom production industry. Every 1kg of edible mushrooms produce 3.5kg to 5kg of SMS. Hence, this review helps to understand the current trends and the potentials of CMM in the poultry industry.

Introduction

In the United States, chicken meat generated about $43 billion in revenue in 2023 (USDA, 2024a). Chicken dominates the U.S. poultry (meat) market—in 2023, nearly 30 million tons of chicken meat was produced, surpassing the combined production of pork and beef (USDA, 2024a; USDA, 2024b). The demand for high-quality chicken meat in the world has also being growing steadily since the middle of the last century, and by 2025, the amount of chicken meat produced worldwide will have increased from 7.56 million tons in 1961 to 139.19 million tons. (Abd El-Hack, 2018). Feed is the primary input that determines the quality of chicken meat and profitability to the producer (Sugiharto and Ranjitkar, 2019, Mohammed and Worku 2019, Kalia et. al. 2019). Feed makes up 60–75% of the total cost of producing chickens (Ojewola et al., 2005). As a result, the cost and efficient use of feed is a top priority for producers.

A number of diseases threaten chicken production, making effective disease management another high priority for the industry (Blake and Tomley., 2014; Van Immerseel et al., 2004). As a result, prophylactic antibiotic use emerged as early as 1910 as an effective tool to prevent disease, thereby increasing feed-use efficiency—promoting poultry growth and other production metrics (Alloui et al., 2014). However, humans face health risks from poultry consumption due to an overreliance on these antibiotics (Shad et al., 2019). The primary risks are attributable to [human] pathogens that have become antibiotic-resistant, a trait that evolves from the continual [over-] administration of feed-based antibiotics (Edens, 2003; Dibaji et al., 2012; Zeryehun et al., 2017; Mahfuz and Piao, 2019). But, eliminating antibiotics as growth promoters reduces feed conversion ratios and increases agitation and the incidence and severity of some poultry diseases (Wierup, 2001). For instance, the intestinal parasite coccidiosis was estimated to have cost US chicken producers $1.5 billion in 2016 alone (Blake et al., 2020). Additionally, respiratory disorders and necrotic enteritis, other conditions that prophylactic antibiotics are used for, result in annual losses exceeding $2 billion (CEAH, 2012; Dahiya et al., 2006; Keyburn et al., 2008). These issues are exacerbated when poultry are raised in suboptimal environments where antibiotics would provide the greatest benefit (Bhushan, 2017). The risks to both human and animal health may be reduced by employing modern nutritional strategies that provide balanced diets, probiotics, including phytobiotics, and other novel interventions, in addition to antibiotics as a treatment for disease (Alkhalf et al., 2010; Khan and Naz, 2013; Kumar et al., 2014). Natural materials—which include organic acids, probiotics, enzymes, essential oils, prebiotics, and naturally-occurring antibiotics (Karangiya et al., 2016)—are generally regarded as safe feed additives to increase feed-use efficiency poultry development performance and (Daneshmand et al., 2012; Adil et al., 2015; Attia et al., 2017). Mushrooms have recently been the subject of research as safe, natural feed additives for poultry diets (Abou-Zeid et al., 2019). Here, we review the relevant literature to identify the [potential] role of culinary and medical mushrooms (CMM), and their byproducts, as part of a viable poultry nutrition strategy.

Methodology:

This review compiles and summarizes information from many sources, primarily journals, reporting research on the impact of culinary and medicinal mushrooms on the wellbeing and productivity of chickens.

Review:

Culinary and medicinal mushrooms

Humans have been using mushrooms as food and/or medicinal for thousands of years. There more than 14,000 known species of mushrooms, and about 2000 are regarded as edible (Chang and Buswell, 2008). Of those, 270 species, across 30 genera are thought to have the potential to hold preventative or curative properties for human health (Wasser, 2002).

CMM has been collected and cultivated for human use since at least the Magdalenian phase of the Upper Paleolithic period (> 12,000 years ago; Power et al., 2015). Today, CMM is growing in popularity due to a number of factors—CMM are fat-free while having high dietary fiber, protein, essential amino and fatty acids, as well as containing bioactive compounds including B-complex vitamins, vitamin D, and phenolic-, flavonoid-, and carotenoid-based antioxidants (Royse, 2005; Sganzerla et al., 2022). CMM has long been used in traditional medicine across various cultures (Wasser, 2010). The polysaccharides, polyphenols, antioxidants, and other bioactive components of CMM have been a subject of interest to researchers exploring their positive effects on both human and animal health (Friedman, 2015; Kothari et al., 2018; Bonanno et al., 2019; Stojkovic et al., 2019; Jana & Acharya, 2020;). Nevertheless, ongoing research continues to uncover new potential uses for mushrooms in human and animal nutrition and medicine (Tang et al., 2016).

CMM as animal feed additives. Due to their antioxidant, immunostimulatory, antimicrobial, anti-inflammatory, hypocholesterolemic, and probiotic activity, medicinal mushrooms are being investigated as natural feed additives for livestock (Willis and Wall, 2013; Bederska-Łojewska et al., 2017; Strong et al., 2022). For instance, incorporating mushroom-derived additives into dairy feed has been shown to improve digestion, enhance immune function, and increase stress-resistance (Bonanno et al., 2019). Fermented king oyster mushroom (*Pleurotus eryngii*) by-products were shown to fatten pigs, enhancing crude protein content, water holding capacity, and muscle lightness (color) while decreasing Warner-Bratzler shear force and cooking loss in the resulting pork (Chu et al., 2012). However, feeding intact mushrooms directly to animals is rarely justified given the much higher value of CMM for human consumption.

Substrates for mushroom production

In many parts of the world, crop residues and other organic wastes are often tilled into the soil, composted, or burned (Kumar et al., 2023). However, some crop residues may provide a source of energy and nutrients, with the potential to increase agricultural sustainability through the effective use of this marginalized resource. For instance, China, Indonesia, Thailand, Bangladesh and Sri Lanka utilize crop residues for bioenergy generation, organic fertilizers, and paper manufacture (Kumar et al., 2023), while India uses more than half of its annual crop residue (686 Mt in 2013) for cattle feed and cooking fuel (Hiloidhari et al., 2014). Barley, rice, and wheat straws, corn stover, and woody materials like sawdust and woodchips may be used as substrates for mushroom cultivation because they are high in cellulose and lignin, primary nutrient sources for some CMM (Mohd et al., 2018; Grimm and Wösten, 2018). These wastes, when used as substrates for mushroom cultivation, may offer financial benefits both in supplying high-nutrient-dense mushrooms as well as byproducts potentially suitable for poultry feed (Israilides and Philippoussis, 2003; Zervakis and Philippoussis, 2000).

Byproducts and waste from mushroom production in livestock feed

Similar to other agricultural products, mushroom cultivation generates a substantial amount of waste (marginalized resources), including non-marketable ("cull") mushrooms, trimmed stems, and spent mushroom substrates (SMS; Lim et al., 2013). These residues pose environmental challenges given the hydrophobic nature of fungal mycelia and the relative lack or incompatibility of large-scale composting and bio-energy facilities to address the volume of waste near large-scale producers (Jiang et al., 2017; Lam et al., 2019).

Stem waste. Due to the abundance of phenolic compounds, increased immune activities, and antioxidants in enoki mushrooms (*Flammulina velutipes*), increased production has produced an excess of waste from stem bases (Zeng et al., 2012). In China alone, the production of *F. velutipes* stem waste exceeds 100,000 tons annually. Developed regions like Europe, America, Japan, and South Korea generate even higher quantities of this waste material due to their larger mushroom cultivation industries (Mahfuz et al., 2018).

Spent mushroom substrate. For every 1 kg of edible fungi cultivated, about 3.25 kg to 5 kg of substrate waste is generated (Zhao et al., 2012). In China alone, there were 8.9 million t of edible mushrooms produced in 2015 (Liu, 2019). That level of production would generate nearly 30 million t of spent mushroom substrate (SMS). CMM species capable of breaking down lignin improve the dry matter digestibility of lignocellulosic materials and enhance the nutritional content of the substrate they are grown on when used as an animal feed additive (Mohd, 2018; Grimm and Wösten, 2018; Galaviz-Rodriguez, 2010). SMS contains polysaccharides, vitamins, and trace minerals like iron, calcium , zinc , and magnesium (Ahlawat, 2002; Hajkova et al., 2008; Zhu et al., 2012) which support animal growth and balance the microbiota in the livestock digestive tracts (Paredes, 2009). SMS has been demonstrated to efficiently replace a component of traditional roughage, like rice straw (widely used in Asian nations) and microbial additives of beef cattle (Kim et al., 2012), enhance rumen bacterial diversity and abundance as well as production efficiency and meat quality in Hu sheep (Huang et al., 2023), and used as roughage for meet goat (Rangubhet et al., 2020). A system that starts with 1) a grain crop, utilizes the residues as substrate to produce 2) a CMM crop, then recycles the exhausted substrate as 3) an animal feed additive, may be more sustainable than systems focused on feeding virgin materials.

Understanding the gut health of poultry and its modulation

Young chicks start to form cooperative associations between the microorganisms in their surrounding environment and their gastrointestinal system as they grow during the post-hatch period (Torok et al., 2011). Bacteria make up the majority of microorganisms found in chicken intestines (Wei et al., 2013). A number of helpful bacteria play key roles, such as competitive exclusion, pre-digestive activities, and defense against pathogen invasion, in a chicken's digestive tract (Schneitz, 2005; Yoon et al., 2015). As a result, intestinal bacteria are now being used as markers of particular health conditions that either favorably or negatively influence the growth and development of chickens (Dawkins et al., 2004; Day et al., 2015). The environment, housing circumstances, and diet may have an impact on the presence and abundance of these health-promoting microbes (Yegani and Korver, 2008).

The cecum, located between the small and large intestines, plays a crucial role in the digestive process of chickens. It provides a unique environment for microbial colonization due to its slower transit time, allowing for extended interaction between the digesta and the resident microorganisms (Guo et al., 2004). This extended contact promotes the fermentation of undigested carbohydrates and fibers, enabling the breakdown of complex compounds that are otherwise indigestible by the host (Guo et al., 2004). According to Barnes et al. (1972), the ceca of a chicken contains approximately 10^{11} bacterial cells/gram (wet weight), with anaerobic bacteria ranging from 10^8 to 10^9 cells/gram. The cecal microbiota possesses the necessary enzymes to degrade complex polysaccharides into simpler molecules, like short-chain fatty acids, which serve as an energy source for the host. Moreover, cecum aid in the synthesis of certain vitamins, such as vitamin K, and contribute to immune modulation (Guo et al., 2004). However, the cecum also hosts pathogenic microbes (Macfarlane and Cummings, 1991). Increasing the quantity of beneficial bacteria to colonize and compete with pathogens (Rolfe, 1991), or using other means (e.g. antibiotics) to inhibit the proliferation of invading pathogenic bacteria will promote gut health, optimize nutrient utilization, and reduce the risk of digestive disorders in poultry production (Rolfe, 1991; Guo et al., 2004).

Understanding the chemical composition of mushrooms and mushrooms residue for utilization as poultry feed:

Silva et al. (2002) measured the potential of the mushroom residue and (A) cotton (*Gossypium hirsutum* L) waste, lemongrass (*Cymbopogon citratus*) leaves (B), and Guinea grass (*Panicum maximum* Jacq [C]) substrates as an ingredient of animal feed. Mushrooms grown on cotton waste, and the resulting SMS, provided the highest concentration of protein, lipids, glucoses and fiber, and grew faster relative to lemongrass and Guinea grass substrates. Further, mushrooms have a high bioconversion efficiency, converting the macronutrients from [minimally digestible] substrates into more bioavailable forms—for inclusion in poultry feeds (Shbeeb et al., 2019). Zied et al. (2023) calculated macronutrient bioconversion efficiency of several CMM species by dividing the dry weight of macronutrient in substrate by the dry weight of macronutrient in finished mushrooms, such that a higher number equals a lower conversion rate. For the conversion of substrate protein to mushroom-based protein, CMM species ranged from about 1.8 (oyster mushroom, *Pleurotus ostreatus*) to 8.5 (enokitake, *Flammulina velutipes*; Zied et al., 2023). This means that for every 8.5 kg of protein in the substrate, enokitake would contain about 1 kg of protein. The conversion of fiber ranged from 15 (enokitake) to 82 (velvet pioppini mushroom, *Agrocybe aegerita*), the ratio of fat conversion was 0.15 (king oyster mushroom, *Pleurotus eryngii*) to about 1.4 (enokitake), carbohydrate conversion ranged from about 60 (button mushroom, *Agaricus bisporus*) to 315 (king oyster mushroom, Zied et al., 2023). Thus, mushrooms scavenge the majority of available lipids, a high to moderate proportion of substrate-based proteins, and a relatively low share of the fiber and carbohydrates.

Mushrooms and health of chickens

CMM as a macronutrient source in poultry. Agaricus and *Pleurotus* mushrooms are low in fat and rich in protein and fiber, making them potential protein substitutes in animal feed (Shbeeb et al., 2019). The study examined the moisture, ash, fiber, protein, fat, and carbohydrate contents of *Pleurotus ostreatus* (oyster mushroom) and *Agaricus bisporus* (button mushroom). *Agaricus* mushrooms had lower carbohydrate contents (47.2 g/100g compared to 61.1 g/100g), and greater ash, protein, and fat contents (8.7, 32.1, and 3.1 g/100g dry wt, respectively) than fresh *Pleurotus* mushrooms (7.7, 20.0, and 2.5 g/100g dry wt, respectively; Shbeeb et al., 2019).

CMM as natural antibiotics. Culinary and medicinal mushrooms may effectively reduce the impact of some viral and bacterial diseases in chickens, thereby reducing antibiotic use and the cost of production. Mushroom polysaccharides enhance the population of intestinal microflora, decreasing the number of harmful bacteria (like *E. coli*), while increasing the number of beneficial bacteria (like *Lactobacillus*; Guo et al., 2004)*).* Though, mushrooms-based polysaccharides were comparable to those of prebiotics (Willis et al., 2009). Mushrooms have been shown to enhance T-cell immune responses—the secretion of interferons to stimulate cytokines and increase macrophage activity (Guo et al., 2003) and stimulate the growth of immune organs in chickens infected with Newcastle disease (Zhang et al., 1998). Chickens stimulated by hot water and methanolic extracts of oyster mushroom (*P. ostreatus*) had improved humoral and cellular immune responses resulting in enhanced protection against infection by mixed species of Eimeria (coccidiosis; Ullah et al., 2015). Willis et al. (2012) found that inclusion of 5% of fungus-myceliated grain to caged, broiler-chick feed was effective in protecting the birds from coccidiosis. Lectin isolated from *Fomitella fraxiena* injected into 18-day-old embryos prevented coccidiosis-related weight loss and decreased oocyst shedding after a post-hatch, orally administered *E. acervulina* infection (Dalloul et al., 2006) . Mushroom lectin effectively stimulated the immune system, promoting growth in coccidiosis-affected chickens.

CMM as antioxidants. Supplements containing extracts from *Lentinus edodes*, *Tremella fuciformis*, and other medicinal mushrooms improved immunological responses in chickens infected with *Emeria spp*. The *Eimeria*-infected groups fed the mushrooms extracts showed noticeably greater production of specific IgA, IgM (at days 14 and 21 postinfection), and IgG (at day 21 postinfection) than the infected group not fed the extracts. (Guo et al., 2004;), which includes a species that causes chicken ceca coccidiosis (Willis et al., 2011). Fungus-myceliated grain, added to chicken feed at 10% (w/w) and at 5% (w/w) in starter-broiler feed provide optimum anticoccidial protection for body weight, respectively. Supplements containing shiitake mushroom (*Lentinus edodes*) have decreased *Salmonella* infections while increasing the populations of beneficial gut microbes like *Bifidobacteria* (Willis et al., 2009).

Supplementing Mealworms with Spent Mushrooms Substrate

Over the years, a number of studies have evaluated the possibility of using insects as a source of protein in the animal feed industry. Currently, insect protein can be utilized to feed fish and legislation is being drafted in the European Union to allow insects to be fed to poultry. In the United States, the Food, Drug, and Cosmetic Act mandates that animal products be safe, hygienic, and free of dangerous or detrimental ingredients. The Association of American Feed Control Officials' (AAFCO) recommendations serve as the foundation for feed restrictions in many states. Only one species of insect has been defined by the AAFCO as an animal feed ingredient for animal feed. Aquaculture feed for salmonids, including salmon, trout, and char, is allowed to contain black soldier fly (*H. illucens*) larvae, including dried entire larvae (since 2016) and black soldier fly meal (since 2018).

It is worthy of note that SMS-containing feed may degrade quickly in damp environments, leading to environmental problems such rancidity in poultry feed and unpleasant stench, but mealworms can feed on the SMS regardless of its states, which can then be applied to animal feed in dry matter without causing rancidity and stench. (Li et al., 2020)

Mycelium or mushrooms are essential to the life cycle of many insects (Vega and Blackwell, 2005). Mealworms' crude protein concentration varies from 45.83% (Hussain et al., 2017) to 54.4% (Biasato et al., 2018), while SMS's crude protein content is only 8.1% (Kim et al., 2007). This variation affects feed quality and production costs. The exhausted mycelia and partially degraded organic material in SMS may provide an inexpensive insect feed source. While considerable amounts of SMS are composted and applied to soil—to increase soil fertility and organic matter content (Dai, Luan & Qi 2017; Lopez et al., 2008)—redirecting this resource to feeding insects bound for poultry feed may improve the economic prospects of CMM, insect, and poultry producers (Onsongo et al., 2018). Insects [as a feed source] provide high-quality proteins, dietary fibers, polyunsaturated fatty acids, and a range of micronutrients (Rumpold and Schlüter, 2013), and many insects rely on mycelium or mushrooms as part of their life cycle (Vega and Blackwell, 2005). The crude protein content of mealworm ranges from 45.83% (Hussain et al., 2017) to 54.4% (Biasato et al., 2018) and only 8.1% in SMS (Kim et al., 2007) which makes the difference in the quality of feed and cost of production.

Further, insects require much less land and have a smaller environmental footprint than the grains grown for poultry feed (Huis et al., 2013).

Tenebrio molitor larvae (TML) feed

The larvae of mealworms (*Tenebrio molitor* L.) may be a sustainable chicken feed because of their high nutritional content and minimal risk of contamination (Melis et al., 2018; Ding et al., 2023). Mealworms are a pest of grains, flour, and other stored foods (Ramos-Elorduy et al., 2002). Mealworms are simple to reproduce and the larval and pupal stages of this pest are high in protein (46 to 60% CP) and they (Ghaly and Alkoaik, 2009). When utilized in poultry diets, the protein quality of mealworms is comparable to Soybean Meal (SBM), but the methionine content may be restrictive (Ramos-Elorduy et al., 2002). However, Silva et al., 2002 showed TML providing higher methionine content than SBM . Inclusion of insect meal to replace soybean and fish meals in poultry diet as accounted for 25.0% better return on investment (Onsongo et al., 2018).

Bovera et al. (2016), showed that complete replacement of SBM with TML is possible in the feed formulation of poultry. In the study, the growth efficiency, nutrition absorption, and broiler carcass and meat characteristics were improved with the replacement of SBM with TML. The carcass characteristics of broilers slaughter are shown in Table 4.4: In comparison to broilers fed TML, broilers fed SBM showed a decreased absolute and relative weight for the complete digestive system ($P < 0.05$). Additionally, the SBM group's weight and spleen % were lower ($P < 0.05$) than those of the TML groups, although there were no differences found between the groups for the other traits shown in the table.

Silva et al., 2002 also show that TML as a main source of protein in poultry feed ingredient has no significant effect on the carcass traits and the growth performance, especially between 30 to 62 days of age. However, the effect of chitin on the TML fed broilers makes it has longer intestine and weight which results in larger spleen weight in broilers, this might not be desirable where the concentration is on growth and meat production, but in places such as Africa, this is desirable as the intestine and spleen are considered a form of variety meat.

From boosting immune function and reducing inflammation to providing antioxidants and essential nutrients, mushrooms offer a natural and holistic approach to enhancing overall well-being of poultry (Bederska-Łojewska et al., 2017). Additionally, SMS may promote weight gain, improve feed efficiency, and even enhance the quality of meat and milk (Huang et al., 2023).

Conclusion:

This review showed the potential inclusion of culinary and medicinal mushrooms (CMM) as an alternative feed/supplement in chicken production. CMM and SMS have potential to benefit chicken production in supporting health growth and combating coccidiosis, *Salmonella* infections, improve digestive tract of the chicken and reduce *E. coli* infestation. Further research is needed on the nutritional and medicinal properties of CMM and SMS products, especially as its relates to the impact of those products on chicken growth and health. U.S. Producers would benefit from research screening all domestically produced species of CMM to evaluate the nutritional and medicinal properties of the waste streams from mushroom production. Future studies should evaluate the potential to treat infectious bronchitis disease, other strains of coccidiosis, Newcastle disease virus and Marex disease, all conditions still reliant on prophylactic antibiotic and vaccine usage.

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Table 4.1 - Percentage of Protein, Lipids, Ash, Glucoses, Fibre and Minerals in the substrates used for mushroom cultivation (n = 3)

Assays/ Substrate	Cotton Waste	Lemongrass	Guinea Grass
Protein (%)	10.63 ± 0.05	7.87 ± 0.10	7.55 ± 0.05
Lipids (%)	4.17 ± 0.13	2.77 ± 0.07	0.91 ± 0.02
Ashes (%)	6.85 ± 0.15	7.40 ± 0.11	5.97 ± 0.12
Glucoses (%)	0.80 ± 0.08	0.67 ± 0.05	0.79 ± 0.04
Fibre (%)	49.02 ± 0.22	28.40 ± 0.10	37.50 ± 0.12
Iron (%)	0.048 ± 0.002	0.039 ± 0.004	0.006 ± 0.001
Magnesium (%)	0.0015 ± 0.0001	0.0012 ± 0.0001	0.1410 ± 0.0014
Potassium (%)	0.0085 ± 0.0002	0.0086 ± 0.0002	0.3100 ± 0.0120
Calcium (%)	0.0065 ± 0.0002	0.0066 ± 0.0003	0.2600 ± 0.0150

n = n^{o}- of repetition

Silva et al. (2002)

Table 4.2 - Percentage of Protein, Lipids, Ashes, Fibre and Minerals in the fruiting bodies of *Pleurotus pulmonarius* (Fr.) Quél., cultivated on different substrates. (n=3)

Assays / Mushroom	Cotton Waste	Lemongrass	Guinea Grass
Protein (%)	20.03 ± 0.04	16.90 ± 0.03	26.82 ± 0.10
Lipids (%)	2.62 ± 0.43	2.02 ± 0.20	1.05 ± 0.10
Ashes (%)	7.33 ± 0.13	5.86 ± 0.13	7.07 ± 0.15
Fibre (%)	9.00 ± 0.01	4.00 ± 0.01	4.94 ± 0.02
Iron (%)	0.0075 ± 0.0001	0.0154 ± 0.0012	0.0105 ± 0.001
Magnesium (%)	0.2593 ± 0.002	0.1011 ± 0.001	0.0707± 0.001
Potassium (%)	2.3709 ± 0.0020	1.9941 ± 0.0030	0.5790 ± 0.0020
Calcium (%)	0.0038 ± 0.0002	0.0684 ± 0.0011	0.0034 ± 0.0002

n = n^0 of repetitions

Table 4.3 - Percentage of Protein, Lipids, Ashes, Glucoses, Fibre and Minerals in the residue after oyster mushroom *(Pleurotus pulmonarius*) cultivation.

Assays / Residue	(A) [i]	(B) [ii]	(C) [iii]
Protein (%)	9.35 ± 0.32	4.24 ± 0.09	5.90 ± 0.41
Lipids (%)	2.03 ±0.33	3.20 ± 0.34	2.05 ± 0.58
Ashes (%)	9.20 ± 0.15	9.55 ± 0.21	7.90 ± 0.20
Glucoses (%)	0.90 ± 0.15	1.35 ±0.07	0.90 ± 0.90
Fibre (%)	37.06 ± 0.90	23.26 ± 2.04	26.66 ± 2.25
Iron (%)	0.0807 ± 0.0026	0.0671 ± 0.0022	0.0186 ± 0.0001
Magnesium (%)	0.1039 ± 0.0006	0.0890 ± 0.0034	0.2496 ± 0.0072
Potassium (%)	0.4324 ± 0.0002	0.4535 ± 0.0044	1.8214 ± 0.0351
Calcium (%)	2.7350 ± 0.2121	1.5712 ± 0.4790	0.7398 ± 0.0435

n = n^0 of repetition

Silva et al. (2002)

i. A is the percentage of Protein, Lipids, Ashes, Glucoses, Fibre and Minerals in the residue after oyster mushroom *(Pleurotus pulmonarius*) cultivation using cotton waste as substate
ii. B is the percentage of Protein, Lipids, Ashes, Glucoses, Fibre and Minerals in the residue after oyster mushroom *(Pleurotus pulmonarius*) cultivation using leaves of Cymbopogon citratus as substate
iii. C is the percentage of Protein, Lipids, Ashes, Glucoses, Fibre and Minerals in the residue after oyster mushroom *(Pleurotus pulmonarius*) cultivation using leaves of Panicum maximum Jacq as substate

Table 4.4 - Live weight and carcass traits of broilers selected for euthanasia fed soybean meal (SBM) or *Tenebrio molitor* larvae meal (TML) at 62 d of age (8 experimental units per group, each consisting of 2 birds)

Item	SBM	TML	RMSE[1]	*P*-value
Weight, g				
Live weight	3,376	3,469	341	0.470
Digestive system	357.3[b]	424.1[a]	67.5	0.015
Head	88.3	84.9	13.2	0.497
Dressed carcass	2,428	2,515	276	0.194
Carcass without internal viscera	2,313	2,361	241	0.601
Liver	59.7	58.1	6.8	0.521
Kidney	16.8	18.1	3.4	0.326
Hearth	16.9	18.5	2.2	0.071
Lungs	24.6	26.7	4.0	0.271
Spleen	4.40[b]	5.57[a]	1.20	0.041
Percentage on live weight, %				
Digestive system	10.4[b]	12.7[a]	2.5	0.021
Head	2.61	2.45	0.58	0.473
Carcass yield	71.2	73.2	8.4	0.379
Eviscerated carcass yield	68.5	68.9	8.1	0.901
Liver	1.77	1.67	0.18	0.146
Kidney	0.50	0.52	0.15	0.395
Hearth	0.50	0.55	0.24	0.239
Lungs	0.73	0.77	0.16	0.961
Spleen	0.12[B]	0.16[A]	0.05	0.008

[a,b]Means within a row with different superscripts differ ($P < 0.05$). [A,B]Means within a row with different superscripts differ ($P < 0.01$). [1]RMSE = root mean square error.

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Chapter 5 - Concluding chapter

Stropharia rugosoannulata (L.), commonly known as the King Stropharia or wine-cap mushroom, is a wood-loving fungus that thrives on organic substrates. Many vegetables also perform well when grown with organic mulches like woodchips or straw. This juxtaposition presents an opportunity for farmers to potentially boost their income by intercropping wine caps with compatible vegetable crops. However, no studies have been identified that report on the feasibility of this approach or its impact on vegetable yields. The foregoing research has provided an insight to the compatibility of intercropping wine caps with asparagus and tomato. Successfully producing wine caps in mulched asparagus represents a proof-of-concept that wine-cap can effectively be grown with a vegetable crop, drawing from an otherwise underutilized component of the production system, the organic mulch. Woodchip mulch limits the competition of weeds with the asparagus plants and is reported to add organic matter and moderate soil temperatures (a benefit in the extreme heat and cold of the northeastern Kansas summers and winters). Using those woodchips as a substrate to grow mushrooms increases the utility of the mulch by creating a potential return to the farm. Unfortunately, the intercrop of wine-cap mushrooms with tomato was not as encouraging. And, while the relative failures of both the tomato and mushroom crops could have had unrelated causes, our tentative conclusion is that wine caps may not be a compatible intercrop with tomato in the field. However, we recommend further research to confirm the incompatibility. Finally, I have reviewed the use of mushrooms and mushroom waste in chicken feed to improve chicken health and production.

Mulching asparagus with woodchips can effectively reduce weed competition, significantly lowering the labor needed for weed management. The yields of asparagus remained largely unaffected by mulching until the mulch depth reached 12 inches, at which point harvests were delayed, and both yield and quality were negatively impacted. The depth of woodchip mulch had only minor effects on wine-cap mushroom production, with a 4-inch mulch layer producing slightly larger, but potentially lower-quality mushrooms compared to deeper mulch treatments. Due to the gregarious spread of wine-cap mushrooms (*Stropharia rugosoannulata*) into uninoculated plots, it was not possible to assess properly whether the presence of mushroom inoculum in the mulch affected asparagus growth directly. However, the presence of wine caps did not appear to cause negative effects compared to bare ground.

For future research on intercropping wine-cap mushrooms with asparagus or other vegetables, we recommend increasing the space between plots to better isolate treatments. Here, mylar insulation was an inadequate barrier to prevent *S. rugosoannulata* mycelium from spreading to non-inoculated plots. Additionally, using separate tools for inoculated and non-inoculated plots or implementing a sterilization protocol for tools after working in inoculated plots could help prevent contaminating non-inoculated plots with viable propagules from inoculated plots. These measures could improve the understanding of the interaction between wine-cap mushrooms and vegetable crops.

Our findings do not support recommending wine-cap mushrooms as a compatible intercrop with tomato. While a few wine caps emerged from woodchips in a buffer plot, indicating that the mushrooms can grow near tomato plants, no mushrooms grew in woodchips, wheat straw, or soy straw in our research plots during either of the two growing seasons after inoculation. We recommend further research to determine if the incompatibility is specific to the tomato species, cultivar, or influenced by the climate and soil conditions of northeast Kansas. While mushroom production was unsuccessful, there appeared to be a slight protective effect of mulch inoculation on tomato yield and marketability during the second year, when a heavy pathogen load severely impacted the crop. If a compatible tomato cultivar or intercrop method is identified, rotating plots out of tomato production may be necessary to maintain fruit yields year after year in a warm, humid environment, like northeast Kansas. Additionally, soy straw appears to be a viable organic mulch, performing similarly to black plastic mulch in most metrics. While soy straw required significantly less time for weeding than other mulches, applying it and planting into it was more time consuming compared to installing plastic mulch. However, organic mulches do not require the capital investment or learning curve associated with using a plastic mulch layer. This highlights soy straw as a cost-effective and sustainable alternative for weed management in tomato production systems.

Culinary and medicinal mushroom (CMM), and byproducts of the cultivation of those mushrooms, may be incorporated into chicken feed. Studies have reported CMM and their byproducts may enhance chicken health by providing antioxidants, like phenolic compounds, to improve immune function. CMM not only supplies essential micro and macronutrients but also offers a viable, cost-effective alternative to expensive ingredients like soybean meal, benefiting poultry farmers with reduced feed costs and potentially higher profits. Additionally, CMM waste, including spent mushroom substrate, may provide a sustainable solution for feeding mealworms, a rich protein source for poultry. By redirecting SMS as mealworm feed, producers can decrease agricultural waste and contribute to a circular, environmentally friendly poultry production system. Integrating CMM and SMS in poultry nutrition not only advances bird health and growth but also promotes eco-friendly practices by repurposing mushroom by-products, aligning with sustainable agriculture objectives.

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Appendix A - More tables of results from tomato chapter

Appendix Table A.1 - Effect of mulch type and inoculation with *Stropharia rugosoannulata* mushroom on size distribution of 'Galahad' tomato in Manhattan, KS in 2021 and 2022[i]

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

		Jumbo [iii]				Extra Large				Large				Medium				Small			
Mulch	**Inoc [ii]**	**2021**		**2022**		**2021**		**2022**		**2021**		**2022**		**2021**		**2022**		**2021**		**2022**	
		No	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**	**No**	**Wt**
Bareground	**No**	7.3	2.39	0	0	30.0	6.83	0	0	17.3	2.76	0.08	0.01	8.3	0.96	0	0	2.8	0.23	0.08	0.01
Black Plastic	**No**	5.3	1.70	0	0	30.5	7.16	0.03	0.01	15.5	2.52	0.14	0.02	6.8	0.77	0.19	0.02	3.0	0.24	0.28	0.02
Wheat Straw	**No**	6.5	2.27	0	0	24.5	6.22	0.07	0.01	15.0	2.42	0.13	0.02	5.0	0.58	0.23	0.03	4.5	0.33	0.57	0.04
Wheat Straw	**Yes**	8.0	2.77	0	0	25.3	7.97	0	0	16.3	2.59	0.03	0	8.0	0.96	0.14	0.01	3.3	0.20	0.59	0.04
Soy Straw	**No**	9.8	3.28	0	0	26.3	5.87	0	0	15.5	2.45	0.10	0.02	5.3	0.63	0.30	0.03	3.8	0.28	0.23	0.02
Soy Straw	**Yes**	10.5	3.62	0	0	33.5	5.91	0	0	15.5	2.50	0	0	6.5	0.87	0.22	0.02	1.5	0.11	0.75	0.05
Woodchips	**No**	6.8	2.27	0	0	15.0	3.53	0	0	5.8	0.94	0.14	0.02	1.8	0.21	0.05	0	0.8	0.06	0.43	0.03
Woodchips	**Yes**	6.8	2.38	0	0	21.5	5.04	0.08	0.02	8.0	1.32	0.19	0.03	4.0	0.48	0.42	0.05	0.5	0.04	0.12	0.01

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)

iii. USDA grading standard of tomato fresh fruit

Appendix Table A.2 - Effect of mulch type and inoculation with *Stropharia rugosoannulata* mushroom on marketable fruits of 'Galahad' tomato in Manhattan, KS in 2022[i].

Treatment		Marketable Fruit % of Total (w/w)	Marketable Fruit Weight per fruit (g)
		2022	2022
Mulch Treatment	**Inoc[ii]**		
Bare ground	**No**	8.96	0.129
Black Plastic	**No**	3.41	0.083
Wheat Straw	**No**	7.93	0.104
Wheat Straw	**Yes**	9.31	0.077
Soy Straw	**No**	2.29	0.098
Soy Straw	**Yes**	4.45	0.090
Woodchips	**No**	4.35	0.091
Woodchips	**Yes**	6.06	0.117

i. All means presented are non-transformed. Means within a column followed by the same letter are not significantly different (P > 0.05) as determined by two-sided tests, with a = 0.05, and Tukey's adjustment applied to pairwise comparisons.

ii. Inoculated with *S. rugosoannulata* sawdust spawn (Field and Forest Products, Peshtigo, Wisconsin, USA)