



Tritrophic interactions with *Silphium integrifolium* at the axis – Elucidating the role of arbuscular mycorrhizal fungal species on plant phenotype

Philipp Hansen

Degree project/Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Agroecology Master's Programme
Alnarp 2023



Tritrophic interactions with *Silphium integrifolium* at the axis – Elucidating the role of arbuscular mycorrhizal fungal species on plant phenotype

Philipp Hansen

Supervisor: Teun Dekker, Swedish university of agricultural sciences,
Department of Plant Protection Biology
Assistant supervisor: Chase Stratton, The Land Institute, Crop Protection Ecology
Assistant supervisor: James Bever, University of Kansas, Department of Ecology and
Evolutionary Biology
Examiner: Björn Bohman, Swedish University of Agricultural Sciences,
Department Plant Protection Biology

Credits: 30
Level: Second Cycle, A2E
Course title: Independent Project in Agricultural Science
Course code: EX0848
Programme/education: Agroecology Master's Programme
Course coordinating dept: Department of Biosystems and Technology
Place of publication: Alnarp
Year of publication: 2023

Keywords: Arbuscular mycorrhiza, mycorrhizal responsiveness, induced plant
volatiles, *Silphium integrifolium* (Silflower), perennial grains, new
crops, ecological intensification

Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Biosystems and Technology

Foreword

Upon reflection, I think that I embarked on the journey of studying agroecology hoping to broaden and deepen my knowledge and looking for some answers, just like the many students before and with me. After a couple of years of running a small-scale, back-breaking vegetable operation, I wondered how to achieve a truly sustainable agricultural vision. While I fully subscribed to the idea of improving the environmental impact of food production, I had started to understand that the social sustainability issues and policy challenges we are facing were equally as important - and potentially more difficult - to address.

Naturally, a field as complex, transdisciplinary and ambitious as agroecology does not lend itself to straight-forward insights and clear-cut answers. Despite this frustrating realization sinking in within the first months of studies, my conviction that I was truly passionate about agriculture and food in general grew stronger. To enrich the study experience for all students and translate theory into action, as it is often put in agroecology, my fellow students and I started looking into opportunities to establish an educational farm. We ended up starting Alnarp's Agroecology Farm, a meeting place for students and the local community to grow, learn, and explore small-scale food production. Now, two years after the we started the first negotiations to get a small piece of land on campus, it is wonderful to see that the farm keeps growing, not only in size, but activities, with people from different universities and the local community involved.

To complete this course of study, I was aiming to gain some first-hand insights into how agricultural research is conducted. I feel were lucky that I know my good friend Anna who works at The Land Institute in Kansas and inspired me to reach out to them to see if I could come over and do my thesis with them. In hindsight, it was perfect for me to spend almost a year there working alongside people with clear goals and ambitious projects, that were welcoming and willing to integrate me and my projects there in their work.

I feel like over the course of the past two and a half years I was able to grow, experiment and reflect on my interests. While I still have to figure out what is next for me, I feel inspired start working in this field that has countless interesting experiences to offer. In sum, I am very grateful for all the interesting people I have met during this journey and the support on both sides of the Atlantic for this thesis.

Abstract

A fundamental understanding of the intricate interactions between crops and their environment may help guide the development of ecologically intensified agriculture. Arbuscular mycorrhizal fungi (AMF) are root symbionts that can increase a plant's access to soil resources and have been found to reprogram plant defensive mechanisms, potentially influencing the performance of plant antagonists. The effect of mycorrhizal association on plant phenotype may thereby depend on fungal genotype, as AMF strains can differ in functional traits. In the presented experiment the impact of native prairie AMF inocula on growth and volatile organic compound (VOC) production of two silflower (*Silphium integrifolium*) accessions was assessed. Plants were grown in the greenhouse with a single species AMF inoculum, a mixture of all strains, or mock-inoculated. To assess if any of the volatile production was mediated by different AMF inocula, plants were stressed with the chewing herbivore *Epicauta funebris* or mechanically wounded prior to VOC measurements. The presence but not the type of native mycorrhizal inoculum was found to be crucial for plant growth as it resulted in a nearly fivefold increase in aboveground biomass compared to mock-inoculated plants. The two silflower accessions also showed marked growth differences in the presence of AMF, indicating that the mycorrhizal responsiveness (MR) of silflower is dependent on plant genotype. Fungal treatment did not lead to distinct VOC profiles, yet the measured VOCs differed among fungal strains and non-mycorrhizal silflower specimen, the latter being found to be more prolific in VOC production per unit biomass than plants grown with some of the AMF strains. Without a direct measure of herbivore performance or attraction of its natural enemies, however, little can be inferred about the actual impact of the mycorrhizal phenotype on crop-antagonist interactions. While further research is required for a better understanding of the role of mycorrhizae in applied settings and if selection for this trait is desirable, these findings suggest that native AMF are an important component of silflower ecology.

Keywords: Arbuscular mycorrhiza, mycorrhizal responsiveness, induced plant volatiles, *Silphium integrifolium* (Silflower), perennial grains, new crops, ecological intensification

Contents

1	Introduction	1
2	Materials and Methods	4
2.1	Study System & Biological Materials	4
2.2	Experimental design	5
2.3	Plant cultivation	5
2.4	Aboveground treatments	6
2.4.1	Herbivory treatment	6
2.4.2	Herbivory mimicry	6
2.4.3	Silphium rust infection	6
2.5	Plant harvest	6
2.6	Assessment of constitutive and induced plant defenses	7
2.6.1	Headspace volatile collection	7
2.6.2	VOC data analysis	8
2.7	Statistical analyses	8
3	Results	10
3.1	Plant growth	10
3.2	VOC analysis	12
4	Discussion	18
4.1	Silflower growth response to mycorrhizal inocula	18
4.2	Mycorrhizal responsiveness by silflower genotype	21
4.3	VOC measurements in response to aboveground treatments	23
4.4	Arbuscular mycorrhizal fungal mediation of VOC measurements	23
4.5	Visioning ecologically intensified agroecosystems with silflower	25
5	Conclusions	26

List of Tables

1	Overview of the mycorrhizal inocula employed	5
2	Analysis of variance results for aboveground biomass	11
3	Overview of the tentatively identified volatile organic compounds	13
4	Permutational analysis of variance results for measured volatile organic compounds	14
5	Analysis of variance results for total and grouped volatile organic compound measurements	18

List of Figures

1	Plant aboveground biomass for mycorrhizal treatments	10
2	Mycorrhizal responsiveness of the two silflower genotypes	12
3	Nonmetric multidimensional scaling plot for volatile organic compound measurements by aboveground treatment	15
4	Nonmetric multidimensional scaling plot for volatile organic compound measurements by mycorrhizal treatment	16
5	Nonmetric multidimensional scaling plot for volatile organic compound measurements by mycorrhizal x aboveground treatment	17
6	Mean measured volatile organic compounds by mycorrhizal x aboveground treatment	19
7	Mean decrease in accuracy estimates derived from the random forest algorithm for classification of volatile organic compound samples by aboveground treatment	20
8	Group membership predictions derived from the random forest algorithm	22

Abbreviations

AMF arbuscular mycorrhizal fungi	1
MR mycorrhizal responsiveness	1
VOCs volatile organic compounds	2
GLVs green leaf volatiles	2
TLI The Land Institute	3
GCMS gas chromatography coupled with mass spectrometry	8
ANOVA analysis of variance	8
PERMANOVA permutational multivariate analysis of variance	9
NMDS nonmetric multidimensional scaling	9
MDA mean decrease in accuracy	9
DMNT 4,8-dimethylnona-1,3,7-triene	12

1 Introduction

The projected increase in global population and changing dietary preferences are thought to induce a 25-75% surge in the demand for global crop production by the year 2050 (Davis et al., 2016; Hunter et al., 2017). With a large share of the world's arable land area already being cultivated and land being lost due to urbanization, this calls for further intensification of cropping systems (Bommarco et al., 2013). While the agricultural sector has seen remarkable productivity gains through key mechanical, chemical and molecular innovations in the past century, yield gains have been stagnating in recent years (Grassini et al., 2013). The industrial agriculture that is common in many developed countries is also highly dependent on foreign inputs of fertilizer and other agrochemicals, leaving these systems vulnerable to input shortages (Altieri, 2018; Cordell and White, 2014). Of further concern are concurrent environmental impacts such as the observed loss of biodiversity and soil degradation, as well as vulnerability to severe weather events that may intensify due to rapid climate change (Evans et al., 2020; Gliessman, 2014; Krupek et al., 2022). As a path towards the reconciliation of crop production and environmental integrity, ecological intensification has been proposed (Bommarco et al., 2013). While ecological intensification is highly context dependent, its practices are generally inspired by natural ecosystems, including the promotion of biodiversity, self-regulation, and perenniality to replace foreign physical and chemical inputs (Tittonell, 2014). Establishing and managing such complex agroecosystems calls for a fundamental understanding of the ecological processes shaping them (Doré et al., 2011).

Organisms that play a crucial role in the ecology of many plant species are arbuscular mycorrhizal fungi (AMF). These ancient endosymbionts belong to the phylum *Glomeromycota* and form associations with an estimated 80% of extant land plants (Meier and Hunter, 2018). They have a range of direct and indirect effects on plant fitness and are thought to have played a pivotal role in the terrestrialization of plants (Rich et al., 2021). Upon infection of the roots, AMF produce an extraradical hyphal network able to access resources beyond root depletion zones (Jacott et al., 2017). Plant and fungus engage in a reciprocal exchange, with the plant providing carbon in the form of hexose sugars in return for phosphates, water and other micronutrients (Van Der Heijden et al., 2006; Wang et al., 2019). Although often thought of as a classical example of mutualism, growth benefits derived from mycorrhization, referred to as mycorrhizal responsiveness (MR), are context dependent (Johnson et al., 1997). In highly amended fields MR may be low, since the efficiency of fungal nutrient acquisition does not exceed the plant's ability to access readily available resources (Gianinazzi et al., 2010; Rillig et al., 2019). Mycorrhizal responsiveness also varies by plant species and has often been found to be low for annual monocots, which encompass some of our most important crops such as wheat and corn (Lehnert et al., 2018; Smith and Smith, 2011). The resulting uncertainty on where crop associations with AMF fall on the mutualism-parasitism continuum has consequently led to the role of AMF having long been neglected in breeding and overlooked in industrial agriculture at large, with proponents persisting that managing for AMF should be of little concern to practitioners (Ryan and Graham, 2018).

Renewed interest stems from the phosphorus provisioning services that AMF render (Thirkell et al., 2022). Not only are phosphorus amendments mined from increasingly scarce rock phosphate reserves (Herrera-Estrella and López-Arredondo, 2016), but this key plant macronutrient is also quickly immobilized (Tinker, 2000). This results in the build-up of plant unavailable 'legacy phosphorus' in agricultural soils (Rowe et al., 2015; Xie et al., 2019). In order for crops to utilize the phosphorus stocks in soils, their mobilization is required, which AMF symbiosis facilitates (Thirkell et al., 2022). Beyond their proposed capacity to increase phosphorus resource use efficiency in conventional systems, AMF are of great interest for backers of ecological intensification that target decreasing dependency on foreign inputs by revamping soil health and biological diversity (Gianinazzi et al., 2010). Arbuscular mycorrhizal fungi are known to improve

for soil aggregation and soil organic carbon stabilization by producing the glycoprotein glomalin (Rillig, 2004). Association with AMF has also been found to be beneficial for drought tolerance of plants by not only increasing access to water resources, but also by reprogramming plant physiology to increase water use efficiency (Augé, 2001; Bernardo et al., 2019; Lehnert et al., 2018). A further non-nutritional effect can be a change in tolerance of or defense against pests and pathogens (Koziol et al., 2019; Rasmann et al., 2017; Vannette and Hunter, 2009). Realizing these potentials should warrant further attention towards the role of AMF in agriculture.

Previous research has shown that the effect of AMF symbiosis on aboveground herbivores can be positive, negative or negligible, both in controlled conditions (Bennett and Bever, 2007; Kempel et al., 2010; Meier and Hunter, 2018; Real-Santillán et al., 2019), as well as in the field (Bernaola et al., 2018; Gange et al., 2003; Gehring and Bennett, 2009). To explain these highly variable outcomes, the following underlying mechanisms for AMF mediation of herbivore performance have been suggested: a) increase in plant quantity b) increase in plant nutritive value, c) increase in plant tolerance, d) regulation of plant defense and e) interference as defense in the rhizosphere (Gehring and Bennett, 2009; Rasmann et al., 2017). These mechanisms are interrelated and may be conducive to either plant or herbivore fitness. Antagonists may benefit from more plentiful and more nutritious plant tissues derived from mutualistic AMF-plant associations, but the plant may also have more resources to allocate towards defense (Pineda et al., 2010; Vannette and Hunter, 2009). Increased resource availability may also facilitate regrowth more effectively after attack, thereby increasing plant tolerance (Gehring and Bennett, 2009). Aside from these effects arising from plant nutritional status, mature symbioses may alter the plant defensive phenotype by regulating metabolic pathways independently of plant nutrition (Vannette and Hunter, 2009). A known mechanism upon herbivory, which may be more pronounced in mycorrhizal plants, is the increased concentration of jasmonic acid at the site of damage (Kaur and Suseela, 2020). This hormone ‘primes’ the plant’s defenses, a change in physiological state that can result in an accelerated and potentially stronger response in the form of defensive secondary metabolite production (Conrath et al., 2006; Pineda et al., 2010).

A component of the plant’s defensive phenotype is volatile organic compounds (VOCs), which are secondary plant metabolites released by plant organs into their ambient environment. These volatiles can have various ecological functions such as pollinator attraction (Quarrell et al., 2022, under review) or plant-plant signaling (Glinwood et al., 2011; Pierik et al., 2014). When it comes to the emission of volatiles, a distinction between constitutive and induced volatile blends is often made (Davidson-Lowe et al., 2021; Dicke et al., 2003). Constitutive emission from vegetative organs, that is the release without an acute stressor, may be limited as it is costly and may lead to attraction of antagonists (War et al., 2012). In contrast, the release of VOCs usually increases in response to stressors such as an arthropod herbivore or a pathogen (Dicke, 2009). These induced VOCs may serve as semiochemicals mediating plant indirect defenses by attracting parasitoids and arthropod carnivores (Huang et al., 2020; War et al., 2012), direct defenses by repelling the attacker (Sharma et al., 2017), as well as potentially signaling a pending attack to undamaged plant organs or other plants (Dicke and Baldwin, 2010). Plant headspace VOCs generally consist of a plethora of volatile metabolites derived from different metabolic pathways such as various terpenes and green leaf volatiles (GLVs) (Holopainen, 2004). The quantity and composition of the VOCs released depend not only on the genotype of the plant as well as its attacker (Pierik et al., 2014), but also on belowground mutualists such as AMF (Sharma et al., 2017). Few studies to date have focused on the effect of AMF on the production of plant volatiles (Gehring and Bennett, 2009; Pineda et al., 2010; Rasmann et al., 2017).

Overall, the intricate interplay between AMF-plant-antagonist actors is far from fully understood. To add to the complexity, interactions are not only specific to the organisms studied, but also the environmental parameters of the experiment, greatly limiting the transferability

of results (Kaur and Suseela, 2020; Meier and Hunter, 2018; Schweiger et al., 2014). Variables that can influence both plant growth and plant defensive phenotype are the abundance, diversity and species identity of mycorrhizal symbionts (Koziol et al., 2018; Vannette and Hunter, 2009). Arbuscular mycorrhizal fungi are not created equal, with the cosmopolitan phylum consisting of several hundred species identified to date, distributed over 14 families and a total of 29 genera (Oehl et al., 2011). Species differ in key functional traits such as in their efficiency of phosphate provision and carbon demand, or investment into extraradical versus intraradical biomass (Chagnon et al., 2013; Munkvold et al., 2004). This is demonstrated by observed differences in mycorrhizal responsiveness (MR) among different AMF (Mensah et al., 2015; Watts-Williams et al., 2019). Similarly, the production of secondary metabolites in mycorrhizal plants has been shown to vary with the genotype of the fungal symbiont (Bennett et al., 2009).

Knowledge on how the plasticity of a plant's phenotype is impacted by AMF may motivate breeding for mycorrhizal responsiveness and ultimately facilitate the design of ecosystem service providing agroecosystems that require less foreign inputs by being more resistant to or tolerant of environmental stresses. A promising perennial candidate for such multifunctional and ecologically intensified cropping systems is silflower (*Silphium integrifolium* (Michaux)). Due to its high seed oil content and desirable composition thereof, as well as its high drought tolerance, this plant species is currently undergoing *de novo* domestication by The Land Institute (TLI) in Salina, Kansas, USA (Turner et al., 2018; Van Tassel et al., 2020). Here it is bred as an oilseed and forage crop, with potential uses for fiber and honey production. Some of the advantages of established perennial crops over annuals are their deep, permanent and dense root networks, which contribute to soil organic carbon formation, increase precipitation use efficiency, mitigate nutrient leaching and erosion (Crews et al., 2018). Whereas practices characteristic of conventional annual agriculture such as frequent tillage, fallow soil periods and fungicide application can decrease the abundance and diversity of AMF taxa, decreased disturbance has been shown to benefit fungal communities (Maurer et al., 2014; Rasche et al., 2017; Säle et al., 2015). This highlights the increased potential of AMF to benefit novel perennial over highly managed annual agroecosystems (Crews et al., 2018). To make silflower a viable crop, seed size, yield and other traits of agronomic significance need to be improved. A further challenge for the breeding program are insect pests and pathogens (Peterson et al., 2022; Turner et al., 2018). Perennial crops such as silflower are grown in the same location for multiple years, providing conducive conditions for the build-up of high pest and pathogen pressures, which are otherwise disrupted by crop rotation in annual cropping systems (Cox et al., 2005). Unravelling the impact that AMF strain identity and diversity have on the ecology of silflower can thus be insightful for breeding programs, AMF inoculum producers and crop management decisions alike.

The aim of this study was to elucidate how different AMF strains affect *Silphium integrifolium* growth and secondary metabolism. Two silflower genotypes were grown from seed, one stemming from a wild collection and the second being a semidomesticated accession provided by The Land Institute, with five single species and one mixed AMF treatment. The mature plant-fungal association was then exposed to aboveground antagonism in the form of chewing herbivory, herbivory mimicry and rust fungus infection. The following questions were addressed: (1) How is the growth of *Silphium integrifolium* influenced by the presence, strain identity, and diversity of AMF? (2) Do wild versus semidomesticated accessions of *S. integrifolium* differ in their growth responses to AMF treatments? (3) Do AMF treatments influence silflowers' reaction to antagonism in the form of herbivory, herbivory mimicry and fungal disease? The plant's response to antagonism was thereby investigated by collecting headspace VOCs upon conclusion of each aboveground treatment.

2 Materials and Methods

2.1 Study System & Biological Materials

Silphium integrifolium (Asterales: Asteraceae), commonly known as silphium, silflower or prairie rosinweed, is a long-lived, late successional grassland species native to 21 states in the central to eastern United States (Van Tassel et al., 2020; Vilela et al., 2018). In their first year the plants form a dense rosette and produce vegetative buds in late summer (Vilela et al., 2020). In the following growing season, internode elongation leads to flowering stems with pairwise leaves and inflorescences 5-7 cm in diameter which resemble the flowers of annual sunflowers (Vilela et al., 2018). Several large vertical roots provide access to resources in deeper soil layers and contribute to the plants' apparent drought tolerance. Silflower has also been shown to be highly beneficial to native pollinator communities (Butters et al., 2022). The *S. integrifolium* used in this study were two types: a wild collection and an accession from The Land Institute (TLI). For the wild collection, seeds were collected from a wild silflower population at a local remnant prairie in Jerry Smith Park (Kansas City, MO, United States; 38°52'48.7"N, 94°34'02.4"W) in the fall of 2021. The TLI accession was a genetically diverse set of silflower germplasm, semi-domesticated for multiple agronomic traits such as improved seed set and increased inflorescence size. The original wild collections for the breeding population were mainly conducted in central Kansas, close to the western limits of silflower's natural range. Seed from this accession was provided by TLI's silflower breeding program.

The major pest challenge for silflower cultivation has been a specialist caterpillar, *Eucosma giganteana* (Riley; Lepidoptera: Tortricidae); however, other generalist chewing herbivores are also known to feed on this plant (Peterson et al., 2022). As *E. giganteana* has not been successfully reared in the laboratory, silflower's response to a generalist herbivore, the margined blister beetle (*Epicauta funebris*, Coleoptera: Meloidae), was tested instead. This beetle has been reported to feed on silflower and other native forbs (Marschalek, 2013). For this experiment, adult *E. funebris* were collected at Jerry Smith Park on the same day as the planned herbivory treatment and placed individually in brown paper bags during transport. After feeding on the plants, most of the beetles were returned to their native environment, while a few specimens were frozen and then later identified to species by Dr. Zack Falin, the entomology collections manager and Coleoptera specialist at the University of Kansas.

Of the multiple pathogens that infect silflower, one of the most serious is silphium rust *Puccinia silphii* (Pucciniales: Pucciniaceae), a microcyclic rust fungi that infects species in the *Silphium* genus (Turner et al., 2018). Severe levels of infection may build up under conducive conditions, resulting in stunted growth and significantly reduced productivity of silflower stands (Turner et al., 2018). The pathogen is a major challenge for breeding at TLI, which has led to renewed silflower collection efforts throughout its native range to screen wild genotypes for rust resistance (Van Tassel et al., 2020). To infect the plants with silphium rust, plant material with developed rust telia were collected from the same *S. integrifolium* population the wild germplasm was obtained from.

For the mycorrhizal treatments, inocula were obtained from single species cultures, which were originally isolated from local prairies. As AMF are obligate biotrophs, the fungi were cultured on mixtures of native mycorrhizal forbs and grasses growing in 19 l buckets filled with an autoclaved 6:10 mix of surface to local prairie soil. Mature cultures were dried and refrigerated to maintain a high spore viability. The five AMF species employed in this experiment were *Rhizophagus clarus*, *Funneliformis mosseae*, an *Ambiospora* species, another *Rhizophagus* species and a further, currently unidentified species. The spores of each single species culture were examined by taking a 50 cm⁻³ culture soil sample, which was blended with tap water and the suspension strained through two sieves, with the second having a 38 μ m mesh width to retain

AMF spores. To further separate the spores, the retained material was suspended in a 60% sucrose solution and centrifuged (Bever et al., 1996). The supernatant was subsequently decanted on to a plate and spores enumerated under the microscope, leading to a spore density estimate for each single species culture (Table 1). In addition to the five AMF treatments with single species inocula, a mixed species inoculum of all AMF species was produced by combining equal parts of each AMF culture soil.

Table 1: Overview and spore density counts of the arbuscular mycorrhizal fungi single species cultures used in this study according to Bever et al. (1996).

Family	Species	Inoculum spore density (per 100 cm ⁻³)
<i>Ambisporaceae</i>	<i>Ambispora sp.</i>	2360
<i>Glomeraceae</i>	<i>Funneliformis mossae</i>	340
<i>Glomeraceae</i>	<i>Rhizophagus clarus</i>	1040
<i>Glomeraceae</i>	<i>Rhizophagus sp.</i>	560
Unknown	Unidentified sp.	1175

2.2 Experimental design

To study tritrophic interactions with silflower at the axis, a greenhouse experiment was conducted at the Kansas Biological Survey in Lawrence, Kansas, United States in 2022. The factors considered and manipulated in this experiment were plant genotype, belowground AMF treatment and aboveground stress. Five single species AMF inocula (Table 1), one mixture of all species and a non-inoculated control served as belowground treatments. To explore the response of plant-AMF associations to stress, plants were exposed to either *Epicauta funebris* chewing herbivory, herbivory mimicry by hole punching, infection with *Puccinia silphii* or an undamaged control treatment. For this multifactorial design, five replicates per germplasm source, AMF and aboveground treatments were planted, resulting in 5 replicates x 2 germplasm sources x 7 AMF treatments x 4 aboveground treatments = 280 plants. Five blocks were designated in the greenhouse, with replicates from all treatments evenly distributed between blocks and treatment replicate locations within each block randomized.

2.3 Plant cultivation

Silflower seeds from the two accessions were spread out on individual trays filled with twice autoclaved Ultimate Organic PRO-MIX[®] (Premier Tech, Quakertown, PA) and cold moist stratified from January 13th until March 24th. The trays were then transferred to the greenhouse for germination. Subsequently, the plants were grown and hand-watered in the trays for 4 weeks. Seedlings were transplanted into individual 983 ml Deepot containers (D60L, Stuewe & Sons, Inc., Tangent, OR) with an autoclaved soil mixture consisting of one part local prairie soil to one part sand as growing substrate. Each container was first filled with 700 ml of the soil:sand mix, then 50 ml of AMF inoculum were amended to the direct rooting zone of the seedling before planting the seedling and topping up the container with 200 ml of growing substrate. Non-inoculated controls received an additional 50 ml of the autoclaved soil:sand mixture instead of AMF inoculum.

To reintroduce a non-fungal soil microbiome to the autoclaved growing medium, 5 ml of a bacterial wash was applied to each pot. This wash was prepared by blending 5 cm³ of soil from

each AMF species culture with water before filtering the suspension through filtering screens with the following mesh sizes, sequentially: 1 mm, 500 μm , 250 μm , 38 μm . 450 ml of the resulting liquid were filtered through WhatmanTM filter paper with 11 μm particle retention (Whatman plc, Maidstone, UK) and then diluted to 2 liters prior to application. This filtration method removes the fungal soil constituents while retaining the unicellular soil microbiome.

All containers were watered with drip irrigation automated to water for two minutes every other day to twice daily, dependent on greenhouse temperature conditions and plant size. 12 weeks post transplanting, 50 ml of a nitrogen fertilizer solution were applied per plant to reduce nitrogen limitations. Seedling transplanting commenced on April 14th and the final aboveground biomass harvest was conducted on August 11th, after 17 weeks of growth with the respective belowground mutualist treatments.

2.4 Aboveground treatments

2.4.1 Herbivory treatment

For the generalist herbivore treatments, stainless steel mesh cages were built around each plant container and secured with a zip tie and hot glue. On August 6, 2022, *E. funebris* adults were collected from the field as previously described, and one beetle was placed in each cage after a starving period of 3-4 hours. After feeding overnight, the beetles were removed and volatile organic compounds (VOC) measurements were conducted on the plants. As three VOC collection rounds had to be staggered to sample all plants, the beetles were feeding for about 18, 20 and 22 hours, respectively. Most beetles were then returned to their native habitat, while a couple were retained and placed on ice for identification purposes.

2.4.2 Herbivory mimicry

Chewing herbivory damage was mimicked using a circular, 1 cm² hole punch. Between 6-12 holes per plant were punched on the afternoon of August 6th and the same procedure was repeated the next day prior to each VOC collection round on August 7th. The total amount of leaf area loss per plant ranged from 15-25 cm².

2.4.3 Silphium rust infection

21 days prior to aboveground biomass harvesting plants were placed in a custom-built, sealed humidity chamber for infection with silphium rust. Collected plant material infected with *Puccinia silphii* were suspended above the plants using a mesh screen, with the lesions facing downwards (Thomas et al., 2021). A humidifier was placed in this chamber to stimulate teliospore germination. The plants were kept in these conditions over night and signs of infection were assessed 10 days after attempted infection.

Despite attempting rust inoculation three times, none of the plants were infected with silphium rust. The protocol used was developed at The Land Institute and also failed to yield any infections there during the summer months. Due to the absence of rust infections, the rust treatment plants were excluded from further data collection and analysis.

2.5 Plant harvest

To assess plant growth and vigour, the number and length of the true leaves were measured at the time of transplanting and AMF inoculation, as well as 8 weeks thereafter. Aboveground biomass harvest took two days and occurred four days after the conclusion of herbivory and

herbivory mimicry treatments, respectively. Plants were cut approximately 1 cm above the soil surface, placed separately in paper bags and transported to The Land Institute, where the shoots were dried in a climate controlled drying room set at 15.5 °C and 13% RH for two weeks. Once the dried matter reached a moisture equilibrium, shoot dry weight was recorded.

As an indicator for AMF mediated growth response, mycorrhizal responsiveness (MR), also referred to as mycorrhizal dependency, was calculated following Menge et al. (1978):

$$MR = \frac{DW_{mycorrhizal}}{\frac{1}{n} \sum_{i=1}^n DW_{control}} * 100\% \quad (1)$$

where $DW_{mycorrhizal}$ and $DW_{control}$ are the dry shoot weight of the mycorrhizal plant and dry shoot weight of the non-mycorrhizal control treatment plants, respectively. This indicator was calculated for each of the two seed sources separately to gauge growth response by plant genotype.

2.6 Assessment of constitutive and induced plant defenses

2.6.1 Headspace volatile collection

Due to the significant workload that comes with collecting headspace volatile organic compounds (VOCs), and limitations on the amount of equipment and time available to collect the volatiles, a subset for VOC analyses had to be chosen. The main question to be answered hinged upon the effect of different AMF treatments on growth and plant defense, therefore one of the two seed sources was excluded. Since volatile emissions vary among conspecific genotypes (Pierik et al., 2014), plants grown from the wild seed source were measured, as they were obtained from a single population.

Three runs of VOC collections with 12 pumps were completed daily between 9am and 4pm. Plants were moved to a designated bench in the greenhouse and individually bagged with oven bags (406x444mm, Kitchens™ Oven Bags, Reynolds, Lake Forest, IL, USA). These bags were prepared in the lab with an air inlet at the bottom consisting of an oven bag lined PET tube fitted with an activated carbon filter to remove ambient VOCs from the air entering the bag (Kigathi et al., 2019; Michereff et al., 2011). An insert at the top of each bag was connected to a custom-built pump for air extraction. The pumps were run for 5 minutes to allow for the inside of the bag to be filled with filtered air and avoid potential contamination. Then a volatile collection trap with Porapak-Q™ (Volatile Collection Systems, Gainesville, FL) was inserted. Air was extracted at a rate of 2 +/- 0.4 l/min for two hours through this trap to collect the VOCs emitted by each plant. Traps were wrapped in aluminium foil and stored at -20°C until volatile elution using 400 μ l dichloromethane, which was pushed through the trap into a vial using research-grade helium. These vials were subsequently sealed with PTFE tape to minimize evaporation and stored at -20°C. Before reusing traps, they were cleaned with 1500 μ l of dichloromethane and purged with helium (Ponce et al., 2022).

Two rounds of VOC collections were conducted. Before any aboveground treatments commenced, constitutive VOC measurements were completed from July 5th to July 9th on all 140 wild silflower genotype plants. The second round of constitutive, beetle herbivory and herbivory mimicry induced volatile collections occurred from August 4th to August 8th, with 105 plants being measured after the rust infection had failed. All samples for the aboveground treated plants were collected within one day due to the time sensitive volatile release response upon herbivory and mechanical leaf damage (Holopainen, 2004). To be able to distinguish and exclude volatiles stemming from the soil, container or greenhouse air from the analysis, background volatile samples were collected throughout the sampling process.

To prepare the samples for gas chromatography coupled with mass spectrometry (GCMS) analysis, the vials were first opened in a fume hood at ambient temperatures for 75 minutes to allow for 50-60% solvent evaporation and concurrent sample concentration. A 150 μl subsample was then transferred into a glass autosampler vial and as an internal standard 1 μl tetradecane solution was added to each vial using a Hamilton syringe (7000 Series Modified Microliter Syringe, Hamilton Company Inc., Reno, NV, USA). This solution had a concentration of 190.5 ng of tetradecane (99% purity, MilliporeSigma, Burlington, MA) per 1 μl of solution. These samples were then analyzed using an Agilent 7890 Gas Chromatograph (GC) coupled with an Agilent 5977B Mass Spectrometer (MS) single quadrupole detector at the USDA-ARS Center for Animal Health and Grain Research in Manhattan, KS (Ponce et al., 2022). The column used was an Agilent Durabond HP-5 (30 m length, 0.250 mm diameter, and 0.25 μm film thickness), with helium as an inert carrier gas flowing at a constant rate of 1.15 $\text{ml}\cdot\text{min}^{-1}$ and 39 $\text{cm}\cdot\text{s}^{-1}$ velocity. A 1 μl aliquot of each sample was injected in split mode at a 15:1 ratio with an injection inlet temperature of 250°C. The oven temperature started at 60°C held for 1 min followed by a 10 $\text{K}\cdot\text{min}^{-1}$ increase to 300°C over 26.5 min, which was then held for 4 min at 300°C. After a 4 minute solvent delay, mass ranges from 50 to 550 atomic mass units were scanned by the MS.

2.6.2 VOC data analysis

Chromatograms from the GCMS runs were processed using Agilent’s MassHunter software package (Agilent Technologies, Santa Clara, CA, USA). The program arrives at tentative compound identifications by comparing sample mass spectra to published spectra in the NIST20 spectral library. To further guide and inform tentative identification, published Kovats retention indices for candidate compounds were checked using the NIST database. All peak data was then exported to R, known contaminants excluded and alignment of tentatively homologous compounds across samples achieved using the R package GCAAlignR on the pre-processed data set (Ottensmann et al., 2018). A 75% presence threshold for any one of the AMF x Aboveground treatments was applied to select focal compound for further analysis (Meier and Hunter, 2018). Peak area was divided by dry shoot weight and the internal standard peaks were used to compare the detected amounts among treatments to arrive at an estimate for volatiles detected in $\text{ng}\cdot\mu\text{l}^{-1}\cdot\text{g shoot dry weight}^{-1}\cdot\text{h}^{-1}$ (Kigathi et al., 2019; Nihranz et al., 2022). Additionally, the relative proportions of compounds measured per sample were calculated for the analysis VOC blends. Samples with no identified peaks as well as samples from herbivory treated plants with no visual herbivory damage were excluded, resulting in n=97 volatile samples.

2.7 Statistical analyses

All statistical analyses were performed in R, version 4.2.1, using the RStudio GUI, version 2022.07.01 (R Core Team, 2022; RStudio Team, 2015). Visualizations were created with the package ‘ggplot2’ and its extensions ‘ggpubr’ and ‘ggsci’.

The effects of AMF treatment, seed source and aboveground treatment on dry shoot weight were tested in a mixed model analysis of variance (ANOVA) using the R package ‘lme4’. Initial leaf length measured at transplanting was included as covariate and a blocking variable was added to the model as a random effect to account for potential spatial heterogeneity of environmental conditions affecting growth within the greenhouse. Assumptions of normality and homoscedasticity were assessed visually by plotting residuals and employing Bartlett’s test, respectively. For each significant effect ($P < .05$), pairwise comparisons were performed with the ‘emmeans’ package using the default Tukey HSD method. A homologous analysis was performed for mycorrhizal responsiveness (MR) to gauge the differences in response to AMF inoculation by seed source, as well as by mycorrhizal treatment.

To assess plant semiochemical blends, the influence of potential environmental variables on VOC release was considered. VOC were measured in three time intervals during each day of collection and plant VOC emissions have been found to be sensitive to abiotic variables such as light intensity (Gouinguéné and Turlings, 2002). Therefore, a categorical time of day variable with three levels was included in the models as a random effect in addition to the random block effect. For the analysis of differences in VOC profiles with all individual compounds identified, permutational multivariate analysis of variance (PERMANOVA) as well as nonmetric multidimensional scaling (NMDS) were performed using the R package ‘vegan’ (Oksanen et al., 2020). PERMANOVA is a non-parametric method based on permutation tests developed for multivariate community ecology data sets, which are often comprised of many skewed and non-normally distributed dependent variables (Anderson, 2001). As VOC profile data exhibits similar qualities, PERMANOVA has been employed in chemical ecology research (Miano et al., 2022; Scieuzo et al., 2021). In this study PERMANOVAs were computed using the ‘adonis2’ function set to 1000 permutations with AMF and aboveground treatment as well as their interaction as independent variables. This was conducted for both the total measured amounts of VOCs emitted and the relative proportions of the different volatiles present in the blends. To further elucidate differences by mycorrhizal treatments, three additional factor levels reducing the degrees of freedom were tested in individual PERMANOVAs - uninoculated versus inoculated plants, mixed versus single species inocula, and differences among single species inocula. The function ‘metaMDS’ was used for NMDS, with a dissimilarity matrix being calculated from the VOC matrix using the Bray-Curtis index. As a convergent solution the lowest number of dimensions with a resulting stress < 0.15 was accepted to allow for reasonable interpretation.

To investigate the complexity of the VOC blends, the number of compounds identified per sample was evaluated using a generalized linear model with a poisson error distribution. Additionally, the measured production of all detected compounds was summed by sample and log transformed to assess the total measured VOCs per gram of aboveground biomass. Upon examination of the NMDS results, the detected VOCs were additionally summed within the identified compound groups and log+1 transformed. Mixed linear models were then produced with AMF treatment, aboveground treatment and their interaction as main effects. Differences were assessed in ANOVAs and significant effects were further investigated in pairwise comparisons.

To identify the most important variables distinguishing treatments from each other, random forest classification algorithms as implemented in the R packages ‘randomForest’ and ‘VarSelRF’ were employed (Breiman, 2001; Díaz-Uriarte and Alvarez de Andrés, 2006). Random forest is a machine learning approach belonging to the data-mining techniques, which is ideally suited for datasets with many dependent variables, such as headspace VOCs (Ranganathan and Borges, 2010). It arrives at a minimal set of predictor variables required to distinguish groups while not overfitting the data. Random forest classifications were conducted for both aboveground and AMF treatments separately. Between group classifications were conducted with the ‘randomForest’ function starting at 1000 trees. Overall variable importance was gauged by obtaining the mean decrease in accuracy (MDA) for leaving out the respective variable from the model using the ‘Importance’ function. In addition, the classification performance of the algorithm was assessed using a bootstrapping approach with 200 iterations. This returned the prediction errors for group membership of each sample, with error rates estimated using the .632+ bootstrap method (Díaz-Uriarte and Alvarez de Andrés, 2006; Ranganathan and Borges, 2010). To select the most important predictor variables for each group, one vs the rest classifications were conducted with the ‘VarSelRF’ function at 500 iterations (Ranganathan and Borges, 2010).

3 Results

3.1 Plant growth

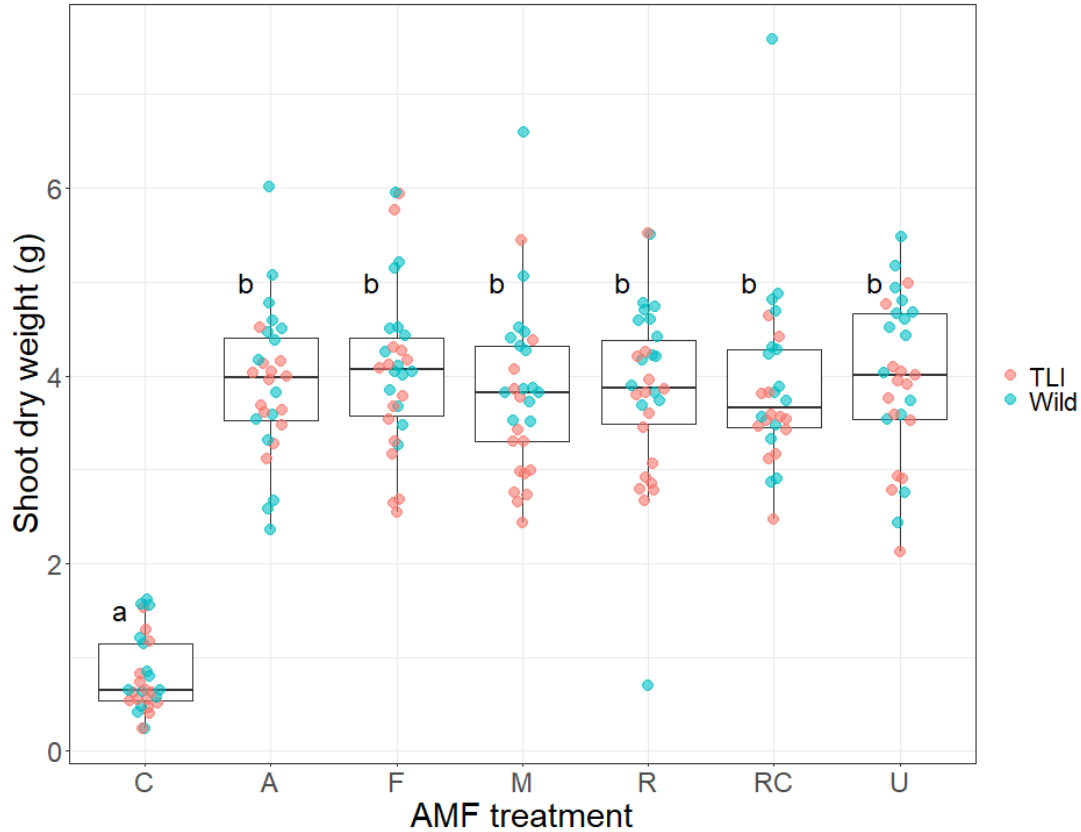


Figure 1: Shoot dry weight by mycorrhizal treatment and seed source. The mycorrhizal treatments were C= Control, A= *Ambispora* species, F= *Funneliformis mossae*, M= Mixture, R= *Rhizophagus* species, RC= *Rhizophagus clarus*, U= Unidentified species. Points populating the boxplots represent the plant individual weights with their color indicating the seed source. Letter annotations denote differences by mycorrhizal treatment attested by Tukey’s HSD test ($P < 0.05$).

Eight weeks after AMF inoculation, visual assessments and leaf length measurements indicated conspicuous differences between AMF inoculated and non-inoculated plants. A fully factorial mixed model ANOVA revealed strong effects for mycorrhizal inoculation ($F_{6,203} = 77.01$, $p < .001$) and seed source ($F_{1,203} = 20.13$, $p < .001$) on shoot dry biomass (Table 2). Further significant effects were found for aboveground treatment ($F_{2,203} = 8.14$, $p < .001$) and initial leaf length ($F_{2,203} = 5.27$, $p = .022$, Table 2). No interactions of variables were significant. Statistically, all mycorrhizal treatments were equally beneficial for growth and led to significantly more foliage production compared to non-inoculated controls ($p_{adj} < .001$). The average increase in biomass when grown with mycorrhizae was 490% (Figure 1). Herbivory by *E. funebris* reduced the biomass when compared to undamaged controls ($p_{adj} < .001$), however the biomass reduction derived from herbivory mimicry was not found to be significant ($p_{adj} = .136$).

The two germplasm accessions employed in this study showed a genotypical variation in plant growth, with offspring stemming from the wild population at Jerry Smith Park generally

Table 2: ANOVA results for effects of mycorrhizal treatment, seed source and aboveground treatment on dry shoot biomass. Initial leaf length was included as a covariate. For each seed source, mycorrhizal responsiveness was calculated by dividing dry shoot weight of mycorrhizal plants by mean dry shoot weight of non-inoculated controls. Treatment effects on mycorrhizal responsiveness (MR) were assessed in another ANOVA. Statistical significance levels are indicated with asterisks (*** $P < 0.001$, ** $P < 0.01$ and * $P \leq 0.05$).

	Shoot dry weight			MR		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
AMF treatment	6, 203	77.07	<.001***	5, 173	0.64	0.667
Seed source	1, 203	20.13	<.001***	1, 173	8.01	0.005**
Aboveground treatment	2, 203	8.14	<.001***	2,173	10.03	<.001***
Initial leaf length	1, 203	5.27	0.022*	1,173	2.09	0.150
AMF: Seed source	6, 203	0.84	0.541	5,173	1.22	0.301
AMF: Aboveground	12, 203	0.95	0.498	10,173	0.85	0.585
Seed source: Aboveground	2, 203	1.19	0.308	2,173	1.47	0.234

growing more vigorously compared to the plants grown from The Land Institute seeds ($p_{adj} < .001$, Figure 1). Calculating growth response by seed source revealed a mean mycorrhizal responsiveness (MR) of 511% for the TLI accession and 475% for the wild collection plants (Figure 2). MR was significantly greater for the TLI seed source ($F_{1,173} = 8.01$, $p = .005$; Figure 2), but no significant differences by AMF treatment ($F_{1,173} = 0.64$, $p = .667$) or the interaction of AMF treatment x seed source ($F_{1,173} = 1.22$, $p = .301$; Table 2) were found.

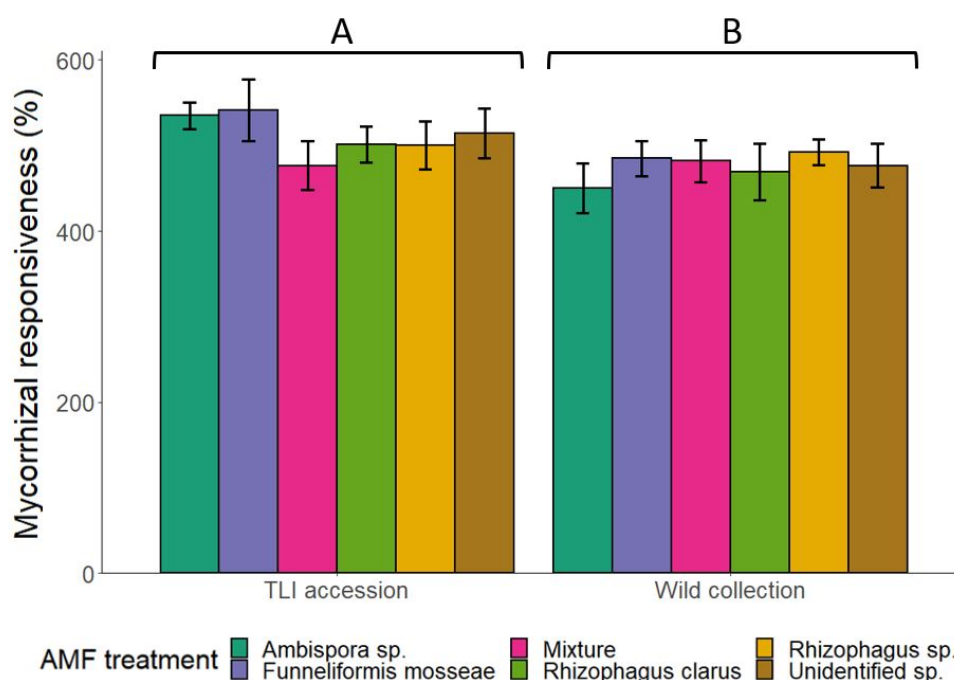


Figure 2: Relative growth facilitation of mycorrhizal treatments compared to mock-inoculated controls - so called mycorrhizal responsiveness (MR). Means for each AMF treatment mean are represented by bars, which are grouped by seed source. Error bars represent one standard error of the mean, while letter annotations indicate significant differences attested by Tukey’s HSD test ($P < 0.05$).

3.2 VOC analysis

Upon review of the chromatograms of the first round of constitutive volatile collections, it became clear that few plant derived compounds were detected in the collected samples. Therefore, the dataset was dropped from the analysis. Across all second round samples, a total of 17 focal compounds were identified (Table 3). Five mono-, one homo and six sesquiterpenes were detected along with two green leaf volatiles (GLVs), whereas the remaining three VOCs could either not be identified or not associated with any of the compound groups above. While retention indices were not computed, published retention times were reviewed to confirm that the tentatively identified compounds followed the correct order. Across all treatments, 4,8-dimethylnona-1,3,7-triene (DMNT), β -caryophyllene and 2-ethyl-1-hexanol were found to be the most abundant compounds, with 20%, 18% and 11% of the measured VOCs, respectively.

PERMANOVAs with all 17 focal compounds as dependent variables were performed to examine if headspace volatile profiles differed by treatment. For the measured VOCs by unit weight of biomass, significant effects were found for aboveground treatment ($F_{2,97} = 16.64$, $p = <.001$), as well as the AMF treatment ($F_{6,97} = 1.65$, $p = .004$; Table 4), but there was no significant AMF:Aboveground treatment interaction effect (Table 4). The composition of the VOC blends, assessed by calculating relative proportions of VOCs present in a sample, was not found to depend on AMF treatment in the fully factorial analysis. Additional contrasts tested for the AMF treatments revealed significant differences for uninoculated vs inoculated plants, both for measured VOCs and the proportions of the detected compounds in the blends (Table 4).

Table 3: Overview of the tentatively identified compounds included in the VOC analysis. Compounds were grouped by class and their mean retention time for the GC-MS runs included.

Compound name	Retention time
<i>Monoterpenes</i>	
α -Thujene	4.67
Camphene	4.87
β -Myrcene	5.23
Limonene	5.90
β -Ocimene	6.11
<i>Green Leaf Volatiles</i>	
3-Hexenyl acetate	5.52
2-Ethyl-1-hexanol	5.81
<i>Homoterpenes</i>	
4,8-Dimethylnona-1,3,7-triene	7.07
<i>Sesquiterpenes</i>	
β -Elemene	11.01
β -Caryophyllene	11.46
β -Bergamotene	11.56
Humulene	11.89
Bicyclosesquiphellandrene	12.23
α -Farnesene	12.38
<i>Other compounds</i>	
2,2,4,6,6-Pentamethylheptane	5.34
Unidentified compound 1	13.29
Unidentified compound 2	14.10

Furthermore, this contrast resulted in a significant AMF:Aboveground treatment interaction effect for measured VOCs ($F_{2,97} = 1.77$, $p = .030$). Both AMF diversity, i.e. mixed versus single strain inocula, as well as fungal species identity, for which single strain inocula were compared among each other, were not found to have a significant effect on VOCs (Table 4).

To visualize differences between treatments, non metric multidimensional scaling (NMDS) was performed. As indicated by the statistical analysis, aboveground treatments led to distinct VOC profiles, with beetle herbivory and the herbivory mimicry treatment diverging clearly in the ordination (Figure 3). Plant volatiles measured in reaction to *Epicauta funebris* herbivory were thereby associated with a release of sesquiterpenes such as β -caryophyllene as well as the homoterpene DMNT, whereas herbivory mimicry treatments were shifted towards GLVs. Constitutive VOC analysis from undamaged plants resulted in chromatograms with smaller and fewer peaks than for the two damage treatments, resulting in a greater scatter of these samples in the ordination (Figure 3). Some monoterpenes such as α -thujene were quite frequently

Table 4: PERMANOVA results for the analysis of measured VOCs from *Silphium integrifolium* individuals. The table shows statistical results for both the measured VOCs ($\text{ng} \cdot \mu\text{l}^{-1} \cdot \text{g leaf tissue}^{-1} \cdot \text{h}^{-1}$) and the relative proportions of the compounds present in the VOC blends (% of the total VOCs measured per sample). AMF treatment, aboveground treatment and their interaction were the independent variables. Separate PERMANOVAs were performed for three AMF treatment contrasts of interest, indicated by indentation. Statistical significance levels are indicated with asterisks (***P < 0.001, **P < 0.01 and *P ≤ 0.05).

	Measured VOCs			VOC proportions		
	<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
AMF treatment	6, 97	1.65	0.004**	6, 97	1.29	0.153
Uninoculated vs inoculated	2, 97	4.07	<.001**	2, 97	3.14	0.008**
Mixture vs single species	2, 84	0.46	0.924	2, 84	0.23	0.965
Among single species	4, 70	1.41	0.079	4, 70	1.10	0.331
Aboveground treatment	2, 97	16.64	<.001***	2, 97	18.13	<.001***
AMF: Aboveground	12, 97	1.18	0.107	12, 97	1.11	0.272
Uninoculated vs inoculated	2, 97	1.77	0.030*	2, 97	1.56	0.121
Mixture vs single species	2, 84	0.94	0.555	2, 84	1.11	0.358
Among single species	8, 70	1.14	0.206	8, 70	1.01	0.458

detected for constitutive samples, whereas other compounds including most sesquiterpenes and the unidentified compounds were rare to completely absent in undamaged plants.

AMF treatment means and standard errors in two dimensions were plotted to examine their divergence in the ordination (Figure 4). Whereas the mycorrhizal treatments were clustered and did not load strongly onto the axes, the uninoculated control plants were shifted towards GLVs and the monoterpene limonene. The same type of graph was produced for the AMF:aboveground interaction effect, since there was a significant interaction for the uninoculated vs inoculated contrast (Figure 5). This graph shows that the observed shift of the non-mycorrhizal plants can mainly be attributed to the specimen of the undamaged control treatment, which clustered with the herbivory mimicry treatments.

To further evaluate the VOC data, ANOVAs on the total measured VOCs and the amount of compounds identified were performed (Table 5). Both undamaged and herbivory mimicry treated plants exhibited lower compound diversity than samples from herbivory treated plants ($F_{2,97} = 37.74$, $p < .001$; Table 5). AMF treatment also had a significant effect on the number of compounds ($F_{6,97} = 2.87$, $p = .014$), but not the AMF:aboveground interaction effect (Table 5). Pairwise comparisons revealed that the non-mycorrhizal controls had a lower headspace chemical diversity compared to the *Ambispora sp.* ($p_{adj} = .029$) and the *Funneliformis mossae* treatment ($p_{adj} = .040$). Compounds that were not detected in the headspace of uninoculated control plants were the monoterpenes camphene and β -myrcene, which were found to be minor constituents in 46% and 42% of the mycorrhizal plant VOC blends, respectively. Similarly, aboveground and AMF treatment had a significant effect on the measured total release of VOCs (Aboveground: $F_{2,97} = 58.35$, $p < .001$; AMF: $F_{6,97} = 5.87$, $p = < .001$), but not their interaction effect ($F_{12,97} = 1.73$, $p = .078$; Table 5). Here the mock-inoculated controls were found to emit significantly more VOCs per unit weight of aboveground biomass than the plants treated with *Rhizophagus clarus*

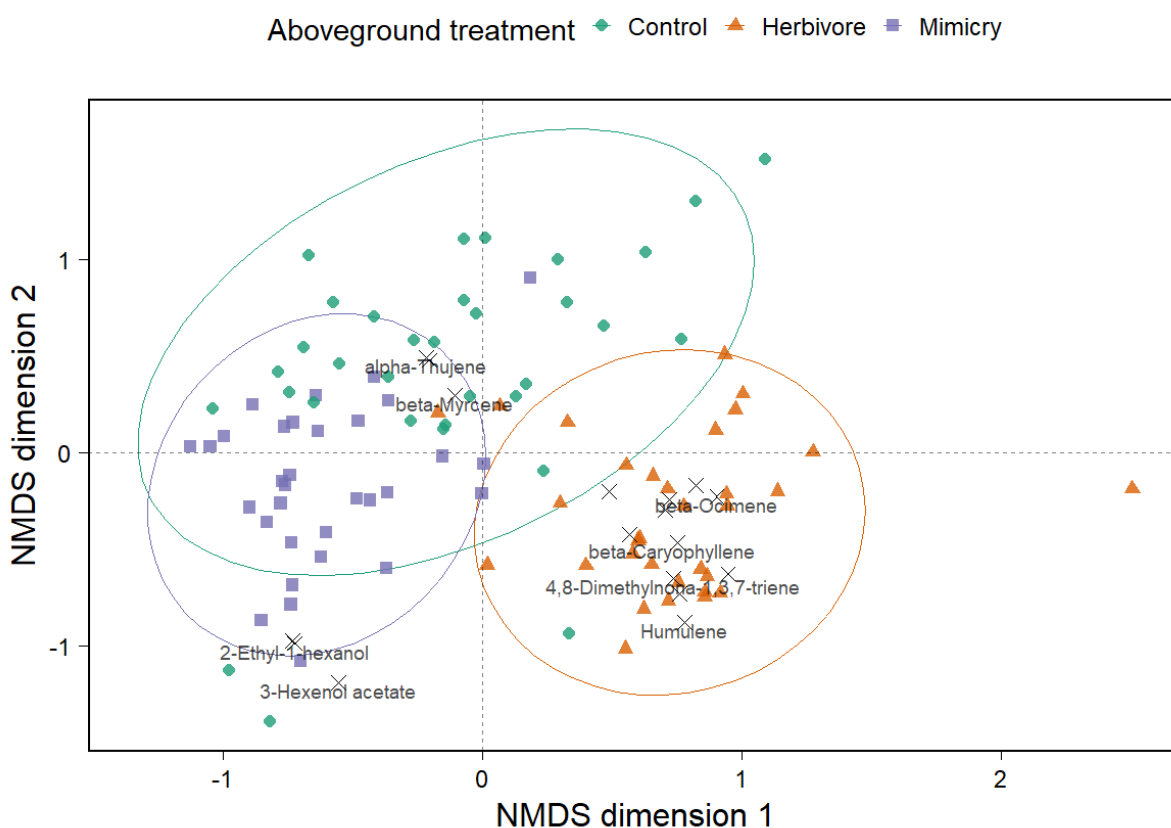


Figure 3: Nonmetric multidimensional scaling (NMS) ordination of measured VOCs ($\text{ng}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) for the compounds present in silflower headspace VOC samples. Aboveground treatment membership of each sample is indicated by point shape and color. Clustering by aboveground treatment is highlighted with ellipses drawn at 95% level.

($p_{adj} = .019$) and the unidentified AMF species ($p_{adj} < .001$). In addition, all other mycorrhizal treatments exhibited a significantly higher measured release of VOCs than silflower grown with the unidentified fungal species.

Since the detection of certain compound groups appeared to correlate with aboveground treatments (Figure 3), further ANOVAs were performed by compound group (Table 5). Analysis of treatment differences for the grouped VOCs resulted in significant effects for all aboveground treatments (Table 5), with homo- and sesquiterpenes almost exclusively produced upon herbivory, whereas green leaf volatiles were found to be most abundant in mechanically damaged plants (Figure 6). Green leaf volatiles differed significantly by AMF treatment ($F_{6,97} = 2.78$, $p = .018$), with pairwise comparisons revealing that the non-mycorrhizal treatment resulted in significantly more GLVs detected than for the unidentified species treatment ($p_{adj} < .003$). For sesquiterpenes, pairwise comparisons of the significant interaction effect (Table 5) returned that for herbivory treated plants, all belowground treatments besides from *Ambispora sp.* resulted in significantly more measured sesquiterpenes than plants associated with the unidentified AMF species (Figure 6). In addition, unidentified fungal species plants were also found to be most conservative in the production of the homoterpene DMNT (Figure 6), but none of the pairwise comparisons were significant (Table 5). Constitutive samples from undamaged control plants were again found to produce few VOCs above the detection threshold besides from two of the non-mycorrhizal plants, which released both the green leaf volatile 2-ethyl-1-hexanol and the

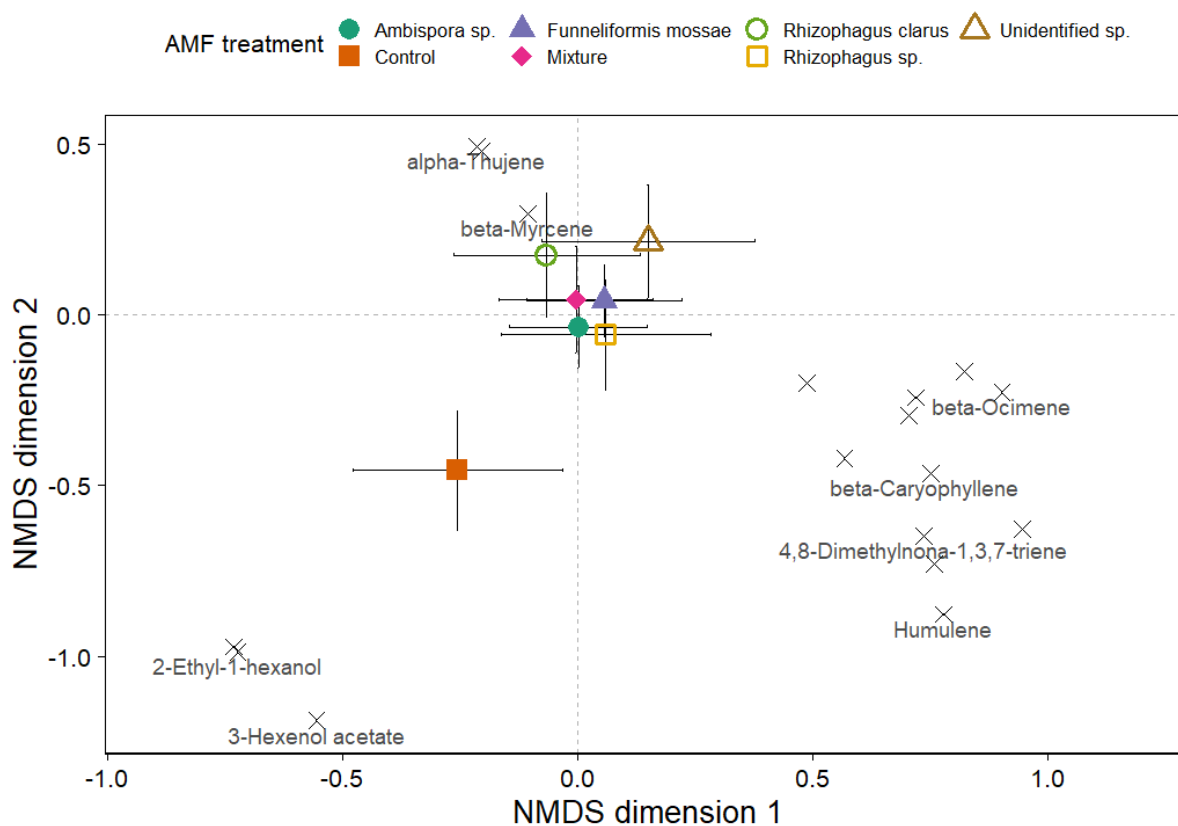


Figure 4: Nonmetric multidimensional scaling (NMS) ordination of measured VOCs ($\text{ng}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) for the compounds present in silflower headspace VOC samples. Data was grouped by AMF treatment and treatment means are indicated by point shape and color with error bars in two dimensions representing the standard error of the mean location.

monoterpene limonene in very high quantities (Figure 6). Overall, VOC production per unit weight of aboveground biomass was highly variable for many treatments and especially for the mock-inoculated plants.

For the distinction between aboveground treatments, random forest classification was conducted, resulting in a measure of variable importance for classification accuracy (Figure 7). This data-mining technique identified the overall most abundant compounds, the homoterpene DMNT (Mean decrease in accuracy (MDA) = 29%), the sesquiterpene β -caryophyllene (MDA = 26%) and the **GLV!** (**GLV!**) 2-ethyl-1-hexanol (MDA = 23%) as the variables with the overall greatest importance for distinguishing beetle herbivory, herbivory mimicry and undamaged control VOC blends. The bootstrapping method selected nine of the 17 compounds in the dataset for aboveground treatment classification and arrived at a .632+ prediction error of 15.5%. Here, the classification accuracy was highest for herbivory VOCs, whereas mimicry and constitutive blends were more frequently confused for each other (Figure 8).

Next, a variable selection procedure was conducted for each treatment using a one versus the rest approach (Ranganathan and Borges, 2010). To distinguish beetle herbivory blends, two VOCs were sufficient, DMNT and β -elemene. The latter was exclusively present in 82% of the beetle herbivory samples, whereas DMNT was detected in 90% of all herbivory headspace samples and only minor quantities in 24% of non-herbivory samples. Herbivory mimicry was distinguished from beetle herbivory and undamaged control treatments using five compounds

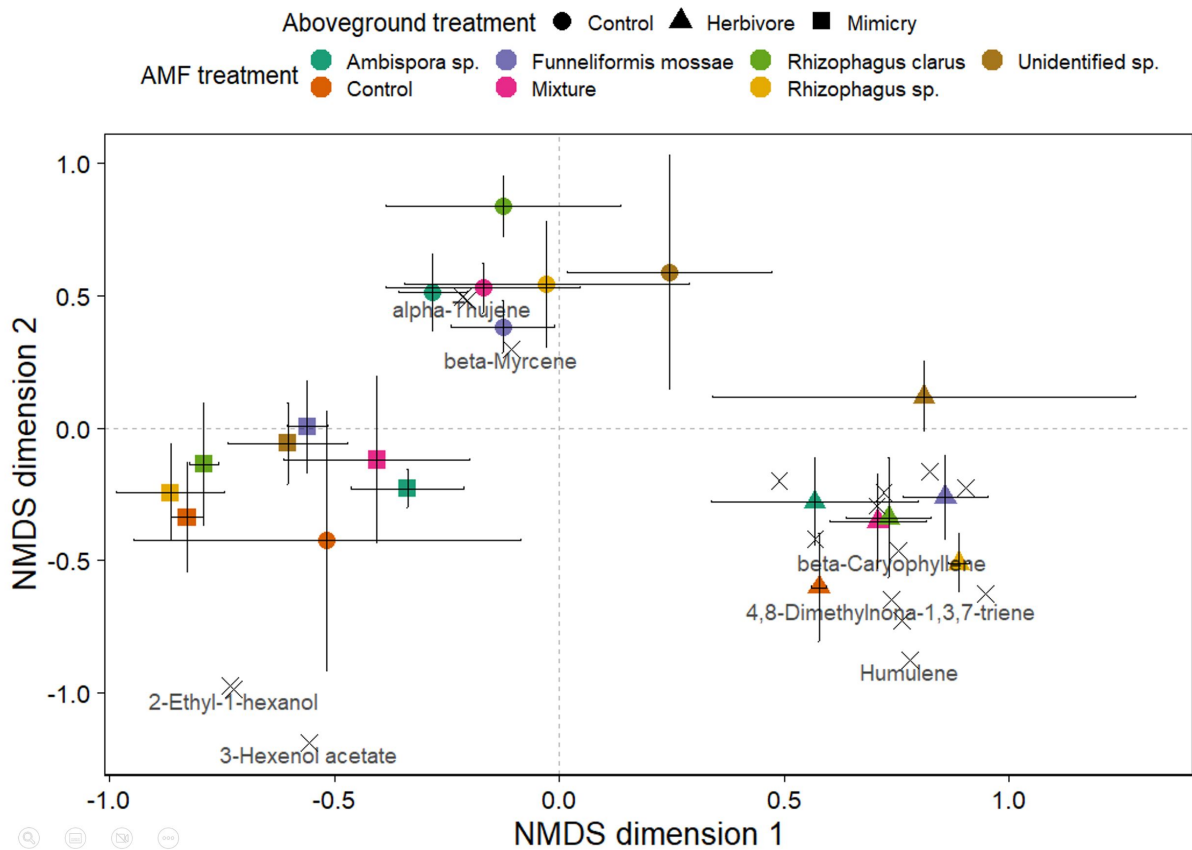


Figure 5: Nonmetric multidimensional scaling (NMDS) ordination of measured VOCs (ng*g⁻¹*h⁻¹) for the compounds present in silflower headspace VOC samples. Data was grouped by AMF:Aboveground treatment and means are represented with a point layer with error bars in two dimensions representing the standard error of the mean location. AMF treatment membership is indicated by point color, whereas point shape denotes aboveground treatment.

- 2-ethyl-1-hexanol, α -thujene, β -caryophyllene, limonene and humulene. 2-Ethyl-1-hexanol, a green leaf volatile typically released upon mechanical damage (Holopainen and Gershenzon, 2010), was present in 85% of mimicry samples, yet it was also detected for herbivory and undamaged control samples. The sesquiterpenes β -caryophyllene and humulene, on the other hand, were most prevalent in herbivory samples and in minor quantities in control samples, but only in 15% of mimicry samples. This exemplifies how the algorithm employs absence of certain volatiles to determine group membership. Constitutive VOC release had the lowest classification accuracy in the bootstrapping approach and no volatiles could be identified that were more prevalent in undamaged controls than plants experiencing a form of aboveground stress.

Random forest classification was attempted for all seven AMF treatments, resulting in an unacceptable prediction error of 87.5%. Due to the clear effect of aboveground treatments on volatile blends, the dataset was split by aboveground treatment and random forest classification attempted again by AMF treatment. Nonetheless, the classification accuracy remained very low at an out-of-bag prediction error between 78.5% and 87.5%. As the previous analyses had hinted that the greatest differences were between plants that were not inoculated and mycorrhizal plants, this contrast was tested with random forest. Low classification accuracy was again indicating that fungal treatments could not be distinguished by the specific VOC components.

Table 5: ANOVA results for VOCs with the main effects AMF inoculation (five single species, one mixed species and one uninoculated control treatment), aboveground treatment (herbivory, herbivory mimicry and undamaged control) as well as their interaction. The number of compounds produced were assessed in a general linear model, whereas mixed linear models were produced for the overall detected VOCs and for the four major compound classes. Statistical significance levels are indicated with asterisks (***P < 0.001, **P < 0.01 and *P ≤ 0.05).

	AMF		Aboveground		AMF: Aboveground	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
# of Compounds	2.87	0.014*	37.74	<.001***	1.27	0.254
VOC total	5.87	<.001***	58.35	<.001***	1.73	0.078
Monoterpenes	1.24	0.297	5.09	0.009**	1.71	0.084
GLVs	2.78	0.018*	9.66	<.001***	0.85	0.602
Homoterpenes	0.89	0.501	88.15	<.001***	2.11	0.027*
Sesquiterpenes	2.07	0.068	63.88	<.001***	4.02	<.001***

4 Discussion

4.1 Silflower growth response to mycorrhizal inocula

Aside from this work there are two more studies on candidate perennial crops that have investigated the mycorrhizal responsiveness (MR) of silflower both in greenhouse (Koziol et al., 2019; McKenna et al., 2020) and for field settings (Koziol et al., 2019). While McKenna et al. (2020) reported an MR range of 600-1000%, Koziol et al. (2019) found that silflower biomass increased by 44% in the greenhouse when grown with a mixture of native prairie AMF. Koziol et al. (2019) also established a positive relationship between silflower aboveground biomass production and the amount of inoculum applied in the field. This range of results exemplifies the variability of MR for a single plant species, which may be due to experimental variables such as abiotic conditions and the duration of the experiment, as well as potentially the plant and fungal genotypes involved.

Mycorrhizal responsiveness has been a topic of interest for decades, with investigations ranging from growth responses among plant species or by crop cultivar to the effects of fungal strains on a select plant genotype (Johnson et al., 1997; Menge et al., 1978; Thirkell et al., 2022). Cheeke et al. (2019) assessed the MR of native late-successional, native early-successional prairie species, and non-native plant species of diverse plant taxa. They observed that native late-successional species, a category that *Silphium integrifolium* falls into, are generally more responsive to AMF and also more sensitive in their response to fungal strain identity. Among AMF isolates differences in key functional traits such as carbon demand and nutrient acquisition efficiency have been hypothesized and empirically established (Chagnon et al., 2013; Koziol et al., 2018; Mensah et al., 2015). For the model plant species *Plantago lanceolata*, Bennett and Bever (2007) found significant differences in biomass production related to inoculation with three single species isolates of native prairie AMF. Watts-Williams et al. (2019) established significant interaction effects between fungal and plant genotype on plant growth for 18 sorghum (*Sorghum bicolor*) accessions. No differential response to the various native prairie AMF strains employed was established for the silflower individuals in this experiment. Silflower may thus not be particularly sensitive to fungal identity despite being a late-successional native prairie

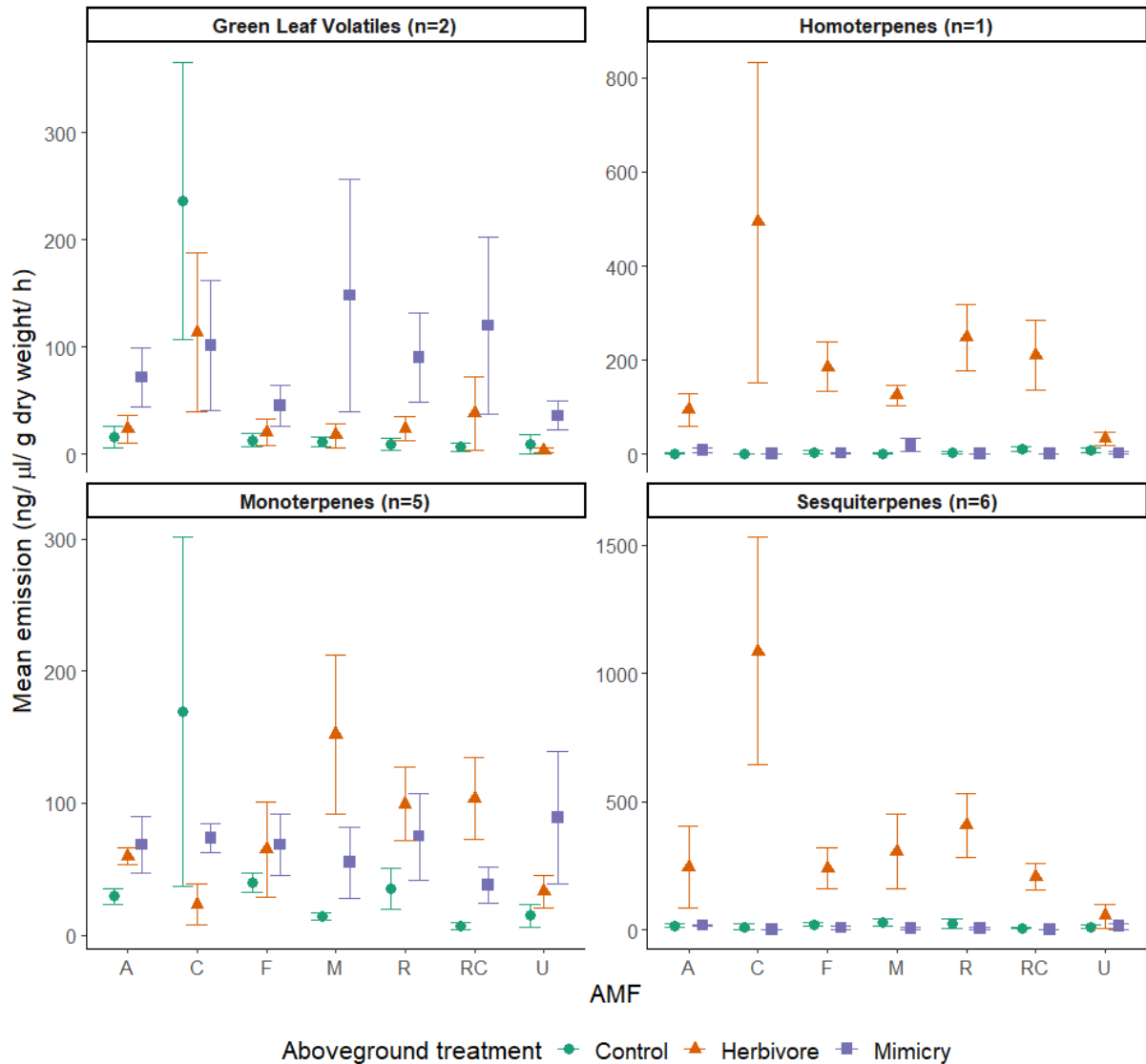


Figure 6: Mean measured VOCs for *Silphium integrifolium* by VOC groups associated with seven belowground and three aboveground treatments. The mycorrhizal treatments were A= *Ambispora* species, C= uninoculated control, F= *Funneliformis mossae*, M= Mixture, R= *Rhizophagus* species, RC= *Rhizophagus clarus*, U= Unidentified species. Aboveground treatments are indicated by point shape and color, error bars indicate one standard errors.

species or the plants may not have not been sensitive in the context of the provided experimental conditions. Alternatively, the fungal strains that were used for inoculation may not have been particularly functionally distinct.

While sensitivity was low in the presented experiment, McKenna et al. (2020) observed that native prairie soil inoculum resulted in greater silflower growth than soil inoculum stemming from a managed field. The field that the inoculum was derived from featured a monoculture of the perennial grass species intermediate wheatgrass (*Thinopyrum intermedium*), which is being bred as a perennial grain crop by The Land Institute (TLI) and commercialized under the name Kernza®. Agricultural soils are known to host a different AM fungal community than

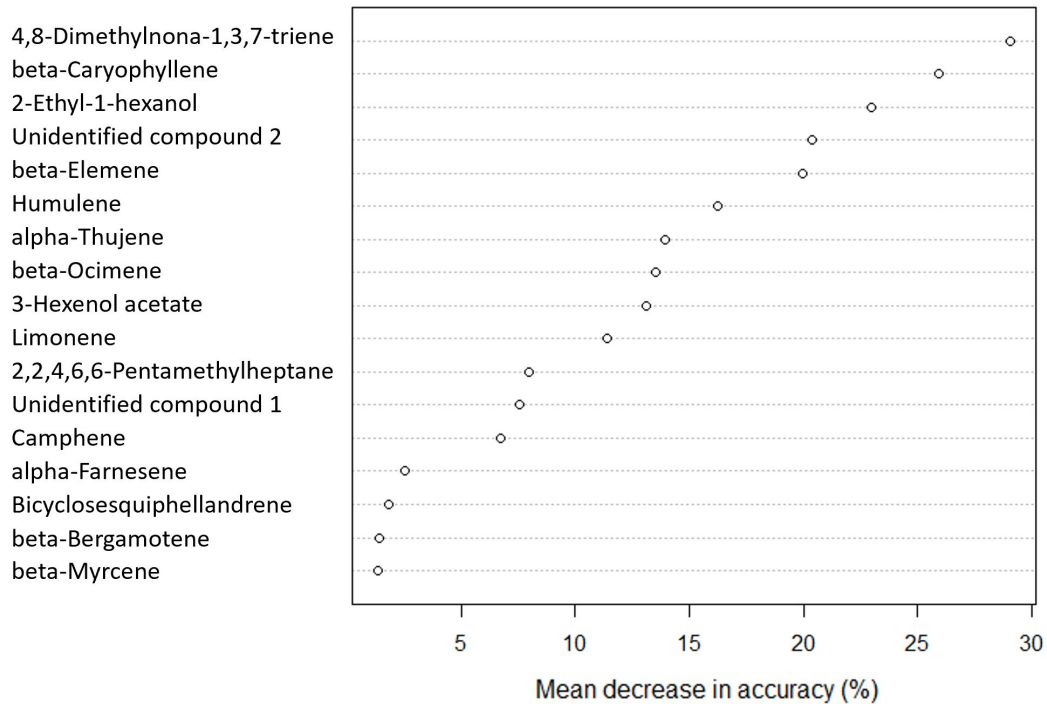


Figure 7: Mean decrease in accuracy (MDA) for group membership classification conducted with a random forest machine learning algorithm. The presented values indicate the importance of each VOC to distinguish between beetle herbivory, herbivory mimicry and undamaged control VOC blends. MDA is estimated by comparing the classification error rate of the original data set to classification errors derived from permuted variables.

soils of native ecosystems (Såle et al., 2015), which often have a higher plant diversity and experience less disturbance than highly-managed systems (Chagnon et al., 2013; Koziol et al., 2019; Middleton et al., 2015). House and Bever (2018) found that the fungal communities of remnant prairie sites differed from adjacent sites that experienced anthropogenic disturbance by either overgrazing or tillage. Of the 181 taxa observed, 25 species increased in abundance with disturbance and 31 decreased strongly. Furthermore, native prairie species have been found to respond more positively to native AMF inoculum compared to commercial AMF mixes, which typically include species with good performance in managed, frequently disturbed systems. (Middleton et al., 2015). When relating those findings to the presented methodology, it becomes clear that potentially not more diverse responses to different fungal strains were captured due to the source of the inocula. This greenhouse experiment exclusively featured AMF isolates from local remnant prairie sites, having evolved in a biome that *S. integrifolium* is native to. Any future explorations into the mycorrhizal responsiveness of silflower should include fungal species that are not locally adapted, such as inocula stemming from agricultural soils or commercial sources, in addition to native prairie AMF. Such work would complete the picture, especially for the agricultural context of cultivating silflower at field scale, and help assess if native AMF amendments to silflower fields are desirable.

Regarding treatments with a mixture of AMF species, it has been suggested that the impact of fungal species richness is small compared to the presence of AMF, and that mixtures are as beneficial to plant growth as the most mutualistic individual species present in the mix (Koziol

et al., 2018; Vogelsang et al., 2006). No significant differences in aboveground biomass gain between mixture and single species AMF treatments were established, so that the results of the presented experiment generally support that assessment.

4.2 Mycorrhizal responsiveness by silflower genotype

The two silflower genotypes employed differed in vigor, with the plants from the wild accession generally growing larger than plants from The Land Institute (TLI) seed for respective treatments. Mycorrhizal responsiveness, however, was significantly greater for TLI accession plants. This result emerged despite the suspected genetic heterogeneity in the TLI breeding population, which mainly stemmed from collections of wild *Silphium integrifolium* in central Kansas and is undergoing a broad hybridization and screening process (Van Tassel et al., 2020). Which mechanisms might be explaining this genotypical response to AMF, and what does this tell us about the selection for MR as a trait in the process of *de-novo* domestication?

A longitudinal precipitation gradient exists in the native range of silphium with aridity increasing towards the Rocky Mountains to the west (Cassetta et al., 2022, in press). Such gradients in abiotic conditions are known to affect natural selection pressures leading to locally adapted plant ecotypes (Alberto et al., 2013; López-Goldar and Agrawal, 2021). AMF have been shown to be more beneficial to plants with increasing water scarcity (Augé et al., 2015; Chitarra et al., 2016), with mycorrhizal plants being more efficient in root water transport (Quiroga et al., 2019) as well as exhibiting changes in physiology improving the aboveground water management (Augé, 2001). This effect has even been demonstrated for silflower by McKenna et al. (2020), with mycorrhization being relatively more beneficial to plant growth in water limited conditions. Greater natural selection for mycorrhizal symbiosis in more arid central Kansas than in more humid western Missouri, where seeds from a wild population were collected, may thus offer a potential explanation for the observed differences in MR by silphium genotype.

Aside from the geographic origin, the selection process that the breeding population at The Land Institute undergoes is another obvious difference between the two accessions used in this study. While Kernza[®] is not highly mycorrhizal and has been found to experience growth inhibition when inoculated (McKenna et al., 2020), a trend towards a more positive response to AMF inoculation has been found for its more recent breeding cycles developed at TLI. Koziol et al. (2019) conducted a greenhouse study on MR including forage type intermediate wheatgrass, the first Kernza breeding cycle and the newer Kernza^{®5} variety. They found a 8% growth facilitation for Kernza^{®5}, whereas the growth of the first cycle Kernza and intermediate wheatgrass was reduced by 11% and 22%, respectively. This indicates a potential untargeted selection for MR by TLI, which has internally been hypothesized to arise from the relative phosphorus deficiency of the soils of the TLI breeding plots. Another challenge for plants in central Kansas, where TLI currently conducts its breeding, is water stress, for which mycorrhizal plants have been shown to perform better (McKenna et al., 2020; Quiroga et al., 2019). Plants more responsive to AMF might thus have more access to limiting resources, resulting in more vigorous growth and concurrent selection for these genotypes by breeders. The same selection for MR could be the case for the early-stage domestication accession of silflower employed in this experiment.

Only two silflower accessions were featured here, with one of them stemming from a wider geographic area. In addition, they were grown in a prairie soil:sand mix, so that these results do not lend themselves to detailed insights regarding the hypotheses outlined above. The observed difference in MR by plant genotype might, however, motivate further research. To fully understand silflower genotypical variability regarding MR, experiments with panels of more and distinct genotypes of silflower would need to be conducted. In addition, an investigation employing growing substrates derived from typical agricultural soils would be more indicative of

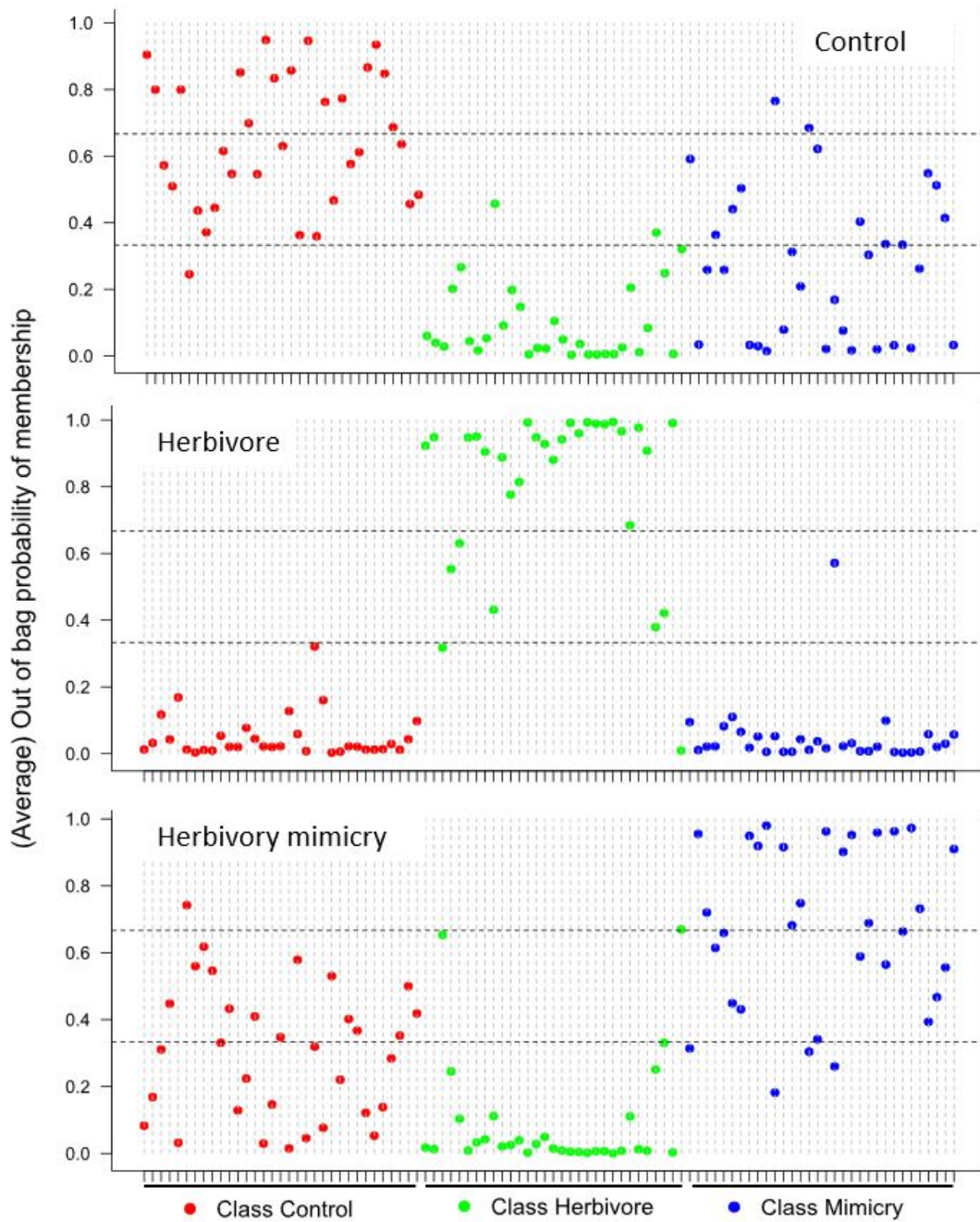


Figure 8: Out-of-bag group membership predictions derived from the random forest bootstrapping algorithm. Aboveground treatments were used for grouping, with values close to one indicating samples with a distinct volatile profile for their respective group. Each graph represents the prediction accuracy of the indicated group compared to the samples in the other groups.

mycorrhizal plant performance in field settings. Soils from the breeding plots at TLI could be used to scrutinize the potential untargeted selection for mycorrhizae symbiosis.

4.3 VOC measurements in response to aboveground treatments

Plants are sessile organisms that produce volatile organic compounds (VOCs) to communicate with their environment (Dicke, 2009). Potential functions of volatile blends are signaling to neighboring plants, attraction of beneficial organisms, repellency or confusion of antagonists and the recruitment of natural enemies upon infestation with insect pests (Dicke and Baldwin, 2010; Holopainen and Gershenzon, 2010; Pierik et al., 2014). While volatile blends are unique to the insect-plant system observed (War et al., 2012), the breadth of research that exists on the emission of VOCs in relation to aboveground stress treatments such as chewing herbivory allows for some tentative interpretations of the general patterns that were observed.

The quite distinct volatile profiles upon *Epicuata funebris* herbivory and herbivory mimicry indicate that the volatile response depends on the type of damage the plant experiences (Dicke, 2009). The two detected green leaf volatiles 2-ethyl-1-hexanol and 3-hexenyl acetate were found to be most abundant in the herbivory mimicry treated plants (Figure 6). GLVs are released from mechanically damaged plant tissue within seconds to minutes of the damage occurring and can make up a significant proportion of biogenic volatiles (Holopainen, 2004). Plants experiencing beetle herbivory were also mechanically damaged, but lower GLV concentrations were detected for those samples compared to herbivory mimicry. Whereas herbivory mimicry treated plants were wounded immediately prior to volatile measurements, beetle damage occurred over the 18-22 hour feeding interval. With the immediate release of GLVs in mind, this is a possible explanation for the lower amounts of detected VOCs for herbivore damaged plants.

Some insect herbivores are thought to elicit a specific response by producing oral secretions and regurgitates, which the plant senses (Dicke, 2009; War et al., 2012). All of the sesquiterpenes and the homoterpene DMNT were detected in much larger quantities in the headspace samples of herbivore damaged plants (Figure 6). Chewing herbivory has been found to primarily activate defense genes related to the jasmonic acid signaling pathway in plants (Acevedo et al., 2015; Ament et al., 2004; Stratton et al., 2022). Terpenes related to this pathway have been reported to be synthesized *de novo* upon herbivory and are typically released within hours after attack (Holopainen, 2004). As the release of DMNT and sesquiterpenes appeared to be induced almost exclusively upon arthropod feeding, these compounds are potential candidates serving some kind of defensive function, such as deterring the herbivore or attracting its natural enemies (Pierik et al., 2014; Sharma et al., 2017).

4.4 Arbuscular mycorrhizal fungal mediation of VOC measurements

Previous work indicates that AMF inoculation can alter plant defensive chemistry (Bennett et al., 2009; Gehring and Bennett, 2009; Meier and Hunter, 2018) and AMF mediated changes in VOC profiles have been investigated for several AMF-plant-insect systems (Rasmann et al., 2017; Sharma et al., 2017). A study on broad bean (*Vicia faba*) with a diverse commercial fungal treatment dominated by *Glomus* sp. resulted in decreased detection of the sesquiterpenes (*E*)-caryophyllene and (*E*)- β -farnesene, concurrent with a greater attraction of aphids to mycorrhizal plants (Babikova et al., 2014). Green beans (*Phaseolus vulgaris*) plants challenged with spider mites produced a different composition of volatiles with increased amounts of β -ocimene and β -caryophyllene when grown with the AMF species *Glomus mossae* (Schausberger et al., 2012). Mycorrhizal beans were also more attractive to predatory mites after six days of spider mite

feeding. For constitutive and jasmonic acid induced tomato volatiles, Asensio et al. (2012) reported little change when analysing terpenes for plants grown with a mix of *Glomus* species as AM fungal inoculum, but an increase in essential non-volatile isoprenoids in tomato leaves. In contrast, Shrivastava et al. (2015) found that tomatoes produced greater amounts of monoterpenes and sesquiterpenes upon inoculation with *Funneliformis mossae*, both constitutively and upon exposure to beet armyworm.

This range of findings exemplifies the many variables and confounding factors when studying the volatile profiles of AMF-plant-herbivore interactions, including fungal genotype, plant genotype, aboveground stress factors, abiotic experimental conditions and data collection approach (Pierik et al., 2014). The measurements in the presented experiment were highly variable, with an overall greater release of VOCs relative to plant biomass detected in the absence of fungal inocula. While nutrient concentrations in the silflower individuals were not analyzed, the more vigorous mycorrhizal plants indicate greater resource limitations for non-mycorrhizal plants. Existing evidence suggests that most abiotic stresses lead to an increase in VOC production (Holopainen and Gershenson, 2010), but nutrient limitation has been connected to a decrease in volatile production in maize (Gouinguéné and Turlings, 2002). The greater release of VOCs upon herbivory per unit biomass for non-mycorrhizal plants could indicate a change defensive strategy due to mycorrhizae. Lack of an alternative strategy such as tolerance to herbivory in a non-optimal environment without a fungal partner has been suggested as a potential mechanism (Bennett et al., 2006; Vannette and Hunter, 2009). Alternatively, the high variability in VOC production that was recorded could also mean that there are additional factors influencing VOC production. While some abiotic factors were taken into account in the statistical models, the amount of damage the plant experienced or the performance of herbivores was not assessed in the presented experiment. This could have played a significant role in amount of defensive VOCs that were produced and may have skewed the results for the much smaller non-mycorrhizal plants in this study in particular.

Overall, the composition of volatile blends did not appear to vary greatly by mycorrhizal treatments. The most apparent difference among AMF treatments was that plants associated with the unidentified AMF species were found to be more conservative in releasing sesquiterpenes and homoterpenes upon herbivory than plants grown with other fungal treatments. This may indicate that the defensive strategy of plants can depend on their fungal partner. In trying to maximize reproduction, plants face a dilemma of how to allocate resources between growth and defense when reacting to herbivores and pathogens (Pierik et al., 2014). Volatile semiochemicals can be effective at attracting arthropod carnivores or repel certain herbivore, but they can also come at an ecological cost by attracting other herbivores (Dicke and Baldwin, 2010; War et al., 2012). As it is costly to increase VOC production, it may be more beneficial to opt for tolerance as a dominant strategy by allocation of resources to primary metabolites and additional growth. Additionally, fungal symbionts rely on the provision of carbon from plants for resource provision, so that allocation to the fungus instead of volatile defenses has been suggested as a potential mechanism (Fontana et al., 2009).

To increase the interpretability of the VOC measurements and draw any conclusions, however, improvements of the methods and further measurements indicative of the efficacy of the plant's defense would be needed. Upon conclusion of the data collection and the GCMS runs, it became obvious that running an alkane standard to calculate a retention index, running the samples on a polar column in addition to the non-polar column used, and employing a slower method with a 5 K*min⁻¹ temperature ramp would have had the potential to significantly improve the quality of the GCMS data and would have been necessary to validate compound identification. Studies with a similar design have usually evaluated the performance of herbivores such as recording weight gain and survival of caterpillars (Bennett et al., 2009; Kempel et al., 2010). Another potentially

informative assessment for function of individual VOCs and VOC blends is the attraction of herbivores or their natural enemies to plants in behavioral assays (Clavijo McCormick et al., 2014; Davidson-Lowe and Ali, 2021). Alternatively, the reaction of insect antennae to volatile compounds can be gauged by performing gas chromatography coupled with electroantennography (Miano et al., 2022). Such measurements of either performance, attraction or reaction are a direct indicators for the ecological significance of observed changes in VOC production.

To complete the picture of the defensive phenotype of silflower in response to inoculation with AMF, it would also be necessary to assess other defensive pathways coupled with VOC measurements. As its alternative common name, prairie rosinweed, suggests, silflower is a resinous forb that enriches antifeedant defensive chemicals in its tissue to fend off herbivores. For the model species *Plantago lanceolata*, Bennett et al. (2009) found that non-mycorrhizal plants produce greater amounts of defensive iridoid glycosates than mycorrhizal plants, and the different mycorrhizal species they employed also varied in the amount of defensive compounds produced constitutively. Interestingly, mycorrhizal plants did not increase the relative abundance of defensive iridoid glycosates, but fungal plants were more effective at allocating their defensive resources to the site of damage (Bennett et al., 2009). Thus, a more detailed analysis of defensive resource allocation would help to parse out if AMF associated plants prioritise direct defenses such as antifeedants, indirect defenses such as VOCs or tolerance and resource allocation to growth and fungal symbiosis as a dominant defensive strategy.

4.5 Visioning ecologically intensified agroecosystems with silflower

Agroecology and the increasing popularity of ecological intensification practices stand for a movement countering the excessive simplification of cropping systems, whose unacceptable ecological disservices are becoming increasingly apparent (Doré et al., 2011). To arrive at the ambitious goals laid out by its proponents, both the creation of a knowledge base through holistic, integrative research and the development of technology to effectively manage the novel and diversified agroecosystems envisioned will be necessary (Gliessman, 2014). Insights into field biological processes and their significance on the performance of crop plants promise sustainable, self-regulating solutions to key issues today. Nutrient provision and pest pressure are typically addressed with silver bullet type solutions in the form of disruptive, exogenous inputs in conventional agroecosystems (Bommarco et al., 2013). Using natural ecosystems as a model, the understanding and management of mutualistic soil biota may help to provide these provisioning and regulating services sustainably (Gianinazzi et al., 2010). The presented study marks an initial exploration on the significance of AM fungal partners for the novel perennial crop silflower, but the insights gained are far from the knowledge required to effectively realize target benefits of these interactions in the field. Both further basic and applied research can help advance our understanding and ultimately allow us to arrive at application. Firstly, establishing the genetic basis of AMF symbiosis in silflower, including identifying the quantitative trait loci (QTL) involved, can inform breeding for silflower genotypes that are highly mycorrhizal (Thirkell et al., 2022). Secondly, applied community ecology research in diversified systems can guide the selection of appropriate companion crops and management techniques for silflower (Tittonell, 2014). This may include research on the dynamics of interspecific common mycorrhizal networks (CMN), which can play a significant role in the performance of intercropping systems (Doré et al., 2011; Gao et al., 2021). Ultimately, a crucial step to implement and popularize novel agroecosystems with silflower will be integrating the practitioner’s perspective by collaborating with farmers and incorporating their local and traditional knowledge (Altieri, 2018; Gliessman, 2014).

5 Conclusions

Silphium integrifolium is currently undergoing *de-novo* domestication to be employed as an oilseed crop in ecologically intensified agroecosystems. To arrive at this goal, many challenges for cultivation need to be addressed, among them insect pests and pathogens. A fundamental understanding of the community ecology of silflower may help guide the development of and inform the management decisions for sustainable silflower cropping systems. This work demonstrates that native prairie AMF are highly beneficial to plant growth regardless of fungal species, whereas further research is required to parse out if any AMF mediated changes in the secondary metabolism of silflower have implications for plant fitness. A follow-up question to be addressed upon these results with native prairie AMF strains is the degree of mutualism between silflower and AMF strains that typically dominate agricultural soils. This would help to determine the importance of management decisions that respect the integrity of the soil's microbiome and if application of native mycorrhizal amendments can be beneficial.

Acknowledgements

First and foremost, I would like to thank Amanda Gehin at the University of Kansas for the outstanding collaboration on this project. I am also very grateful for everyone at The Land Institute involved in this effort for their continuous and dedicated guidance and advice. This includes my supervisors Ebony Murrell and Chase Stratton, as well as everyone that has lent an ear and discussed this project with me. Furthermore, the invaluable advice and generosity of Jim Bever and his lab at the University of Kansas, as well as Rob Morrison at the USDA-ARS Center for Animal Health and Grain Research made this work possible. Additional funding came from The Land Institute and the Perennial Agriculture Project. I would also like to thank Teun Dekker for the supervision of this thesis, as well as Johanna Entrup and Andrea Sweeting for their help with collecting data.

References

- Acevedo, F. E., Rivera-Vega, L. J., Chung, S. H., Ray, S., and Felton, G. W. (2015). Cues from chewing insects - the intersection of DAMPs, HAMPs, MAMPs and effectors. *Current opinion in plant biology*, 26:80–86.
- Alberto, F. J., Aitken, S. N., Alía, R., González-Martínez, S. C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., and Savolainen, O. (2013). Potential for evolutionary responses to climate change – evidence from tree populations. *Global Change Biology*, 19(6):1645–1661.
- Altieri, M. A. (2018). *Agroecology: The Science Of Sustainable Agriculture, Second Edition*. CRC Press, Boca Raton, FL, 2nd edition.
- Ament, K., Kant, M. R., Sabelis, M. W., Haring, M. A., and Schuurink, R. C. (2004). Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. *Plant physiology*, 135(4):2025–2037.
- Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, 26(1):32–46.
- Asensio, D., Rapparini, F., and Peñuelas, J. (2012). AM fungi root colonization increases the production of essential isoprenoids vs. nonessential isoprenoids especially under drought stress conditions or after jasmonic acid application. *Phytochemistry*, 77:149–161.
- Augé, R. (2001). Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza*, 11:3–42.
- Augé, R. M., Toler, H. D., and Saxton, A. M. (2015). Arbuscular mycorrhizal symbiosis alters stomatal conductance of host plants more under drought than under amply watered conditions: a meta-analysis. *Mycorrhiza*, 25(1):13–24.
- Babikova, Z., Gilbert, L., Bruce, T., Dewhurst, S. Y., Pickett, J. A., and Johnson, D. (2014). Arbuscular mycorrhizal fungi and aphids interact by changing host plant quality and volatile emission. *Functional Ecology*, 28(2):375–385.
- Bennett, A. E., Alers-Garcia, J., and Bever, J. D. (2006). Three-Way Interactions among Mutualistic Mycorrhizal Fungi, Plants, and Plant Enemies: Hypotheses and Synthesis. *The American Naturalist*, 167(2):141–152.
- Bennett, A. E. and Bever, J. D. (2007). Mycorrhizal species differentially alter plant growth and response to herbivory. *Ecology*, 88(1):210–218.
- Bennett, A. E., Bever, J. D., and Deane Bowers, M. (2009). Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory. *Oecologia*, 160(4):771–779.
- Bernaola, L., Cosme, M., Schneider, R. W., and Stout, M. (2018). Belowground inoculation with arbuscular mycorrhizal fungi increases local and systemic susceptibility of rice plants to different pest organisms. *Frontiers in Plant Science*, 9(June):1–16.
- Bernardo, L., Carletti, P., Badeck, F. W., Rizza, F., Morcia, C., Ghizzoni, R., Roupheal, Y., Colla, G., Terzi, V., and Lucini, L. (2019). Metabolomic responses triggered by arbuscular mycorrhiza enhance tolerance to water stress in wheat cultivars. *Plant physiology and biochemistry*, 137:203–212.
- Bever, J. D., Morton, J. B., Antonovics, J., and Schultz, P. A. (1996). Host-Dependent Sporulation and Species Diversity of Arbuscular Mycorrhizal Fungi in a Mown Grassland. *The Journal of ecology*, 84(1):71–82.

- Bommarco, R., Kleijn, D., and Potts, S. G. (2013). Ecological intensification: Harnessing ecosystem services for food security. *Trends in Ecology and Evolution*, 28(4):230–238.
- Breiman, L. (2001). Random Forests. *Machine Learning*, 45(1):5–32.
- Butters, J., Murrell, E., Spiesman, B. J., and Kim, T. N. (2022). Native Flowering Border Crops Attract High Pollinator Abundance and Diversity, Providing Growers the Opportunity to Enhance Pollination Services. *Environmental Entomology*, 51(2):492–504.
- Cassetta, E., Peterson, K., Bever, J., Brandvain, Y., Van Tassel, D., Lubin, T., Alexander, H., Byers, D., Schiffner, S., and Turner, M. K. (2022). Adaptation of pathogens to their local plant host, *Silphium integrifolium*, along a precipitation gradient. *Ecosphere*.
- Chagnon, P. L., Bradley, R. L., Maherali, H., and Klironomos, J. N. (2013). A trait-based framework to understand life history of mycorrhizal fungi. *Trends in Plant Science*, 18(9):484–491.
- Cheeke, T. E., Zheng, C., Koziol, L., Gurholt, C. R., and Bever, J. D. (2019). Sensitivity to AMF species is greater in late-successional than early-successional native or nonnative grassland plants. *Ecology*, 100(12):e02855.
- Chitarra, W., Pagliarani, C., Maserti, B., Lumini, E., Siciliano, I., Cascone, P., Schubert, A., Gambino, G., Balestrini, R., and Guerrieri, E. (2016). Insights on the Impact of Arbuscular Mycorrhizal Symbiosis on Tomato Tolerance to Water Stress. *Plant Physiology*, 171(2):1009–1023.
- Clavijo McCormick, A., Gershenzon, J., and Unsicker, S. B. (2014). Little peaks with big effects: Establishing the role of minor plant volatiles in plant-insect interactions. *Plant, Cell and Environment*, 37(8):1836–1844.
- Conrath, U., Beckers, G. J. M., Flors, V., García-Agustín, P., Jakab, G., Mauch, F., Newman, M.-A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L., and Mauch-Mani, B. (2006). Priming: Getting Ready for Battle. *Molecular Plant-Microbe Interactions MPMI*, 19(10):1062–1071.
- Cordell, D. and White, S. (2014). Life’s Bottleneck: Sustaining the World’s Phosphorus for a Food Secure Future. *Annual Review of Environment and Resources*, 39:161–188.
- Cox, C., Garrett, K., and Bockus, W. (2005). Meeting the challenge of disease management in perennial grain cropping systems. *Renewable Agriculture and Food Systems*, 20(1):15–24.
- Crews, T. E., Carton, W., and Olsson, L. (2018). Is the future of agriculture perennial? Imperatives and opportunities to reinvent agriculture by shifting from annual monocultures to perennial polycultures. *Global Sustainability*, 1:1–18.
- Davidson-Lowe, E. and Ali, J. G. (2021). Herbivore-induced plant volatiles mediate behavioral interactions between a leaf-chewing and a phloem-feeding herbivore. *Basic and Applied Ecology*, 53:39–48.
- Davidson-Lowe, E., Ray, S., Murrell, E., Kaye, J., and Ali, J. G. (2021). Cover Crop Soil Legacies Alter Phytochemistry and Resistance to Fall Armyworm (Lepidoptera: Noctuidae) in Maize. *Environmental Entomology*, 50(4):958–967.
- Davis, K. F., Gephart, J. A., Emery, K. A., Leach, A. M., Galloway, J. N., and D’Odorico, P. (2016). Meeting future food demand with current agricultural resources. *Global Environmental Change*, 39:125–132.

- Díaz-Uriarte, R. and Alvarez de Andrés, S. (2006). Gene selection and classification of microarray data using random forest. *BMC Bioinformatics*, 7(1):1–13.
- Dicke, M. (2009). Behavioural and community ecology of plants that cry for help. *Plant, Cell and Environment*, 32(6):654–665.
- Dicke, M. and Baldwin, I. T. (2010). The evolutionary context for herbivore-induced plant volatiles: beyond the ‘cry for help’. *Trends in Plant Science*, 15(3):167–175.
- Dicke, M., Van Poecke, R. M. P., and De Boer, J. G. (2003). Inducible indirect defence of plants: from mechanisms to ecological functions Basic and Applied Ecology. Technical report.
- Doré, T., Makowski, D., Malézieux, E., Munier-Jolain, N., Tchamitchian, M., and Tittone, P. (2011). Facing up to the paradigm of ecological intensification in agronomy: Revisiting methods, concepts and knowledge. *European Journal of Agronomy*, 34(4):197–210.
- Evans, D. L., Quinton, J. N., Davies, J. A., Zhao, J., and Govers, G. (2020). Soil lifespans and how they can be extended by land use and management change. *Environmental Research Letters*, 15(9):0940b2.
- Fontana, A., Reichelt, M., Hempel, S., Gershenson, J., and Unsicker, S. B. (2009). The effects of arbuscular mycorrhizal fungi on direct and indirect defense metabolites of *Plantago lanceolata* L. *Journal of Chemical Ecology*, 35(7):833–843.
- Gange, A. C., Brown, V. K., and Aplin, D. M. (2003). Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids. *Ecology Letters*, 6(12):1051–1055.
- Gao, D., Pan, X., Khashi u Rahman, M., Zhou, X., and Wu, F. (2021). Common mycorrhizal networks benefit to the asymmetric interspecific facilitation via K exchange in an agricultural intercropping system. *Biology and Fertility of Soils*, 57(7):959–971.
- Gehring, C. and Bennett, A. (2009). Mycorrhizal fungal-plant-insect interactions: The importance of a community approach. *Environmental Entomology*, 38(1):93–102.
- Gianinazzi, S., Gollotte, A., Binet, M. N., van Tuinen, D., Redecker, D., and Wipf, D. (2010). Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza*, 20(8):519–530.
- Gliessman, S. R. (2014). *The Ecology of Sustainable Food Systems*. CRC Press, Boca Raton, FL, 3rd edition.
- Glinwood, R., Ninkovic, V., and Pettersson, J. (2011). Chemical interaction between undamaged plants - Effects on herbivores and natural enemies. *Phytochemistry*, 72(13):1683–1689.
- Gouinguéné, S. P. and Turlings, T. C. (2002). The effects of abiotic factors on induced volatile emissions in corn plants. *Plant Physiology*, 129(3):1296–1307.
- Grassini, P., Eskridge, K. M., and Cassman, K. G. (2013). Distinguishing between yield advances and yield plateaus in historical crop production trends. *Nature Communications*, 4(1):1–11.
- Herrera-Estrella, L. and López-Arredondo, D. (2016). Phosphorus: The Underrated Element for Feeding the World. *Trends in plant science*, 21(6):461–463.
- Holopainen, J. K. (2004). Multiple functions of inducible plant volatiles. *Trends in Plant Science*, 9(11):529–533.
- Holopainen, J. K. and Gershenson, J. (2010). Multiple stress factors and the emission of plant VOCs. *Trends in Plant Science*, 15(3):176–184.

- House, G. L. and Bever, J. D. (2018). Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient. *Ecological Applications*, 28(3):736–748.
- Huang, D., Sun, M., Han, M., Zhang, Z., Miao, Y., Zhang, J., and Yao, Y. (2020). Volatile organic compounds (VOCs) regulate the spatial distribution of Lepidoptera insects in an orchard ecosystem. *Biological Control*, 149.
- Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., and Mortensen, D. A. (2017). Agriculture in 2050: Recalibrating targets for sustainable intensification. *BioScience*, 67(4):386–391.
- Jacott, C. N., Murray, J. D., and Ridout, C. J. (2017). Trade-Offs in Arbuscular Mycorrhizal Symbiosis: Disease Resistance, Growth Responses and Perspectives for Crop Breeding. *Agronom*, 7(4):75.
- Johnson, N. C., Graham, J. H., and Smith, F. A. (1997). Functioning of mycorrhizal associations along the mutualism-parasitism continuum. *New Phytol*, 135:575–585.
- Kaur, S. and Suseela, V. (2020). Unraveling arbuscular mycorrhiza-induced changes in plant primary and secondary metabolome. *Metabolites*, 10(8):1–30.
- Kempel, A., Schmidt, A. K., Brandl, R., and Schädler, M. (2010). Support from the underground: Induced plant resistance depends on arbuscular mycorrhizal fungi. *Source: Functional Ecology*, 24(2):293–300.
- Kigathi, R. N., Weisser, W. W., Reichelt, M., Gershenson, J., and Unsicker, S. B. (2019). Plant volatile emission depends on the species composition of the neighboring plant community. *BMC Plant Biology*, 19(1).
- Koziol, L., Crews, T. E., and Bever, J. D. (2019). Benefits of native mycorrhizal amendments to perennial agroecosystems increases with field inoculation density. *Agronomy*, 9(7):353.
- Koziol, L., Schultz, P. A., House, G. L., Bauer, J. T., Middleton, E. L., and Bever, J. D. (2018). The Plant Microbiome and Native Plant Restoration: The Example of Native Mycorrhizal Fungi. *BioScience*, 68(12):996–1006.
- Krupek, F. S., Redfearn, D., Eskridge, K. M., and Basche, A. (2022). Ecological intensification with soil health practices demonstrates positive impacts on multiple soil properties: A large-scale farmer-led experiment. *Geoderma*, 409(4):115594.
- Lehnert, H., Serfling, A., Friedt, W., and Ordon, F. (2018). Genome-Wide Association Studies Reveal Genomic Regions Associated With the Response of Wheat (*Triticum aestivum* L.) to Mycorrhizae Under Drought Stress Conditions. *Frontiers in plant science*, 9:1728.
- López-Goldar, X. and Agrawal, A. A. (2021). Ecological Interactions, Environmental Gradients, and Gene Flow in Local Adaptation. *Trends in Plant Science*, 26(8):796–809.
- Marschalek, D. A. (2013). *Blister Beetles (Coleoptera: Meloidae) of Wisconsin: Distribution and Ecology*. PhD thesis, University of Wisconsin-Madison.
- Maurer, C., Rüdy, M., Chervet, A., Sturny, W. G., Flisch, R., and Oehl, F. (2014). Diversity of arbuscular mycorrhizal fungi in field crops using no-till and conventional tillage practices. *Agrarforschung Schweiz*, 5(10):398–405.
- McKenna, T. P., Koziol, L., Bever, J. D., Crews, T. E., and Sikes, B. A. (2020). Abiotic and biotic context dependency of perennial crop yield. *PLoS ONE*, 15(6):e0234546.
- Meier, A. R. and Hunter, M. D. (2018). Mycorrhizae Alter Toxin Sequestration and

- Performance of Two Specialist Herbivores. *Frontiers in Ecology and Evolution*, 6(33).
- Menge, J. A., Johnson, E. L. V., and Platt, R. G. (1978). Mycorrhizal Dependency of Several Citrus Cultivars Under Three Nutrient Regimes. *The New Phytologist*, 81(3):553–559.
- Mensah, J. A., Koch, A. M., Antunes, P. M., Kiers, E. T., Hart, M., and Bücking, H. (2015). High functional diversity within species of arbuscular mycorrhizal fungi is associated with differences in phosphate and nitrogen uptake and fungal phosphate metabolism. *Mycorrhiza*, 25(7):533–546.
- Miano, R. N., Ayelo, P. M., Musau, R., Hassanali, A., and Mohamed, S. A. (2022). Electroantennogram and machine learning reveal a volatile blend mediating avoidance behavior by *Tuta absoluta* females to a wild tomato plant. *Scientific Reports*, 12(1).
- Michereff, M. F. F., Laumann, R. A., Borges, M., Michereff-Filho, M., Diniz, I. R., Neto, A. L. F., and Moraes, M. C. B. (2011). Volatiles Mediating a Plant-Herbivore-Natural Enemy Interaction in Resistant and Susceptible Soybean Cultivars. *Journal of Chemical Ecology*, 37(3):273–285.
- Middleton, E. L., Richardson, S., Koziol, L., Palmer, C. E., Yermakov, Z., Henning, J. A., Schultz, P. A., and Bever, J. D. (2015). Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere*, 6(12).
- Munkvold, L., Kjølner, R., Vestberg, M., Rosendahl, S., and Jakobsen, I. (2004). High functional diversity within species of arbuscular mycorrhizal fungi. *The New phytologist*, 164(2):357–364.
- Nihraz, C. T., Helms, A. M., Tooker, J. F., Mescher, M. C., De Moraes, C. M., and Stephenson, A. G. (2022). Adverse effects of inbreeding on the transgenerational expression of herbivore-induced defense traits in *Solanum carolinense*. *PLoS one*, 17(10):e0274920.
- Oehl, F., Sieverding, J., Palenzuela, K., Ineichen, K., and Silva, G. (2011). Advances in Glomeromycota taxonomy and classification. *IMA Fungus*, 1:191–199.
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O’hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., and Maintainer, H. W. (2020). *vegan: Community Ecology Package*. Technical report.
- Ottensmann, M., Stoffel, M. A., Nichols, H. J., and Hoffman, J. I. (2018). GCalignR: An R package for aligning gas-chromatography data for ecological and evolutionary studies. *PLOS ONE*, 13(6):e0198311.
- Peterson, K., Cheremond, E., Brandvain, Y., Van Tassel, D., and Murrell, E. (2022). Weight Gain of *Spodoptera frugiperda* Larvae (Lepidoptera: Noctuidae) on Leaf and Floral Tissues of *Silphium integrifolium* (Asterales: Asteraceae) Differs by Plant Genotype. *Environmental Entomology*, 51(2):397–404.
- Pierik, R., Ballaré, C. L., and Dicke, M. (2014). Ecology of plant volatiles: Taking a plant community perspective. *Plant, Cell and Environment*, 37(8):1845–1853.
- Pineda, A., Zheng, S. J., van Loon, J. J., Pieterse, C. M., and Dicke, M. (2010). Helping plants to deal with insects: The role of beneficial soil-borne microbes. *Trends in Plant Science*, 15(9):507–514.
- Ponce, M. A., Lizarraga, S., Bruce, A., Kim, T. N., and Morrison, W. R. (2022). Grain Inoculated with Different Growth Stages of the Fungus, *Aspergillus flavus*, Affect

- the Close-Range Foraging Behavior by a Primary Stored Product Pest, *Sitophilus oryzae* (Coleoptera: Curculionidae). *Environmental Entomology*, 51(5):927–939.
- Quarrell, S. R., Weinstein, A. M., Hannah, L., Bonavia, N., Borrello, O. d., Flematti, G. R., and Bohman, B. (2022). Critical pollination chemistry: Specific sesquiterpene floral volatiles in carrot inhibit honey bee feeding. *bioRxiv*.
- Quiroga, G., Erice, G., Ding, L., Chaumont, F., Aroca, R., and Ruiz-Lozano, J. M. (2019). The arbuscular mycorrhizal symbiosis regulates aquaporins activity and improves root cell water permeability in maize plants subjected to water stress. *Plant, Cell & Environment*, 42(7):2274–2290.
- Ranganathan, Y. and Borges, R. M. (2010). Reducing the babel in plant volatile communication: Using the forest to see the trees. *Plant Biology*, 12(5):735–742.
- Rasche, F., Blagodatskaya, E., Emmerling, C., Belz, R., Musyoki, M. K., Zimmermann, J., and Martin, K. (2017). A preview of perennial grain Agriculture: Knowledge gain from biotic interactions in natural and agricultural ecosystems. *Ecosphere*, 8(12).
- Rasmann, S., Bennett, A., Biere, A., Karley, A., and Guerrieri, E. (2017). Root symbionts: Powerful drivers of plant above- and belowground indirect defenses. *Insect Science*, 24(6):947–960.
- Real-Santillán, R. O., del Val, E., Cruz-Ortega, R., Contreras-Cornejo, H. A., González-Esquivel, C. E., and Larsen, J. (2019). Increased maize growth and P uptake promoted by arbuscular mycorrhizal fungi coincide with higher foliar herbivory and larval biomass of the Fall Armyworm *Spodoptera frugiperda*. *Mycorrhiza*, 29(6):615–622.
- Rich, M. K., Vigneron, N., Libourel, C., Keller, J., Xue, L., Hajheidari, M., Radhakrishnan, G. V., Le Ru, A., Diop, S. I., Potente, G., Conti, E., Duijsings, D., Batut, A., Le Faouder, P., Kodama, K., Kyojuka, J., Sallet, E., Bécard, G., Rodriguez-Franco, M., Ott, T., Bertrand-Michel, J., Oldroyd, G. E., Szövényi, P., Bucher, M., and Delaux, P. M. (2021). Lipid exchanges drove the evolution of mutualism during plant terrestrialization. *Science*, 372(6544):864–868.
- Rillig, M. C. (2004). Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Science*, 84(4):355–363.
- Rillig, M. C., Aguilar-Trigueros, C. A., Camenzind, T., Cavagnaro, T. R., Degrune, F., Hohmann, P., Lammel, D. R., Mansour, I., Roy, J., van der Heijden, M. G., and Yang, G. (2019). Why farmers should manage the arbuscular mycorrhizal symbiosis. *The New phytologist*, 222(3):1171–1175.
- Rowe, H., Withers, P. J., Baas, P., Chan, N. I., Doody, D., Holiman, J., Jacobs, B., Li, H., MacDonald, G. K., McDowell, R., Sharpley, A. N., Shen, J., Taheri, W., Wallenstein, M., and Weintraub, M. N. (2015). Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security. *Nutrient Cycling in Agroecosystems*, 104(3):393–412.
- Ryan, M. H. and Graham, J. H. (2018). Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. *The New phytologist*, 220(4):1092–1107.
- Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., van der Heijden, M. G., and Oehl, F. (2015). Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 84:38–52.
- Schausberger, P., Peneder, S., Jürschik, S., and Hoffmann, D. (2012). Mycorrhiza changes

- plant volatiles to attract spider mite enemies. *Functional Ecology*, 26(2):441–449.
- Schweiger, R., Baier, M. C., Persicke, M., and Müller, C. (2014). High specificity in plant leaf metabolic responses to arbuscular mycorrhiza. *Nature Communications*, 5(1):1–11.
- Scieuzo, C., Nardiello, M., Farina, D., Scala, A., Cammack, J. A., Tomberlin, J. K., Vogel, H., Salvia, R., Persaud, K., and Falabella, P. (2021). *Hermetia illucens* (L.) (diptera: Stratiomyidae) odorant binding proteins and their interactions with selected volatile organic compounds: An in silico approach. *Insects*, 12(9).
- Sharma, E., Anand, G., and Kapoor, R. (2017). Terpenoids in plant and arbuscular mycorrhiza-reinforced defence against herbivorous insects. *Annals of Botany*, 119(5):791–801.
- Shrivastava, G., Ownley, B. H., Augé, R. M., Toler, H., Dee, M., Vu, A., Köllner, T. G., and Chen, F. (2015). Colonization by arbuscular mycorrhizal and endophytic fungi enhanced terpene production in tomato plants and their defense against a herbivorous insect. *Symbiosis*, 65(2):65–74.
- Smith, A. F. and Smith, S. E. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? *Plant and Soil*, 348(1-2):63–79.
- Stratton, C. A., Ray, S., Bradley, B. A., Kaye, J. P., Ali, J. G., and Murrell, E. G. (2022). Nutrition vs association: plant defenses are altered by arbuscular mycorrhizal fungi association not by nutritional provisioning alone. *BMC Plant Biology*, 22(1):1–10.
- Thirkell, T. J., Grimmer, M., James, L., Pastok, D., Allary, T., Elliott, A., Paveley, N., Daniell, T., and Field, K. J. (2022). Variation in mycorrhizal growth response among a spring wheat mapping population shows potential to breed for symbiotic benefit. *Food and Energy Security*, 11(2):e370.
- Thomas, S. E., Evans, H. C., Cortat, G., Koutsidou, C., Day, M. D., and Ellison, C. A. (2021). Assessment of the microcyclic rust *Puccinia lantanae* as a classical biological control agent of the pantropical weed *Lantana camara*. *Biological Control*, 160:104688.
- Tinker, P. B. P. B. (2000). *Solute movement in the rhizosphere*. Topics in sustainable agronomy. Oxford University Press, New York, 2nd ed. edition.
- Tittonell, P. (2014). Ecological intensification of agriculture-sustainable by nature. *Current Opinion in Environmental Sustainability*, 8:53–61.
- Turner, M. K., Ravetta, D., and Van Tassel, D. (2018). Effect of *Puccinia silphii* on yield components and leaf physiology in *Silphium integrifolium*: Lessons for the domestication of a perennial oilseed crop. *Sustainability*, 10(3):696.
- Van Der Heijden, M. G., Streitwolf-Engel, R., Riedl, R., Siegrist, S., Neudecker, A., Ineichen, K., Boller, T., Wiemken, A., and Sanders, I. R. (2006). The mycorrhizal contribution to plant productivity, plant nutrition and soil structure in experimental grassland. *New Phytologist*, 172(4):739–752.
- Van Tassel, D. L., Tesdell, O., Schlautman, B., Rubin, M. J., DeHaan, L. R., Crews, T. E., and Streit Krug, A. (2020). New Food Crop Domestication in the Age of Gene Editing: Genetic, Agronomic and Cultural Change Remain Co-evolutionarily Entangled. *Frontiers in Plant Science*, 11:789.
- Vannette, R. L. and Hunter, M. D. (2009). Mycorrhizal fungi as mediators of defence against insect pests in agricultural systems. *Agricultural and Forest Entomology*, 11(4):351–358.

- Vilela, A., González-Paleo, L., Turner, K., Peterson, K., Ravetta, D., Crews, T. E., and Van Tassel, D. (2018). Progress and bottlenecks in the early domestication of the perennial oilseed *Silphium integrifolium*, a sunflower substitute. *Sustainability*, 10(3):638.
- Vilela, A. E., González-Paleo, L., Ravetta, D. A., Murrell, E. G., and van Tassel, D. L. (2020). Balancing forage production, seed yield, and pest management in the perennial sunflower *silphium integrifolium* (Asteraceae). *Agronomy*, 10(10).
- Vogelsang, K. M., Reynolds, H. L., and Bever, J. D. (2006). Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *The New phytologist*, 172(3):554–562.
- Wang, G., Ye, C., Zhang, J., Koziol, L., Bever, J. D., and Li, X. (2019). Asymmetric facilitation induced by inoculation with arbuscular mycorrhizal fungi leads to overyielding in maize/faba bean intercropping. *Journal of Plant Interactions*, 14(1):10–20.
- War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., and Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores.
- Watts-Williams, S. J., Emmett, B. D., Levesque-Tremblay, V., MacLean, A. M., Sun, X., Satterlee, J. W., Fei, Z., and Harrison, M. J. (2019). Diverse *Sorghum bicolor* accessions show marked variation in growth and transcriptional responses to arbuscular mycorrhizal fungi. *Plant Cell and Environment*, 42(5):1758–1774.
- Xie, Z., Li, S., Tang, S., Huang, L., Wang, G., Sun, X., and Hu, Z. (2019). Phosphorus Leaching from Soil Profiles in Agricultural and Forest Lands Measured by a Cascade Extraction Method. *Journal of Environmental Quality*, 48(3):568–578.

Popular science summary

As global population is on the rise and dietary preferences are changing, crop production will need to increase to meet future food demand. Meanwhile, adverse environmental effects of industrial practices employed in agriculture today become increasingly apparent and calls for them to be addressed are growing louder. Perennial agroecosystems – growing more perennial crops instead of annuals – are a suggested pathway to increase the provision of ecosystem services such as carbon sequestration and conservation of biodiversity while providing food, feed and fibers sustainably.

Silflower (*Silphium integrifolium*) is a perennial sunflower relative that is bred to become a future oilseed crop. Aside from increasing yield, further challenges for growing silflower at larger scales are insect pests and pathogens. In order to breed for resistance and to develop agricultural systems that are resilient to these stressors, the ecology of the plant needs to be better understood.

In the presented study the importance of arbuscular mycorrhizal fungi for silflower growth and defense against an insect pest was explored. These fungi are symbionts that associate with the plant roots and improve access to soil resources. In addition, they have been found to change the plants defensive strategy. While further research is needed to understand if these fungi change the defensive strategy of the plant significantly, the results of the greenhouse experiment show that arbuscular mycorrhizal fungi from native prairie soils are highly beneficial for silflower growth. In addition, silflower plants stemming from two geographical areas were found to differ in their responsiveness to mycorrhizae, potentially indicating the selecting for silflower cultivars that are highly mycorrhizal is possible.

Future explorations in the field and more complex settings will be necessary to further elucidate the ecology of silflower. Such efforts will help facilitate the development of diverse, perennial and sustainable agricultural systems with novel perennial crops.

Publishing and archiving

Approved students' theses at SLU are published electronically. As a student, you have the copyright to your own work and need to approve the electronic publishing. If you check the box for YES, the full text (pdf file) and metadata will be visible and searchable online. If you check the box for NO, only the metadata and the abstract will be visible and searchable online. Nevertheless, when the document is uploaded it will still be archived as a digital file. If you are more than one author, the checked box will be applied to all authors. Read about SLU's publishing agreement here:

- <https://www.slu.se/en/subweb/library/publish-and-analyse/register-and-publish/agreement-for-publishing/>.

YES, I/we hereby give permission to publish the present thesis in accordance with the SLU agreement regarding the transfer of the right to publish a work.

NO, I/we do not give permission to publish the present work. The work will still be archived and its metadata and abstract will be visible and searchable.