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The Role of Mycorrhizal Associations in Modulating Poplar Growth, Phytohormone Responses, and Mortality under Elevated CO₂ and Temperature Conditions

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Biology

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Abstract

Climate change poses significant challenges to forests worldwide, particularly the Canadian boreal forest. *Populus* spp. are ecologically and economically important tree species that have had declining growth and survival due to elevated temperatures and droughts associated with climate change. Symbiotic microbes, such as mycorrhizal fungi, may increase plant growth under climate change conditions by altering tree metabolic profiles and increasing tree access to water and nutrients. My thesis explores the relationship between mycorrhizal fungi and a *Populus* hybrid (*Populus x canadensis*) grown under a range of future climate scenarios: ambient CO₂ (400 ppm) or elevated CO₂ (750 ppm) with either ambient temperatures or a +4 °C or +8 °C warming treatment. My primary objective is to assess how mycorrhizae influence growth, stress phytohormone concentrations, and stress tolerance of hybrid poplar under predicted future climatic scenarios. Additionally, I identify compounds exuded by mycorrhizal fungi and evaluate their potential to enhance plant growth. My findings reveal that different boreal mycorrhizal fungi produce similar profiles of phytohormones, amino acids, and organic acids in their exudates. The exudates of some mycorrhizal fungi enhanced plant growth, while others caused mortality. In my hybrid poplar growth study, the colonization of poplar roots by mycorrhizal fungi increased with elevated temperature and CO₂. Inoculation with mycorrhizal fungi did not increase tree height or mass, with the exception of trees grown under +4 °C warming, where total biomass increased by ~15% compared to control trees. Unexpectedly, inoculation with mycorrhizal fungi almost always increased hybrid poplar mortality. To understand why mycorrhizal inoculation increased mortality but improved the growth of surviving hybrid poplars, I investigated the impacts of climate change and mycorrhizal inoculation on plant stress hormone concentrations. Mycorrhizal inoculation generally increased leaf concentrations of the

stress hormone jasmonic acid, while the stress hormones salicylic acid and abscisic acid had reduced leaf concentrations across elevated temperature and CO₂ treatments. Overall, my research contributes valuable insights into the intricate connections between mycorrhizal fungi, trees and climate change, offering a better understanding of forest ecosystem resilience in the face of environmental challenges.

Keywords: Climate change, Boreal Forest. *Populus*, mycorrhizal associations, elevated temperatures and CO₂, phytohormones, mortality.

Summary for Lay Audience

In our changing world, the health of forests is at risk due to climate change, especially in the Canadian boreal forest. One important group of trees, *Populus* (poplars), is crucial for understanding how these changes affect trees. Almost all plants, including poplars, form partnerships with soil fungi called mycorrhizal fungi, and to date, little research has been done on how climate change could impact this relationship. These fungi establish a mutually helpful relationship with tree roots, giving them water and nutrients in exchange for sugars from the trees. This relationship is thought to increase resilience to environmental stresses, such as elevated temperatures that accompany climate change. My research focused on exploring this association between mycorrhizae and a poplar hybrid in the face of climate change. I studied how associating with these fungi would affect tree growth and stress tolerance to high CO₂ and temperatures. I also looked into the substances released by these fungi, checking if they could help plants grow. I found that the fungi release certain substances that, when applied to plants, can either improve growth or harm them. Even though warming alone didn't affect tree growth much, 4 °C warming combined with high CO₂ made the trees without fungi larger, but increased tree death rates. I also discovered that fungi increased their association with tree roots in warmer conditions but at the cost of higher tree mortality. In summary, my research helps us understand the association between mycorrhizal fungi and trees under climate change. By figuring out how these organisms work together, we can learn how to better protect our forests in a warming world.

Co-Authorship Statement

This thesis includes three manuscripts. Chapter 2 is a version of a manuscript near submission for the journal *Molecules*. I am the first author, and Tod Ramsfield, Mamdouh Abou-Zaid and Danielle Way are co-authors. Tod Ramsfield, Mamdouh Abou-Zaid, Danielle Way and I contributed to the experimental design of the project, conceived ideas on data collection, and discussed ideas on data analyses. I conducted the experiments and collected/analyzed all of the data. Mamdouh Abou-Zaid helped to generate, analyze, and interpret all of the HPLC data. Tod Ramsfield, Danielle Way, and I wrote the manuscript, and Mamdouh Abou-Zaid provided editorial advice.

A version of Chapter 3 is near submission for the journal *Mycorrhiza*. I am the first author, and Tod Ramsfield and Danielle Way are co-authors. Tod Ramsfield, Danielle Way, and I contributed to the experimental design of the project, conceived ideas on data collection, and discussed ideas on data analyses. I conducted the experiments and analyzed all of the data. Tod Ramsfield, Danielle Way, and I wrote the manuscript.

Chapter 4 is a version of a manuscript to be submitted to the journal *Molecules*. I am the first author, and Tod Ramsfield, Mamdouh Abou-Zaid and Danielle Way are co-authors. Tod Ramsfield, Mamdouh Abou-Zaid, Danielle Way, and I contributed to the experimental design of the project, conceived ideas on data collection, and discussed ideas on data analyses. I conducted the experiments and analyzed all of the data. Mamdouh Abou-Zaid generated, analyzed and interpreted all of the HPLC data. Tod Ramsfield, Danielle Way, and I wrote the manuscript, and Mamdouh Abou-Zaid provided editorial advice.

Acknowledgments

Embarking on a Ph.D. journey is a monumental undertaking, seldom navigated in solitude. The successful completion of this colossal task owes its accomplishment to the invaluable support and guidance of numerous individuals. I am profoundly grateful to those who have played a pivotal role in shaping my Ph.D. years, extending their wisdom and assistance.

Foremost, my sincere gratitude goes to my supervisor, Dr. Danielle (Dani) Way. I want to thank you for allowing me to pursue my ideas and lead my own research project, even though none of my experiments or ideas were based on the ecophysiology of photosynthesis that our lab is so well known for. Your encouragement for me to pursue every opportunity to learn and acquire new skills as well as put up with my extreme excitement (and let's be honest, contain it) about the impacts of mycorrhiza on plant growth and their use to help mitigate the impacts of climate change was greatly appreciated. I am indebted to you for advocating a balanced work-life dynamic and emphasizing the importance of mental well-being. Your steadfast support during both the triumphs and tribulations of my doctoral journey; from supporting me when I got married, welcoming both my daughters into the world, when my father, aunt and grandmother passed away, when **all** my poplar trees died, when we had a global pandemic, and when my wife got ill and landed in the hospital multiple times; has been invaluable. Reflecting on this journey, I am grateful for your guidance, and I couldn't have picked a better supervisor to work with and support me through all this.

In tandem, I extend my gratitude to my co-supervisor, Dr. Tod Ramsfield. Your constant encouragement and support, especially during moments of self-doubt, have been instrumental. I really appreciated your hospitality when I visited Edmonton at the start of my Ph.D. to meet you,

collect my 14 fungal species and learn how to maintain fungal cultures. Your willingness to always meet and help me understand fungal and general plant pathology concepts was very much appreciated. Much like Dani, your presence during significant life events and emphasis on prioritizing family underscored the profound impact you've had on my academic and personal growth. Collaborating with and learning from you has been a highlight of my Ph.D.

Special thanks to Dr. Mamdouh Abou-Zaid for his enduring help, guidance, and mentorship. Your enthusiasm for phytochemistry and scientific philosophy has left an indelible mark on my scientific thinking, propelling me towards excellence. Your consistent support throughout my thesis projects is deeply appreciated.

I am very thankful to my dear Way Lab members (Dr. Joseph Ronald Stinziano, Dr. Mirindi Eric Dusenge, Dr. André Galvao Duarte, Alejandra Rey Rincon, Sasha Madhavji, Bridget Kathleen Murphy, Sarah McDonald, Kristyn Bennett, Jacob Hauger, Kumkum Azad, Kiana Jada Lee, Samuel Woolsey, Andrew David Matthew Cook, Julia Hammer and the army of undergrad volunteers) for their moral support, help on my Ph.D. projects, biomass harvests and their feedback on my talks for conferences during lab meetings. I would like to specifically thank the undergraduate volunteers who have been instrumental in ensuring an efficient and effective harvest of all plant material at the end of the growth experiments. In particular, I would like to thank Holly Nevison, an undergraduate thesis student who was instrumental in weighing most of my plant biomass, helping me grind plant tissue for hormone extraction, and helping me score fungal colonization of plant roots.

I am grateful to the following people for offering technical support, service, and advice at various times: Karen Nygard (at the Biotron), Elizabeth Myscich, and Colin Myrholm (Northern Forestry Center, Edmonton, Alberta).

I also thank my advisors Dr. Greg Thorn, Dr. Mamdouh Abou-Zaid, and Dr. Ben Rubin. Dr. Thorn, in particular, deserves my deepest thanks for his ideas, encouragement, and invaluable guidance during Dani's transition to Australia. His support in refining my thesis work has been indispensable.

I would like to thank my family. My mother (Debra) and grandmother (Marjorie) for all the support and encouragement since I was 3 right up to 33. If it were not for your encouragement and sacrifice over the years, I would never have made it this far. I would also like to thank my two sisters, Cassie and Taylor, as well as my uncles, Robert, Shawn and Mark, for their continued encouragement and support during these years. I would also like to thank the people who are no longer with me but who nonetheless helped shape my journey: Robert Taylor (Grandfather), Jim Frank (Father), and Lynn Frank (Grandmother). I would also like to thank my mother and father-in-law, Dr. Patricia Sealy and Keven Webb, for their continued support and guidance during this milestone in my life. The penultimate acknowledgment goes to my two daughters, Aurora, and Elouise, who have contributed a great deal of typos to my chapter drafts and always put a smile on the faces of my lab mates and supervisors when they interrupted the many Zoom calls. Last but certainly not least, I thank my wife, Eliza. Doing a Ph.D. while trying to raise a family is not easy, yet every step of the way, you were there to hold me up and keep me going, even when I thought I had no more to give. Your unwavering support and sacrifices during this challenging journey have been the cornerstone of my success in attempting to achieve my dream. Thank you, with all my love.

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List of Abbreviations

Ψ – water potential

0T – Ambient temperature treatment

4T – Plus °C temperature treatment

8T – Plus 8 °C temperature treatment

ABA – Abscisic acid

AC – Ambient carbon dioxide concentration

AM - Arbuscular mycorrhiza

ANOVA - analysis of variance

C3 – photosynthesis where the first stable product of CO₂ fixation is a three-carbon sugar

Cis-JA - Cis-jasmonate

CO₂ – Carbon dioxide

DSE - Dark septate endophyte

EC – Elevated carbon dioxide concentration

ECM – Ectomycorrhiza

ERM - Ericoid mycorrhiza

FACE – Free Air CO₂ Enrichment

GA - Gibberellic acid

g_s - Stomatal conductance

IAA - Indole-3-acetic acid

IPCC - Intergovernmental Panel on Climate Change

JA – Jasmonic acid

KIN - Kinetin

Meth-JA - Methyl jasmonate

PPM – Parts per million

SA – Salicylic acid

SAR - Systemic acquired resistance

VOC- Volatile organic compound

VPD - Vapour pressure deficit

Chapter 1

1 Climate Change and the Boreal Forest

1.1.1 Climate Change

Human activities have increased greenhouse gas concentrations such as carbon dioxide (CO₂), methane, and nitrous oxides in the atmosphere, resulting in rising global temperatures (IPCC, 2014; IPCC, 2021; IPCC, 2023). Specifically, CO₂ concentrations have increased from 280 parts per million (ppm) before the Industrial Revolution to current levels of 420 ppm, primarily due to the burning of fossil fuels and land use changes (Keeling et al., 1976; Thoning et al., 1989; Etheridge et al., 1996; Ciais et al., 2013; IPCC, 2021; Iz, 2022; IPCC, 2023). The increase in CO₂ concentrations, along with those of other greenhouse gases, has already caused the global mean surface air temperature to rise by 1.09 °C (IPCC, 2021). Moreover, 2023 saw daily average mean surface temperatures exceeding 1°C above pre-industrial levels, with 50% of days exceeding 1.5°C, and two November days exceeding 2°C for the first time (NOAA, 2024; Copernicus; 2024). Without significant reductions in greenhouse gas emissions, global surface temperatures will continue increasing by 1-4°C by 2100 (IPCC, 2021; IPCC, 2023). Higher latitudes, which include the boreal forest, are expected to experience an increase in surface temperatures of up to 8°C by the turn of the century (Price et al., 2013; Stocker et al., 2013; IPCC, 2021; IPCC, 2023). This temperature change could potentially disrupt precipitation patterns, leading to more frequent and severe regional droughts occurring around the globe (Trenberth, 2011; IPCC, 2021; Hammond et al., 2022; Abbass et al., 2022; IPCC, 2023).

1.1.2 Boreal Forests

Spanning Canada, Northern Europe, and Russia, the boreal forest covers 552 million hectares and serves as a critical source of food and water for humans, an essential habitat for wildlife, and a large carbon sink (Brandt et al., 2013; Ciais et al., 2013; Natural Resource Canada, 2016; Le Quere et al., 2018; Astrup et al., 2018). The boreal forest, the largest land-based biome and a crucial component of the Canadian forestry industry, is highly vulnerable to climate change (Brandt, 2009; Price et al., 2013; Berner & Goetz, 2022). Substantial forest losses in North America since 2008 provide clear evidence of this vulnerability (Raffa et al., 2008; Hogg et al., 2008; Bentz et al., 2009), despite efforts focused on reforestation involving genetically improved hybrid tree species like poplar or spruce (Larchevêque et al., 2010). While increased temperatures and CO₂ concentrations can extend the growing season and enhance plant growth, they also disrupt precipitation patterns, contribute to heatwaves, and increase plant mortality and forest fire frequency and intensity (Stocker et al., 2013; Marchand et al., 2019; Day et al., 2020; Enbrecht et al., 2021). These changes, coupled with ongoing shifts in vegetation and species composition, impact the stability and resilience of the boreal forest community (Foster et al., 2019; Berner & Goetz, 2022). The shift towards warmer temperatures may also favour more deciduous broadleaf species over conifers, transforming the forest's structure and function (Massey et al., 2023; Malla et al., 2023). Furthermore, the boreal forest's role as a carbon sink is increasingly at risk as changing temperature and precipitation patterns affect its capacity to absorb CO₂. Implementing effective management strategies is vital for mitigating these impacts and preserving the boreal forest's ecological integrity and economic value.

1.1.2.1 The Significance of Poplar in Boreal Forest Ecosystems

Populus spp. hold ecological importance and economic value and are widely distributed in the boreal biome, with *Populus balsamifera* L. and *Populus tremuloides* Michx. being the most widely distributed species within the boreal (Cooke, 2007; Worrall et al., 2013; Nlungu-Kweta et al., 2017). Additionally, poplars provide vital ecosystem services, such as sequestering CO₂ and supporting biodiversity by acting as a food source and habitat for small animals and birds (Isebrands, & Karnosky, 2001; Cooke, 2007; Riccioli et al., 2020). *Populus* trees are perennial, characterized by rapid vegetative growth and a reproductive phase (sexual and asexual propagation) once established (Bradshaw et al., 2000; Hsu et al., 2011). *Populus* trees that reproduce asexually often do so through the root collars of dead trees or broken branches that are buried in the soil (Bradshaw et al., 2000). Some *Populus* trees can reproduce by sending out sucker shoots from roots, particularly in the aftermath of a fire, resulting in the formation of clonal stands that can cover several hectares (Bradshaw et al., 2000). *Populus* species exhibit considerable genetic diversity, adapting to varying environmental conditions and contributing to their ecological versatility across North America (Bradshaw et al., 2000; Cooke, 2007). This, in part, is due to the ability of *Populus* to hybridize naturally (Eckenwalder, 1996; Bradshaw et al., 2000). For example, the hybrid *Populus x canadensis* Moench formed through the natural hybridization of *Populus deltoides* W. Bartram ex Marshall, after its introduction to Europe in the 18th century (Eckenwalder, 1996). These hybrid poplars are utilized in various industries, such as pulp, dimension lumber, and plywood; conservation, afforestation and reforestation efforts; ornamental plantings; and phytoremediation applications (Pearson et al., 2010). In addition, poplars are generally recognized for their production of secondary metabolites,

including salicylates and volatile organic compounds (VOCs), which influence tree interactions with herbivores and other organisms (Müller et al., 2015). Research into poplar trees encompasses; genomics and breeding for the development of improved cultivars, investigations into their capacity to adapt to climate change, assessment of their contribution to ecosystem services (including carbon sequestration), and studies on their phytochemistry and interactions with insects (Cooke, 2007; Hsu et al., 2011; Müller et al., 2015; Riccioli et al., 2020).

1.1.3 Impacts of Elevated Temperature and CO₂ Concentrations on Plant Growth

The distribution of plants across the Earth is primarily determined by precipitation and temperature (Prentice et al., 1992). Boreal tree species, often considered cold-limited, are expected to benefit from warming temperatures, resulting in increased growth (Tucker et al., 2001). However, the relationship between temperature and tree growth is more complex. Moderate warming can enhance tree carbon balance (i.e. the balance between CO₂ uptake in photosynthesis and CO₂ losses via respiration) and promote growth, while extreme warming can suppress photosynthesis and stimulate respiratory carbon loss, leading to decreased growth (Way & Oren, 2010; Wang et al., 2012; Way et al., 2014). The responses of boreal tree species to increased temperature are complex and species-specific. Moderate warming can generally, stimulate growth in many tree species, such as *Populus tremula* L. and *P. tremuloides*, *Pinus taeda* L., *Liriodendron tulipifera* L., and *Betula ermanii* Cham. (Way et al., 2013; Constable & Ratzlaff, 2000; Sobuj et al., 2018; Du et al., 2021), while extreme warming events can have detrimental effects on tree growth (Constable & Ratzlaff, 2000; Juday & Alix, 2012; Girardin et al., 2016; Stinziano & Way, 2017; Sobuj et al., 2018; Cahoon et al., 2018; Nicklen et al., 2018;

Trugman et al., 2018; Du et al., 2021). Generally, warming tends to suppress growth in evergreen trees but enhances growth in deciduous species (Way and Oren, 2010). In addition to temperature, an extended growing season induced by elevated spring and/or fall temperatures has been suggested to promote tree growth within the boreal forest, as this allows trees more time for photosynthesis and carbon assimilation, resulting in increased biomass production (Gu et al., 2022; Wang et al., 2023). Phenological shifts, including earlier spring greening and alterations in vegetative activity, have been observed in response to extended growing seasons (Cleland et al., 2007; Wang et al., 2015), significantly affecting carbon assimilation, energy exchange, and the overall productivity of boreal forests.

Elevated CO₂ concentrations enhance plant growth by increasing the availability of CO₂ for photosynthesis and suppressing photorespiration (Ainsworth & Long, 2005; Bonan, 2008; Allen et al., 2010; Gardner et al., 2022). This CO₂ fertilization effect has been observed in various plant species, including boreal trees (Girardin et al., 2016; Kaiser et al., 2017; Song et al., 2020). Studies have demonstrated that crops like *Cucumis sativus* L. and *Fragaria x ananassa* Duchesne. have increased yield and growth when exposed to elevated CO₂ levels (Enoch et al., 1976; Ainsworth & Long, 2005). Similarly, *Populus* spp. exhibited enhanced photosynthetic rates and improved water use efficiency under elevated CO₂ conditions (Battipaglia et al., 2013). However, prolonged exposure to elevated CO₂ concentrations can lead to the accumulation of non-structural carbohydrates (NSC) and the downregulation of photosynthetic processes, limiting the long-term benefits of CO₂ enrichment (Moore et al., 1998; Zhou & Quebedeaux, 2003; Ainsworth & Roger, 2007; Wang et al., 2010).

The combined effects of temperature and CO₂ concentrations on plant growth are intricate, leading to outcomes that depend on the plant species, treatment conditions, and the interactions between temperature and CO₂ levels (Lee et al., 2023). While some studies have observed stimulation of plant growth when temperature and CO₂ are both increased (Sobuj et al., 2018; Dusenge et al., 2020; Murphy & Way, 2021), other research suggests that global models may overestimate the positive effects of increasing temperature and CO₂ concentrations on terrestrial photosynthesis and growth (Laffitte et al., 2022).

1.1.4 Impacts of Elevated Temperature and CO₂ Levels on Plant Mortality

Climate change is already impacting trees, leading to widespread tree deaths in forests across North American, Asian, Equatorial and European forests (Worrall et al., 2013; Stephenson et al., 2018; Hartmann et al., 2022). Upwards of ~ 20 million hectares of forest in North America have been lost over the last 20 years, in part, due to the detrimental effects of climate change (Raffa et al., 2008; Hogg et al., 2008; Bentz et al., 2009; Rotbarth et al., 2023). Climate change-induced factors such as heat stress, drought, increased insect outbreaks, fires, and pathogens have taken a severe toll on various tree species, including *Populus* spp., *Acer saccharum* Marshall, and *Picea rubens* Sarg. (Brandt, 2009; Brandt et al., 2013). The rise in growth temperatures, coupled with reduced water availability, has been linked with increased xylem cavitation and damage to roots and foliage, resulting in increased dieback in some boreal species (Brandt, 2009; Menezes-Silva et al., 2019).

Hardwood species like *Populus tremuloides* cope with the increased vapour pressure deficit caused by warmer temperatures by reducing stomatal conductance to limit water loss (Hogg et al., 2000; Frey et al., 2004). However, this adjustment comes at the cost of carbon fixation (McDowell et al., 2008; Dusenke et al., 2019). One of the potential consequences of warming conditions is carbon starvation, where trees cannot supply enough carbon to sustain their hydraulic function and metabolic processes. This carbon deficit can accelerate tree mortality (Zeps et al., 2017; Murphy & Way, 2021). Furthermore, the depletion or loss of carbon (Murphy & Way, 2021) and water pools within the trees due to drought or high VPD can compromise defensive mechanisms against biotic attacks (Ruess et al., 2021), further intensifying physiological stress, increasing tissue damage, and eventually death (Hogg et al., 2000, 2008). These findings underscore the urgent need to address the impacts of climate change on northern forests. The loss of such vast forest areas due to climatic stresses not only threatens biodiversity and ecosystem services but also has profound socio-economic implications (Malhi et al., 2020).

Although elevated atmospheric CO₂ concentrations are positively associated with plant growth (Kaiser et al., 2017; Dusenke et al., 2019; Song et al., 2020), when coupled with elevated growth temperatures, higher CO₂ levels may not be able to offset the detrimental effects of warming, leading to stress and increased mortality (D'Arrigo et al., 2004; Juday & Alix, 2012; Girardin et al., 2016; Cahoon et al., 2018; Nicklen et al., 2018; Trugman et al., 2018). These impacts can have far-reaching consequences for boreal forest ecosystems, including reduced ecosystem function, decreased resilience, and an increased risk of forest conversion to alternative ecosystems (Worrall et al., 2013; Natural Resource Canada, 2015, 2016). Furthermore, the rise in atmospheric CO₂ concentrations may be linked to increased tree mortality through increased

water demand, nutrient limitations, early maturity and reproduction, and stand self-thinning dynamics (Pugh et al., 2020; Maschler et al., 2022). The concept of survival-growth trade-offs is well-established in plant ecology, and it is widely recognized that higher growth rates under elevated CO₂ can come at the expense of increased mortality risk (Bigler & Veblen, 2009; McDowell et al., 2018, 2020). This negative correlation between elevated CO₂ and mortality can be attributed to factors such as reduced wood density, diminished investment in defences, increased vulnerability to environmental stressors, and hydraulic failure in larger plants (McDowell et al., 2008; Maschler et al., 2022). Moreover, while elevated CO₂ levels can enhance photosynthesis, they can also disrupt evaporative cooling processes during heat events, leading to leaf senescence and heat-induced tissue damage, further limiting the beneficial effects of elevated CO₂ (Wang et al., 2018; Maschler et al., 2022). However, elevated CO₂ may also play a role in facilitating tissue regeneration following heat or drought stress (Pan et al., 2017; Maschler et al., 2022). These findings highlight the intricate interactions between plant growth and mortality under changing environmental conditions and emphasize the need for further research to enhance our understanding of these dynamics. Such knowledge is crucial for implementing effective forest management and conservation practices that can help mitigate the impacts of climate change on boreal forests. One potential opportunity for mitigating climate change effects on northern forests is to identify and utilize beneficial mycorrhizal fungi that may reduce heat and drought stress on plants, as discussed in Section 1.2.

1.2 Mycorrhizal Symbiosis

Fungi can be classified into three main nutritional groups: 1) saprobes, which decompose material and play a role in nutrient cycling; 2) pathogenic and parasitic fungi, which feed on living tissues, including plants; and 3) mycorrhizal fungi, which form associations with plant roots. Albert Frank (1885) first identified the association between plant roots and mycorrhizal fungi, which are found in almost all terrestrial ecosystems. Two major mycorrhizal types have been described based on their structure and function: ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM; Martin et al., 2015). ECM develop associations between woody plants and fungi, mainly from the phyla Ascomycota and Basidiomycota and consist of a Hartig net of hyphae encircling plant cells in the root cortex and a mantle or hyphal sheath covering the root tip (Figure 1.1; Martin et al., 2015). AMs form structures such as arbuscules, vesicles, and intraradical hyphae within plant cells, occupying a significant portion of root length in upper soil layers (Soudzilovskaia et al., 2015, Figure 1.1). Mycorrhizal fungi have diverse interactions with plants, providing benefits such as increased mineral nutrition, greater water availability, and disease reduction for the host plant, while also obtaining carbon sources for their own growth (Martin & Nehls, 2009; Bonfante & Genre, 2010; Kipfer et al., 2012). Mycorrhizae are widespread among land plants, formed by over 90% of species, including many trees (Brundrett et al., 1996; Bonfante & Genre, 2010). These associations create an extensive network of fungal hyphae, connecting individual plants and forests. This underground network allows for the exchange of nutrients and signals between plants (Brundrett et al., 1996; Soudzilovskaia et al., 2015; Bonfante & Genre, 2010; Sharma et al., 2022).

Mycorrhizae significantly enhance nutrient availability (nitrogen and phosphorus) to plants, through the upregulation of nutrient transporters after association with plant roots (Martin & Nehls, 2009; Bonfante & Genre, 2010; Wu, 2016) in exchange for photosynthates. In addition, mycorrhizae also play a role in plant water relations, with some ECMs improving shoot water potential (Ψ_w) in plants under both moist and dry conditions (Kipfer et al., 2012). Additionally, mycorrhizae increase root hydraulic conductance and shoot water potential in certain plant species (Lehto & Zwiazek, 2011).

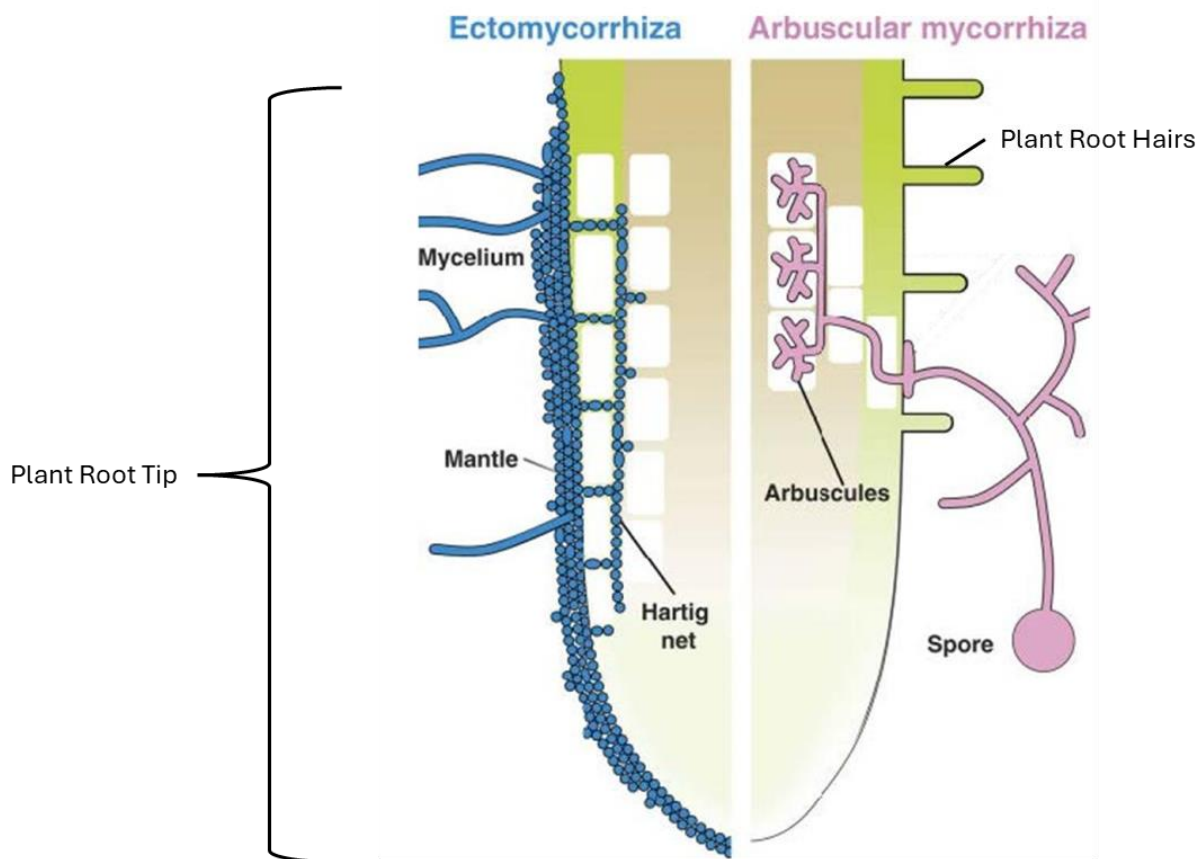


Figure 1.1. Representation of ectomycorrhizal (blue) and arbuscular mycorrhizal (pink) associations with a plant root and root cells (Modified from Bonfante & Genre, 2010).

Hormones play a role in the interaction between plants and mycorrhizal fungi. Studies have observed changes in gene expression related to auxin metabolism and root development in plants associated with mycorrhizal fungi (Barker & Tagu, 2000; Jing et al., 2022). Moreover, mycorrhizal fungi can synthesize or induce a wide diversity of plant phytohormones, such as indole-3-acetic acid, gibberellic acid, and cytokinin, which may directly stimulate plant growth and induce stress tolerance (Tudzynski, 2005; Huang et al., 2014; Pozo, 2015; Hinsch et al., 2015). Moreover, studies have shown strong interactions between mycorrhizal symbiosis and plant hormones like jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA) (Blilou et al., 2000; Herrera-Medina et al., 2007; Golldack et al., 2013; Plett et al., 2014a; Lioa et al., 2018). Furthermore, mycorrhizal fungi release compounds into the rhizosphere, including phytohormones that influence soil chemistry and plant growth, as discussed in the next section.

1.2.1 Mycorrhizal Exudates

Fungal exudates, the compounds released by fungi, play crucial roles in mediating interactions between fungi, plants, and other microorganisms. Mycorrhizal fungi release enzymes that break down complex soil compounds to release nutrients like potassium and nitrogen in the soil and release organic acids into the rhizosphere to increase the bioavailability of essential minerals like iron for plant uptake (Ray & Adholeya, 2008; Nadeau, 2015; Wong-Bajracharya et al., 2020; El-Gendi et al., 2022). Fungal exudates also act as signalling molecules, regulating interactions with other organisms that can inhibit or stimulate microbial growth, while others induce changes in plant growth and development (Zhang et al., 2011; Qin et al., 2018; Jamil et al., 2022). These exudates can also decrease phytohormone-mediated defence mechanisms, increase root sugar

and amino acid exudation, and prime root cells for infection (Medina et al., 2003; Cameron et al., 2013; Banasiak et al., 2020). Amino acids released by mycorrhizal fungi may boost plant growth and pro-symbiotic pathways by stimulating beneficial phytochemical synthesis *in planta* (Tohge et al., 2013; Kaur & Suseela, 2020). Mycorrhizal and all other fungal species have an active phenylpropanoid pathway, which allows them to produce a variety of phenolic chemicals that assist in symbioses with plant hosts and minimize plant stress responses to external stresses (Seshime et al., 2005; Kaur & Suseela, 2020). Some species of root-associated fungi produce and release auxins, gibberellic acid, and cytokinin, which can boost plant growth and stress tolerance (Tudzynski, 2005; Hinsch et al., 2015; Numponsak et al., 2018). Furthermore, fungal exudates shape microbial communities by acting as antimicrobial compounds and influencing community composition (Xu et al., 2023). Additionally, fungal exudates have potential applications in agriculture and biotechnology, including enhancing plant growth, suppressing plant pathogens, and providing valuable compounds.

1.2.2. Effects of Climate Change on Plant-Mycorrhizal Interactions

Changes in temperature and precipitation patterns, key drivers of climate change, can disrupt the availability of crucial resources like water and nutrients, affecting the fitness of both plants and mycorrhizal fungi (Tedersoo et al., 2014; Barceló et al., 2019; Sharma et al., 2022). Like plants, fungi are often limited by water availability and changes in precipitation patterns could have profound consequences for the stability and sustainability of the mutualistic relationship (Tedersoo et al., 2014; Sharma et al., 2022). Climate change can also alter the spatial distribution and diversity of mycorrhizal fungal communities (Větrovský et al., 2019; Barceló et al., 2019;

Sharma et al., 2022). As environmental conditions shift, certain fungal species that are better suited to the new conditions may thrive, leading to the displacement and potential loss of local fungal species (Barceló et al., 2019; Sharma et al., 2022). Moreover, specific plant species and their associated mycorrhizal fungi may respond differently to climate change due to their varying physiological, ecological, and evolutionary traits (Singh & Shourie, 2021; Sharma et al., 2022). It has been shown that elevated CO₂ conditions increase both root exudation and the abundance of mycorrhizal fungi within the rhizosphere (Terrier et al., 2018). Furthermore, warming within the boreal forest may reduce the abundance of mycorrhizal fungi such as *Cenococcum geophilum* (Defrenne et al., 2021). Therefore, understanding the combined effects of elevated temperature and CO₂ on interactions between plants and mycorrhizal fungi is crucial for many plant species' long-term survival and resilience. As suggested by Duarte & Maherali (2022), further research is necessary to elucidate the consequences of climate change on mycorrhizae fully and to develop effective strategies for managing and preserving these interactions in the face of a changing climate.

1.2.3. Impacts of Mycorrhizae on Plant Growth and Survival

Mycorrhizal fungi can enhance plant growth and alleviate abiotic stresses such as heat stress (Wahab et al., 2023). Mycorrhizae also facilitate stress signalling and resource sharing between trees, promoting resilience to stresses and disturbances (Song et al., 2015). Under drought conditions, mycorrhizal fungi improve stomatal function and mineral uptake, increasing tree seedling survival (Ouledali et al., 2019). They also enhance plant resilience against pathogens and osmotic stress (Mena-Violante et al., 2006; Hao et al., 2019). Certain mycorrhizal species

significantly improve the growth of tree seedlings. For example, *Hebeloma crustuliniforme*, *Tricholoma scalpturatum*, and *Hyaloscypha finlandica* enhance the growth of *Picea glauca* (Moench) Voss seedlings (Nadeau, 2015). Inoculation with mycorrhizal fungi boosts the growth of *Pinus sylvestris* L. seedlings, and mycorrhizal trees grown under elevated CO₂ levels show increased biomass compared to non-inoculated controls (Alberton et al., 2010). Moreover, under elevated CO₂ trials, Terrer et al. (2016) found that plants with ECM increased plant biomass by 28% under low-nitrogen environments. However, mycorrhizal associations can be detrimental to plant growth and survival under certain stress conditions such as drought or low nutrients (Odokonyero et al., 2016; Wang et al., 2023). Further research is needed to fully understand how mycorrhizal fungi affect trees under changing climate conditions (Alberton et al., 2010; Nadeau, 2015; Hortal et al., 2016).

Hormones are critical for the interaction between plants and mycorrhizal fungi. Differentially expressed genes related to auxin metabolism and root morphogenesis have been observed in plants associated with mycorrhizal fungi (Felten et al., 2009). Mycorrhizal fungi produce many phytohormones like ABA and cytokinins (Pons et al., 2020). Fungal-synthesized or -induced phytohormone synthesis *in planta* can contribute to increased plant growth, and stress tolerance, highlighting the significance of mycorrhizae in plant ecology and ecosystem functioning. Since mycorrhizae can mitigate climatic stresses in plants, and this could be, in part, due to changes in plant hormone concentrations, I will review the functioning of some hormones in the following section (Section 1.3).

1.3 Plant Stress Response and Stress Hormones

To gain insights into the biochemical and molecular mechanisms underlying plant defence against biotic and abiotic stress, researchers have employed various approaches, including genetic studies, transcriptomics, and metabolomics analyses, utilizing different model species (Wang et al., 2017; Manickam et al., 2023; Roychowdhury et al., 2023). These investigations have provided valuable information on the complex signalling pathways and molecular interactions involved in plant defence responses. One crucial aspect of plant defence is the activation of hormonal signalling pathways that coordinate the plant's response to stressors. Hormones such as JA, SA, and ABA, play pivotal roles in regulating defence responses (Verma et al., 2016). These hormones interact with each other and with growth-promoting hormones to modulate plant defences.

1.3.1 Jasmonic Acid

Jasmonic acid (JA) is a key player in the plant's response to herbivory and necrotrophic pathogens. It is involved in activating defence genes, synthesizing defence compounds, and modulating plant growth and development (Verma et al., 2016; Ruan et al., 2019). Upon herbivore attack or pathogen infection, JA levels increase, triggering a cascade of signalling events that lead to the activation of defence-related genes (Ruan et al., 2019). This results in the production of compounds such as proteinase inhibitors and VOC's that deter herbivores and inhibit pathogen growth (Verma et al., 2016; Yang et al., 2018; Ruan et al., 2019). JA also influences plant growth responses, including root growth and branching, to optimize resource allocation under stressful conditions (Ruan et al., 2019).

1.3.2 Salicylic Acid

Salicylic acid (SA) is predominantly associated with the plant's response to biotrophic pathogens, which depend on living host tissues for their survival. SA signalling activates defence responses that restrict pathogen growth and spread (Verma et al., 2016). SA acts as a signal molecule, triggering the expression of defence-related genes, including those encoding pathogenesis-related proteins and enzymes involved in synthesizing antimicrobial compounds (Klessig et al., 2000). Additionally, SA plays a role in systemic acquired resistance (SAR), a long-lasting defence response that occurs in uninfected parts of the plant following localized pathogen infection that provides enhanced resistance to subsequent pathogen attacks (Klessig et al., 2000; Verma et al., 2016).

1.3.3 Abscisic Acid

Abscisic acid (ABA) is a hormone involved in various aspects of plant growth, development, and stress responses (Verma et al., 2016). In the context of plant defence, ABA regulates responses to abiotic stressors such as drought, salinity, and cold (Verma et al., 2016). ABA helps plants cope with water deficit by promoting stomatal closure, thus reducing transpiration and conserving water (Zhang et al., 1987; Klein, 2014; Filho et al., 2018). It also plays a role in seed dormancy and germination, initiating leaf abscission, and helps enable plants to respond to changing environmental conditions (Rodríguez-Gacio et al., 2009).

1.3.4 Plant Hormone Interactions and Crosstalk

These three hormones (JA, SA, and ABA) collectively contribute to the complex defence responses exhibited by plants (Figure 1.2). The hormones interact with each other and with other signalling pathways to coordinate and modulate the plant's defence strategies (Zandalinas et al., 2016). The interplay between these hormones allows plants to fine-tune their responses to different types of stressors, striking a balance between growth promotion and defence resistance. The SA pathway suppresses JA-responsive genes, resulting in an antagonistic relationship between the two pathways (Takahashi et al., 2004; Verma et al., 2016; Zandalinas et al., 2016). Interestingly, at low concentrations, SA and JA can synergistically induce defence responses, highlighting the complexity of hormone interactions (Mur et al., 2006). Moreover, the ABA and JA signalling pathways often collaborate in regulating plant responses to herbivory, influencing plant growth and development concurrently (Per et al., 2018; Yang et al., 2018). This crosstalk allows for the monitoring and fine-tuning of plant metabolism and growth in response to external stimuli, maintaining a delicate balance between growth promotion and defence resistance. In addition to hormonal signalling, recent research has highlighted the role of epigenetic mechanisms in shaping plant defence responses. Epigenetic modifications, including DNA methylation and histone modifications, can regulate the expression of defence-related genes and influence plant stress tolerance (Wang et al., 2017). These modifications can be influenced by environmental cues, such as temperature, light, and pathogen attack, and can result in heritable changes in gene expression patterns. Understanding the complex interconnections between hormone signalling pathways and epigenetic regulation provides insights into the underlying mechanisms that enable plants to mount effective defence responses.

1.3.5 Impacts of Elevated Temperatures and CO₂ Levels on Plant Stress Hormones

Research on the involvement of the JA signalling in plant heat resistance is limited, but JA has been suggested to be induced under heat stress (Kumar & Verma, 2018). JA has also been identified as a crucial signalling molecule for the production of sesquiterpenes induced by heat shock (Clarke et al., 2009; Xu et al., 2016; Ruan et al., 2019). However, under elevated CO₂ concentrations, foliar JA levels were reduced in tomato plants (Goa et al., 2012), while in lima beans, increased CO₂ levels were linked to enhanced levels of JAs (Ballhorn et al., 2011).

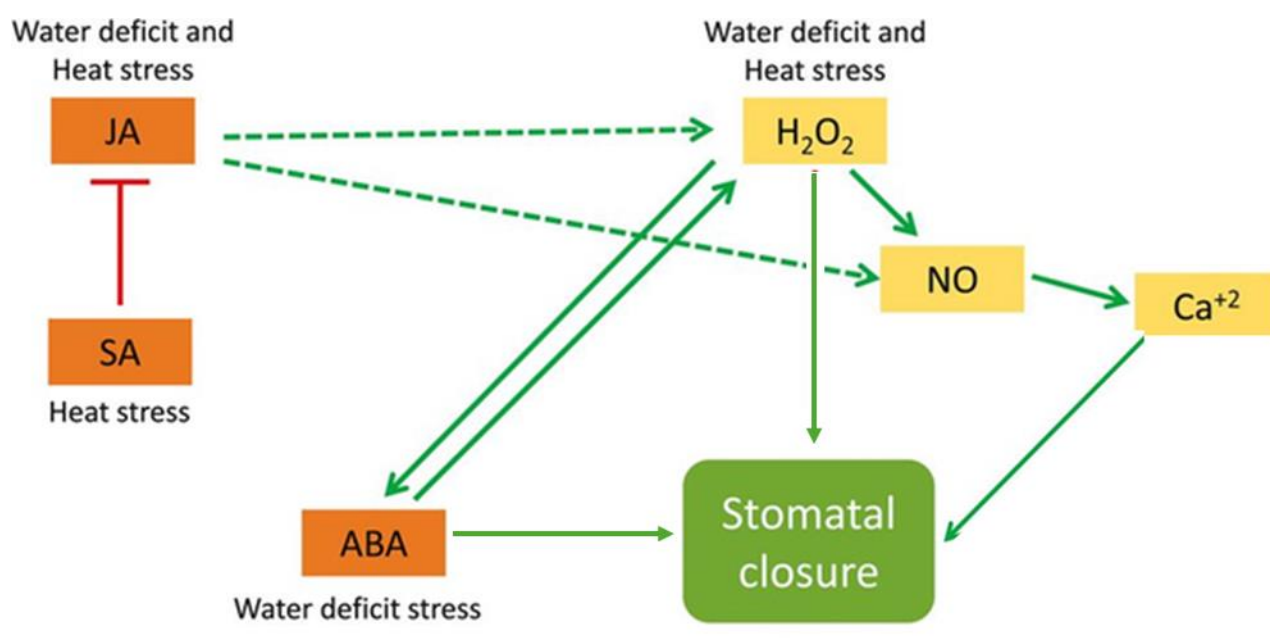


Figure 1.2. A model for the signalling crosstalk of JA, SA, and ABA during drought and heat stress and its relation to stomatal regulation (Modified from Zandalinas et al., 2016). Dotted lines indicate hypothetical interactions, while solid lines and arrows indicate positive (green arrows) and negative (red lines) regulation, respectively. Abbreviations: ABA, abscisic acid; JA, jasmonic acid; NO, nitric oxide; SA, salicylic acid; H₂O₂, hydrogen peroxide and Ca²⁺, intracellular calcium.

SA exhibits diverse effects under warming conditions, including promoting thermogenesis, extending flower longevity, inhibiting ethylene biosynthesis and seed germination, and countering the effects of ABA (Wassie et al., 2020; Li et al., 2021). Exogenous application of SA mitigates the adverse effects of heat shock by inducing heat shock proteins and contributing to thermo-protection and recovery of photosynthesis in plants like *Medicago sativa* L. and *Solanum lycopersicum* L. (Verma et al., 2016; Shah et al., 2019; Wassie et al., 2020). Elevated CO₂ levels have been found to increase SA concentrations in *Glycine max* (L.) Merr. cultivars, suggesting a potential role of SA in managing plant carbon balance under stress conditions (Casteel et al., 2012).

Increased ABA levels enhance heat tolerance by increasing reactive oxygen species levels and inducing antioxidant capacity, with plants lacking key components of ABA signalling pathways displaying impaired heat stress tolerance (Larkindale, et al., 2005; Verma et al., 2016; Wang et al., 2023). ABA also acts as a thermo-priming hormone, enabling rapid and efficient responses to heat stress (Verma et al., 2016). ABA contributes to drought acclimation and enhances resistance to abiotic stressors (Verma et al., 2016; Aslam et al., 2022). Additionally, ABA levels are linked to stomatal responses to changes in atmospheric CO₂, suggesting its involvement in CO₂ sensing and signal transduction mechanisms (Engineer et al., 2016; Zhang et al., 2020). However, further research is needed to understand the long-term effects of elevated CO₂ on stomatal regulation and ABA-controlled drought response (Verma et al., 2016).

1.3.6 Impacts of Mycorrhizae on Plant Stress Hormones

Mycorrhizal fungi can alter plant hormone concentrations, which, combined with providing plants greater access to water and nutrients, may improve plant growth and reduce stress. The ECM fungus *Laccaria bicolor* produces small effector peptides that target the host's defence mechanisms, such as JA, SA, and ethylene, during the colonization of the host plant (Plett et al., 2014a; 2014b). The role of ABA in mycorrhizal plants is still uncertain, but genetic evidence suggests a positive regulatory role in AM establishment (Herrera-Medina et al., 2007). The relationship between JA and mycorrhizal colonization varies among plant species, with *S. lycopersicum* plants having elevated JA concentrations, while grey poplar (*Populus x canescens* (Aiton) Sm. = *Populus tremula x Populus alba* L.) have decreased JA levels after inoculation with mycorrhizal fungi (Luo et al., 2009; Duc et al., 2018). The role of SA in mycorrhizal symbiosis is not yet clear; however, it seems that elevated plant SA is required for the root establishment of some AM species (Garg & Bharti, 2018), though high SA levels may also inhibit root colonization of others (Blilou et al., 2000).

1.4 Study Outline and Objectives

There has been a decline in growth and an increase in mortality among *Populus* species in recent decades, which raises concerns about their resilience to climate change (Worrall et al., 2013; Natural Resource Canada, 2015; 2016). As *Populus* spp. represent 13% of the trees in the North American boreal forest (Canada's National Forest Inventory, 2022), studying their ability to tolerate future climate conditions and the extent to which mycorrhizae can mitigate climate stress

on this widespread tree genus, would provide valuable insights into improving the resilience of *Populus* spp. under changing climatic conditions.

The model tree system used for my thesis project was the hybrid *Populus x canadensis*, a hybrid variety of *Populus nigra x Populus deltoides*, neither of which originates from the boreal. This may result in *Populus x canadensis* potentially thriving in the boreal forest due to climate change (Ferus et al., 2020). Poplar hybrids, including *Populus x canadensis*, are fast-growing and often used in reforestation projects (Larchevêque et al., 2010). Poplars, along with other boreal trees, establish root associations with various microbes, including mycorrhizal and other root-associated fungi, which have the potential to mitigate heat and drought stress associated with climate change (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Liu et al., 2014). These findings provide further support for the use of hybrid poplars as a model organism to study the interactions between trees and mycorrhizal fungi in the context of climate change, plant growth and survival (Peterson & Peterson, 1992; Landhäusser et al., 2002; Hacquard & Schadt, 2015). Investigating the interactions between hybrid poplars, climate change, and mycorrhizal fungi will enhance our understanding of how these symbiotic relationships can support tree growth and enhance ecosystem resilience in the face of climate change.

Most studies investigating the interactions between mycorrhizal fungi and trees in the context of climate change have focused on either elevated CO₂ or temperature effects, while the combined impacts of elevated temperature and CO₂ on interactions of trees with mycorrhizal fungi have received limited attention (Cheng et al., 2012; Pischl & Barber, 2016; Terrer et al., 2017).

Understanding the complex dynamics between mycorrhizal fungi and trees under changing climatic conditions is essential for accurate prediction and effective mitigation of the impacts of climate change on boreal forests. By addressing the combined effects of elevated temperature and CO₂ in studies examining the impacts of mycorrhizal fungi on trees, we can develop a more comprehensive understanding of the interactive and cumulative impacts of these climate factors on tree growth, productivity, and carbon cycling. This knowledge is critical for refining models and developing management strategies that enhance the resilience and sustainability of boreal forests in the face of climate change (Cheng et al., 2012; Pischl & Barber, 2016; Terrer et al., 2017).

1.4.1 Goals and Objectives

During my Ph.D. research, I aimed to investigate the benefits of mycorrhizae on enhancing poplar growth under predicted future elevated temperatures and CO₂ conditions. Additionally, I sought to understand how mycorrhizal fungi influenced poplar biology and hormone regulation across climate scenarios. My secondary goal was to determine what compounds are secreted by various mycorrhizal fungi and other poplar root fungi in pure culture to test whether these fungal exudates could improve plant growth, thus identifying potential chemical growth stimulators for use in the field.

These goals were accomplished by meeting three objectives:

- 1) Determine what compounds different mycorrhizal fungi and root fungi exude in pure culture and determine whether these exudates improve plant growth (Chapter 2).

2) Evaluate the effects of temperature and CO₂ levels on poplar growth and survival while assessing whether mycorrhizal fungi could enhance tree growth and reduce mortality rates under current and projected future climate conditions (Chapter 3).

3) Investigate how elevated temperatures and CO₂ levels impact poplar stress hormone concentrations and whether mycorrhizal fungi can mitigate tree stress induced by future temperature and CO₂ conditions (Chapter 4).

1.5 References

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Chapter 2

2 Characterizing root-associated fungal exudate profiles and investigating their impacts on plant growth

2.1 Introduction

Soil fungi are a diverse group of eukaryotes that play vital roles in nutrient cycling, carbon sequestration, and promoting plant growth (Frac et al., 2018). There are estimated to be 2.2-3.8 million different species of soil fungi, with many taxa having high structural and functional diversity (Hawksworth & Lücking, 2017). Soil fungi are classified into three distinct nutritional groups: saprotrophs that decompose organic matter, pathogens, and symbionts, such as mycorrhizal fungi. Mycorrhizal fungi and rhizosphere fungi form a community of soil-dwelling fungi that work with plants to enhance plant growth and resilience by providing them greater access to nutrients, improved water absorption, and increased resistance to pathogens; all in exchange for sugars from the plant (Brundrett et al., 1996; Martin & Nehls, 2009; Bonfante & Genre, 2010; Kipfer et al., 2012; Kuang et al., 2021).

Mycorrhizal fungi can have profound impacts on their host plants, soil geochemistry and rhizosphere dynamics through their ability to secrete compounds into the environment (Van Schöll et al., 2008; Huang et al., 2014; Vignale et al., 2018). In addition, mycorrhizal fungi begin to condition the host plant for root colonization by releasing compounds into the soil (Mónica et al., 2018; Banasiak et al., 2020). These fungal exudates alter plant physiology by suppressing phytohormone-mediated defence pathways, increasing plant root exudation of sugars and amino

acids, and priming root cells for infection (Medina et al., 2003; Banasiak et al., 2020).

Mycorrhizal fungi can improve plant growth by releasing compounds such as organic acids, which increase the bioavailability of trace minerals or essential nutrients such as phosphorus, iron, and magnesium from the soil (Huang et al., 2014; Pozo, 2015; Anwar et al., 2020).

Mycorrhizal fungi can also exude amino acids into the rhizosphere, which can serve as communication molecules and attractants, potentially influencing the composition of the soil microbiome and promoting plant resilience to stresses such as salt or biotic stress (Tohge et al., 2013; Krain & Siupka, 2021). Moreover, certain amino acids, like phenylalanine, are linked to the synthesis of secondary metabolites in plants, stimulating and enhancing stress tolerance and defence mechanisms (Tohge et al., 2013; Kaur & Suseela, 2020). The release of such amino acids by mycorrhizal fungi may stimulate the production of these beneficial compounds within host plants, ultimately stimulating plant growth and initiating pro-symbiotic pathways in the host (Bonfante & Genre, 2010; Kaur & Suseela, 2020). All fungal species, including mycorrhizal ones, have an active phenylpropanoid pathway, which enables them to synthesize a broad range of phenolic compounds, some of which help establish symbioses with plant hosts and act as chemical messengers to reduce plant stress responses to environmental stressors (Seshime et al., 2005; Kaur & Suseela, 2020). More recently, it has been shown that some mycorrhizal fungi synthesize and release plant growth hormones such as auxins (Numponsak et al., 2018), gibberellic acid (Tudzynski, 2005) and cytokinin (Hinsch et al., 2015), which may directly stimulate plant growth and induce stress tolerance (Huang et al., 2014; Pozo, 2015).

Plant associations with soil fungi may ameliorate heat and drought stress in plants and enhance plant growth (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Liu et al., 2014) through

increased access to nutrients and altered plant stress physiology (Peterson & Peterson, 1992; Landhüsser et al., 2002; Hacquard & Schadt, 2015). However, it is unclear whether these growth-promoting and stress-relieving abilities that many mycorrhizal fungi provide to host plants can be achieved with mycorrhizal exudates alone. It has been suggested that mycorrhizal establishment or even the presence of fungal exudates can trigger systemic resistance in plants, bolstering defence mechanisms and priming systemic responses to combat diverse pathogens (Jung et al., 2012). Moreover, exogenous application of phytohormones has a stimulatory effect on plant growth and defence, so the same could hold true for fungal-derived phytohormones. The few studies that have examined the effects of fungal exudates on plant growth have found that exudates from endophytes such as *Epichloe* and arbuscular mycorrhiza can promote root development and plant growth (Fusconi, 2014; Vignale et al., 2018). Further research is required to elucidate the effects of exuded hormones, amino acids, and other compounds on plant growth, development, and the initiation of symbiotic relationships with mycorrhizal fungi, as this remains a substantial knowledge gap (Fusconi, 2014).

Understanding the variability in fungal exudate effects on plant growth is relevant for agriculture, forestry, and ecosystem management, and can inform decisions about which fungal symbionts or mycorrhizal species to promote or control in specific agricultural or ecological settings. This investigation aimed to determine baseline exudation profiles of organic acids, plant hormones, and amino acids from 14 mycorrhizal or root endophytes isolated from boreal trees. My goals were to determine: 1) fungal exudate profiles from diverse mycorrhizal isolates from a common host; and 2) if fungal exudates can improve plant growth and enhance plant biomass. I hypothesized that:

- 1) With the high diversity observed within mycorrhizal fungi and their well-documented ability to secrete organic acids, amino acids, and plant growth hormones in the rhizosphere, all my fungal isolates from aspen roots will also have organic acids, amino acids, and phytohormones within the secretion profiles. There will also be significant variability in the composition and concentration of these compounds among different fungal isolates.
- 2) Mycorrhizal fungi secretions have been shown to improve nutrient bioavailability and promote plant growth; therefore, introducing exudates from my fungal isolates to *Arabidopsis* will significantly increase plant biomass and leaf area.

2.2 Methodology

2.2.1. Fungal Cultures and Maintenance

Fungi were obtained from the roots of trembling aspen trees (*Populus tremuloides* Michx.) in Cold Lake, AB, Canada (2014) by collaborators at the Northern Forestry Centre in Edmonton, AB, Canada or the Northern Forestry Centre Culture Collection collected from *Pinus sylvestris* L., Oslo, Norway, 1984 (*Paxillus involutus*). The fungal isolates used in this investigation, which underwent ITS sequencing for identification by my collaborators at the Northern Forestry Centre, are listed in Table 2.1. These fungal isolates were selected because they are known as, or could be, beneficial mycorrhizal fungi that promote plant growth (Tedersoo et al., 2014). While not directly isolated from *P. tremuloides*, *P. involutus* is a well-studied ectomycorrhizal fungus that is widespread (found in Europe, Asia, North America, Australia, and New Zealand) and is recognized for its ability to establish symbiotic associations with coniferous and hardwood tree

species (Wallander & Söderström, 1999; Jargeat et al., 2014; Sayyed & Hussain, 2020). Fungal isolates were initially grown on modified Melin-Norkrans (MMN, Appendix 2.1 for recipe) agar plates at room temperature in the dark for 18 days (to establish a culture) and then stored at 4 °C. Isolates were then grown in sterile 20 mL glass test tubes containing 15 mL of liquid MMN. A 1 cm square of fresh fungal mycelium grown on MMN agar was added to the sterile room temperature MMN liquid media to inoculate it. Two rounds of liquid cultures (n = 3 for each isolate per round for a total of n = 6) were grown for 21 days in the dark at room temperature on a rotary shaker set to 75 rpm.

2.2.2. Fungal Exudates: Isolation and Measurement

Fungal cultures grown in liquid MMN for 21 days were centrifuged at 7500 rpm for 5 minutes to separate fungal mycelia from the media. The supernatant (medium with fungal exudates) was decanted into a new sterile 20 mL Falcon tube and then re-centrifuged to ensure fungal biomass was removed, with the resultant supernatant decanted into a new 20 mL Falcon tube. To prepare for analysis, 5 mL of 100% ethyl acetate was added to 10 mL of spent MMN to extract non-polar molecules from polar molecules through phase separation (Beninger et al., 2004). The extraction took place at room temperature for 10 min, inverting the tubes every few minutes. After extraction, the organic phase was decanted, and both fractions were evaporated for five days at room temperature in a fume hood. Dried fungal media evaporites were then hydrated with 1 mL

Table 2.1. List of the mycorrhizal fungi or root endophytes used for the exudate profiling and Arabidopsis bioassay study, with their corresponding GenBank reference number and the Northern Forestry Centre Culture Collection number. All fungi are ascomycetes isolated from aspen saplings (Cold Lake, Alberta) except for a basidiomycete (*P. involutus*), which came from collected from *Pinus sylvestris*, Oslo, Norway, 1984. The biological functional type is based on classification conducted by Tedersoo et al. (2014). (ECM= ectomycorrhizal, ERM= ericoid mycorrhizal, DSE= dark septate endophyte)

Fungal Species	GeneBank #	NoF Culture #	Biological Functional Type
<i>Paxillus involutus</i>	Not Sequenced	2339	ECM
<i>Cenococcum geophilum</i>	MT276004	3129	ECM
<i>Cladophialophora chaetospora</i>	MT294404	3119	ERM, DSE
<i>Oidiodendron pilicola</i>	MT294418	3120	ERM
<i>Cryptosporiopsis ericae</i>	MT294405	3122	DSE
<i>Hyaloscypha finlandica</i> isolate A	MT294403	3116	ECM, ERM, DSE
<i>Hyaloscypha finlandica</i> isolate C	MT276005	3138	ECM, ERM, DSE
<i>Phialocephala fortinii</i> isolate C	MT276009	3139	ERM, DSE, ECM?
<i>Phialocephala fortinii</i> isolate D	MT276010	3124	ERM, DSE, ECM?
<i>Leptodontidium orchidicola</i>	MT294412	3121	ECM, ERM, DSE
<i>Lachnum pygmaeum</i> isolate A	MT276007	3126	root endophyte
<i>Lachnum pygmaeum</i> isolate B	MT294411	3127	root endophyte
<i>Rhizoscyphus ericae</i>	MT294421	3128	ERM, DSE
<i>Phialocephala fortinii</i> isolate A	MT294419	3123	ERM, DSE, ECM?

of high-performance liquid chromatography (HPLC) grade acetonitrile to re-suspend compounds for analysis. After 5 minutes of vortexing, samples were filtered (0.22 μm) to remove any particulate matter and stored at 4 °C until analyses could be done. Extracts were pooled to ensure enough sample could be used for statistical analysis.

To do a targeted analysis of fungal exudates, the following chemicals were used as standards: gibberellic acid (GA; Bioshop, Burlington, ON, Canada), kinetin (KIN; Bioshop), citric acid (CA; Bioshop), succinic acid (SUC; Bioshop), an amino acid standard set which included 17 reference standards at 2.5 μM (L-Alanine, L-Arginine, L-Aspartic acid, L-Cystine, L-Glutamic acid, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine, L-Valine; Sigma-Aldrich, St. Louis, Missouri, United States), jasmonic acid (JA; Sigma-Aldrich), indole-3-acetic acid (IAA; Sigma-Aldrich), cis-jasmonate (Cis-JA; Sigma-Aldrich), methyl jasmonate (M-JA; Sigma-Aldrich), abscisic acid (ABA; Sigma-Aldrich), salicylic acid (SA; Sigma-Aldrich), and oxalic acid (OA; Sigma-Aldrich). HPLC-MS (mass spectroscopy) was performed using a Thermo Scientific LTQ Orbitrap Discovery (MS 2.5.5) equipped with an Autosampler Accela AS 2.2.1, and pump 1.04.05. The instrument was equipped with a CORTECS C18+ column (Waters Limited, Mississauga, ON, Canada), 50 mm length, 2.1mm I.D., and 1.6 μm particle size, operated at room temperature. The injection volume was 10 μL . A solvent gradient was employed in this study with a flow rate of 0.4 mL/min. Solvent A was composed of acetonitrile acidified with 0.1 vol% of formic acid, whereas solvent B was composed of water acidified with 0.1 vol% of formic acid. The gradient was programmed as follows: solvent A 2 vol%, increased to 10 vol% at 2 min, increased to 25 vol% at 6 min, increased to 50 vol% at 10 min, increased to 75 vol% at

14 min, increased to 95 vol% at 18 min, decreased to 2 vol% at 20 min, followed by 2 min of isocratic elution with 2% of solvent A (total elution time 22 min). The LTQ Orbitrap MS was equipped with an electrospray ionization (ESI) source operating in positive ionization mode using the following operating parameters: electrospray voltage of 3.1 kV, sheath gas flow rate of 8 abu (arbitrary unity), auxiliary gas flow rate of 1 abu, capillary temperature of 270 °C, capillary voltage set to 49.00 V, and tube lens offset at -148.43 V. Instrument calibration was performed externally before each run sequence, employing the Thermo Scientific Pierce LTQ Velos ESI positive ion calibration solutions. Accurate mass spectra of [MM+H]⁺ ions were recorded from 100 to 1000 m/z, the mass resolution power of the mass analyzer was set to 30,000 (m/m) at m/z 400. Nitrogen gas (purity 99.95%) was used both as sheath gas and auxiliary gas to serve as the co-collision gas in the HCD cell and the bath gas in the C-trap.

2.2.3. Exudate Growth Effect Assay

This experiment was conducted to test whether fungal exudates impact plant growth.

Arabidopsis was chosen as a model plant system for the growth assay due to its fast development and ease of propagation. *Arabidopsis thaliana* (Col-0) seeds were surface-sterilized by soaking them in 70% ethanol for 5 min, then in 1.5% sodium hypochlorite for 10 min, followed by 3 rinses in sterile deionized water. Seeds were then stored at 4 °C in the dark for three days to stratify them to synchronize seed germination. Seeds were transferred to agar plates (3 seeds per plate) containing half-strength Murashige-Skoog (MS) media with 1% sucrose. Plates were sealed with Parafilm® (Bemis Company, Inc) and placed in a growth chamber (16:8-hour light: dark cycle maintained at 22°C and 60% relative humidity) for 7 days. To each plate containing *Arabidopsis* seedlings, 5 mL of spent fungal media (14 isolates x 4 biological replicates) or

sterile MMN medium or distilled water was added (N= 64) in a randomized block. Plants were placed back into the growth chamber for four weeks, with spent fungal media, or MMN media, or distilled water added every seven days under sterile conditions to the same cohort of plants. After four weeks, plants were imaged for the leaf area using a digital camera (Canon EOS Rebel T7 DSLR with 18-55mm IS lens) and a scale bar; images were analyzed for total leaf area using Image J (Schneider et al., 2012). After the imaging, all plants were harvested for dry biomass.

2.2.4. Data and Statistical Analysis

To determine the concentration of each detected compound, raw peak values and concentrations for known compound standards were compared to those compounds detected within experimental samples using the following:

$$\frac{\text{Peak Area of Sample}}{\text{Peak Area of Standard}} * \text{Standard Concentration} \quad \text{Equation 2.1}$$

All sample concentrations were then subjected to a media correction to remove the influence of the baseline medium (sterile MMN) from detected compounds that were found within the spent fungal media to rule out screened-for compounds that may also be present in the original MMN media. All detected compound concentrations are presented as micromolar. Furthermore, based on histogram analysis, one data point from each of the following fungal exudate groups were removed due to being outliers from the total sample size: JA (one data point from each of *C. geophilum* and *P. fortinii* isolate C), SA (one data point from *L. pygmaeum* isolate B), GA (one data point from *H. finlandica* isolate B), ABA (one data point from *C. chaetospora*), alanine (one data point from *C. geophilum*), arginine (one data point from *C. chaetospora*), citric acid (one data point from *R. ericae*), and oxalic acid (two data points from *P. fortinii* isolate A).

Due to the data not being normally distributed ((and thus unsuitable for a one-way analysis of variance (ANOVA)), a Kruskal-Wallis ANOVA on Ranks was conducted on all fungal exudate concentrations (Table 2) and on *Arabidopsis* mean leaf area ($H = 37.950$, $DF = 14$, $p < 0.001$), followed by a post-hoc Tukey's test. *Arabidopsis* biomass data were analyzed using a one-way ANOVA ($F = 14.222$, $SS = 144038$, $MS = 10288.45$, $DF = 14$, $p < 0.001$), followed by a post-hoc Holm-Sidak test. All statistical tests were performed using SigmaPlot version 13.0 to detect treatment effects (ANOVA) and significant differences among treatment means (post-hoc tests) at $p \leq 0.05$.

2.3 Results

2.3.1. Fungal Exudates

To identify compounds released by the fungal isolates, chromatograms from the MMN media and the reference standards were compared to those from the fungal isolates. A summary of detected compounds from each fungal isolate is reported in Appendix 2.2 and 2.3 with a sample chromatogram Appendix 2.4. The standard amino acids aspartic acid, glutamic acid, glycine, isoleucine leucine, serine or valine were not detected by our HPLC-MS experiment setup and thus were not used for our targeted analysis. Taken together, the overall exudate profile of any given fungal isolate is statistically similar to that of another, largely due to the high variability in concentrations across the dataset (Table 2.2). Despite this general overarching similarity in exudate composition, the amount of each compound exuded varied considerably between and within fungal isolates (Figure 2.1-2.3). Most fungi could synthesize and exude most of the

screened phytohormones into the media, with isolates exhibiting variability in the amount of each phytohormone that was synthesized and excreted (Figure 2.1). In particular, 28% of fungi did not exudate cis-jasmonate, while 21% did not exude GA. The diversity of amino acids released by different fungi varied significantly. Among the amino acids screened, only histidine, phenylalanine, and proline were universally exuded by all examined fungi (Figure 2.2). Furthermore, only *P. involutus* and *P. fortinii* isolate D exuded all the screened amino acids, with isolate-specific differences observed in the amino acid exudate profile of *H. finlandica*, *L. pygmaeum*, and *P. fortinii*. In terms of exuded organic acids, all fungi released citric acid and succinic acid except *C. ericae*, which had no detectable succinic acid (Figure 2.3 A,C). However, only ~80% of fungal isolates had detectable levels of oxalic acid in the spent media (Figure 3B).

Table 2.2. Summary report of a one-way analysis of variance (ANOVA) on ranks for each targeted compound within the fungal exudates, indicating H-values, degrees of freedom (DF), and p-values; bolded numbers represent $p < 0.05$. Phytohormone (plant hormone) abbreviations: jasmonic acid (JA); methyl jasmonate (M-JA); cis-jasmonate (Cis-JA); salicylic acid (SA); abscisic acid (ABA); gibberellic acid (GA); kinetin (KIN); and indole-3-acetic acid (IAA).

	<i>H</i>	<i>DF</i>	<i>p-value</i>
<i>Phytohormones</i>			
<i>JA</i>	5.519	13	0.962
<i>MethJA</i>	6.366	13	0.932
<i>CisJA</i>	18.557	13	0.137
<i>SA</i>	8.481	13	0.811
<i>ABA</i>	12.228	13	0.509
<i>GA</i>	14.121	13	0.365
<i>IAA</i>	14.801	13	0.320
<i>Kin</i>	7.541	13	0.872
<i>Amino Acids</i>			
<i>Alanine</i>	17.346	13	0.184
<i>Arginine</i>	14.036	13	0.371
<i>Cystine</i>	8.265	13	0.826
<i>Histidine</i>	10.088	13	0.687
<i>Lysine</i>	21.463	13	0.064
<i>Methionine</i>	12.970	13	0.450
<i>Phenylalanine</i>	15.411	13	0.282
<i>Proline</i>	7.521	13	0.873
<i>Threonine</i>	20.883	13	0.075
<i>Tyrosine</i>	13.206	13	0.432
<i>Organic Acids</i>			
<i>Citric Acid</i>	9.469	13	0.737
<i>Oxalic Acid</i>	9.141	13	0.762
<i>Succinic Acid</i>	18.385	13	0.143

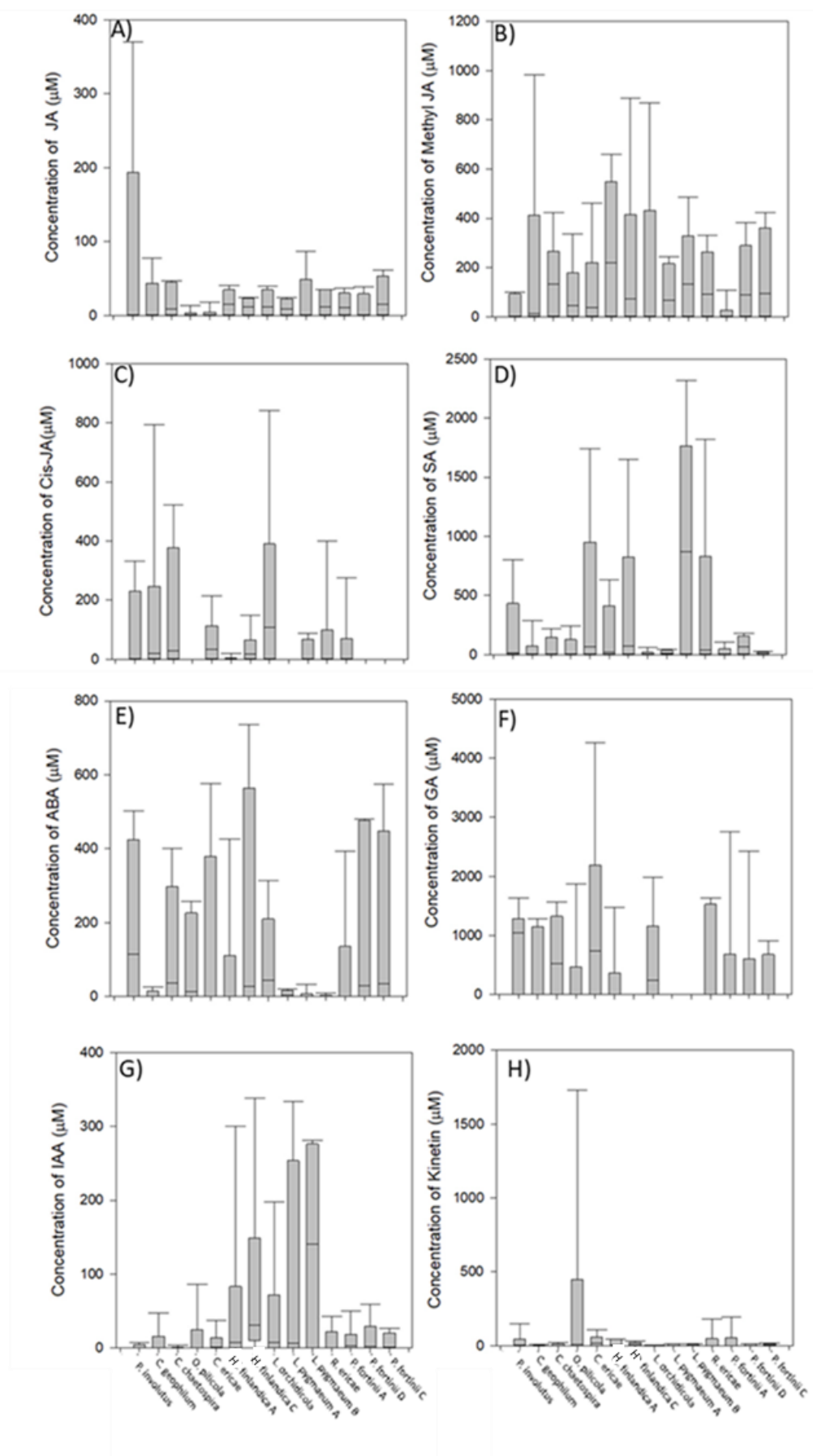
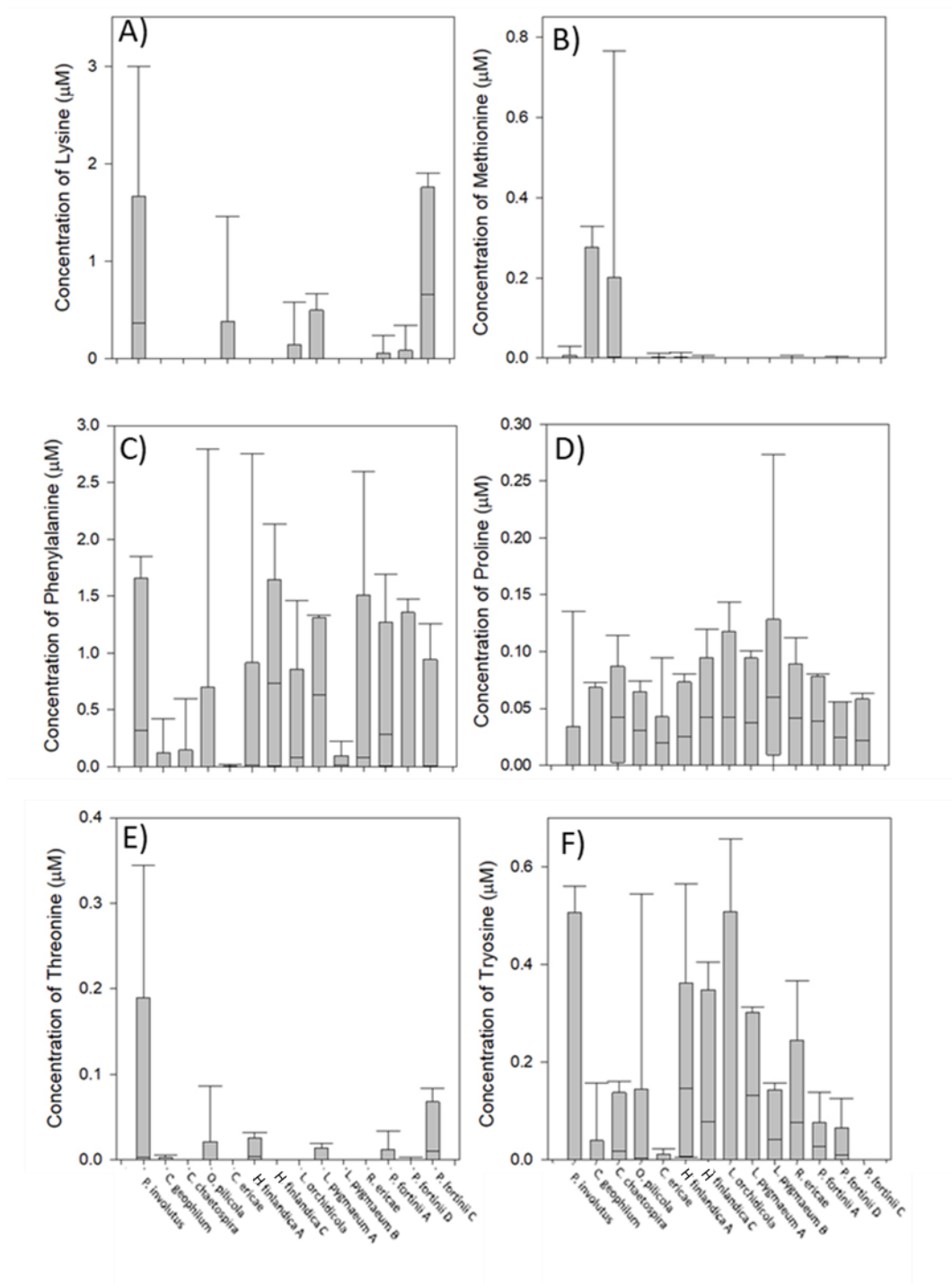


Figure 2.1. Phytohormones secreted by fungal isolates. Fungal media from: *P. involutus*, *C. geophilum*, *C. chaetospora*, *O. pilicola*, *C. ericae*, *H. finlandica* isolate A and C, *L. orchidicola*, *L. pygmaeum* isolate A and B, *R. ericae*, and *P. fortinii* isolate A, C and D analyzed using HPLC-MS to determine the concentrations of jasmonic acid (A), methyl jasmonate (B), cis-jasmonate (C), salicylic acid (D), abscisic acid (E), gibberilic acid (F), indole-3-acetic acid (G), kinetin (H). The box plots span the first to the third quartiles segmented by the median; the whisker above the box is the maximum (n = 5-6).



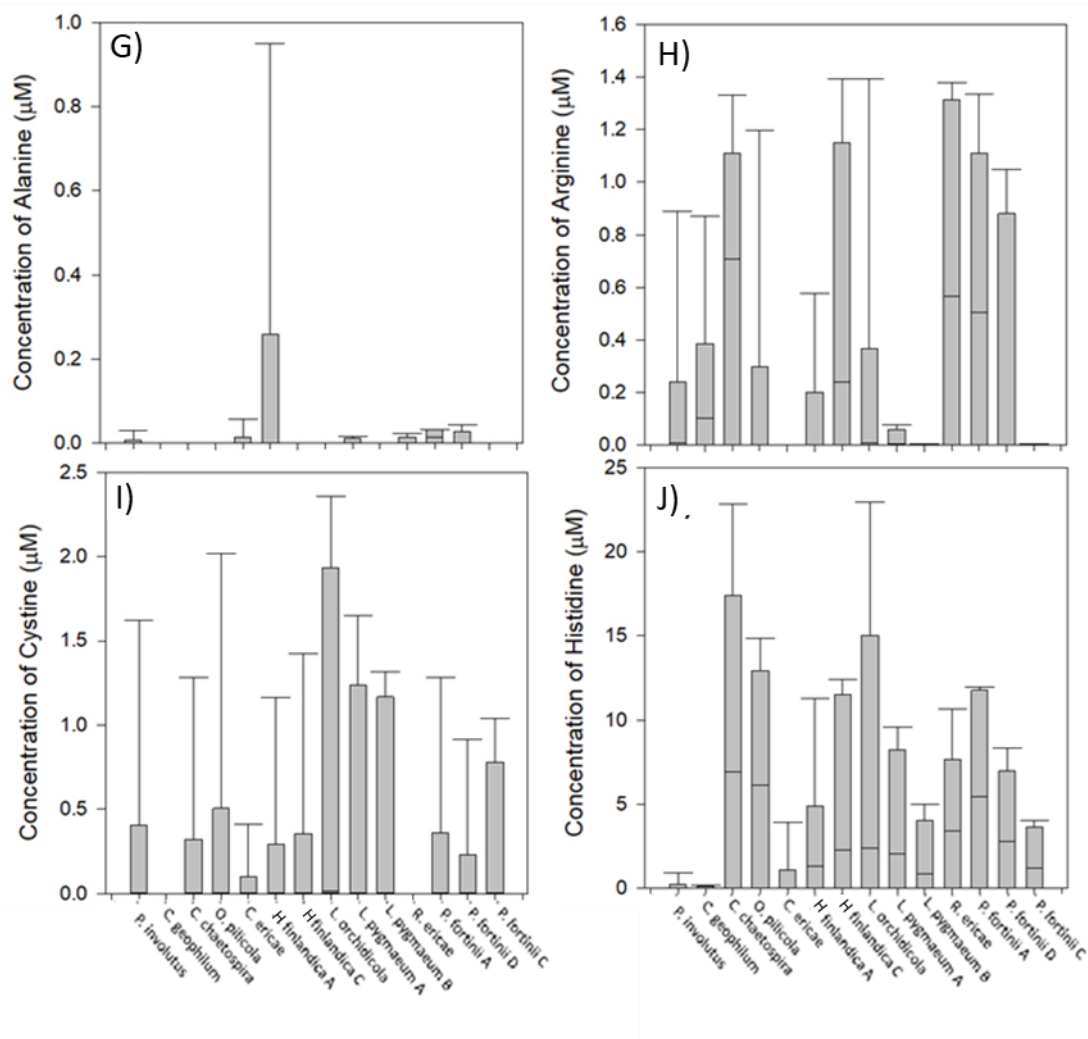


Figure 2.2. Exuded amino acids by fungal isolates. Fungal media from: *P. involutus*, *C. geophilum*, *C. chaetospora*, *O. pilicola*, *C. ericae*, *H. finlandica* isolate A and C, *L. orchidicola*, *L. pygmaeum* isolate A and B, *R. ericae*, and *P. fortinii* isolate A, C and D; where analyzed using HPLC-MS to determine the concentrations of lysine (A), methionine (B), phenylalanine (C), proline (D), threonine (E), tyrosine (F), alanine (G), arginine (H), cystine (I), and histidine (J). The box plot spans the first to the third quartile segmented by the median; the whisker above the box, is the maximum (n = 5-6).

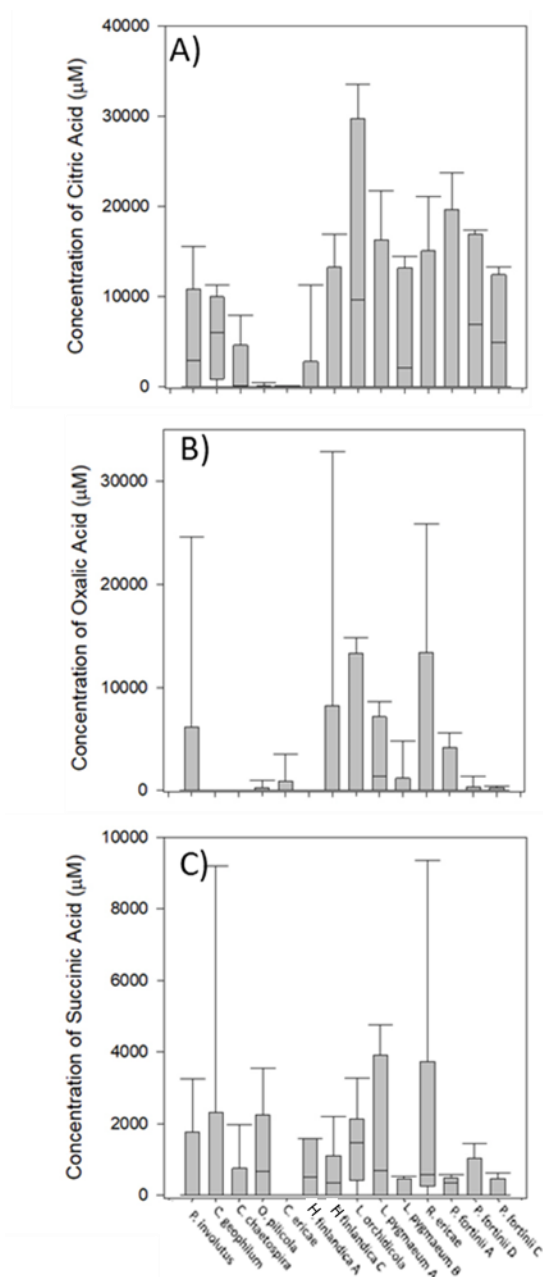


Figure 2.3. Organic acids secreted by fungal isolates. Fungal media from: *P. involutus*, *C. geophilum*, *C. chaetospora*, *O. pilicola*, *C. ericae*, *H. finlandica* isolate A and C, *L. orchidicola*, *L. pygmaeum* isolate A and B, *R. ericae*, and *P. fortinii* isolate A, C and D; where analyzed using HPLC-MS to determine the concentrations of citric acid (A) oxalic acid (B) and succinic acid (C). The box plot spans the first to the third quartile segmented by the median; the whisker above the box, is the maximum (n = 5-6).

2.3.2. *Arabidopsis* Bioassay

The addition of media containing fungal-derived exudate compounds significantly affected *Arabidopsis* biomass ($F= 14.222$, $p < 0.001$). Fungal exudates generally increased mean dry plant mass by 28-115% above the median value of the control plants (Figure 2.5A), with exudates from *L. orchidicola* having the greatest biomass-promoting effect on *Arabidopsis*. However, when *Arabidopsis* seedlings were grown with exudates, the rosette area was significantly reduced by ~ 8-77% below the median value of the control plants ($H = 37.950$, $df = 14$, $P < 0.001$; Figure 2.4, 2.5B). While the overall effect of exudates was a decline in leaf area, only exudates from *R. ericae* produced a significant reduction in leaf area. Interestingly, exudates from *H. finlandica* isolate C (Figure 2.4, 2.5) resulted in 100% mortality in *Arabidopsis* seedlings three to five days post exudate application.

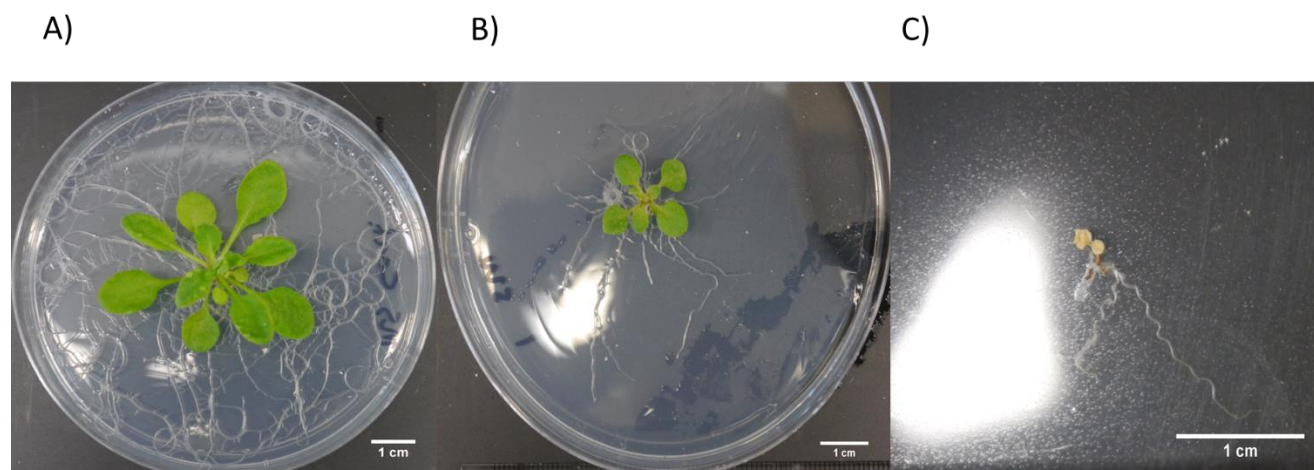


Figure 2.4. Effects of fungal exudates on *Arabidopsis* development exposed to fungal exudates had reduced rosettes, with some fungal exudates causing 100% mortality. Control plants (A) were larger than plants inoculated with fungal exudates (*C. geophilum* (B), and *H. finlandica* isolate C (C). Exudates from *H. finlandica* isolate C killed all *Arabidopsis* seedlings after 3 days of application (C).

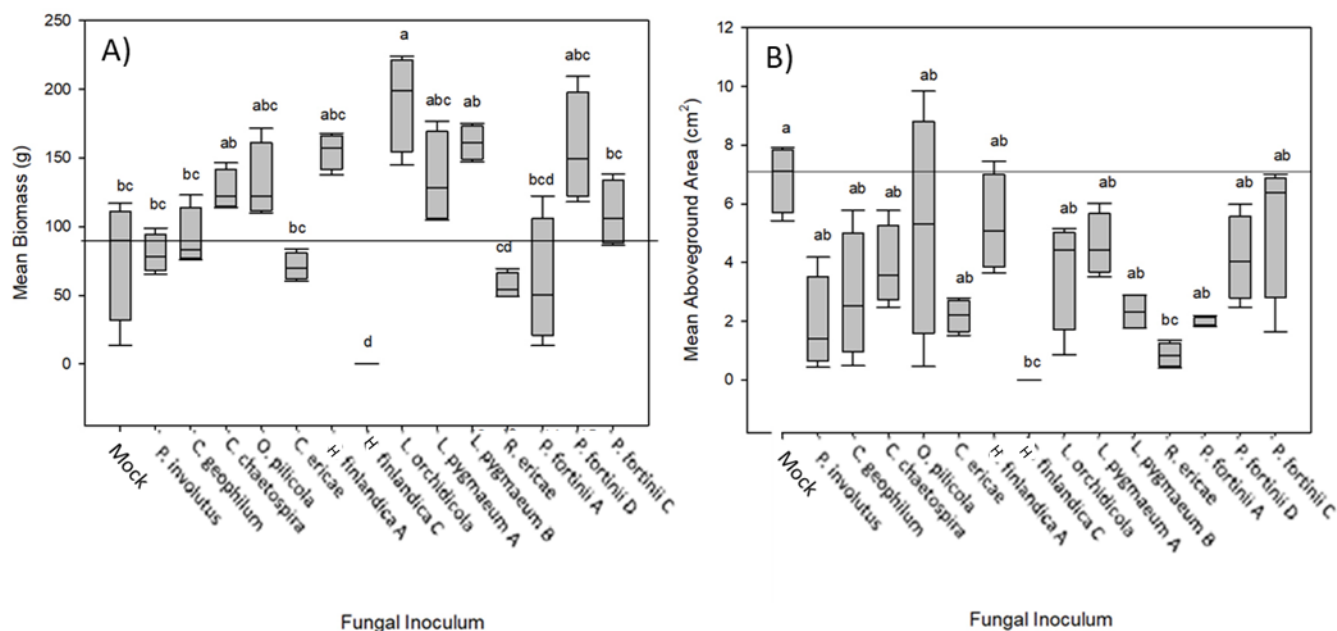


Figure 2.5. The effects of fungal exudates on *Arabidopsis* growth. 14-day-old *Arabidopsis* grown in 0.8% agar and 1% sucrose had their mean A) dry biomass and B) aboveground area (B) measured. Seedlings were inoculated with either MMN media (mock inoculation) or one of the 14 fungal isolates and grown for 14 days. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box is the maximum. Bars with different letters represent differences across the six treatments. Panel A. one-way ANOVA followed by a Holm-Sidak post-hoc test ($p < 0.001$, $n = 4$). Panel B. Kruskal-Wallis ANOVA on Ranks followed by a post-hoc Tukey's test ($p < 0.001$, $n = 4$).

2.4 Discussion

In this study, I investigated the production of phytohormones, amino acids, and organic acids of 14 different fungal isolates and their effects on the growth of *Arabidopsis* seedlings. My results demonstrate that the mycorrhizal isolates do secrete a diverse profile containing organic acids, amino acids, and phytohormones, supporting my first hypothesis. Mycorrhizal isolates synthesized and exuded the phytohormones: JA, methyl jasmonate, cis-jasmonate, SA, ABA, GA, and IAA; with approximately 20% of mycorrhizal isolates failing to exude cis-jasmonate and GA. Moreover, I demonstrated that the amino acid profiles of fungal isolates are diverse, with some amino acids like phenylalanine being exuded by all mycorrhizal fungi, while some amino acids were only exuded by specific mycorrhizal isolates. Furthermore, mycorrhizal isolates could exude organic acids with some fungi having variations in the types of organic acids produced in their secretion profiles. The *Arabidopsis* bioassay demonstrated that the application of secreted fungal compounds significantly affected *Arabidopsis* biomass and aboveground growth. The observed increase in mean dry plant mass and reduction in aboveground area in response to fungal exudates indicate that these compounds can modulate plant growth and development. Therefore, the mixed growth results only partially support my second hypothesis that fungal exudates would promote growth on both a biomass and aboveground area basis.

2.4.1. Exudation Profiles of Mycorrhizal Fungi

This study adds to the growing body of work on fungal exudate production, with a specific interest in screening mycorrhizal fungi isolated from aspen roots for selected organic acids, phytohormones and amino acids. Looking across the various screened compounds reveals that

the tested fungal isolates seem to have heterogeneous exudate profiles in support of my hypothesis that mycorrhizal isolates would have differences in their exudate profiles. This observation supports the notion that mycorrhizal fungi have evolved diverse ways for engaging with their environment and host plants, to exploit varying nutrient reservoirs in the rhizosphere or to modulate plant responses for symbiosis, growth or environmental stress (Van Schöll et al., 2006; Johnson & Gehring, 2007; Courty et al., 2010; Nadeau, 2015; Ortas et al., 2019). This heterogeneity within mycorrhizal exudate profiles unexpectedly extends to the strain level: *L. pygmaeum* strain A, for example, produced alanine, lysine and threonine, while *L. pygmaeum* strain B did not. This result is supported by the work of Christensen et al. (1991) and Patchett & Newman (2021), who determined that different fungal strains within the same species can have varying exudate profiles and even virulence levels (Latgé & Chamilos, 2020). My work adds to this idea that fungal strain-specific differences can be detected in the exudation profile of *L. pygmaeum*, *H. finlandica* and *P. fortinii* strains obtained from aspen in the boreal forest. Moreover, given that mycorrhizal fungi have unique exudation profiles, this could be used alongside genomic-based approaches to identify mycorrhizal or other root endophyte fungi from one another and/or different strains within the same species.

Mycorrhizal fungi, which form a symbiotic relationship with plants, play a crucial role in plant growth, nutrient acquisition, soil fertility, and stress tolerance (Wang et al., 2018; Mahmoudi et al., 2019; Field et al., 2020; Tedersoo et al., 2020; Hai-xi et al., 2022). They synthesize and release phytohormones into the rhizosphere and plant root tissues to enhance communication with the host and promote host plant growth, and reduce stress (Garcia et al., 2015; Wang et al., 2018; Pons et al., 2020; Wong-Bajracharya et al., 2020; Ma et al., 2022). As expected, all 14

fungus isolates released a broad suite of phytohormones, although four of the fungi did not produce detectable cis-jasmone. However, the relative similarity of the fungal phytohormone profiles was surprising, as most metabolic profiles for mycorrhizal fungal species show variation in the amounts of each metabolite produced (Christensen et al., 1991; Patchett & Newman, 2021). For example, methyl-jasmonate can act as a signalling molecule in plants to induce alkaloid synthesis and local plant defence under herbivory (Zhang et al., 2011; Qin et al., 2018). Producing methyl-jasmonate and other phytohormone may allow mycorrhizal fungi to alter their host plants' physiology and defences directly, thus initiating (or priming) the symbiotic relationship and eliciting associated pathways (Simons et al., 2008; Qin et al., 2018). The plant phytohormones SA and JA have been well documented to be antagonists of each other in terms of growth and development (Yang et al., 2018), yet it seems that the 14 isolates used in my study are producing both hormones, with SA being exuded at the same or greater concentration than JA and its two derivatives. My observations align with the claim that many ectomycorrhizal symbioses are negatively regulated by JA (Plett et al., 2014) and thus antagonistically counteracting this plant defence pathway with elevated SA at the start of colonization allows for hyphal development into the plant root system. The finding that all mycorrhizal isolates were producing and exuding IAA was not surprising as IAA is a key regulator of root development (mycorrhizal short roots) and root cell elongation, which can increase plant root exudation required for fungal growth and establishment (Fu et al., 2015). Moreover, the homogeneity of the phytohormone profiles obtained in my study aligns well with the hypothesis that many mycorrhizal fungi share a convergent infection pathway to colonize plant roots, damp down plant immune responses and establish a symbiotic relationship with their host (Garcia et al., 2015). Although our understanding on how mycorrhizal fungi and their prospective host plants initiate,

establish and maintain symbiosis is in its infancy, the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and the ectomycorrhizal fungus *Laccaria bicolor* produce effector molecules (phenolics, lipids, and phytohormones) and small secreted peptides that target host defence mechanisms and alter plant physiology in the early stages of root infection (Kloppholz et al., 2011; Plett & Martin, 2012; Garcia et al., 2015) leading credence to the common infection pathway hypothesis.

Amino acids are the fundamental building blocks of proteins and play crucial roles in several plant processes, such as growth and development, cell signalling, and as the starting material of many secondary metabolites (Lopez & Mohiuddin, 2022). Moreover, exuded fungal amino acids can be communication molecules and attractants, particularly in the role of mycorrhizal symbioses (Idrees et al., 2020). All 14 fungal isolates analyzed in my study exuded proline, phenylalanine, and histidine. Proline can act as a chemoattractant when released, potentially attracting other microorganisms into the rhizosphere to strengthen the soil microbiome (Webb et al., 2014). Proline can also act as an antioxidant, which may help mitigate oxidative stress within the host and other microorganisms in the rhizosphere (Signorelli et al., 2014). The aromatic amino acids phenylalanine and tyrosine are central products in the shikimic acid pathway for plants and fungi (Tohge et al., 2013). Moreover, both aromatic amino acids can be used to synthesize phenolic compounds such as flavonoids and tannins to increase stress tolerance (Tohge et al., 2013). Therefore, the exudation of these phenolic amino acids by mycorrhizal fungi may stimulate the biosynthesis of phenolic compounds, improving plant growth, stress resilience and mycorrhizal infection success in the host plants (Kaur & Suseela, 2020). To illustrate this potential, the exogenous application of proline and phenylalanine on *Moringa*

oleifera Lam. trees improved growth, stress tolerance, antioxidant capacity and osmoregularity (Atteya et al., 2022). Furthermore, all but one isolate in my study was able to exude the amino acid tyrosine, which can be taken up by plants and used in the biosynthesis of alkaloids, nitrogen-containing secondary metabolites that act as defence molecules and antifeedant compounds (Tohge et al., 2013; Kaur & Suseela, 2020). Exogenous histidine application induces ethylene production, and the ubiquitous exudation of histidine by all tested isolates might suggest the use of histidine in priming ethylene synthesis in plant roots during early colonization (Khatabi & Schäfer, 2012; Yariyama et al., 2019). Furthermore, 57% of mycorrhizal isolates released methionine, the precursor to plant ethylene production, which could be used to induce localized ethylene synthesis to help increase mycorrhizal colonization success (Khatabi & Schäfer, 2012).

Most soil fungi, including mycorrhizal fungi, can exude organic acids to accelerate the chemical weathering of rocks and increase the bioavailability of minerals like iron, calcium and potassium (Van Schöll et al., 2006; 2008; Ray & Adholeya, 2009; Nadeau, 2015; Wong-Bajracharya et al., 2020). My results align with this well-established result, implying that these fungi can increase the availability of mineral nutrients. Moreover, in my study, all isolates exuded citric acid, implying that this organic acid may be produced to increase the bioavailability of minerals; however, a more diverse pool of organic acid standards would be required to confirm this possibility.

2.4.2. *Arabidopsis* Growth Bioassay

Mycorrhizal fungi and root endophytes colonize plant roots and improve plant growth (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Liu et al., 2014). What is less clear is whether the same growth benefits could be achieved without associating mycorrhizal fungi with plants, but rather by only applying mycorrhizal exudates to plants. Thus, a bioassay was developed using the model plant *Arabidopsis* to test this idea. My study demonstrated that fungal exudates have variable effects on plant growth. Previous research has shown that fungal exudates improve root development and plant growth (Fusconi, 2014; Vignale et al., 2018). In line with this, I determined that mycorrhizal exudates generally increased *Arabidopsis* biomass compared to the control treatment. Interestingly, however, mycorrhizal exudates also reduced the aboveground area of the same plants, suggesting that the exudates may alter biomass allocation toward root production over control plants. This may improve plant resilience to drought stress, improve access to soil nutrient pools, improve plant resistance to uprooting and provide more contact area for mycorrhizal or beneficial microbial colonization (Ryan et al., 2016). While all plants experienced the same light levels, temperature and CO₂ levels, and were grown in MS media with similar nutrient and water levels, the concentrations of organic acids, phenolics, amino acids, and phytohormones within the fungal exudates may themselves have increased nutrient and water availability leading to greater root growth, as suggested by Fusconi (2014).

An unexpected result was that exudates applied to *Arabidopsis* from *H. finlandica* strain C resulted in 100% mortality of the seedlings. Although statistically, the exudate profile of *H. finlandica* was not different from the other isolates, it is possible that having the right mix of phytohormones and amino acids could have resulted in a severe stress response that led to 100%

mortality. For example, in the right proportions, IAA, ABA, and proline can induce programmed cell death in plants (Young & Gallie, 2000; Verslues & Sharma, 2010; Kacprzyk et al., 2022). Moreover, tyrosine can act as an allelochemical that impacts the growth and development of plants (Tyminski et al., 2021). However, it is more likely that one of the non-screened compounds like phenolics, lipids, enzymes, and a wider pool of organic acids and phytohormones like strigolactones that can act as mycotoxins or allopathic chemicals that induce plant stress, resulted in the plant death in my study.

2.5 Conclusion

The present investigation explored the exudation of phytohormones, amino acids, and organic acids by 14 fungal isolates (including 13 taken from aspen roots and one common, well-characterized mycorrhizal fungus as a benchmark) and their influence on plant growth using a bioassay. The results revealed remarkable heterogeneity in the exudate profiles of the tested mycorrhizal isolates, highlighting the diversity in their strategies for interacting with the environment and possibly host plants, yet also a strong similarity in the overall biochemical profile of these diverse fungi. These findings align with the idea that mycorrhizal fungi have evolved distinct mechanisms for nutrient exploitation in the rhizosphere and modulation of plant responses, as observed in previous research (Genre et al., 2020; Figueiredo et al., 2021). Additionally, my study adds more candidate chemicals for the communication between mycorrhizal fungi and their host plants and reveals commonalities in what mycorrhizal fungi, in general, exude into the rhizosphere. The bioassay demonstrated variable effects of fungal exudates on plant growth, with enhancements in biomass and alterations in aboveground growth patterns observed. This research contributes to understanding the intricate interactions between

mycorrhizal exudates and plants, offering potential insights into their roles in nutrient acquisition, stress tolerance, and plant growth promotion. My study also emphasizes the need for more investigations into plant-microbe interactions, including fungi and their exudates, to gain insights into the specific properties and mechanisms that fungal exudates may provide to plants and soil health.

2.6 References

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Chapter 3

3 Complex Effects of Mycorrhizae on Poplars Under Elevated Temperature and CO₂

3.1 Introduction

Carbon dioxide (CO₂) concentrations have increased from 280 ppm before the Industrial Revolution to current levels of 420 ppm, mainly due to the anthropogenic burning of fossil fuels and land use change (Ciais et al., 2013; IPCC, 2014; IPCC, 2021; Iz, 2022; IPCC, 2023). This increase in CO₂ levels has already increased global mean surface air temperatures by 1.09 °C, and global mean annual temperatures are predicted to warm a further 1-4 °C by the year 2100 (IPCC, 2021; 2023). Northern regions, including the boreal forest, may experience even greater warming of up to ~8 °C (Price et al., 2013; Stocker et al., 2013; IPCC, 2021;2023). This warming can alter precipitation patterns, leading to more frequent and extreme regional droughts globally (Trenberth, 2011; Hammond et al., 2022; Abbass et al., 2022). How climate change will impact the growth and productivity of plants (Tucker et al., 2001; Way & Oren, 2010; Kreuzwieser & Gessler, 2010), plant communities (Day et al., 2020), and pests and pathogen interactions with plants (Ayres & Lombardero, 2000; Allen et al., 2010) has yet to be fully resolved.

The world's forests cover approximately 30% of the earth's surface and play a critical role in providing renewable products and services for humans while serving as repositories of biodiversity (FAO, 2001; Brandt, 2013). Moreover, global forest ecosystems absorb

~30% of anthropogenic CO₂ emissions, providing a critical carbon sink and limiting the effects of anthropogenic climate change (Ciais et al., 2013; Le Quere et al., 2018).

Among these forests, the boreal forest stands out as one of the largest biogeoclimatic regions, spanning high northern latitudes in Canada, Northern Europe, and Russia (Brandt, 2009). In Canada alone, the boreal forest covers ~552 million hectares and provides essential ecosystem services such as producing food and fresh water, wood and peat resources for fuel, regulating the climate, water filtration, carbon storage, providing cultural value for Indigenous and non-Indigenous communities, recreational and spiritual opportunities, soil and nutrient cycling, and creating a broad range of habitats for many plant, fungal, and animal species; all of which have an estimated market value of \$50.9 billion (Brandt et al., 2013; Natural Resource Canada, 2016). The boreal region is dominated by forests, peatlands, wetlands, and rivers, primarily consisting of cold-hardy tree species such as *Abies*, *Larix*, *Picea*, *Pinus*, and *Populus* (Ruckstuhl et al., 2008; Brandt, 2009; ACIA, 2013; Brandt et al., 2013). Moreover, through boreal forest replanting efforts, genetically improved native and non-native tree species have been introduced, such as *Picea glauca* Moench and *P. abies* L., respectively. Furthermore, hybrid tree species, mainly *Populus* spp. clones, which are most often a mix of native *Populus* spp. crossed with non-native species have also been introduced to the boreal plant communities (Larchevêque et al., 2010).

The boreal forest is particularly vulnerable to climate change, with mean annual air temperature increases of up to 3 °C in recent years, and further warming projected by 2100 (Price et al., 2013). Such warming trends, coupled with changes in water

availability, may lead to forest losses and gains along the warmest and coolest margins of the boreal forest biome, respectively (Berner & Goetz, 2022). Moreover, elevated temperatures and CO₂ concentrations can potentially extend the growing season, boosting plant growth and carbon assimilation (Beck et al., 2011; Ju & Masek, 2016; Hember et al., 2017; Stinziano & Way, 2017). However, these higher temperatures are also expected to disrupt precipitation patterns, increase the frequency and severity of heatwaves, and contribute to elevated plant mortality and more frequent and severe forest fire regimes (Stocker et al., 2013; Marchand et al., 2019; Day et al., 2020; Enbrecht et al., 2021). Observations from both field studies and satellite data indicate that changes in vegetation and biome shifts are already underway in the boreal forest, corroborating predictions from ecosystem models regarding future ecological changes and shifts in species composition (Foster et al., 2019; Berner & Goetz, 2022). The impacts of elevated temperature and CO₂ on boreal forest community stability and ecosystem function may compromise tree species' health and fitness, alter species composition, and reduce resilience to biotic and abiotic stresses.

Given the potential impacts of climate change on boreal forests, it is essential to examine the effects of temperature on tree growth (Worrall et al., 2013; Natural Resources Canada, 2015; 2016). Moderate warming can stimulate height, stem diameter and biomass production in deciduous tree species (Way and Oren, 2010, Du et al., 2021). Indeed, elevated temperature increased growth 12-54% in *Populus tremula* and *Betula ermanii* compared to control trees (Constable & Ratzlaff, 2000; Sobuj et al., 2018; Du et al., 2021). However, moderate to extreme warming events of 4-8 °C have been associated

with reduced tree growth in the same species (McDowell et al., 2018). For example, *Picea mariana* (Mill.) Britton and *Larix laricina* (Du Roi) K. Koch grown at +8°C had 63% and 44% less biomass, respectively than control trees (Dusenge et al., 2020). Furthermore, Zeps et al. (2017) found that a 4 °C increase in growth temperature reduced growth and led to a 35% decrease in *P. tremula* seedling survival. Elevated growth temperatures in boreal tree species can thus also have detrimental effects on productivity, leading to stress and tree mortality (D'Arrigo et al., 2004; Juday & Alix, 2012; Girardin et al., 2016; Cahoon et al., 2018; Nicklen et al., 2018; Trugman et al., 2018). Dieback events have been observed in various species, including *Populus* spp., *Acer saccharum*, and *Picea rubens*, due to increased growth temperatures and late winter or early spring thaw-freeze cycles (De Hayes, 1992; Auclair et al., 2005; Brandt, 2009). Additionally, *L. laricina* seedlings grown under an 8 °C warming treatment had 40% higher mortality than control trees (Murphy & Way, 2021). Trees, including poplars, reduce stomatal conductance in the increased vapour pressure deficit (VPD) caused by warmer temperatures, thereby maintaining leaf water status at the expense of carbon fixation (Siau, 1971; Hogg et al., 2000; Lopez et al., 2021). Taken together, the literature suggests that moderate temperature increases can stimulate growth and productivity, especially in deciduous trees, while extreme warming and heat waves often reduce growth and cause physiological damage.

All boreal tree species are C3 plants, and thus, elevated CO₂ levels can enhance photosynthesis and their growth. These effects are primarily due to reduced stomatal conductance and photorespiration, coupled with increased photosynthetic rates, resulting

in improved carbon balance and growth (Kaiser et al., 2017; Dusenge et al., 2019; Song et al., 2020). This phenomenon is commonly referred to as the CO₂ fertilization effect. Tree growth is stimulated by 30% on average across a broad range of Free Air CO₂ Enrichment studies (FACE) (Ainsworth & Long, 2005). In *Populus* spp., elevated CO₂ increased photosynthetic rates and water use efficiency, leading to enhanced growth (Zhang et al., 2018; Shanker et al., 2022). Nevertheless, longer-term exposure to elevated CO₂ concentrations leads to the accumulation of non-structural carbohydrates, which can downregulate photosynthetic processes and limit the benefits of CO₂ enrichment (Moore et al., 1998). For example, despite an initial growth stimulation under elevated CO₂ conditions, this effect became less pronounced over the growing season in *P. tremula* (De Roo et al., 2020). Some studies have even demonstrated a negative impact of elevated CO₂, such as a reduction in height in *P. tremula* and reduced growth in poplar clones (Sobuj et al., 2018; Kim et al., 2021). Clearly, elevated CO₂ may improve growth, yield, and photosynthesis; however, the beneficial effects of longer-term elevated CO₂ are not universal. Moreover, while the increase in plant growth rates under elevated CO₂ is well-documented, its long-term implications for carbon storage in plant biomass remain unclear (Körner, 2015).

Increased growth temperature and elevated atmospheric CO₂ concentrations are often associated with enhanced growth in many plant species (Beck et al., 2011; Ju & Masek, 2016). However, current global models may overestimate the effects of increasing temperature and atmospheric CO₂ concentrations on promoting terrestrial photosynthesis and growth (Laffitte et al., 2022). In the case of apple trees, elevated CO₂ initially

inhibited shoot growth but later promoted it, while the impact of elevated temperature was more varied; the combined elevated CO₂ and temperature treatment showed reduced or no growth enhancement and lower average fresh fruit weight (Lee et al., 2023). In another study, when the elevated temperature was combined with elevated CO₂ concentration, *P. tremula* height was stimulated by 41% over control plants (Sobuj et al., 2018). Moreover, in the boreal tree species *Picea mariana* and *L. laricina*, the combined effects of elevated and CO₂ concentrations had a ~80% reduction in tree biomass when compared to controls (Dusenge et al., 2020; Murphy & Way, 2021). The relationship between growth temperature, atmospheric CO₂ concentrations, and plant growth is complex and can have different outcomes depending on the temperature and CO₂ treatment conditions used and the studied plant species. It is thus crucial to consider these interactions to accurately assess the potential impacts of climate change on plant growth and ecosystem dynamics.

Mycorrhizal fungi are a diverse group that colonize over 90% of terrestrial plant species and are broadly classified based on their interaction with plant roots (Brundrett et al., 1996; Bonfante & Genre, 2010). Mycorrhizae develop a network of hyphae through which they interact with soil and plant roots, where up to 45% of the root may be colonized (Brundrett et al., 1996; Soudzilovskaia et al., 2015). These mycorrhizal microbiomes impact plant growth and productivity through various mechanisms, including metabolic cooperation, signalling responses, and microbial dysbiosis (Sharma et al., 2022). Understanding the interactions between plants and microorganisms, particularly in the context of climate change and environmental stress, is crucial for

elucidating their contribution to ecological resilience.

Elevated soil temperatures caused by climate change directly affect mycorrhizal fungi by reducing their growth, spore production and fruiting body sizes, potentially limiting tree growth and carbon allocation to mycorrhizal symbionts (Fernandez et al., 2017).

Meanwhile, elevated CO₂ levels can stimulate the development of mycorrhizal fungi, increasing infectivity, colonization, and mycelium growth (Rillig & Allen, 1999; Fitter et al., 2000). In turn, mycorrhizal fungi have been shown to have the ability to enhance plant growth and alleviate heat stress in trees (Nadeau, 2015). Although controversial (Karst et al., 2023), weak evidence suggests that mycorrhizal networks may facilitate stress signalling in planta and resource sharing between trees, indicating the potential for fungal-mediated resilience (Song et al., 2015). In addition, mycorrhizae can improve stomatal function, maintain turgor pressure, and enhance mineral uptake under drought conditions, contributing to greater tree seedling survival (Ouledali et al., 2018).

Furthermore, mycorrhizae can enhance plant resilience against pathogens and improve osmotic stress tolerance (Hao et al., 2019; Mena-Violante et al., 2006). Many trees, including *Populus* spp., form root associations with mycorrhizal fungi, which may reduce the effects of heat and drought stress associated with climate change (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Liu et al., 2014). Moreover, mycorrhizae may benefit trees under elevated CO₂ conditions, with mycorrhizal inoculation increasing tree seedling growth by 18% at ambient CO₂ conditions but by 70% at elevated CO₂ (700 ppm) (Alberton et al., 2010). However, mycorrhizae can be detrimental to tree growth and survival under some conditions. For example, trees experiencing abiotic stress that

were inoculated with the ECM *Paxillus involutus* had 27% less root and 21% less stem biomass than those grown without mycorrhizae (Luo et al., 2009).

Although the precise mechanisms underlying the positive effects of mycorrhizal fungi under changing climatic conditions remain to be fully elucidated, their potential to mitigate climate stress and promote plant growth is evident (Alberton et al., 2010; Nadeau, 2015; Hortal et al., 2016). Understanding the potential management implications of harnessing mycorrhizae to ameliorate climate stress can contribute to climate change mitigation efforts (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Liu et al., 2014). By identifying specific tree-fungus combinations that are better adapted to changing conditions and unravelling the mechanisms that prevent detrimental effects caused by warming, we can fully harness the benefits of mycorrhizal fungi in supporting plant growth and strengthening ecosystem resilience (Fernandez et al., 2017; Treseder et al., 2021; Usman et al., 2021). Additionally, while most climate change studies look at the impacts of either elevated CO₂ concentrations (Cheng et al., 2012) or temperature (Pischl & Barber, 2017) on mycorrhizal-tree interactions, the effects of both elevated temperature and CO₂ on mycorrhizal-tree interactions are rarely studied. Therefore, I investigated: 1) how elevated CO₂ concentrations and temperatures will alter poplar growth; and 2) whether inoculation with mycorrhizal fungi would enhance poplar growth under future climate change. I tested the following hypotheses using hybrid poplar cuttings:

- 1) Non-inoculated hybrid poplar cuttings will exhibit reduced height, biomass, and

canopy greenness (an index of leaf health) under extreme warming (+8 °C treatment), regardless of CO₂ concentration, but will have enhanced growth under moderate warming (+4 °C) and elevated CO₂ concentrations, consistent with what has been found with other boreal tree species.

2) Based on the observation that increased growth temperatures, especially extreme warming, can increase plant stress and mortality in boreal tree species, non-mycorrhizal hybrid poplar cuttings will have increased mortality under future warming conditions, with the highest mortality observed in the +8 °C treatment.

3) With the potential of mycorrhizal fungi to enhance plant growth and resilience, hybrid poplar cuttings inoculated with mycorrhizal fungi will have greater growth, leaf health, and survival under all treatments, with the strongest effects of mycorrhizal inoculation observed in the most extreme future climatic conditions (+8 warming and elevated CO₂).

3.2. Methods

3.2.1. Fungal Cultures and Maintenance

Mycorrhizal fungi were obtained from the roots of trembling aspen trees (*Populus tremuloides*) in Cold Lake, AB, Canada (*Cenococcum geophilum* (NoF # 3129; GeneBank # MT276004) and *Hyaloscypha finlandica* (NoF # 3117; GeneBank # MT276006) or the Forestry Culture Collection (*Paxillus involutus* (NoF # 2339) collected from *Pinus sylvestris* L., Oslo, Norway, 1984). These fungal isolates were selected because they are ectomycorrhizal fungi that form beneficial associations with *Populus* spp. roots (Garbaye & Churin, 1997; Luo et al., 2009; Alberton et al., 2010; Kipfer et al., 2012; Szuba et al.,

2017). Although *P. involutus* was not isolated from *P. tremuloides* directly, it is a known model mycorrhizal fungus that lives in diverse habitats (found in Europe, Asia, North America, Australia, and New Zealand) and is associated with different hardwood and coniferous tree species, ranging from *Picea abies*, *Populus* spp. *Betula pendula*, and *Pinus contorta* (Wallander and Söderström, 1999; Jargeat et al., 2014; Sayyed & Hussain, 2020). Mycorrhizae from *P. tremuloides* were isolated by surface sterilizing roots and aseptically transferring infected root pieces onto modified Melin-Norkrans (MMN, Appendix 3.1) agar. In the laboratory, fungal isolates were initially grown on MMN agar plates at room temperature in the dark for 18 days and then stored at 4 °C. Isolates were then grown in sterile glass test tubes containing liquid MMN. To inoculate the liquid media, a cube of fresh fungal mycelium was added to the sterile MMN media. Liquid cultures were grown for 21 days in the dark at room temperature on a rotary shaker.

3.2.2. Poplar Growth Conditions and Inoculation

Populus x canadensis [a hybrid variety of *Populus nigra* L. x *Populus deltoides*] is fast-growing and readily forms mycorrhizal associations, making it ideal for studying climate change impacts on plant-mycorrhizal interactions (Liu et al., 2014). One-year-old *Populus x canadensis* clones from Somerville Seedlings (Everett, ON), which share both a similar climate and photoperiod to London, ON, were used for inoculation. Using plant material from a single *Populus x canadensis* clone removed genetic variability among the experimental plants, allowing me to focus on environmental and mycorrhizal effects on plant growth.

Bare root seedlings were cut to a length of 30 cm, roots were removed, and the cut surface was sterilized with 70% ethanol and 30% bleach solution and rinsed with autoclaved water to remove any fungi. Cuttings were then rooted in sterilized Spencer Lemaire trays filled with sterile (autoclaved at 121°C) Promix® (Premier Tech Horticulture, Riviere-du-Loup, QC) and slow- release fertilizer (Slow-Release Plant Food, 12N-4P-8K per 0.204 L/m², Miracle-Gro, The Scotts Company, Mississauga, ON, Canada) and placed for 4 weeks at 18 °C at natural light intensity in a climate- controlled glasshouse at the University of Western Ontario's Biotron Experimental Climate Change Research Centre in London, ON, Canada (Figure 3.1).



Figure 3.1. Biotron glasshouses. Taken in July 2020.

Cuttings that generated new root tissue were either left uninoculated (as controls) or inoculated with one of three mycorrhizal isolates (*Paxillus involutus*, *Cenococcum geophilum* or *Hyaloscypha finlandica*) by placing an inoculated agar plug into the soil next to the roots and adding macerated liquid fungal suspension to the roots. Cuttings were then potted in 11.6 liter pots with Promix®. The pots with the control plants and the three mycorrhizal treatments were then randomized across six glasshouses in one of six treatments (generating 24 mycorrhizal x climate treatments): either ambient CO₂ (AC, 410 ppm) or elevated CO₂ concentrations (EC, 750 ppm) with either ambient temperature (0T), ambient temperature +4 °C (4T), or ambient temperature +8 °C (8T) temperatures (n = 8 plants per fungal x climate treatment; N=192; Figure 3.2).

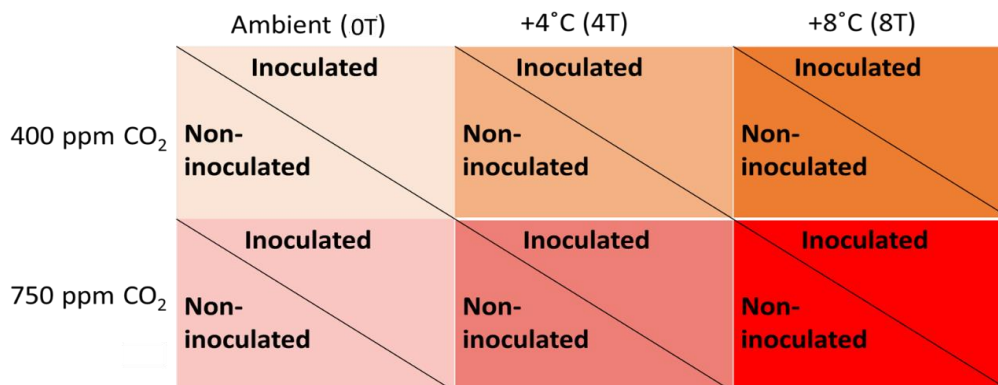


Figure 3.2. Poplar growth conditions schematic within the Biotron glasshouses.

The temperature treatments were chosen to represent a realistic range of possible future temperatures under the Representative Concentration Pathway (RCP) 4.5 and RCP8.5 by 2100 in the Great Lakes Basin (McDermid et al., 2014). The 0T temperature treatment was determined from a five-year mean average hourly temperature value for each day of the growing season (using data from 2012-2016) from the London, ON International Airport's meteorological station (Environment Canada). For example, if the 5-year average temperature on May 26 from 10 AM- 11 AM was 10.2 °C, then each 0T, 4T, and 8T treatment plant would experience 10.2 °C, 14.2 °C, and 18.2 °C respectively for that hour.

CO₂ concentrations were measured in each glasshouse every 10 minutes using an infrared gas analyzer and an Argus control system (Argus Control Systems, Surrey, Canada) and were controlled by injecting pure CO₂ as needed to maintain the EC treatments. Daily temperature and CO₂ levels across all six glasshouses over the duration of this experiment have been previously reported by Murphy and Way (2021). Irradiance matched outdoor

light conditions, varying with natural light conditions over the study period. Humidity was controlled at 60%, and seedlings were watered daily to maintain a moist growth medium. Cuttings were allowed to grow for five months (until October 2, 2017).

3.2.3. Growth and Health Measurements

Height was measured from the soil surface to the top of the tallest stem on each plant every four weeks. Throughout the growing period, any mortality was recorded. From September 30 - October 2, 2017, the number of leaves produced on each plant was counted to quantify the total leaf number. All trees were harvested and divided into leaves, stems, and roots within the first week of October. The plant material was then allowed to dry to a constant mass at 65 °C, after which each tissue type was weighed.

To determine the effect of mycorrhizae on tree health, the number of healthy green leaves, and the number of yellow or brown leaves were recorded. To quantify chlorosis and necrosis from leaf scorching, leaf health was scored based on the colour of the leaves. Green leaves were categorized as healthy, leaves with 50-100% of the leaf surface turned yellow were categorized as stressed, and leaves with greater than 50% of the leaf surface brown were categorized as dying or dead. These categories were used to determine the proportion of green leaves per tree to evaluate the impacts of elevated temperature and CO₂ on leaf health and whether fungal inoculation impacted these health indices.

3.2.4. Mycorrhizal Colonization

A modified version of the gridline intercept method from Brundrett et al. (1996) was used to evaluate the presence of mycorrhizae on roots across the treatments. Briefly, fresh poplar roots were gently washed with autoclaved de-ionized water to remove any potting material attached to the root. Then, 100 g of root tissue was removed from five plants per mycorrhizal x climatic treatment (N = 96). Root samples were cleared by placing roots in 10% KOH for 15-20 minutes, followed by a wash in autoclaved DI water. Cleared root samples were stained with trypan blue to reveal fungal colonization of the root. Stained roots were placed in a Petri dish with a sheet of 1 cm x 1 cm grid-lined paper underneath. Roots that intercepted (crossed) each horizontal and vertical grid line were counted while recording the number of mycorrhizal roots (dyed blue). The percentage of mycorrhizal roots out of the total roots (the sum of all mycorrhizal and non- mycorrhizal roots that crossed the gridlines) was calculated to assess the extent of mycorrhizal colonization among the different mycorrhizal species and climatic conditions.

3.2.5. Statistical Analysis

Data from non-inoculated control cuttings were analyzed using a two-way analysis of variance (ANOVA) to detect growth temperature and CO₂ concentration effects on growth and tree health indices, and Holm-Sidak post-hoc tests were used to assess significant differences among climate treatment means ($p < 0.05$). Mortality was measured as the percentage of deaths in each group and reported as a distractive binary

outcome (dead or alive) due to the small sample size, which was thus not suitable for statistical analysis. Data on biomass and the proportion of green leaves were transformed (with a square-root transformation and a $(x^4)/5$ function, respectively) to satisfy normality assumptions.

To examine the effect of mycorrhizal species on hybrid poplar growth under future climatic conditions, I measured growth and health indicators of inoculated plants. The data are presented as the percentage change compared to the non-inoculated controls within each CO₂ and temperature treatment, emphasizing the impact of the mycorrhizal fungi on trees within each climate treatment. This was calculated as follows:

$$\text{Percent Change} = \frac{\text{mycorrhizal poplar measure} - \text{mean measure of non-inoculated poplar}}{\text{mean measure of non-inoculated poplar}} \times 100$$

Equation 3.1

The effect of increased warming and CO₂ on mycorrhizal colonization of poplar roots and poplar growth measurements were examined using a two-way ANOVA and Holm-Sidak posthoc tests. This was done to identify the effects of elevated growth temperatures and CO₂ concentrations within each fungal treatment, as well as significant differences among treatment means ($p < 0.05$) due to an uneven sample size across treatments (mortality) and the fact that the assumptions of a 3-way ANOVA were not satisfied. To satisfy the normality assumption of the ANOVA: 1) biomass and percentage of green leaves for *P. involutus* were transformed with $\arcsin(\cos(x))$ and $\arcsin x$ functions, respectively; 2) root: shoot ratio and percentage of green leaves for *C. geophilum* were transformed using $\sin x$ and $1/\arcsin x$ functions, respectively; 3) and height and

percentage of green leaves for *H. finlandica* were transformed using $\sin x$ and $1/\sin x$ functions, respectively. To see how growth and health measures are different between the inoculated and non-inoculated trees within each climate treatment, a one-way ANOVA was performed; Holm-Sidak post-hoc tests were run to identify significant differences among treatment means ($p < 0.05$, Appendix 3.1-3.4). All analyses were performed using SigmaPlot statistical software version 13.0.

3.3. Results

3.3.1. Growth and health of non-mycorrhizal control poplars

Under ambient CO₂ (AC) conditions, warming did not affect non-inoculated plant height, while warming stimulated plant height under elevated CO₂ (EC) (Figure 3.3). Elevated CO₂ also increased plant height (Figure 3.3). The EC4T plants were 38% taller than the EC0T plants, and the EC8T trees were 29% taller than the EC0T plants (Figure 3.3). Similarly, biomass in non-mycorrhizal AC poplars was unaffected by growth temperature; however, biomass was significantly increased by 200% in EC4T trees and 93% in EC8T trees when compared to EC0T trees or any AC treatment ($p < 0.001$; Table 3.1; Figure 3.4). Neither the temperature nor the CO₂ treatments nor their interactions affected the root: shoot ratios (R/S) of non-mycorrhizal poplars ($p = 0.058$; Table 3.1; Figure 3.5). There were significant effects of temperature, CO₂, and a temperature x CO₂ interaction on the proportion of green leaves (Table 3.1), driven by the 2.5-fold decrease in the number of healthy, green leaves in AC8T plants compared to the other five treatments (Figure 3.6). Mortality increased with warming in both CO₂ treatments (Figure 3.7).

Table 3.1. Effects of elevated CO₂ and temperature growth conditions on the growth and health of non-inoculated, control poplars. Summary report of a two-way analysis of variance (ANOVA) in non-mycorrhizal poplars, indicating F-values and P-values for the main effects of temperature and CO₂ treatments. Bolded numbers represent p < 0.05.

<i>Parameter</i>	<i>T</i>		<i>CO₂</i>		<i>T X CO₂</i>	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
<i>Height</i>	0.956	0.396	11.347	0.002	6.399	0.005
<i>Biomass</i>	28.212	<0.001	87.633	<0.001	45.360	<0.001
<i>Root: Shoot Ratio</i>	0.317	0.732	1.504	0.232	3.216	0.058
<i>Proportion of Green Leaves</i>	17.069	<0.001	5.157	0.030	11.386	<0.001

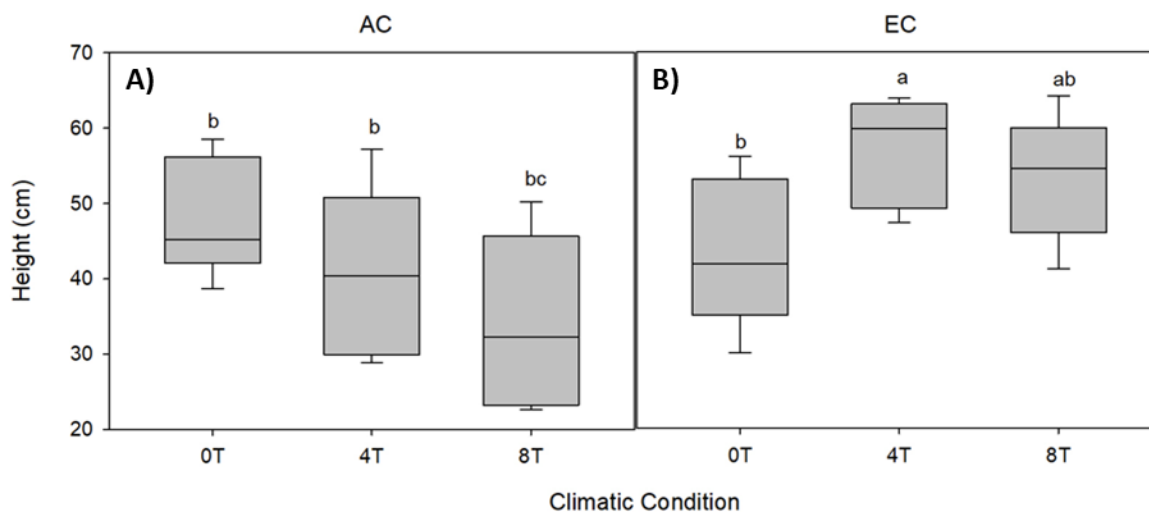


Figure 3.3. Height responses to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box is the maximum. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n \geq 4$.

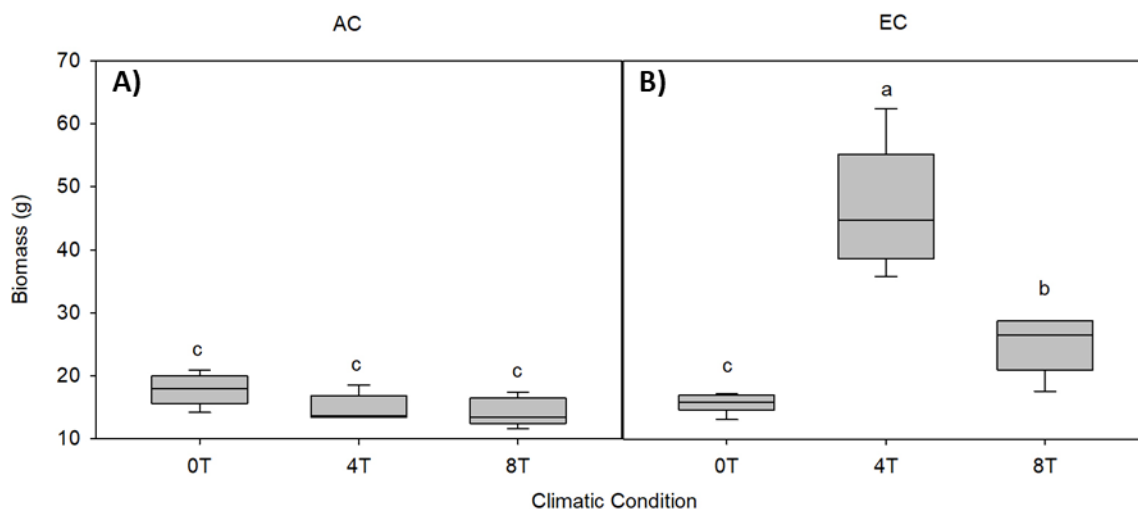


Figure 3.4. Dry biomass responses to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box is the maximum. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n \geq 4$.

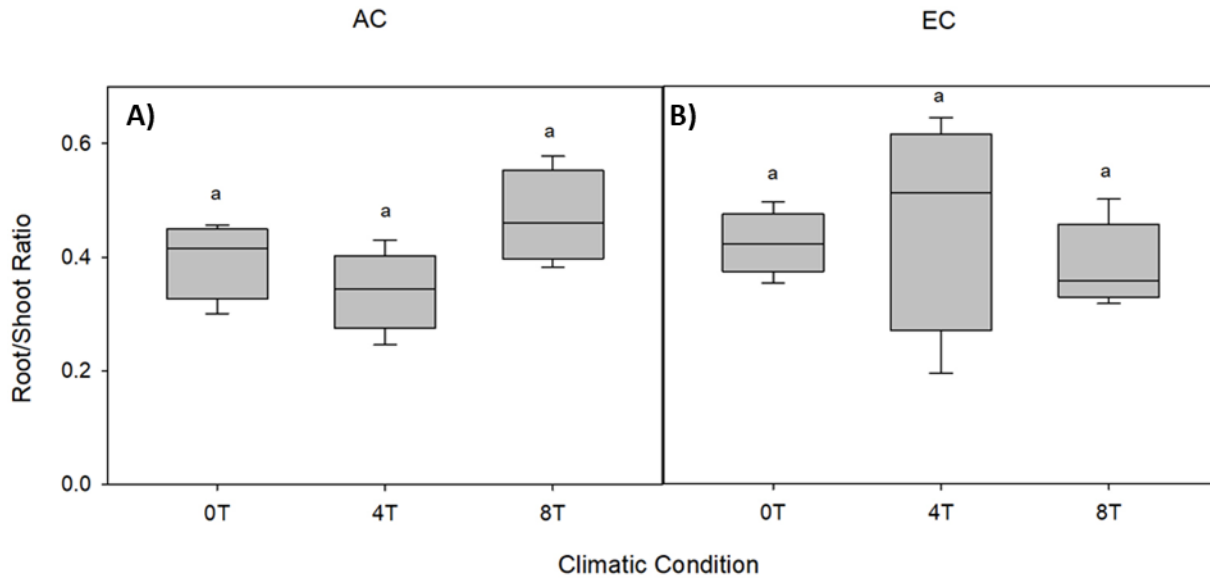


Figure 3.5. Root-to-shoot responses to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box is the maximum. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n \geq 4$.

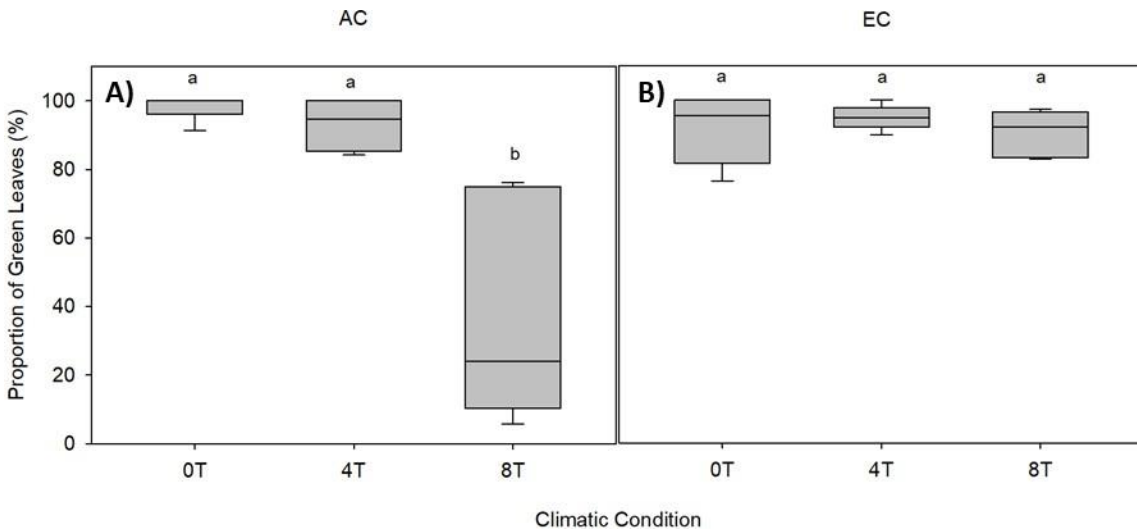


Figure 3.6. The proportion of green leaves in response to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n \geq 4$.

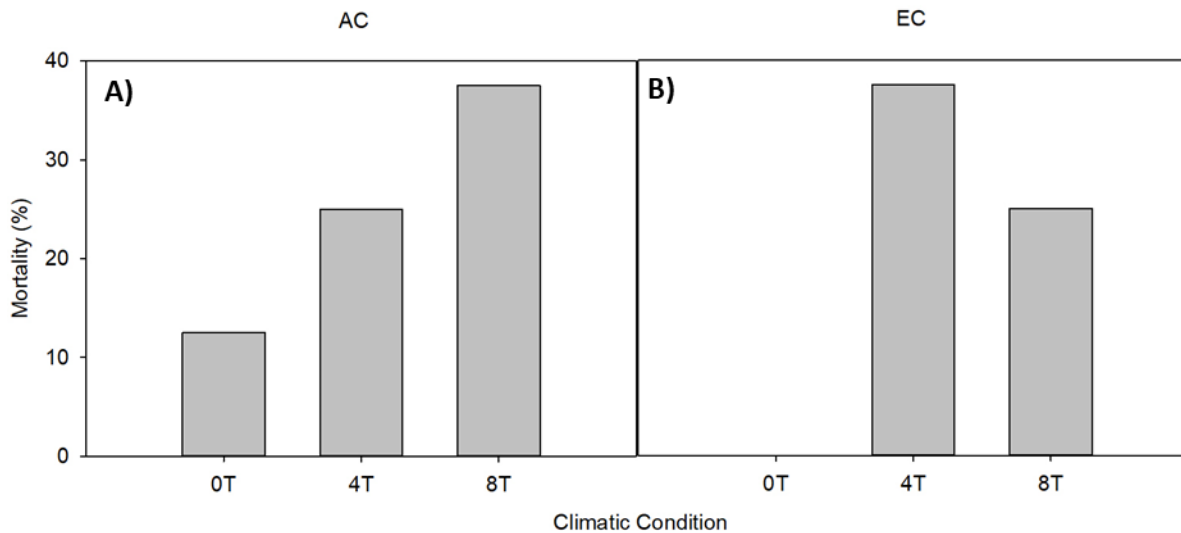


Figure 3.7. Impacts of varying temperature treatments in AC (A) and EC (B) growth conditions on mortality of non-mycorrhizal poplars. AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C.

3.3.2. Mycorrhizal root colonization

All observed mycorrhizal poplar roots showed successful colonization. Root colonization of the three mycorrhizal species (*P. involutus*, *C. geophilum* or *H. finlandica* strain C) was affected by growth temperature, growth CO₂, as well as a temperature x CO₂ (p <0.05, Table 3.2). Generally, *C. geophilum* had greater success colonizing roots, especially when compared to *P. involutus* under AC conditions (Figure 3.8). Increasing growth temperature had no effect on the colonization of roots in AC plants (Table 3.2). However, warming stimulated colonization in EC-grown trees: EC4T poplars had more than twice the degree of mycorrhizal colonization than EC0T poplars, and EC8T poplars had ~33% greater colonization than EC0T poplars.

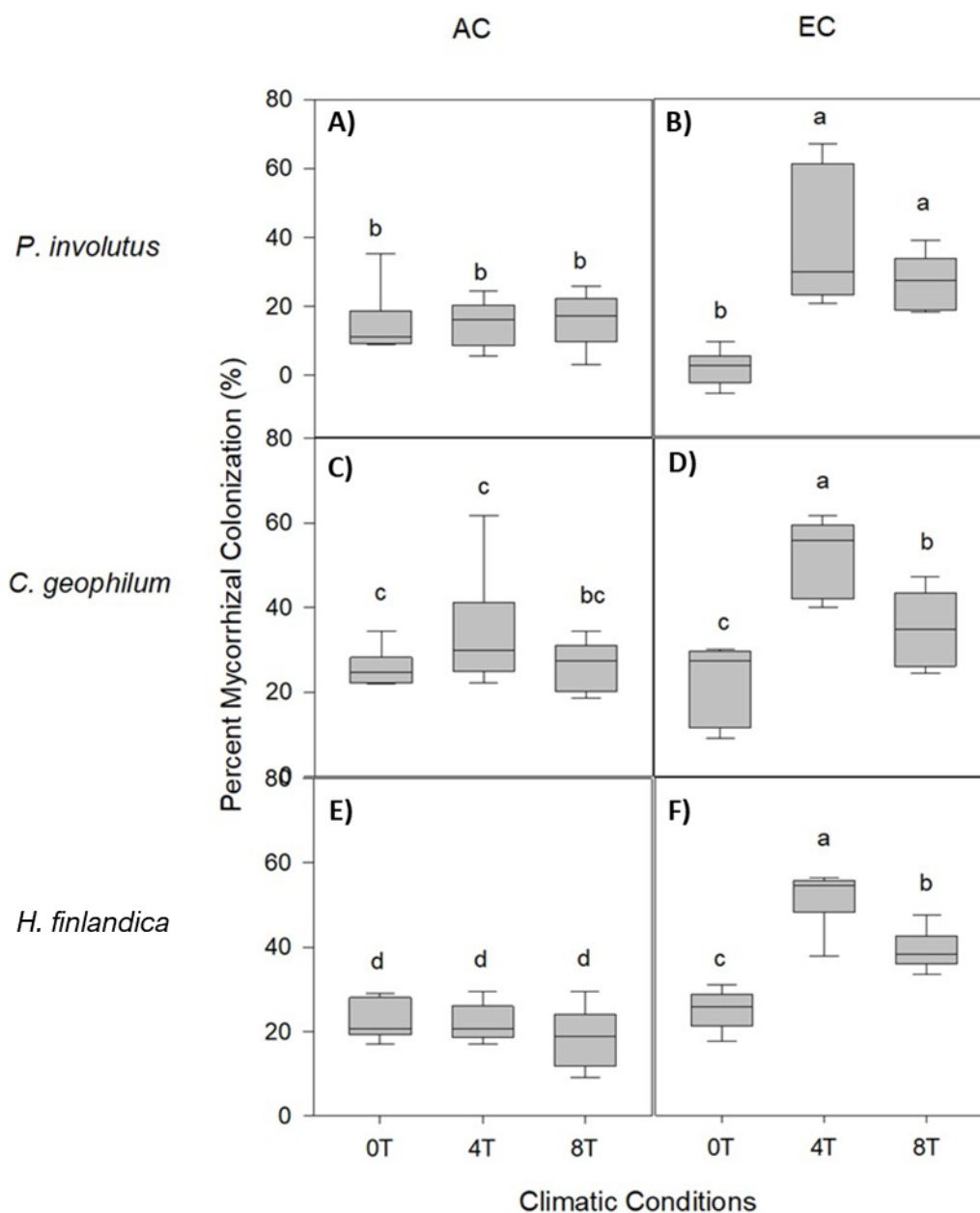


Figure 3.8. Influence of temperature under AC (A, C, E) and EC (B, D, F) conditions on the percent mycorrhizal colonization of poplar roots. *Paxillus involutus* (A,B), *Cenococcum geophilum* (C,D), or *Hyaloscypha finlandica* (E,F) were used to inoculate poplar roots. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C; $n = 4$.

Table 3.2. Impacts of climate change on growth parameters and leaf health of plants inoculated with mycorrhizae. Summary report of a two-way analysis of variance (ANOVA), showing F values and P values for the main effects of temperature and CO₂, within each fungal species treatment. Parameters measured were fungal colonization of roots, plant height, biomass, root- shoot ratio, and proportion of healthy leaves. Bolded numbers represent P-values <0.05.

	T		CO ₂		T X CO ₂	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
A) Fungal Colonization						
P. involutus	10.677	<0.001	33.158	<0.001	8.788	<0.001
C. geophilum	11.504	<0.001	5.520	0.026	3.612	0.041
C. finlandica	11.653	<0.001	11.693	0.002	16.720	<0.001
B) Growth and Health Parameters						
P. involutus						
Height	3.140	0.057	9.887	0.004	6.927	0.003
Biomass	0.887	0.423	16.522	<0.001	12.332	<0.001
Root-Shoot Ratio	12.791	<0.001	19.560	<0.001	22.948	<0.001
Proportion of Healthy Leaves	7.977	0.002	0.289	0.594	9.456	<0.001
C. geophilum						
Height	1.477	0.246	4.155	0.051	2.644	0.089
Biomass	13.619	<0.001	4.703	0.039	2.085	0.144
Root-Shoot Ratio	1.020	0.374	31.375	<0.001	15.950	<0.001
Proportion of Healthy Leaves	0.780	0.469	2.664	0.114	1.030	0.371
H. finlandica						
Height	0.195	0.824	5.455	0.026	3.499	0.042
Biomass	4.475	0.021	2.819	0.105	12.246	<0.001
Root-Shoot Ratio	0.152	0.860	5.696	0.025	3.134	0.060
Proportion of Healthy Leaves	0.170	0.844	0.890	0.353	0.136	0.873

3.3.3. Impacts of future warming and CO₂ on the growth of mycorrhizal-inoculated plants

The effect of mycorrhizal inoculation on plant height varied with growth temperature, growth CO₂, and there was a temperature x CO₂ interaction ($p < 0.05$, Table 3.2). Under AC, inoculation had little effect on the height at current temperatures, but increased height growth above that of the non-inoculated control trees in the warming treatments. Under EC, inoculation had little effect on height at any treatment temperature.

Generally, inoculated poplars grown under either AC or EC at lower growth temperatures (0T or 4T) had similar biomass as non-inoculated poplars from that same treatment (Figure 3.10). However, the percent increase in biomass due to inoculation increased in the AC8T treatment, especially in plants inoculated with *C. geophilum* and *H. finlandica*, and in the EC8T treatment in plants inoculated with *P. involutus* (Figure 3.8).

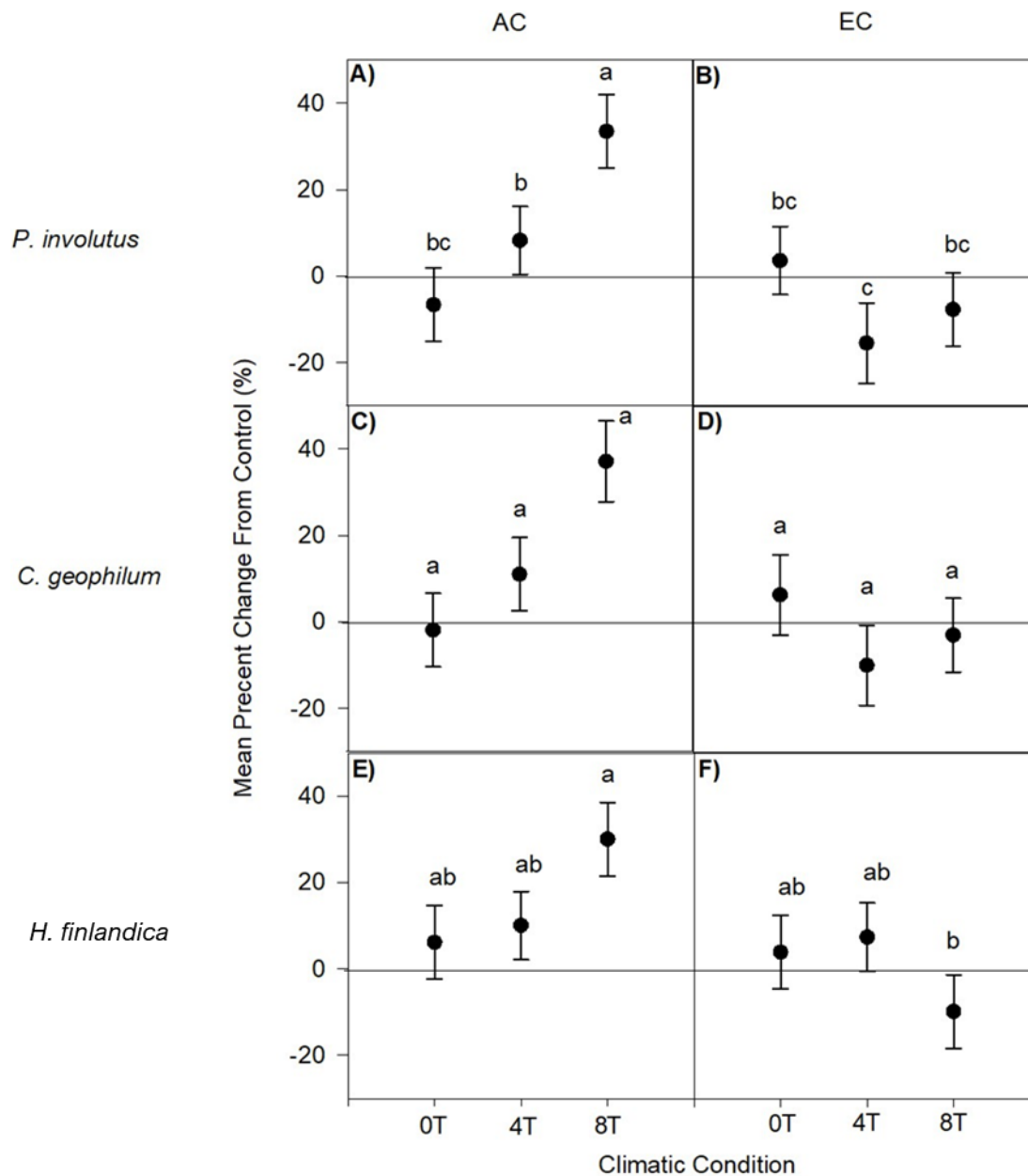


Figure 3.9. Effect of mycorrhizal inoculation on tree height across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum* (C, D) and *Hyaloscypha finlandica* (E, F), and mean percent change from control was determined. Dots represent the Mean \pm SE around the mean of percent change from control. Different letters on dots represents significant differences across the six treatments with a mycorrhizal species (Holm-Sidak post hoc test, $p < 0.05$), $n > 5$. AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4°C; 8T ambient temperature +8°C, $n \geq 4$.

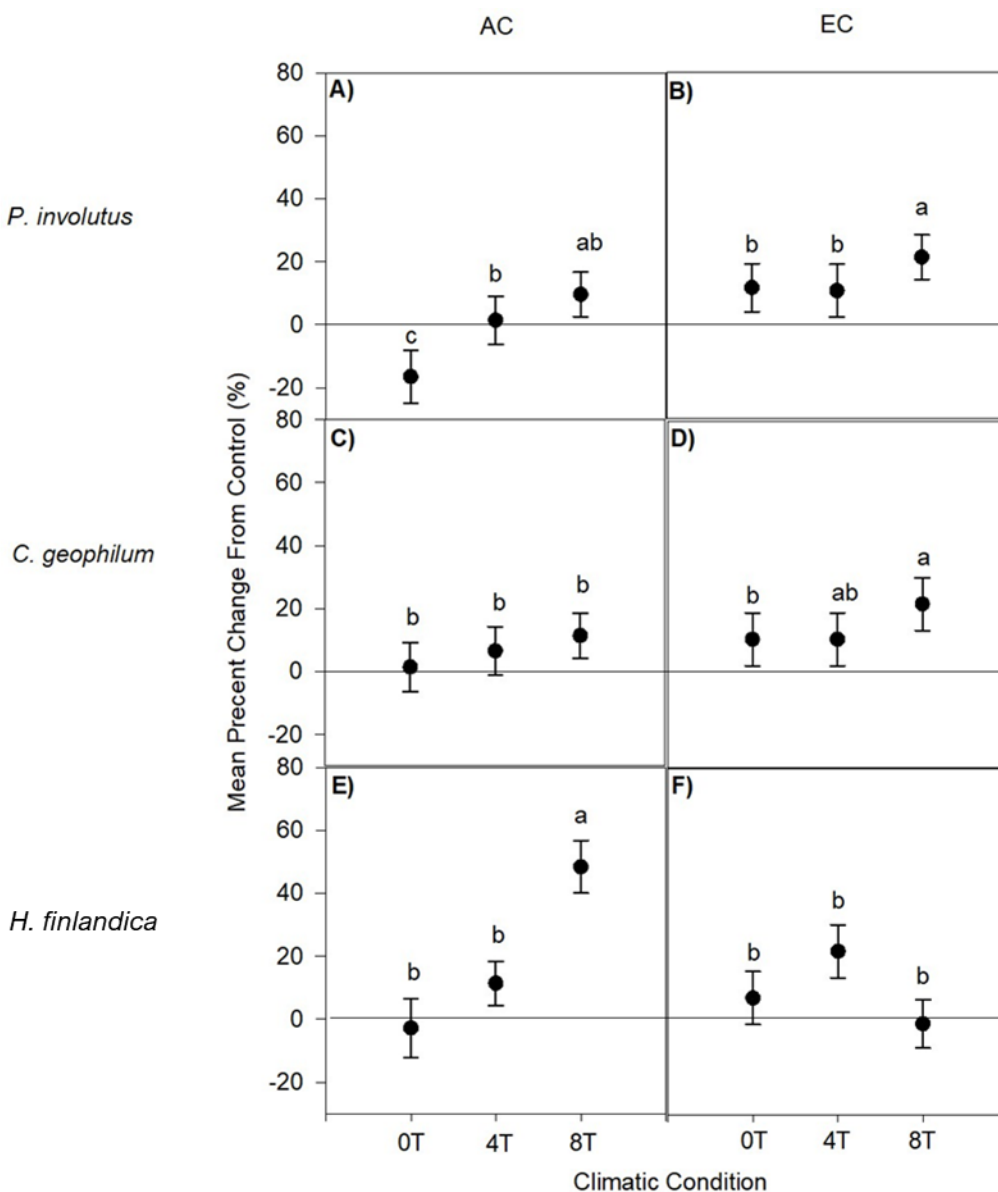


Figure 3.10. Effect of mycorrhizal inoculation on tree biomass across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum* (C, D) and *Hyaloscypha finlandica* (E, F), and mean percent change from control was determined. Dots represent the Mean \pm SE around the mean of percent change from control. Different letters on dots represents significant differences across the six treatments within a mycorrhizal species (Holm-Sidak post hoc test, $p < 0.05$), $n > 5$. AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n \geq 4$.

The effect of inoculation on the root: shoot ratio (R/S) was affected by most treatments and their treatment interactions (Table 3.2). Inoculated AC poplars showed relatively little change in the R/S from the non-inoculated plants across the temperature treatments (Figure 3.11). In contrast, in EC plants, there was a small increase in the R/S in inoculated trees grown at 0T and a large increase in inoculated poplars at 8T, compared to their non-inoculated counterparts. For EC8T poplars, a 2.25-fold increase in the R/S was observed in plants inoculated with *P. involutus* compared to non-inoculated EC8T trees, the largest increase among the tested fungal species. The AC poplars inoculated with *C. geophilum* showed similar R/S results to that of AC trees with *P. involutus*, although with greater R/S loss 8T conditions than non-inoculated controls (Figure 3.11C). EC poplars inoculated with *C. geophilum* had up to a 91% increase in R/S when compared to non-inoculated poplars, except for 4T plants, where there was a significant decrease in R/S when compared to the other temperature treatments. The AC poplars inoculated with *H. finlandica* generally had a lower R/S than non-inoculated poplars, (Figure 3.11E). Under EC, poplars with *H. finlandica* strain C had up to a 35% increase in their R/S, compared to non- inoculated poplar controls, but there was no temperature effect.

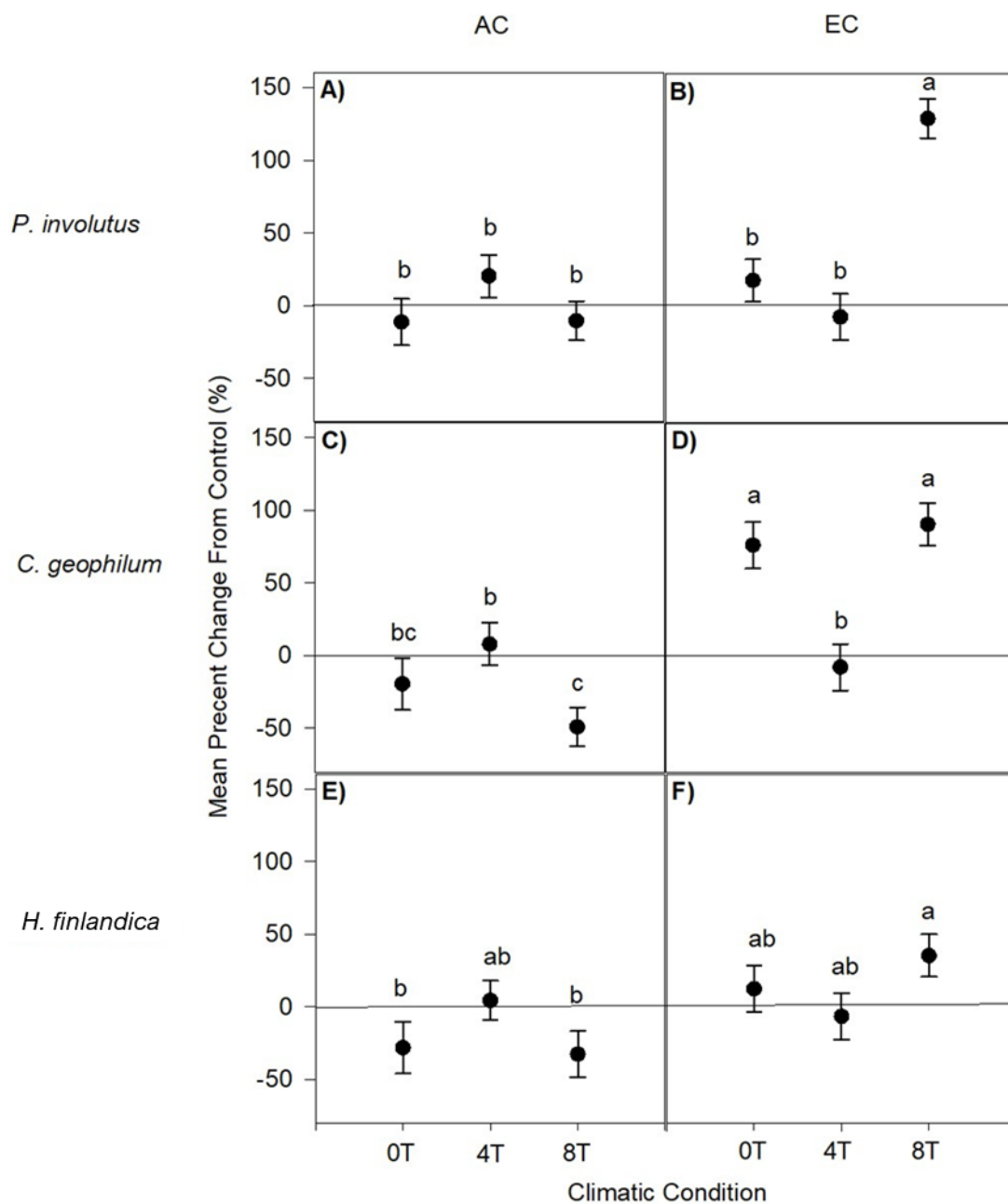


Figure 3.11. Effect of mycorrhizal inoculation on tree root-shoot ratio across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum*, (C, D) and *Hyaloscypha finlandica* (E, F), and mean percent change from control was determined. Dots represent the Mean \pm SE around the mean of percent change from control. Different letters on dots represents significant differences (Holm-Sidak post hoc test, $p < 0.05$), $n > 5$. AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n \geq 4$.

3.3.4. Elevated temperature and CO₂ effects on leaf health and mortality of mycorrhizal poplars

In general, inoculation had little impact on the number of green leaves across the climatic treatments (Figure 3.12). The only exception to this was for AC8T poplars, where inoculation increased the percentage of green leaves compared to non-inoculated AC8T trees. In contrast, inoculation generally increased mortality, except in EC8T trees inoculated with *P. involutus*, which showed a 48% reduction in mortality (Figure 3.13).

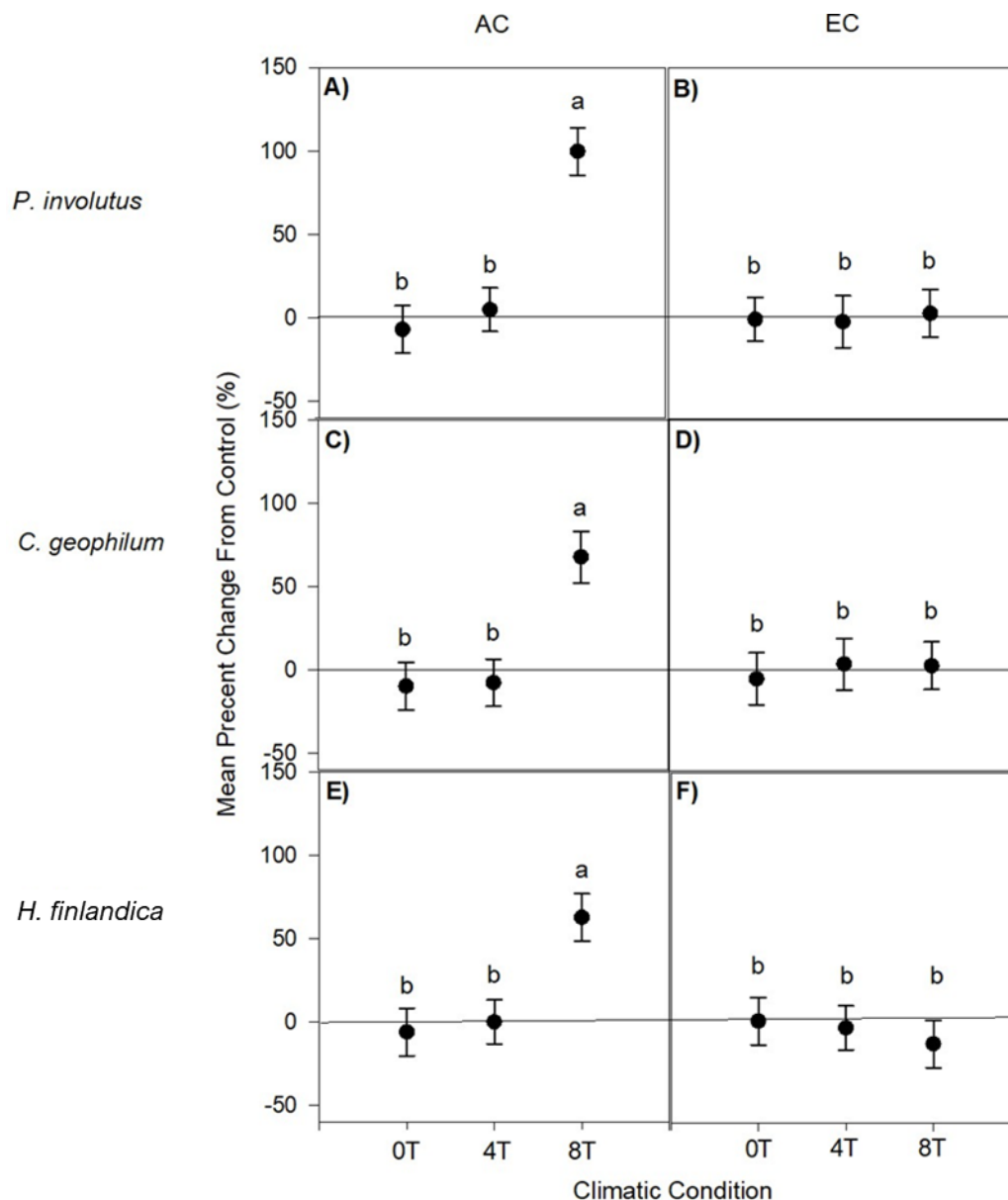


Figure 3.12. Effect of mycorrhizal inoculation on tree leaf greenness across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum* (C, D) and *Hyaloscypha finlandica* (E, F) and mean percent change from control was determined. Dots represent the Mean \pm SE around the mean of percent change from control. Different letters on dots represents significant differences (Holm-Sidak post hoc test, $p < 0.05$), $n > 5$. AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n \geq 4$.

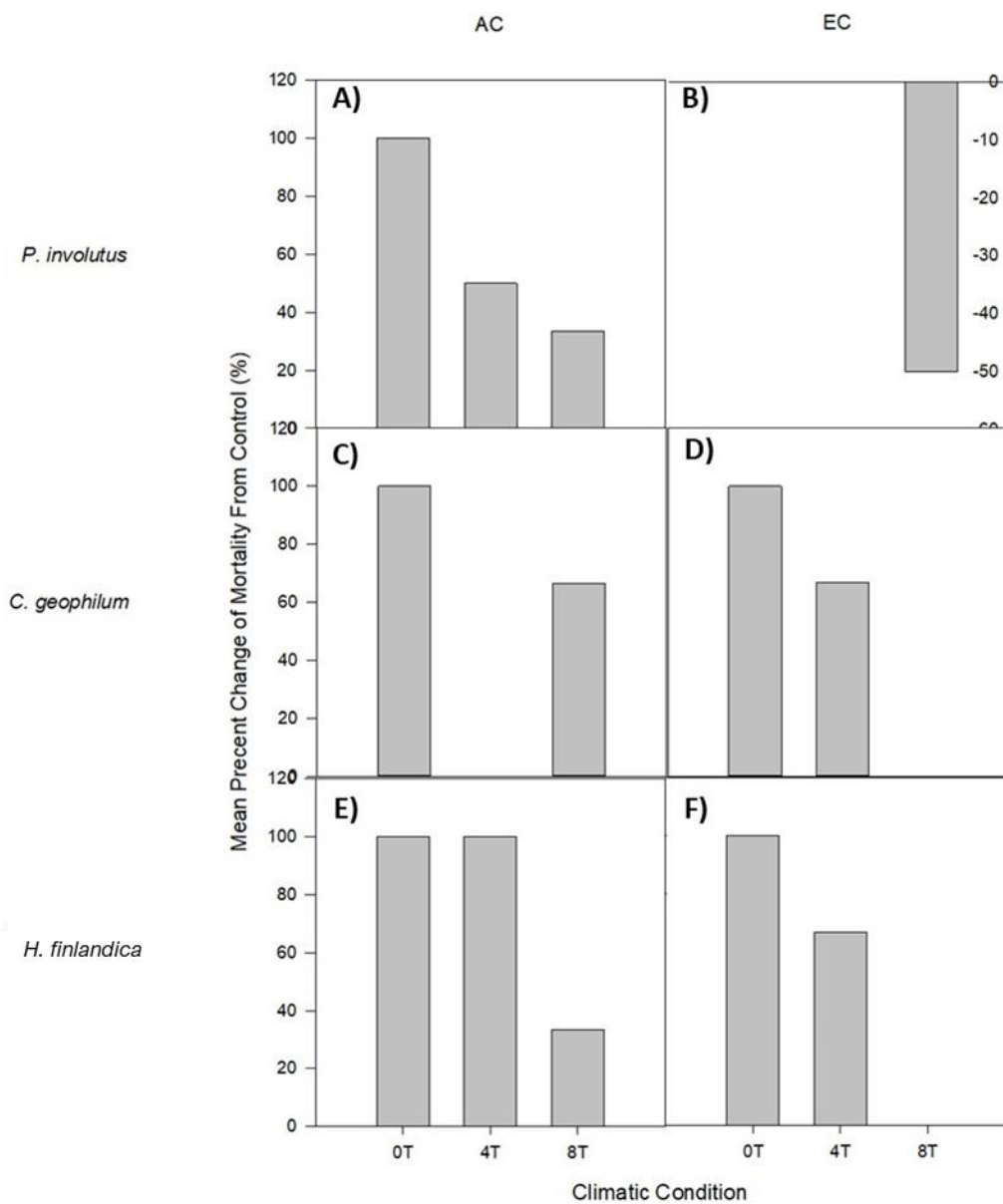


Figure 3.13. Effects of temperature and CO₂ treatments on mortality in mycorrhizal poplar. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum* (C, D), and *Hyaloscypha finlandica* (E, F). Mean percent change from control was determined for mortality for each fungal treatment. AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T, ambient temperature +8 °C.

3.4. Discussion

This study investigated the effects of elevated growth temperature and CO₂ on the growth, health, and survival of non-inoculated and mycorrhizal *Populus x canadensis*. As hypothesized, there were significant temperature and CO₂ effects on non-mycorrhizal tree growth and health measures. However, my prediction of extreme warming (+8) reducing poplar growth and health was only partially supported. Warming had a detrimental effect on poplar health under AC conditions, as evidenced by increased mortality and leaf damage, though warming had no impact on tree growth under AC. However, warming enhanced growth and had no effect on leaf health in EC trees, implying that elevated CO₂ can offset the negative effects of warming on tree health. In mycorrhizal trees, cuttings grown under elevated CO₂ and warming had increased mycorrhizal infection. Regardless of the mycorrhizal species used, height and biomass were stimulated by mycorrhizal infection as growth temperature increased under AC conditions, supporting my hypothesis that mycorrhizae will promote growth in hybrid poplars. However, mycorrhizae had little effect on poplar growth under EC conditions, suggesting there is a limit to the beneficial growth-promoting ability of these mycorrhizae under elevated CO₂, in contrast to my initial hypothesis. Inoculation with mycorrhizal fungi improved growth and leaf health in the most heat-stressed AC trees, as hypothesized, but had little effect on the growth and health of the less stressed EC4T and EC8T trees, despite greater root colonization.

3.4.1. Elevated temperature and CO₂ effects on growth and health of control poplars

The lack of a temperature effect on tree growth seen here is unexpected, as growth typically increases with warming up to ~13°C above control temperatures (Way & Oren, 2010). For example, *Populus tremuloides* grown under 5 °C warming were 22% taller and had 84% more biomass than control trees (Way et al., 2013). Furthermore, in *Populus tremula*, biomass increased by up to 340% under warming (Sivadasan et al., 2018). However, the impact of temperature on plant growth is often based on plant functional groups and thermal niches. Way and Oren (2010) showed that, generally, evergreen species show a more conservative response to temperature changes compared to deciduous species, with the latter exhibiting exponential growth responses to warming. Furthermore, warming suppressed biomass accumulation in tropical and subtropical species but enhanced growth in temperate and boreal species (Way & Oren, 2010), such as the one studied here. Even in species that benefit from some degree of warming, growth can be suppressed if temperatures become stressful. The high VPDs typically seen at warmer temperatures can also directly suppress height and growth (Lopez et al., 2021), so a warming-induced growth stimulation could be offset by a VPD-induced suppression of growth. This trade-off has been documented in many species, such as pine (Samuelson & Teskey, 1991), black locust (Mebrahtu et al., 1991), soybean (Walker et al., 2016), and wheat (Walker et al., 2016). The lack of a warming-induced growth response in my study may thus be due to a trade-off between the effects of warming and higher VPD.

Elevated CO₂ concentrations increase plant growth by increasing CO₂ assimilation (Ainsworth & Long, 2005; van der Kooi et al., 2016). However, my poplars grown under ambient temperatures and elevated CO₂ concentrations were similar in mass and height to AC0T trees. In contrast to other studies, elevated CO₂ increased plant growth by up to 60% in poplar, 133% in tamarack, and 200% in black spruce (Ceulemans et al., 1995; Dusenge et al., 2020). Interestingly, elevated CO₂ in conjunction with elevated temperature also increased plant height and biomass in these studies, although the root:shoot ratio remained unchanged. This may suggest that under elevated temperature and CO₂ conditions, my hybrid *Populus x canadensis* might not suppress photorespiration as effectively, resulting in similar growth to ambient grown trees; even though, in theory, the increased availability of CO₂ should lead to a reduction in the occurrence of photorespiration as the rate of RuBP carboxylation is favoured over RuBP oxygenation leading to enhanced growth (Dusenge et al., 2019).

Heat stress due to elevated growth temperatures can profoundly impact plant leaves, affecting their physiology, morphology, and overall health (Bita & Gerats, 2013; Wang et al., 2018). These impacts are particularly relevant for poplar trees, commonly found in riparian zones and other environments that may experience high temperatures (Ferus et al., 2020). Some of the consequences of heat stress include wilting, loss of chlorophyll, decreased photosynthesis, increased oxidative stress, and changes in leaf shape and size, which, if not corrected, may result in the necrosis of plant tissues (Bita et al., 2016). This can be particularly evident in plant leaves where chlorosis and/or leaf scorching may occur under heat stress. In my study, the AC8T poplars showed more chlorosis and

senescent leaves than other treatments, suggesting that the trees were stressed and unable to acclimate to the extreme (8T) warming even when watered daily. However, elevated CO₂ provided a protective effect to leaf health under these extreme warming conditions. Unfortunately, this added protection did not translate into lower mortality, as poplars under both CO₂ conditions had higher mortality at the warmer +4 and +8 °C conditions than ambient temperatures. It is possible that the increased damage to poplar leaves under extreme warming, especially in AC conditions, reduced photosynthesis, resulting in carbon starvation and leading to higher mortality. Additionally, plants grown under elevated temperatures typically also experience a higher VPD, resulting in increased evapotranspiration and thus potentially leading to increased stomata closure, resulting in limited gas exchange and a further reduction in photosynthesis (Erickson & Markhart, 2001).

3.4.2. Impacts of future warming and CO₂ on the performance of mycorrhizal poplars

When plants are exposed to stressful conditions, mycorrhizal fungi can help mitigate the stress and improve the survival of the plant host by increasing root access to water and nutrients while also providing plants with growth-promoting compounds (Daniels Hetrick & Bloom, 1984; Bonfante & Genre, 2010; Kipfer et al., 2012; Leppälampi-Kujansuu et al., 2013). This might lead to the hypothesis that roots would have increased mycorrhizal colonization under warmer conditions, as seen in ectomycorrhizal fungi in Norway spruce roots (Leppälampi-Kujansuu et al., 2013) and arbuscular mycorrhizal fungi in winter wheat (Daniels Hetrick & Bloom, 1984). However, in my study, fungal

colonization of roots was not affected by elevated temperatures in the AC treatment. If the increased heat stress and VPD experienced by these poplars reduced net CO₂ assimilation rates, resulting in fewer photosynthates available for the mycorrhizae, this may have limited fungal colonization. This idea is supported by the result that root colonization by all three mycorrhizal species increased under EC conditions (and especially under moderate warming with EC) where plant carbohydrate availability was most likely to increase. Hortal et al. (2016) also found that the proportion of mycorrhizal root colonization increased with elevated temperature under elevated CO₂, whereas a similar phenomenon was seen in *Pinus abies* colonized by dark septate root endophytes when grown at elevated CO₂ concentrations (Alberton et al., 2010), lending credence to this explanation.

Inoculation with mycorrhizal fungi nearly always increased poplar growth under ambient CO₂ conditions compared with non-inoculated poplars, in line with many studies (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Alberton et al., 2010; Leppälammii-Kujansuu et al., 2013; Liu et al., 2014). Both *P. involutus* and *C. geophilum* had similar effects on poplar growth, whereas the effect of *H. finlandica* was somewhat different. All three mycorrhizal species promoted plant height, while trees inoculated with *H. finlandica* saw the greatest increase in biomass, especially under extreme warming. My results demonstrate that mycorrhizae can promote poplars' growth and mitigate the impact of climatic stress on growth. Interestingly, inoculation with mycorrhizal fungi either had no or a detrimental effect on height in poplars grown under elevated CO₂ conditions, although inoculation with *P. involutus* or *C. geophilum* enhanced biomass under elevated

CO₂.

Based on the high proportion of colonized roots, it was unsurprising that inoculated EC poplars had greater biomass allocation towards roots than control poplars, especially under extreme warming. This increase in biomass allocation towards the roots not only allows for more mycorrhizal associations (as increased root mass is available for colonization) but also increases plant access to nutrients and water, which could be more limiting under a warmer, high CO₂ environment. In contrast to my results, *Eucalyptus grandis* grown under AC or EC conditions and inoculated with *Pisolithus albus* had 60% and 36% increases in the root-shoot ratio, respectively (Wong-Bajracharya et al., 2020). Poplars grown under moderate warming with elevated CO₂ experienced the highest amount of mycorrhizal colonization; however, a slight increase in shoot biomass allocation was observed rather than an increase in root biomass allocation. This may suggest that to support the higher amounts of fungi on the roots and thus a higher carbon sink, poplars are increasing the amount of their photosynthetic tissues to meet this demand as well as to increase or maintain their biomass.

Inoculation of poplar with the mycorrhizal fungi tested here had minimal impacts on leaf health. The only exception was in AC8T poplar, where inoculation increased leaf health when compared to non-inoculated poplars. This result may indicate that mycorrhizae can mitigate the impacts of severe heat stress (and or leaf drought stress induced by high VPD) by upregulating protective responses such as heat shock proteins, antioxidants, and/or altering plant stress hormone concentrations within leaf tissues, leading to greater

cellular stability by preventing thermal, oxidative and osmotic damage to the photosynthetic machinery, cell organelles, cellular membranes, DNA and proteins (Landhäusser et al., 2002; Kivlin et al., 2013; Huang et al., 2014; Pozo, 2015). Reduced damage to cells and lower physiological stress in host plants could then enhance the carbon fixation needed to supply carbohydrates to the mycorrhizae. However, the increased sink demand of having mycorrhizal fungi associated with their roots nearly always increased mortality compared to non-inoculated poplars. Although mycorrhizae are known to improve plant growth and survival under stressful conditions, it has been proposed that some mycorrhizal fungi, under the right conditions, become pathogenic, increasing mortality for their host species, especially if the host plant is not able to provide enough carbon sources for fungal survival (Rai & Agarkar, 2016).

3.5. Conclusions

My study is one of the few to evaluate the dual effects of elevated CO₂ and temperature on mycorrhizal associations and assess whether mycorrhizae can ameliorate future climatic stresses and promote tree growth. My findings suggest that the interaction between temperature and CO₂ can significantly affect plant growth and health, and that mycorrhizae may play a role in improving the growth and health of trees under heat stress. However, the energetic cost and demand for carbohydrates associated with fungal symbiosis must be considered in any attempt to improve plant survival under future climatic conditions, especially those that may suppress photosynthesis.

As atmospheric CO₂ concentrations and average temperatures increase, their independent

and combined effects on boreal species such as poplar will depend on the severity of the warming, the amount of precipitation, and the availability of soil nutrients. In the short term, my results show that elevated growth temperatures will have minimal effects on poplar growth in the absence of mycorrhizae. However, based on my results, there may be more mortality in poplar stands, especially under extreme warming events that increase heat-associated damage to leaf tissues. Growing poplars with mycorrhizal fungi under current atmospheric conditions generally promoted growth, especially under warming conditions, suggesting that mycorrhizal fungi provide a protective effect under heat stress, although the increasing carbon and energy demand for maintaining mycorrhizal interactions may increase tree mortality. Thus, in the short term, mycorrhizal-associated poplars will likely continue to experience dieback as growth temperatures increase, but those that survive may be taller and have more biomass. As atmospheric CO₂ levels continue to increase, poplars may experience enhanced growth, especially under moderate warming, but will likely still experience greater mortality because of high temperatures and droughts in the field (Price et al., 2013). Association with mycorrhizal partners under future CO₂ and temperature regimes should increase poplar biomass, and biomass allocation will likely be towards root structures rather than aboveground biomass, potentially improving drought tolerance. Under extreme future warming conditions, mycorrhizae do provide a protective effect, increasing poplar survival, possibly by increasing water availability, nutrients, and or altering stress responses to trees, which may increase the chance of ECM- colonized poplars persisting under future climatic conditions (Peterson & Peterson, 1992; Landhäusser et al., 2002; Hacquard & Schadt, 2014; Ouledali et al., 2018).

3.6. References

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Chapter 4

4 Exploring Mycorrhizal Interactions in Stress Hormone Dynamics of Hybrid Poplars Under Changing Climatic Conditions

4.1 Introduction

Earth's climate has undergone significant changes in the last century, characterized by rising global temperatures and increasing concentrations of atmospheric CO₂ (Ciais et al., 2013; Price et al., 2013; Stocker et al., 2013; IPCC, 2014; Iz, 2022; IPCC, 2021; IPCC, 2023). The boreal forest, which covers 14% of Earth's land mass, is highly vulnerable to climate change, with temperatures rising more rapidly than most of the globe (Price et al., 2013; Foster et al., 2019; Berner & Goetz, 2022). While elevated temperatures associated with climate change can extend the growing season and enhance plant growth in these northern regions, they also disrupt long-term precipitation patterns, increase heatwave severity and frequency, and contribute to tree mortality (Stocker et al., 2013; Ju & Masek, 2016; Hember et al., 2017; Stinziano & Way, 2017; Day et al., 2020; Enbrecht et al., 2021). These impacts compromise tree health, alter species composition, and reduce resilience to stresses (Hogg et al., 2000; 2008; Foster et al., 2019; Day et al., 2020; Murphy & Way, 2021; Enbrecht et al., 2021; Berner & Goetz, 2022). Understanding how trees respond metabolically and physiologically to future elevated temperature and CO₂ concentrations is crucial for assessing climate change's overall impacts on the trees that support the ecological processes and economic productivity of the boreal forest.

The elevated temperatures expected under climate change can disrupt vital cellular and physiological processes within plants. High temperatures induce heat stress, which can lead to protein denaturation, membrane damage and oxidative stress (He et al., 2018). High temperatures also often co-occur with high vapour pressure deficits (VPD), which increase leaf-level water loss by imposing atmospheric drought on leaves (Lopez et al., 2021). Plants, such as *Populus* spp., regulate stomatal conductance in response to high VPD, thereby maintaining their leaf water potential by decreasing transpiration (Tardieu & Simonneau, 1998; Beeson, 2011). However, rising temperatures will occur in an atmosphere with elevated CO₂ levels, which will lead to further reductions in stomatal conductance, but also an increase in photosynthesis, thus improving water use efficiency (Paudel et al., 2018) and plant carbon status.

Plants, as sessile organisms, have evolved mechanisms to withstand environmental stressors (such as high temperatures) that affect plant growth (He et al., 2018). These stresses induce various physiological and biochemical responses in plants, including increased flavonoid synthesis, alterations in stomatal conductance, the expression of protective proteins (e.g., heat-shock proteins (HSPs) and antioxidants) and alterations in phytohormone levels (Fich et al., 2016; Wang et al., 2017; Goraya et al., 2017; Lippmann et al., 2019, Li et al., 2021). These stress responses are general and conserved among plants, as they protect against membrane injury, damage caused by reactive oxygen species (ROS), protein denaturation, and osmotic stress induced by multiple abiotic stresses (He et al., 2018). The regulation of these stress responses involves a complex network that encompasses various upstream signalling molecules, including ROS, hydrogen sulfide, and nitric oxide, and stress-related phytohormones such as abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) (He et al., 2018). These interactions establish

the downstream activation of various effector proteins, particularly transcription factors, to modify gene expression and regulate protein/enzyme activities, thereby initiating an appropriate response to the environmental stress imposed.

One strategy that plants have evolved for increasing stress tolerance is to associate with fungi via mycorrhizal symbiosis. In mycorrhizal associations, there is a mutual exchange of material, with plants providing carbon to mycorrhizal partners and fungi aiding in the uptake of water and various nutrients for the plant host (Ek et al., 1997; Genre et al., 2020; Tedersoo et al., 2020). This symbiotic exchange enhances plant growth and resilience to stressors such as elevated temperature or drought (Genre et al., 2020; Tedersoo et al., 2020). The enhanced resilience of mycorrhizal plants to environmental stresses can be attributed to the fungi's ability to modulate their host plants' responses to biotic and abiotic stresses. This modulation involves alterations in stress hormone responses, the induction of stress-alleviating genes, and the induction of stress tolerance mechanisms (Jajoo et al., 2021; Li et al., 2021). The ability of mycorrhizae to alter plant host hormone dynamics is intriguing, as the application of exogenous phytohormones can alleviate heat-induced damage and enhance plant heat tolerance (Wassie et al., 2020; Li et al., 2021). However, our understanding of how mycorrhizal associations alter plant hormone dynamics, especially under future elevated growth temperatures and rising CO₂ concentrations, is still unclear.

Three major plant hormones, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA), play crucial roles in mediating heat stress responses in plants. JA leads to the induction of

proteinase inhibitors against pathogens and herbivores (Yang et al., 2011; Verma et al., 2016), but also helps plants cope with abiotic stresses like heat and drought by triggering the production of secondary metabolites and defence-related proteins (Yang et al., 2018). Recent data suggest that JA can be induced under heat stress and contribute to plant thermotolerance (Sharma & Laxmi, 2015; Kumar & Verma, 2018, Li et al., 2021). Elevated CO₂ concentrations can either decrease or increase foliar JA levels (Ballhorn et al., 2011; Goa et al., 2012), suggesting a potential link between CO₂ levels and JA signalling. SA is vital for pathogen resistance by stimulating pathogenesis-related proteins (Verma et al., 2016) and promoting systemic acquired resistance. SA can also mitigate the adverse effects of heat shock by inducing protective antioxidant proteins (Shah et al., 2019; Wassie et al., 2020), improving plant water content, and enhancing antioxidant enzyme activities (Khan et al., 2015; Ogunsiji et al., 2022). Elevated CO₂ levels can increase SA concentrations by up to 50% (Casteel et al., 2012), indicating the potential use of SA in managing temperature extremes under elevated CO₂ concentrations (Lv et al., 2011; Khan et al., 2013; Haydari et al., 2019; Shah et al., 2019; Wassie et al., 2020). Lastly, ABA is essential in regulating stomatal closure, plant growth, and stress responses, making it a key player in understanding stress tolerance in plants (Verma et al., 2016). Elevated temperatures often lead to high VPD, resulting in increased leaf transpiration and reduced leaf water status, which triggers a rapid accumulation of ABA, in turn leading to stomatal closure and the activation of defence genes (Zhang et al., 1987; Brodribb & Cochard, 2009; Klein, 2014; Filho et al., 2018). The application of exogenous ABA enhances thermotolerance by boosting the activity of ROS-scavenging enzymes and antioxidants (Wang et al., 2023). *Arabidopsis* mutants with impaired ABA biosynthesis and signalling pathways show reduced thermotolerance, further supporting ABA's role in responding to elevated temperatures (Larkindale, et al., 2005). ABA is

also linked to stomatal responses to elevated CO₂ and VPD, suggesting its involvement in CO₂ sensing and signal transduction mechanisms (Merilo et al., 2018; Hsu et al., 2018). However, the long-term effects of elevated CO₂ on stomatal regulation and ABA-controlled drought responses require further investigation (Li et al., 2020; Zamora et al., 2021).

Studies evaluating the response of plant hormones to mycorrhizal fungi have shown strong interactions between mycorrhizal symbiosis and JA, SA and ABA dynamics. The mycorrhizal fungus *Laccaria bicolor* produces effector peptides that target the host's hormone dynamics, including JA, SA, and ethylene, during host plant colonization (Plett et al., 2014a; 2014b). In the interaction between *L. bicolor* and *Populus trichocarpa* Torrey & A. Gray, plant-produced flavonoids induce the expression of mycorrhiza-induced small-secreted protein 7 (MiSSP7), which is imported into the plant nucleus, where it interacts with JA-initiating proteins, suppressing the plant's JA immune response (Plett et al., 2014a). Additionally, genetic evidence from plants with defective ABA biosynthesis suggests a positive regulatory role for ABA in arbuscular mycorrhizal development (Herrera-Medina et al., 2007). In the case of *Medicago truncatula* Gaertn, mycorrhizal inoculation impacts ABA in a concentration-dependent manner mediated through Myc factors (Charpentier et al., 2014). ABA has also been observed to stabilize DELLA proteins in the presence of gibberellic acid (GA), suggesting a potential mechanism through which ABA may control arbuscular mycorrhizal symbiosis (Golldack et al., 2013; Lioa et al., 2018). Exogenous application of SA to *Oryza sativa* L. roots has been shown to reduce root mycorrhizal colonization, indicating that certain mycorrhizal fungi might suppress SA in host plants to enhance root colonization (Blilou et al., 2000). This suggests a complex role for SA in mycorrhizal symbiosis, which has yet to be fully resolved. By influencing these stress-

related processes, mycorrhizal fungi contribute to the improved ability of plants to cope with adverse environmental conditions. However, the degree to which mycorrhizae alter plant hormone dynamics under the warmer, high CO₂ conditions of the future is unknown.

Despite extensive research on stress phytohormone signalling pathways, our understanding of their role in promoting tolerance to climatic stresses remains limited, and the role that mycorrhizae play in modulating these hormone signals under climate change scenarios are limited. Therefore, studies are critically needed to evaluate the interactive effects of elevated temperature and CO₂ on mycorrhizal associations and their influence on plant hormone dynamics. To address this knowledge gap, I investigated the individual and interactive effects of elevated growth temperature and CO₂ concentrations on stress hormone concentrations in poplars grown with and without mycorrhizal partners, specifically focusing on JA, SA, and ABA. I hypothesized that:

- 1) In non-inoculated control trees, increased growth temperatures will induce the production of ABA, JA, and SA as a response to abiotic stresses. The highest concentrations of these stress hormones will be observed under the warmest growth condition.
- 2) Inoculated trees, when compared to non-inoculated trees, will have lower concentrations of plant stress hormones (JA, SA, and ABA) under warming and elevated CO₂ conditions due to mycorrhizal fungi reducing plant-induced stress hormones under climatic stresses.

- 3) Both inoculated and non-inoculated poplars grown under elevated CO₂ concentrations will have higher phytohormone concentrations due to increased carbon availability for phytohormone synthesis compared to those grown under current CO₂ levels.

4.2 Methods

4.2.1. Fungal Cultures and Maintenance

Mycorrhizae were obtained from the roots of trembling aspen trees (*Populus tremuloides*) in Cold Lake, AB, Canada (*Cenococcum geophilum* (NoF # 3129; GeneBank # MT276004) and *Hyaloscypha finlandica* (NoF # 3117; GeneBank # MT276006)) or the Forestry Culture Collection (*Paxillus involutus* (NoF # 2339) collected from *Pinus sylvestris*, Oslo, Norway, 1984. These fungal isolates were selected because they are ectomycorrhizal fungi that form beneficial associations with aspen roots (Garbaye & Churin, 1997; Luo et al., 2009; Alberton et al., 2010; Kipfer et al., 2012; Szuba et al., 2017). While *P. involutus* was not directly isolated from *P. tremuloides* like *C. geophilum* and *H. finlandica*, it has been found to form associations with a range of hardwood and coniferous tree species, including *Picea abies*, *Populus* spp., *Betula pendula*, and *Pinus contorta* (Wallander and Söderström, 1999; Jargeat et al., 2014; Sayyed & Hussain, 2020).

To culture the mycorrhizal isolates, the roots were surface sterilized and infected root pieces were transferred to modified Melin-Norkrans (MMN; Marx; 1969). agar plates. The fungal isolates were initially grown on these agar plates at room temperature in the dark for 18 days and then stored at 4 °C. They were later grown in liquid MMN media in sterile glass test tubes. A

cube of fresh fungal mycelium was added to the sterile media for inoculation, and the liquid cultures were grown for 21 days in the dark at room temperature on a rotary shaker.

4.2.2. Poplar Growth Conditions and Inoculation

Trees were grown as in Chapter 3. One-year-old *Populus x canadensis* clones from Somerville Seedlings in Everett, ON, Canada, were used for the plant growth experiments. These clones share a similar climate and photoperiod to London, ON, where the experiment was run. The poplar seedlings were cut into 30 cm pieces and surface sterilized with a 70% ethanol: 30% bleach solution. The cuttings were then rooted in sterilized trays filled with autoclaved Promix® (Premier Tech Horticulture, Riviere-du-Loup, QC) and slow-release fertilizer (Slow-Release Plant Food, 12N-4P-8K, Miracle-Gro, The Scotts Company, Mississauga, ON, Canada). They were placed in a climate-controlled glasshouse at the University of Western Ontario's Biotron Experimental Climate Change Research Centre in London, ON, Canada, where they were exposed to natural light intensity and maintained at 18°C for 4 weeks. The cuttings that developed new root tissue were either left uninoculated (as controls) or inoculated with one of the three mycorrhizal isolates (*Paxillus involutus*, *Cenococcum geophilum* or *Hyaloscypha finlandica*). Inoculation was done by placing an inoculated agar plug into the soil next to the roots and adding macerated liquid fungal suspension to the roots. The cuttings were then potted in 11.6-liter pots with autoclaved Promix®.

The pots with control plants and the three mycorrhizal treatments were randomly distributed across six glasshouses to study the effects of different climate conditions. There were six

different treatments, resulting in 24 combinations of mycorrhizal isolate and climate conditions. The climate treatments included ambient CO₂ (410 ppm) or elevated CO₂ concentrations (750 ppm) with either ambient temperature (0T), ambient temperature +4 °C (4T), or ambient temperature +8 °C (8T; n = 10 plants per fungal x climate treatment; N=240). These temperature treatments were chosen to represent a realistic range of future temperatures based on climate change scenarios. The 0T temperature treatment was determined from a five-year mean average hourly temperature for each day of the growing season (using data from 2012-2016) from the London, ON International Airport's meteorological station (Environment Canada). CO₂ concentrations were monitored and controlled using an infrared gas analyzer and an Argus control system (Argus Control Systems, Surrey, Canada) and were controlled by injecting pure CO₂ as needed to maintain the EC treatments. Daily temperature and CO₂ levels across all six glasshouses over the duration of my experiment have been previously reported by Murphy and Way (2021). Irradiance matched outdoor light conditions, varying with natural light conditions over the study period. Humidity was controlled at 60%, and seedlings were watered daily to maintain a moist growth medium.

4.2.3. Poplar Metabolic Extraction and Measurement

Poplar leaves were harvested on October 2, 2017 (after 5 months of growth), flash-frozen in liquid nitrogen to preserve phytohormone concentrations and stored at -80 °C. Phytohormones were extracted following a modified protocol from Forcat et al. (2008). Leaves from 3 trees per treatment (n = 72) were frozen using additional liquid nitrogen, ground using a mortar and pestle, and 200 mg of leaf tissue was placed into a 1.7 mL Eppendorf tube. Then, 800 µL of 10% methanol containing 1% acetic acid was added to each Eppendorf tube to extract the hormones.

Each treatment included an extraction control containing no plant material. Samples were vortexed for 1 min and placed on ice for 30 min, followed by centrifugation at 13,000 g for 10 min at 4°C. The supernatant was collected into a separate Eppendorf tube, the sample was re-extracted following the same procedure as stated above, and the 2 extracted supernatants were pooled. Pooled extracts were vortexed for 5 minutes, filtered (0.22 µm) to remove any particulate matter, and placed at -20°C until analysis.

A targeted analysis of poplar phytohormones was conducted using the following chemicals as standards: jasmonic acid (JA; Sigma-Aldrich), abscisic acid (ABA; Sigma-Aldrich), and salicylic acid (SA; Sigma-Aldrich). HPLC-MS (mass spectroscopy) was performed using a Thermo Scientific LTQ Orbitrap Discovery (MS 2.5.5) equipped with an Autosampler Accela AS 2.2.1, and pump 1.04.05. The instrument was equipped with a CORTECS C18+ column (Waters), 50 mm length, 2.1 mm ID, and 1.6 µm particle size, which was operated at room temperature. The injection volume was 10 µL. A solvent gradient was employed in this study with a flow rate of 0.4 mL/min. Solvent A was composed of acetonitrile (AcN) acidified with 0.1 vol% of formic acid whereas solvent B was composed of water acidified with 0.1 vol% of formic acid. The gradient was programmed as follows: solvent A 2 vol%, increased to 10 vol% at 2 min, increased to 25 vol% at 6 min, increased to 50 vol% at 10 min, increased to 75 vol% at 14 min, increased to 95 vol% at 18 min, decreased to 2 vol% at 20 min, followed by 2 min of isocratic elution with 2% of solvent A (total elution time 22 min). The LTQ Orbitrap MS was equipped with an electrospray ionization (ESI) source operating in positive ionization mode using the following operating parameters: electrospray voltage of 3.1 kV, sheath gas flow rate of 8 abu (arbitrary unity), an auxiliary gas flow rate of 1 abu, a capillary temperature of 270 °C, capillary voltage set

to 49.00 V, and tube lens offset at -148.43 V. Instrument calibration was performed externally before each run sequence, employing the Thermo Scientific Pierce LTQ Velos ESI positive ion calibration solutions. Accurate mass spectra of $[MM+H]^+$ ions were recorded from 100 to 1000 m/z, the mass resolution power of the mass analyzer was set to 30,000 (m/m) at m/z 400. Nitrogen gas (purity 99.95%) was used both as sheath gas and auxiliary gas to serve as the co-collision gas in the HCD cell and the bath gas in the C-trap.

4.2.4. Statistical Analysis

Non-mycorrhizal (non-inoculated controls) cuttings were analyzed using a two-way analysis of variance (ANOVA) to detect growth temperature and CO₂ concentration effects on poplar stress hormones; Holm-Sidak post-hoc tests were performed to identify significant differences among climate treatment means ($p < 0.05$).

To investigate the impact of mycorrhizal species on hybrid poplar foliar stress hormone concentrations, data are presented as percentage change from non-inoculated poplars as shown in Chapter 3 (Equation 3.1) in each CO₂ and temperature treatment. For all inoculated cuttings, a two-way ANOVA was performed to detect growth temperature and CO₂ concentration effects on each plant stress hormone for each poplar inoculated with mycorrhizal fungi; Holm-Sidak post-hoc tests were run to identify significant differences among treatment means ($p < 0.05$) This was necessary due to the uneven sample size across treatments (caused by mortality) and the fact that the assumptions of a three-way ANOVA were not satisfied. To see how the hormones (JA, SA, and ABA) are different between the inoculated and non-inoculated trees within each climate

treatment, a one-way ANOVA was performed; Holm-Sidak post-hoc tests were run to identify significant differences among treatment means ($p < 0.05$, Appendix 4.1-4.3). All analyses were performed using SigmaPlot version 13.0.

4.3. Results

4.3.1. Impacts of elevated temperature and CO₂ on non-inoculated, control poplar hormone concentrations

Significant growth temperature effects and a temperature x CO₂ interaction were observed on jasmonic acid (JA) leaf concentrations. Non-inoculated AC poplars had 180% higher JA levels under 8T conditions than 0T trees ($p = 0.01$, Table 4.1, Figure 4.1 A). In contrast, there was no temperature effect in the EC poplars for JA concentrations (Figure 4.1 B). Thus, JA levels were 95% higher in EC0T than AC0T trees, similar in AC4T and EC4T leaves, and 34% lower in 8TEC trees compared to 8TAC trees.

Poplar leaf SA concentrations were significantly higher in EC trees than in AC trees, largely due to the non-significant but 36% higher SA concentrations in the EC0T leaves compared to the AC0T leaves (Table 4.1, Figure 4.2).

Leaf concentrations of ABA rose as the growth temperature increased (Table 4.1). In AC poplars, ABA levels were up to 245% higher in 8T trees when compared to 0T trees. In EC poplars, there was a 350% higher ABA concentration in 8T trees than in 0T poplars (Figure 4.3).

Table 4.1. Effects of elevated CO₂ and temperature growth conditions on phytohormones in non-mycorrhizal poplars. Summary report of a two-way analysis of variance (ANOVA) in non-mycorrhizal poplars, indicating F-values and P-values (DF= 2 for all statistical tests) for the main effects of temperature (T) and CO₂ treatments(CO₂). Parameters analyzed were the concentrations of Jasmonic acid (JA), Salicylic acid (SA), and Abscisic acid (ABA), Bolded numbers represent P-values less than 0.05 (p <0.05).

	<i>T</i>		<i>CO₂</i>		<i>T X CO₂</i>	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
<i>Stress</i>						
<i>Phytohormones</i>						
<i>JA</i>	5.070	0.025	0.0857	0.775	6.941	0.010
<i>SA</i>	0.290	0.753	5.778	0.033	3.526	0.062
<i>ABA</i>	144.567	<0.001	0.562	0.468	1.495	0.263

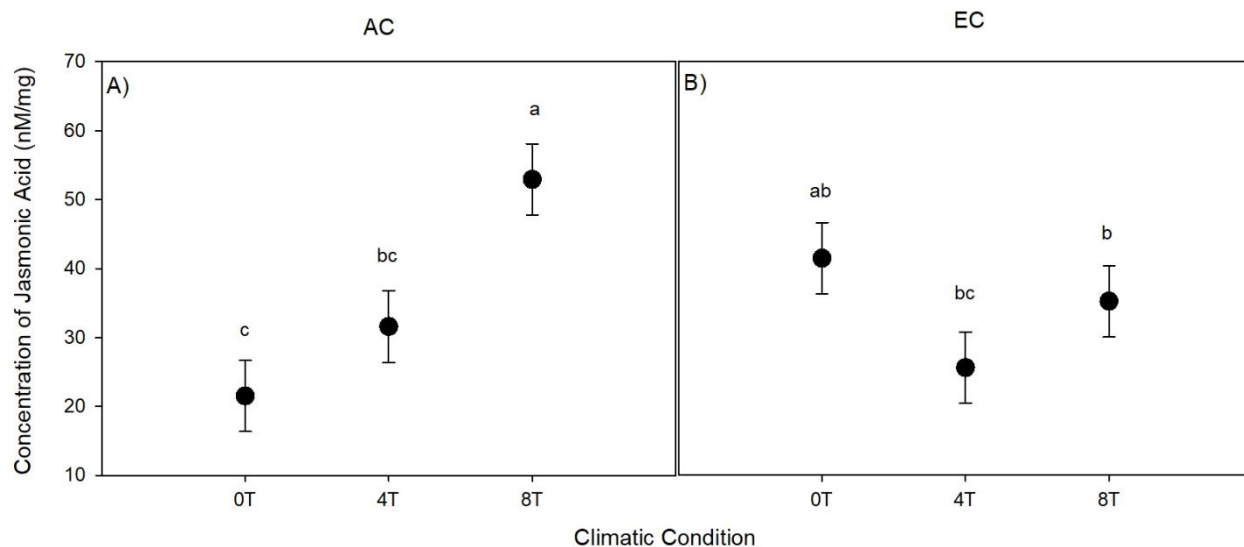


Figure 4.1. Foliar jasmonic acid (JA) concentrations response to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.

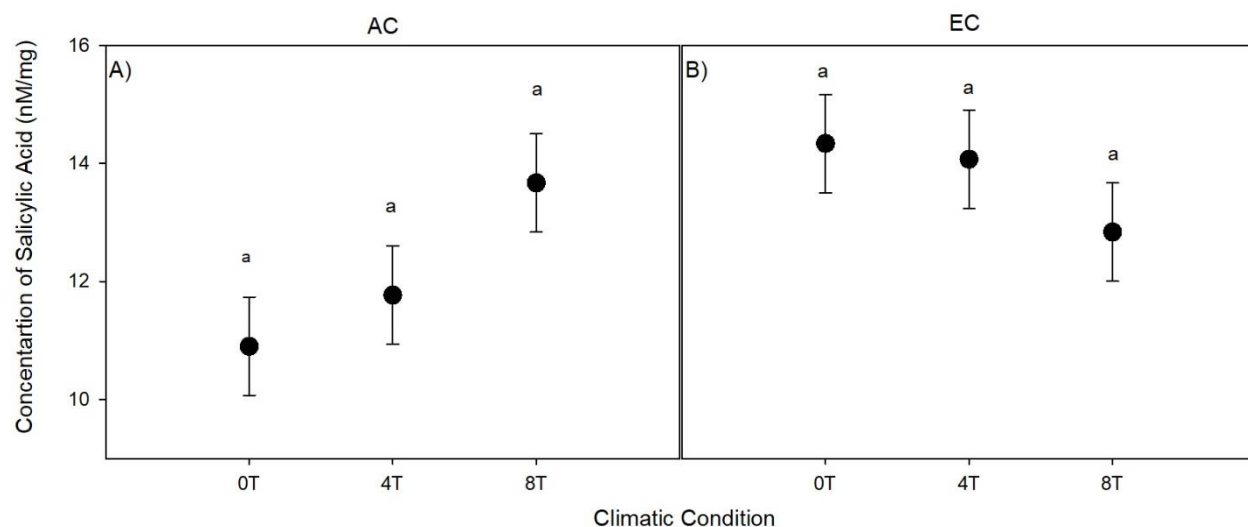


Figure 4.2. Foliar salicylic acid (SA) concentrations response to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.

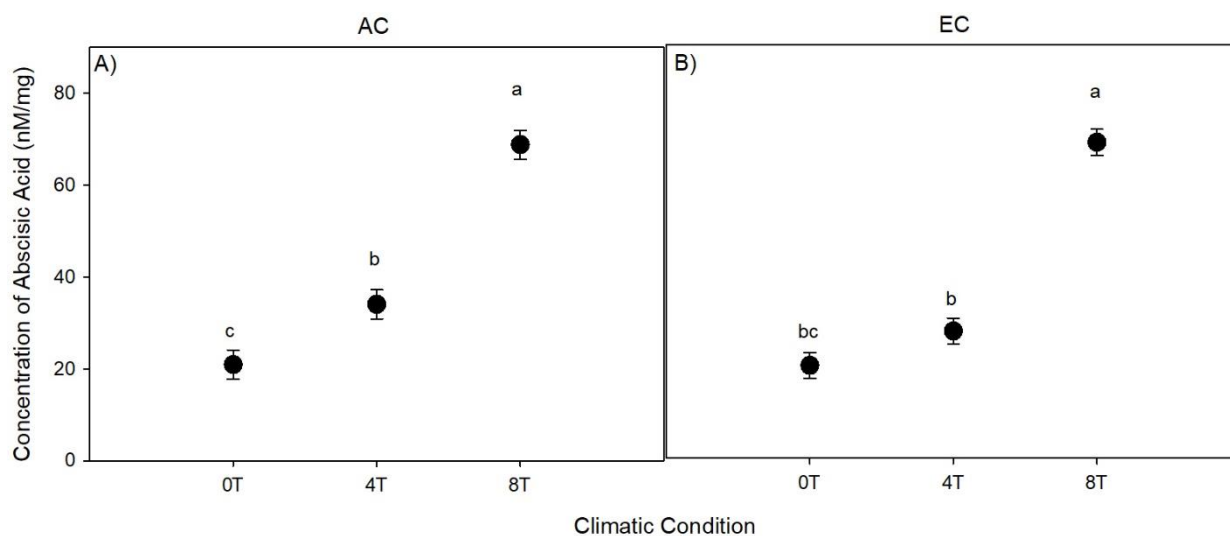


Figure 4.3. Foliar abscisic acid (ABA) concentrations response to A) ambient CO₂ (AC) and B) elevated CO₂ (EC) across temperature treatments in non-inoculated poplars. The box plot spans the first to the third quartile segmented by the median. Bars with different letters represent differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.

4.3.2. Impacts of future warming and CO₂ on stress hormone concentrations in inoculated poplar

Hormone levels in leaves from inoculated poplars were measured to investigate the influence of mycorrhizae on stress phytohormone concentrations under future climatic conditions. All data are presented as the percentage change from the non-inoculated controls within each treatment to emphasize the effects of the mycorrhizae within a specific climate treatment.

Inoculation with mycorrhizal fungi significantly impacted JA concentrations compared to non-inoculated trees within each climate treatment (Table 4.2). In all three mycorrhizal species, JA concentrations declined with increasing growth temperature in the AC poplars, with JA concentrations tending to be higher in the inoculated poplar than in the control trees (Figure 4.4). In the case of EC trees, poplars inoculated with any of the three mycorrhizal fungi had slightly lower JA concentrations than non-inoculated trees at 0T, but significantly higher JA concentrations than non-inoculated trees under warming treatments (Figure 4.4).

Table 4.2. Effects of elevated CO₂ and temperature on stress and growth hormones in mycorrhizal poplars. Summary report of a two-way analysis of variance (ANOVA) in mycorrhizal poplars, showing F values and P values (DF =2 for all statistical tests) for the main effects of temperature, CO₂, and fungal species (impact of fungal species on poplar growth) treatments. Parameters analyzed were the concentrations of Jasmonic acid (JA), Salicylic acid (SA), and Abscisic acid (ABA), Bolded numbers represent P-values less than 0.05 (p <0.05).

	<i>T</i>		<i>CO₂</i>		<i>T X CO₂</i>	
	F Ratio	P Value	F Ratio	P Value	F Ratio	P Value
<i>P. involutus</i>						
<i>JA</i>	6.519	0.012	0.482	0.501	21.995	<0.001
<i>SA</i>	3.807	0.052	5.954	0.031	6.008	0.016
<i>ABA</i>	62.747	<0.001	1.821	0.202	16.889	<0.001
<i>C. geophilum</i>						
<i>JA</i>	23.884	<0.001	0.101	0.757	32.731	<0.001
<i>SA</i>	0.848	0.452	4.472	0.056	0.895	0.434
<i>ABA</i>	14.871	<0.001	12.703	0.004	16.305	<0.001
<i>H. finlandica</i>						
<i>JA</i>	26.383	<0.001	0.903	0.361	10.179	0.003
<i>SA</i>	6.064	0.015	0.970	0.344	12.153	0.001
<i>ABA</i>	97.884	<0.001	3.198	0.099	1.318	0.304

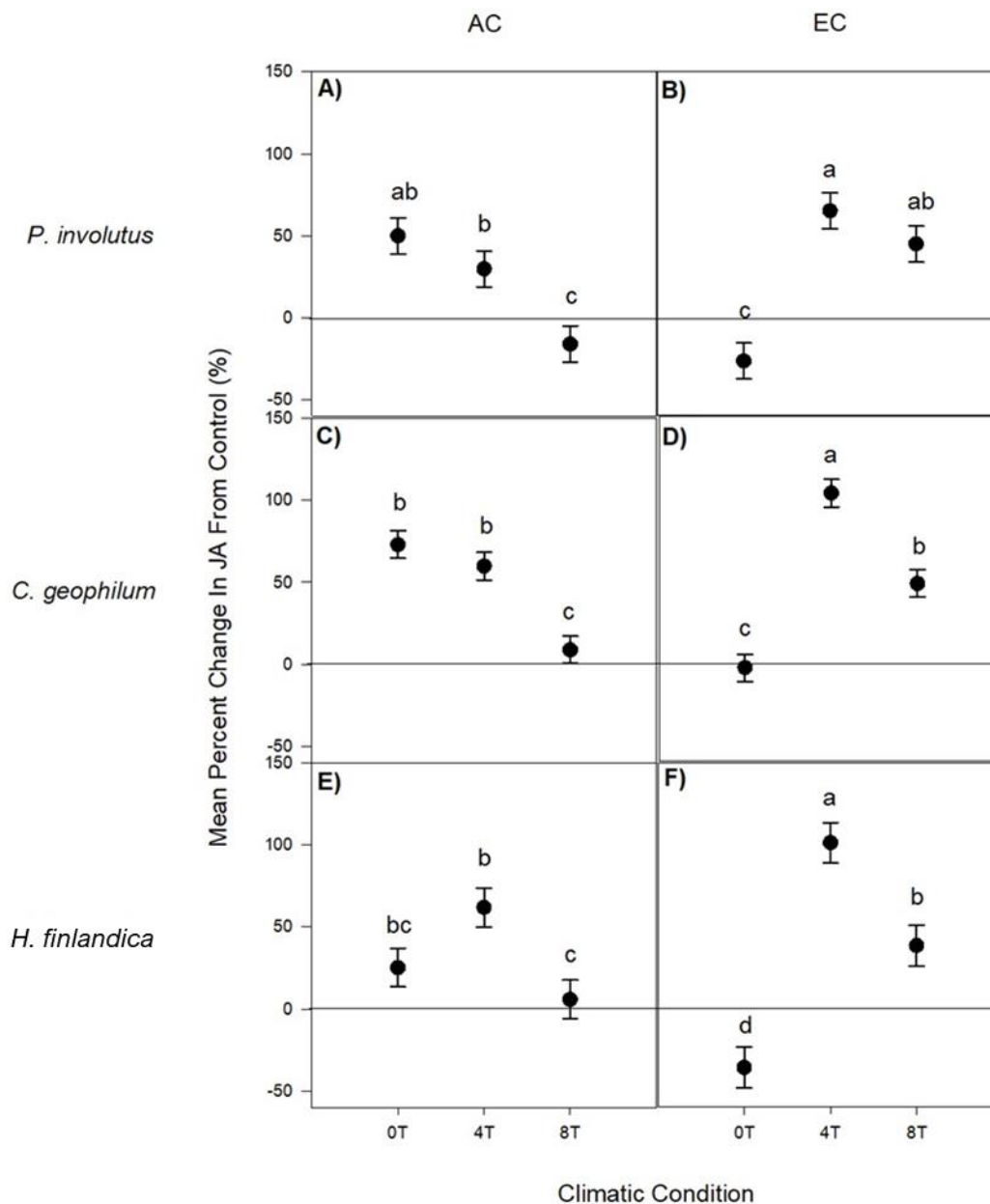


Figure 4.4. Effect of mycorrhizal inoculation on mean percent change in foliar jasmonic acid (JA) concentrations from the control trees across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum*, (C, D) or *Hyaloscypha finlandica* (E, F). Dots represent the Mean \pm SE around the mean percent change from control. Different letters on dots represents significant differences (Holm-Sidak post hoc test, $p < 0.05$), AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n = 3$.

The effects of future climate change on SA concentrations were significantly influenced by mycorrhizal inoculation (Table 4.2). Poplars inoculated with *P. involutus* or *C. geophilum* exhibited similar or up to a 50% reduction in SA concentrations across growth temperature and CO₂ treatments compared to non-inoculated poplars. The only significant CO₂ effect was observed in poplars inoculated with *P. involutus*, where plants grown under moderate warming conditions had lower SA levels in the EC treatment than in the AC treatment (Figures 4.5A, B). In contrast to the first two mycorrhizal fungi, poplars inoculated with *H. finlandica* had SA levels up to 100 times higher than non-inoculated poplar controls (Figure 4.5). Moreover, in poplars inoculated with *H. finlandica*, warming of +4°C had little effect on SA levels in AC plants (compared to the 0T plants), with additional warming to +8°C reducing the stimulation of SA concentrations induced by mycorrhizae. However, in the EC plants inoculated with *H. finlandica*, warming increased the stimulation of SA levels induced by mycorrhizal association (Figure 4.5).

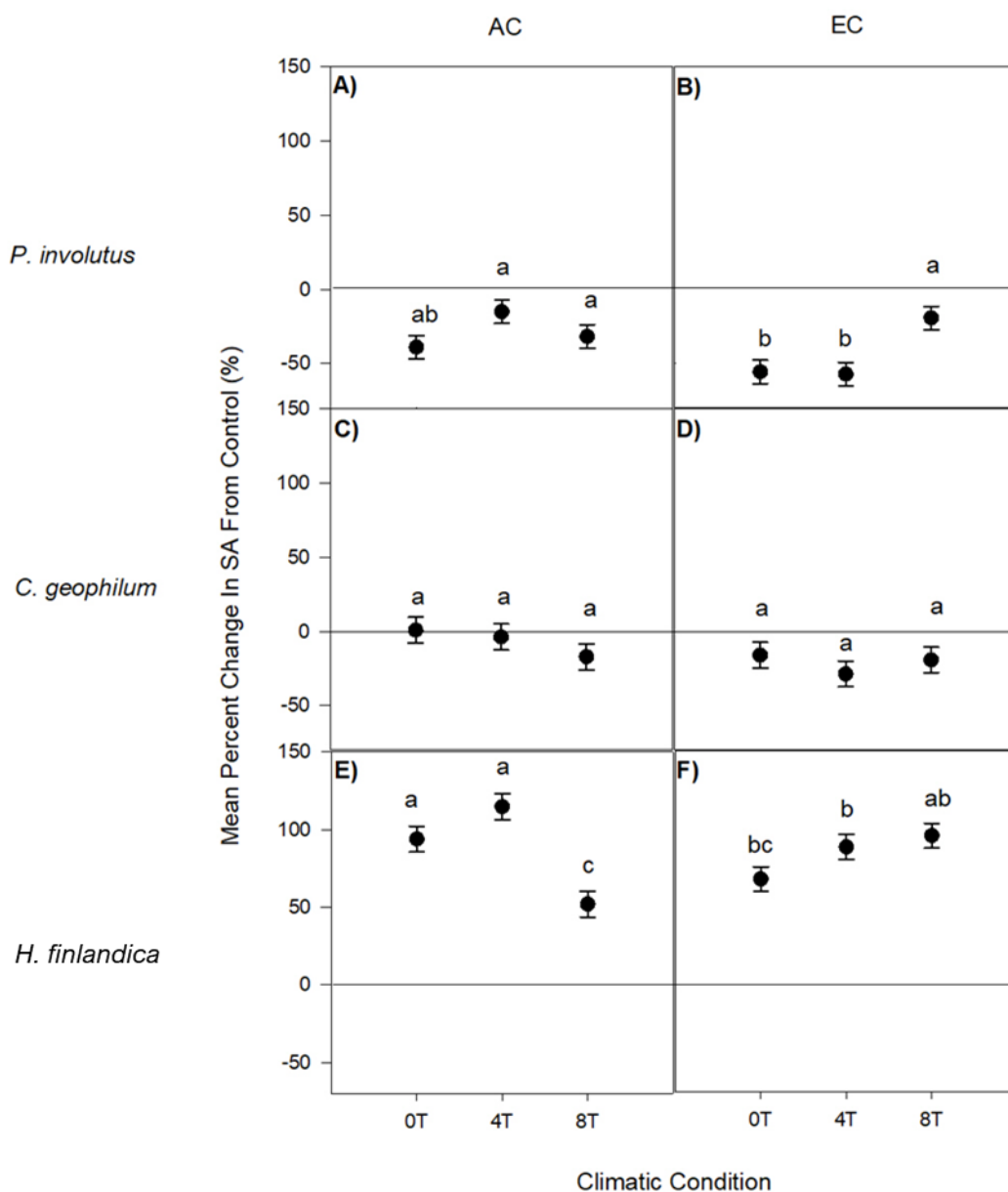


Figure 4.5. Effect of mycorrhizal inoculation on foliar salicylic acid (SA) across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum* (C, D) and *Hyaloscypha finlandica* (E, F), and mean percent change from control was determined. Mean \pm SE around the mean of percent change from control. Different letters on dots represents significant differences (Holm-Sidak post hoc test, $p < 0.05$), AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.

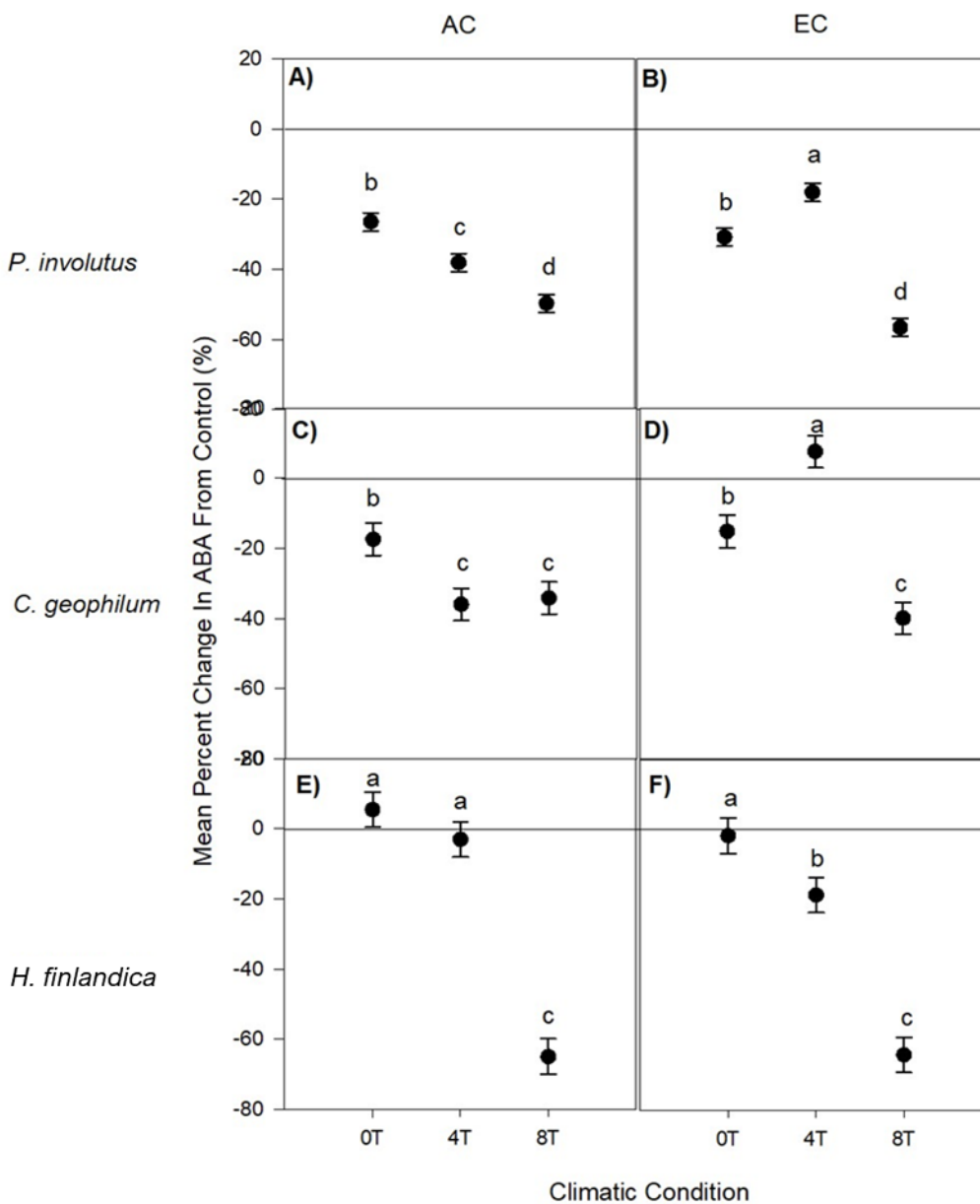


Figure 4.6. Effect of mycorrhizal inoculation on foliar abscisic acid (ABA) across the temperature and CO₂ treatments. Poplars inoculated with either *Paxillus involutus* (A, B), *Cenococcum geophilum*, (C, D) and *Hyaloscypha finlandica* (E, F) and mean percent change from control was determined. Dots represent the Mean \pm SE around the mean of percent change from control. Different letters on dots represents significant differences (Holm-Sidak post hoc test, $p < 0.05$), AC, ambient CO₂; EC, elevated CO₂; 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n = 3$.

ABA concentrations were affected by warming in all mycorrhizal species, with additional CO₂ and interactive effects seen within specific mycorrhizal treatments (Table 4.2). In general, poplars inoculated with any mycorrhizal species exhibited up to a 70% decrease in ABA leaf concentrations, particularly under extreme warming conditions, compared to non-inoculated poplar controls (Figure 4.6). Poplars inoculated with either *P. involutus* or *C. geophilum* showed similar patterns in the reduction of ABA leaf concentrations when grown under AC conditions, and a similar pattern in EC plants of suppressed ABA levels at 0T, less of a suppression of ABA levels at 4T, and a strong suppression of ABA levels at 8T. In contrast, trees inoculated with *H. finlandica* showed little effect of mycorrhizal inoculation on ABA levels at 0T, but an increasing suppression of ABA concentrations as the growth temperature increased (Figures 4.6E, F).

4.4. Discussion

This study explored stress hormone concentrations in hybrid poplar (*Populus x canadensis*) under varying growth conditions: growth temperature, CO₂ levels, and mycorrhizal inoculation. My results partially supported the hypothesis that elevated temperature and CO₂ would increase stress hormone levels in non-inoculated poplars. Leaf concentrations of JA (jasmonic acid) increased with warming in the AC trees, with AC poplars displaying a 180% increase in JA under +8T conditions compared to 0T trees. ABA (abscisic acid) concentrations also positively correlated with growth temperature in both AC and EC poplars, peaking at +8T. Additionally, SA (salicylic acid) concentrations were higher in EC trees. However, not all of the results supported the hypothesis. There were no temperature effects on JA concentrations in trees grown

in EC conditions or on SA concentrations in either CO₂ treatment. There were no overall CO₂ effects on JA or ABA levels.

The hypothesis that mycorrhizal inoculation of poplar roots would reduce stress hormone concentrations was also only partially supported. Inoculation generally increased JA concentrations relative to non-inoculated trees, but the effect diminished with higher temperatures (83% lower JA concentrations in inoculated 8T leaves compared to inoculated 0T and 4T leaves). EC trees inoculated with mycorrhizal fungi had slightly lower JA levels at 0T but higher concentrations of JA in the warming treatments. Mycorrhizal inoculation led to a reduction in SA levels, particularly in trees associated with *P. involutus* or *C. geophilum*, while *H. finlandica*-inoculated poplars showed exceptionally high SA levels. ABA concentrations were substantially affected by the mycorrhizal treatment, with inoculated poplars showing up to a 70% decrease in leaf ABA levels, especially under the +8°C warming conditions.

4.4.1. The Dynamic Responses of Stress Hormones in Poplars

The relationship between plant stress hormones, such as JA, ABA, and SA, and their induction under climatic stressors to sustain plant growth and development are integral to the plant stress response. Although JA, ABA and SA are considered stress hormones that are induced by biotic and abiotic stresses, each also plays a vital part in the regulation, maintenance and stimulation of plant growth, contributing to the observed variations in their responses to different treatments (Fujita et al., 2006; Wasternack C., 2007; Vicente & Plasencia, 2011; Yang et al., 2018; Ali et al., 2020). Jasmonates, known for their involvement in plant defence against pathogens and

herbivory, helps regulate plant growth, seed germination, abscission, tuber formation, and fruit ripening (Koetje, 2003; Davise, 2010; Yang et al., 2018; Ali et al., 2020). Furthermore, SA plays a vital role in conferring pathogen resistance but also promotes flower longevity, inhibiting ethylene biosynthesis and regulating seed germination (Wasternack, 2007; Davise, 2010; Verma et al., 2016). The role of ABA has been well established in regulating stomatal closure, plant development, growth and stress responses (Zhang et al., 1987; 2006; Davise, 2010; Zamora et al., 2021).

While these hormones each play an important role in plant development and physiological regulation, crosstalk between these plant hormone pathways in activating defence and growth responses under environmental stresses enables plants to finely adjust their physiological processes to maximize both growth and stress tolerance under ever-changing environmental conditions (Fujita et al., 2006; Yang et al., 2018). For example, it has been suggested that EC conditions favour heightened SA levels (Foyer & Noctor, 2020), which synergistically induce ABA stress responses and stomatal closure (Parwez et al., 2022). However, the findings of my study challenge this notion, revealing a nuanced interaction between rising temperatures, EC levels, and SA synthesis in poplar trees. This result demonstrates the contextual nature of plant responses to multiple environmental factors and how plants strike a balance through hormonal cross-talk to drive increased growth at high CO₂ levels while also maintaining water balance and reducing heat stress through elevated ABA and JA levels. Moreover, JA and SA represent pivotal plant hormones in defense responses against diverse pathogenic agents, entailing intricate crosstalk mechanisms that regulate their respective signalling pathways (Takahashi et al., 2004). Elevated levels of JA have been associated with an enhanced defensive mechanism against

herbivores and necrotrophic infections (Tamaoki et al., 2013). Conversely, greater levels of SA are widely recognized for their role in combating biotrophic pathogens (Tamaoki et al., 2013). The increase in JA concentrations observed in non-inoculated AC poplars under higher growth temperatures may signify an augmentation of defence mechanisms against herbivores and necrotrophic pathogens, potentially in response to perceived heat stress or to preserve as much undamaged tissue as possible to speed up recovery after heat shock. Conversely, elevated SA concentrations detected in EC poplars might hint at a strategic realignment of defence tactics, possibly reflecting an emphasis on safeguarding against biotrophic pathogens in the context of elevated CO₂ levels. The interplay between JA and SA under evolving climatic conditions could denote an intricate balancing act, where the plant allocates resources and prioritizes distinct defence strategies to counteract various stressors. Furthermore, the ABA and JA signalling pathways frequently collaborate to regulate plant responses to herbivory while concurrently influencing diverse aspects of plant growth and development (Per et al., 2018; Yang et al., 2018). This coordination between different stress hormone pathways allows dynamic regulatory responses to multiple environmental stresses. Moreover, it demonstrates the possible plasticity that these poplar hybrids have in adjusting to varying environmental conditions while concurrently optimizing growth and defence strategies in the face of climate change.

4.4.2. The Impact of Warming on JA, SA, and ABA Concentrations

Higher growth temperatures consistently elevate JA and ABA concentrations, while SA concentrations were largely unaffected by growth temperature under both AC and EC conditions. These results, corroborated by previous studies (Clarke et al., 2004; Clarke et al., 2009; Hu et al., 2013; Xu et al., 2016; Ruan et al., 2019; Su et al., 2021; Zhu et al., 2021), establish further

evidence of temperature-induced changes in stress hormone levels. Notably, Clarke et al. (2009) and Balfagón et al. (2019) reported a 1500% and 100% increase in JA concentrations, respectively, in *Arabidopsis* Col-0 grown at elevated temperatures. Jia et al. (2017) intriguingly detected no discernible difference in foliar JA concentrations in *Populus simonii* (Carrière) subjected to a 5°C increase above ambient conditions. This finding aligns with the lack of change in JA concentrations observed in AC poplars in the 0T and 4T treatments. This result, in conjunction with the findings of Jai et al. (2017), suggests the potential of a high-temperature threshold requisite for eliciting a protective effect of JA during heat stress in poplars. Conversely, Jai et al. (2016) observed a 25% reduction in foliar JA concentrations in the poplar hybrid *P. alba* x *P. tremula* var. *glandulosa* when exposed to a +5°C warming. The culmination of these studies underscores the critical significance of accounting for the dynamic JA response between different study plant species and their varying growth conditions.

Unexpectedly, the outcomes of my study indicate no impact of growth temperature on SA levels in non-inoculated poplars. This contradicts the findings of Jai et al. (2016), who observed a 25% decline in leaf SA concentrations in poplar hybrids subjected to elevated growth temperatures. Similarly, Balfagón et al. (2019) reported a significant 64% decrease in SA concentrations in *Arabidopsis* Col-0 under elevated temperatures. Although my findings did not show a temperature effect on SA levels in non-inoculated poplars, SA may still play a role in improving the thermotolerance of poplar hybrids. Treatment with exogenous SA can mitigate heat-shock-induced damage to growth and photosynthesis in plants (Wassie et al., 2020), and SA-pretreated leaves maintain high levels of SA and SA-induced heat-shock proteins during the recovery phase

after a heat-shock event, preserving photosynthesis and inducing thermotolerance (Li et al., 2021).

There exists a considerable body of research that supports the notion of ABA induction in response to elevated temperatures (Larkindale et al., 2005; Filho et al., 2018; Balfagón et al., 2019; Gietler et al., 2020; Li et al., 2021). For example, Balfagón et al. (2019) demonstrated a 25% increase in ABA concentrations in *Arabidopsis* exposed to 42 °C compared to those maintained at 23 °C. This induction aligns with the observed induction in the 8T poplars in my study. Intriguingly, the findings by Jai et al. (2016), where elevated growth temperatures did not increase ABA concentrations in hybrid poplars, stand in contrast to my results.

4.4.3. The Influence of CO₂ on JA, SA, and ABA on Poplars

Growth CO₂ levels had a nuanced effect on JA and SA levels and little effect on ABA levels in hybrid poplars. Under EC conditions, an increase in JA concentrations in 0T poplars was observed, with an EC modulating effect seen as growth temperatures rise. This modulation is accentuated by the revelation that EC8T trees have a lower JA content compared to AC8T trees, revealing how EC may reduce JA-induced stress responses. The complex relationship between EC concentrations and JA levels has yielded varying and sometimes contradictory outcomes across different studies. A study by Casteel et al. (2012) unveiled a diverse array of sensitivities of JA to EC concentrations among distinct soybean cultivars, exemplifying the intricate nature of this interaction. Some cultivars displayed up to a ~50% decrease in JA foliar content in response to EC exposure, while others exhibited no discernible effect on JA levels. Similarly, research

conducted by Guo et al. (2012) showed a ~20% reduction in JA levels in wildtype tomato plants subjected to EC, contrasting with the lack of change in JA levels observed in jasmonate-deficient mutants. Further, under EC conditions, SA foliar content increased in non-inoculated poplars, particularly in EC0T trees compared to AC0T trees. Similar patterns emerge in soybean cultivars grown under EC, where SA levels increased 50% across all six cultivars (Casteel et al., 2012). Likewise, in *Arabidopsis*, an increase in SA concentration under EC conditions was observed, contributing to the regulation of stomatal closure, as documented by He et al. (2020). While my results do not show a substantial CO₂ effect on ABA levels in non-inoculated poplars, the intricate relationship between EC and elevated ABA levels has been shown in prior studies (Chater et al., 2015; Hsu et al., 2018; Zamora et al., 2021). Nevertheless, the pronounced reduction in ABA concentrations under the EC8T treatment implies a potential synergistic impact of elevated growth temperature and the heightened vapour pressure deficit (VPD) often encountered in warmer conditions (Brodribb & Cochard, 2009; Klein, 2014; Filho et al., 2018), which EC conditions were not able to remediate, even when poplars were well-watered to prevent the induction of ABA in response to drought (Zhang et al., 2006; Gietler et al., 2020; Li et al., 2021).

4.4.4. The Effects of Mycorrhizal Inoculation on Stress Hormones

Mycorrhizal inoculation exerts a profound influence on stress hormone concentrations, yielding insights that unveil the complex interplay between plant-microbe interactions and environmental cues. The stimulation of JA levels in inoculated trees decreases with warming, suggesting an interactive effect between mycorrhizal inoculation and temperature. Inoculation with mycorrhizal fungi generally leads to increased JA concentrations, especially in 0T and 4T trees,

compared to non-inoculated trees. However, the magnitude of the response varies between different mycorrhizal species, with *P. involutus* eliciting a slightly lesser response than the others. Previous studies have shown that mycorrhizal plants have increased expression of JA biosynthesis genes under stress conditions, suggesting that JA plays a role in the plant's response to stress by activating defence-signalling pathways to protect the host from perceived stress. For example, tomato seedlings inoculated with *Funneliformis constrictus* showed a significant increase in JA synthesis, while those experiencing elevated growth temperatures showed similar JA gene induction to that of non-inoculated controls, which is what I found in inoculated poplar (Duc et al., 2018). In contrast to my results, colonization by *P. involutus*, resulted in the accumulation of the JA antagonist SA in root tissues of grey poplar (*Populus x canescens*) and a decrease in JA biosynthesis (Luo et al., 2009). However, it is worth noting that there are variations in the response of JA to mycorrhizal inoculation, as some studies have reported no significant change (Kebert et al., 2023) or even a decrease (Luo et al., 2009) in JA levels. The specific response of JA is likely dependent on the plant-fungus interaction and requires further investigation. Furthermore, EC can suppress the JA pathway (Noctor & Mhamdi, 2017; Foyer & Noctor, 2020). Moreover, it has been demonstrated that the effector protein MiSSP7 produced by the mutualistic ECM fungus *L. bicolor* targets JA-inducing proteins and transcription factors in the plant nucleus, leading to the repression of JA-induced gene expression and promoting fungal proliferation in plant tissues (Plett et al., 2014a; 2014b). While my results did not show a CO₂ effect on JA levels in leaves of inoculated poplars, the JA pathway is significantly inhibited by EC, resulting in higher susceptibility to biotrophic pathogens and herbivory (Zhang et al., 2015; Hu et al., 2020).

In the case of SA, mycorrhizal inoculation influenced the response to growth temperature and CO₂, resulting in reduced SA concentrations compared to non-inoculated trees (except for poplars inoculated with *H. finlandica*, which saw an increase in SA levels regardless of the CO₂ condition). The effects of growth CO₂ on SA concentrations are most pronounced in trees inoculated with *P. involutus* under moderate warming conditions. However, the effects of fungal inoculation on SA levels are varied in the literature, with some studies showing an increase in SA levels (Lopez-Raez et al., 2010) while others report no significant change (Kebert et al., 2023) or a decrease (Luo et al., 2009), a variation clearly demonstrated within my results. These discrepancies may be attributed to factors such as the specific fungal species, plant species, and stress conditions, which can modulate the SA response. It has been proposed that the response of plants to EC levels includes a transient rise in SA levels, which may serve as a shock response to induce stress genes rather than a prolonged influence (Noctor & Mhamdi, 2016). The precise mechanism by which EC modifies the SA pathway remains unclear, but it has been proposed that this influence is associated with EC's inhibitory effect on photorespiration, impacting the generation of reactive oxygen species (Noctor & Mhamdi, 2017; Williams et al., 2018). When plants are exposed to EC, the accumulation of SA in leaves is commonly observed in various plant species such as tobacco, soybean, tomato, *Arabidopsis*, common bean, and wheat, while barley does not exhibit this response (Casteel et al., 2012; Huang et al., 2012; Noctor & Mhamdi, 2016; Kazan, 2018).

Similarly, ABA concentrations are significantly affected by mycorrhizal inoculation, with inoculated trees exhibiting reduced ABA levels under extreme warming conditions, particularly in trees inoculated with *H. finlandica*. In hybrid larch (*Larix kaempferi* (Lambert) Carr. × *L.*

decidua Mill) inoculation with either *L. bicolor* or *C. geophilum* increased ABA foliar content by 25% in non-stressed conditions when compared to non-inoculated plants (Rincón et al., 2005). This contrasts with my study, where I saw a ~ 17% reduction in ABA leaf content in non-stressed hybrid poplars inoculated with *C. geophilum*. Moreover, the only poplars that showed a 10% increase in foliar ABA were those inoculated with *H. finlandica*. Taken together, this may suggest there is a species-specific ABA response to each mycorrhizal inoculum and requires further study. However, when the hybrid larch was exposed to osmotic stress, trees inoculated with *L. bicolor* or *C. geophilum* saw an increase in ABA concentrations by 33% and 10%, respectively, when compared to non-inoculated plants (Rincón et al., 2005). This again clearly contrasts with my results, which show that under a stress condition, inoculation with mycorrhizal fungi significantly reduces ABA concentrations in leaf tissues when compared to non-inoculated controls. This difference may be due to the different abiotic treatment stressors involved in eliciting an ABA stress response, with my study using elevated temperature and CO₂ concentrations with well-watered trees and Rincón et al. (2005) using polyethylene glycol to induce osmotic stress. Interestingly, ABA biosynthetic genes were transcriptionally downregulated in tomato roots inoculated with the arbuscular mycorrhizal fungus *Septoglomus constrictum* under drought however, it remained unchanged under non-stress and heat stress conditions (Duc et al., 2018). This may suggest that my poplars (experiencing higher VPD with warming) experienced a simulated drought, causing higher ABA within leaves to induce stomata closure and thus reduce water loss; however, inoculation with mycorrhizal fungi mitigates this effect. This was seen in both *Acer rubrum* L. (Bauerle et al., 2004) and sunflowers (Cardoso et al., 2020), with higher VPD-induced ABA concentration, resulting in stomatal closure.

4.4.5. Fungal Specific Responses: Exploring Variability in Poplar Hormonal Responses

The complicated interaction between mycorrhizal species and poplar tree stress hormones reveals how unique these symbiotic associations can be between mycorrhizal-partner species and host tree species. Each mycorrhizal species tested demonstrates distinct regulatory effects on poplar stress hormones, offering possible insights into the intricate balance of hormonal dynamics within the boreal ecosystem.

The observed variability in the responses of JA levels provides compelling evidence for the influence of mycorrhizal species on the regulation of stress hormones in poplar trees. Poplar inoculated with *P. involutus* or *C. geophilum* demonstrate similar JA responses to elevated temperatures, showcasing distinct patterns further influenced by varying CO₂ conditions. In particular both fungal species decrease JA foliar concentrations with increasing growth temperature under AC conditions, while maintaining relatively higher JA levels than non-inoculated trees. Under EC conditions, JA concentrations exhibit a unique response, initially lower than non-inoculated trees at 0T but significantly higher under warming treatments. A contrasting theme emerges with *H. finlandica*, which significantly increases JA concentrations in poplars, especially in 0T and 4T trees. This may suggest that *H. finlandica* could enhance poplar's resistance to thermal stress from climate change more than the other two mycorrhizal species (Clarke et al., 2009; Kebert et al., 2022). Concomitantly, poplars inoculated with *P. involutus* or *C. geophilum* elicit reductions in SA concentrations, while in sharp contrast, again, *H. finlandica* induces an increase in SA levels, which EC does not mitigate. These divergent effects shown by my study illuminate the intricate interplay between mycorrhizal species used to infect poplar roots and SA signalling, accentuating the potential of mycorrhizae as tools to alter

plant stress resilience under future climate change scenarios and management practices. For instance, the impact of elevated SA on enhancing resiliency against biotrophic and hemibiotrophic pathogens is widely recognized (Tamaoki et al., 2013; Ding & Ding, 2020). However, it has been proposed that mycorrhiza fungi (AM) may elicit a localized response mediated by a rise in SA to activate plant defence pathways in a process called mycorrhiza-induced resistance (Cameron et al., 2013). In my work with ectomycorrhiza, the activation of the mycorrhiza-induced resistance in host plants may be species-specific. For example, the mycorrhizal species *H. finlandica* may augment SA-induced resistance, while *P. involutus* or *C. geophilum* may diminish it, possibly resulting in reduced plant defence responses against pathogens. The pervasive effect of mycorrhizal species on diminishing ABA levels in poplars, particularly under warming conditions, was also consistent in trees inoculated with *P. involutus* and *C. geophilum* and may be related to the ability of mycorrhizal species to provide trees with increased access to water (Ek et al., 1997; Genre et al., 2020; Kakouridis et al., 2022).

4.5. Conclusion

My results demonstrated that elevated growth temperatures generally increased JA and ABA concentrations, while SA concentrations remained unaffected by temperature in non-inoculated trees. The interaction between temperature and CO₂ levels also influenced stress hormone concentrations, particularly in high CO₂ conditions. Furthermore, mycorrhizal inoculation had a significant impact on stress hormone concentrations, with varying effects depending on the mycorrhizal species. Specifically, mycorrhizal inoculation generally increased JA concentrations, but the stimulatory effect diminished with increasing growth temperature. SA

levels were reduced in response to mycorrhizal inoculation under elevated temperature and CO₂ conditions, although some mycorrhizal species showed exceptions to this trend. ABA concentrations were also affected by mycorrhizal inoculation, with decreased levels observed under extreme warming conditions. These findings collectively highlight the intricate interplay between mycorrhizal associations, climate conditions, and stress hormone dynamics in plants.

My findings contribute to our understanding of how environmental factors and mycorrhizal symbiosis influence stress hormone signalling in poplar trees. The results suggest that higher temperatures and EC levels can induce stress hormone synthesis in poplars, which may play a role in their response to environmental stress. Furthermore, mycorrhizal inoculation can modulate stress hormone concentrations, potentially enhancing the stress tolerance and resilience of the trees. Overall, my study emphasizes the importance of considering multiple environmental factors and the influence of mycorrhizal symbiosis when studying stress hormone dynamics in plants. Further research is needed to elucidate the underlying mechanisms and to explore the potential applications of these findings in improving the stress tolerance of crops and woody plants in the face of changing environmental conditions.

4.6. References

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Chapter 5

5 General Discussion

5.1. Introduction

Climate change poses significant challenges to the sustainability and health of the Canadian boreal forest (Price et al., 2013; Worrall et al., 2013; IPCC, 2023). These forests are vulnerable to climate change, including changes in temperature, precipitation, and atmospheric carbon dioxide (CO₂) levels (Stocker et al., 2013; Marchand et al., 2019; Day et al., 2020; Enbrecht et al., 2021; Berner & Goetz, 2022). Moderate increases in temperatures and CO₂ concentrations can increase photosynthesis and carbon fixation leading to growth promotion in trees (Dusenge et al., 2019). However, extreme warming can suppress photosynthesis and stimulate respiratory carbon loss, leading to decreased growth and higher tree mortality (Dusenge et al., 2019).

Mycorrhizal fungi that associate with plant roots significantly improve plant nutrient availability and water relations (Huey et al., 2020). Mycorrhizal fungi can alleviate abiotic stresses like heat stress, facilitate plant stress signalling, and could promote plant resilience and resistance to climate change (Bonfante & Genre, 2010). The genus *Populus* includes several species that can serve as models for understanding the complex interactions between plants and mycorrhizal fungi (Liu et al., 2014) in shifting climatic conditions. Elevated temperatures and CO₂ concentrations may enhance the plant-mycorrhizal relationship, resulting in enhanced nutrient and water uptake and improved stress resilience (Nadeau, 2015; Sharma et al., 2022).

Despite growing concerns regarding the impacts of climate change on boreal forests, the understanding of how mycorrhizal symbiosis may mitigate these effects on tree species remains largely underexplored. This thesis investigates the impact of elevated CO₂ and temperature on

the resilience of *Populus* hybrid (*Populus x canadensis*) trees with and without mycorrhizal inoculation, which is an unexplored area. In light of anticipated climatic shifts, the principal objective was to assess the influence of mycorrhizal associations on the growth, metabolic profiles, and stress tolerance of poplar trees. Moreover, this study aimed to document the chemicals released by various mycorrhizal fungi, examining their capacity to enhance plant development.

5.2. Thesis Summary

In Chapter 2, I discussed how 14 different types of mycorrhizal fungi released substances such as plant hormones, amino acids and organic acids. While most of these secretions generally supported plant growth, some had varying effects, like stunting *Arabidopsis* growth or causing plant death, showcasing the impacts of these fungal secretions on plants.

As revealed in Chapter 3, I explored the impacts of warming and elevated CO₂ concentrations on the growth and health of inoculated and non-inoculated poplar trees with mycorrhizal fungi. I found that elevated CO₂ conditions (EC) resulted in taller, non-inoculated trees with increased biomass while warming alone did not significantly affect tree height or growth under ambient CO₂ (AC) conditions. However, warming facilitated mycorrhizal colonization, especially in EC conditions. Mycorrhizal inoculation produced mixed effects; it promoted tree height in AC conditions with warming, but had a minimal impact under EC. Interestingly, inoculation generally increased mortality, except for EC-grown trees inoculated with *P. involutus*, which had reduced mortality.

In Chapter 4, I investigated how elevated temperature and CO₂ levels influenced stress hormone concentrations in poplar trees, with a specific focus on jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). My findings showed that both temperature and CO₂ levels affected hormone concentrations, with notable variations in response to climate conditions and mycorrhizal inoculation. Generally, EC led to higher JA levels without a temperature influence, while AC conditions had a temperature-dependent increase in JA concentrations. SA concentrations were higher in EC trees, and ABA levels increased with temperature in both CO₂ conditions. Mycorrhizal inoculation significantly affected hormone concentrations, often modulating the apparent stress response in inoculated trees. For example, inoculated trees typically showed lower ABA levels under warming conditions, suggesting a potential regulatory role of mycorrhizal fungi in mitigating stress responses.

5.3. Impacts of Climate Change on Poplar Growth and Survival

Higher growth temperatures can lead to substantial declines in productivity, increased stress levels, decreased photosynthesis rates, and a rise in tree mortality in boreal tree species (D'Arrigo et al., 2004; Juday & Alix, 2012; Girardin et al., 2016; Cahoon et al., 2018; Nicklen et al., 2018; Trugman et al., 2018; Hartmann et al., 2022). My findings on a hybrid *Populus* spp. align with these broader observations. I observed worsening leaf health (greater degree of chlorosis and leaf scorching) and higher mortality with increased temperatures under ambient CO₂ conditions, alongside increased concentrations of JA and ABA, which are indicative of heightened plant stress and mortality risk (Verma et al., 2016). My work also demonstrates that *Populus x canadensis* growth is unaffected by temperature under AC (within the bounds of the

temperatures used here), even though *Populus* growth is usually stimulated by rising temperatures (Sivadasan et al., 2018). For example, *Populus tremuloides* exposed to a 5°C warming exhibited a 22% increase in height and an 84% increase in biomass (Way et al., 2013).

The observed lack of a temperature-growth response in my trees might be attributed to the 'growth-defence trade-off,' where resources diverted to defence, particularly through the induction of JA and ABA under AC conditions, could limit growth by enhancing secondary metabolite synthesis and cellular protection at the cost of growth (Coley et al., 1985; Herms & Mattson, 1992; Huot et al., 2014; Sharma & Laxmi, 2016; Kumar & Verma, 2018; Yang et al., 2019; Ruan et al., 2019; Li et al., 2021; Zhu et al., 2021). Under EC conditions, despite increased growth and unchanged leaf health, higher CO₂ did not mitigate the elevated mortality due to warming. This scenario suggests a nuanced interaction between increased ABA, indicating stress tolerance, and decreased JA, which could diminish protective responses against warming, influenced by CO₂'s variable effects on JA levels (Ballhorn et al., 2011; Goa et al., 2012). Thus, JA and ABA play key roles in climate stress adaptation, with SA's impact on biomass or leaf health appearing minimal, without implying direct causality.

Hardwood trees, such as *Populus*, may utilize the ABA-mediated stress pathway under future warming to reduce stomatal conductance when faced with increased high vapour pressure deficit (VPD), thereby maintaining leaf water content and minimizing xylem cavitation at the expense of gas exchange and carbon fixation (Siau, 1971; Hogg et al., 2000). The loss of carbon sequestration and the synthesis of primary metabolites needed for maintenance and growth

increase mortality risk in many boreal forest trees, including *Populus* spp. (Hogg et al., 2000; Hogg et al., 2008; Brandt, 2009; Wotherspoon et al., 2023). Thus, the induction of the JA pathway may be a secondary protective pathway working in tandem with the ABA pathway to induce heat shock proteins and antioxidant defence systems to protect cellular proteins, lipids and DNA from warmer temperatures (Devireddy et al., 2021; Pérez-Llorca, et al., 2023). It is also possible that carbon starvation, the inability to supply enough carbon to maintain hydraulic function and other metabolic processes, may contribute to tree mortality under warming conditions. This is best illustrated by Zeps et al. (2017), who found that a 4°C increase in growth temperature was associated with reduced growth and a 35% decrease in tree seedling survival. Furthermore, a study in *Larix laricina* seedlings showed that extreme warming of 8°C increased mortality by 40% (Murphy & Way, 2021). While elevated growth temperatures and higher atmospheric CO₂ concentrations have been associated with increased growth rates of many boreal tree species during the first 50 years of life (Bigler & Veblen, 2009), this accelerated growth comes at a trade-off in terms of life expectancy and greater mortality (McDowell et al., 2018, 2020). For example, *Abies lasiocarpa* (Hooker) Nuttall, *Picea engelmannii*, and *Picea abies* have experienced reduced lifespans of 35%, 30%, and 47%, respectively, due to the effects of increased growth (Bigler and Veblen, 2009). It has also been suggested that the depletion or loss of carbon or water pools and fluxes can further compromise defensive functions against biotic attack, exacerbating physiological stress and damage to tree tissues, resulting in significant mortality (Hogg et al., 2000; 2008; Murphy & Way, 2021).

Elevated CO₂ concentrations from human activities have significant implications for forest ecosystems, particularly in terms of tree mortality (Pugh et al., 2020). However, existing global

models exhibit considerable variation in their incorporation of mortality, leading to uncertainties in projecting the future ecology and functioning of forest communities (Yu et al., 2022). In plant ecology, survival-growth trade-offs are well-established (Bigler & Veblen, 2009; Brienen et al., 2020; Maschler et al., 2022). It is commonly accepted that higher plant growth rates under EC should coincide with an increased risk of mortality (McDowell et al., 2018, 2020). This negative correlation can be attributed to several underlying mechanisms, including increased susceptibility to wind damage due to reduced wood density, reduced investment in chemical defences, and heightened vulnerability to environmental stressors (Maschler et al., 2022). The larger plant size resulting from EC may also contribute to hydraulic failure and limit stress adaptation options, further impacting survival rates (McDowell et al., 2008; Bigler & Veblen, 2009; Mantova et al., 2022). Rising CO₂ levels allow plants to reduce stomatal conductance without negatively affecting photosynthetic rates, enhancing photosynthesis during non-drought periods (Maschler et al., 2022). However, reduced evaporative cooling during heat events can lead to early leaf senescence and tissue damage. EC may also promote carbon allocation to root production over aboveground growth or facilitate the regeneration of damaged tissues following heat or drought stress (Pan et al., 2017; Maschler et al., 2022). The increased damage to poplar leaves under extreme warming, especially in AC conditions, may reduce canopy photosynthesis, resulting in lower carbon availability and higher mortality. Additionally, plants grown under elevated temperatures typically experience higher VPD, resulting in increased evapotranspiration (Massmann et al., 2019). Thus, a trade-off between water conservation and carbon gain becomes an issue for plants grown under elevated temperatures as they regulate stomata to balance water loss with carbon assimilation required for growth.

In my study, EC poplars should better balance water loss with carbon assimilation than their AC counterparts, as there is more CO₂ available for plants to utilize. Moreover, net CO₂ assimilation rates often increase under moderate warming, especially under EC, resulting in more carbon availability and enhanced growth (Centritto et al., 2011; Way et al., 2013). However, as demonstrated in *Populus nigra* (one of the hybrid parents of *P. x canadensis*), elevated growth temperatures also lead to higher respiration and photorespiration rates relative to photosynthetic rates, resulting in increased carbon loss (Centritto et al., 2011). It is proposed that, under both CO₂ conditions in my study, increasing growth temperatures and VPD resulted in greater carbon loss through respiration, photorespiration and resource diversion to JA-ABA pathways to mitigate stress than was gained through photosynthesis, resulting in no enhanced growth in AC trees and no reduction in mortality in EC trees under elevated temperatures.

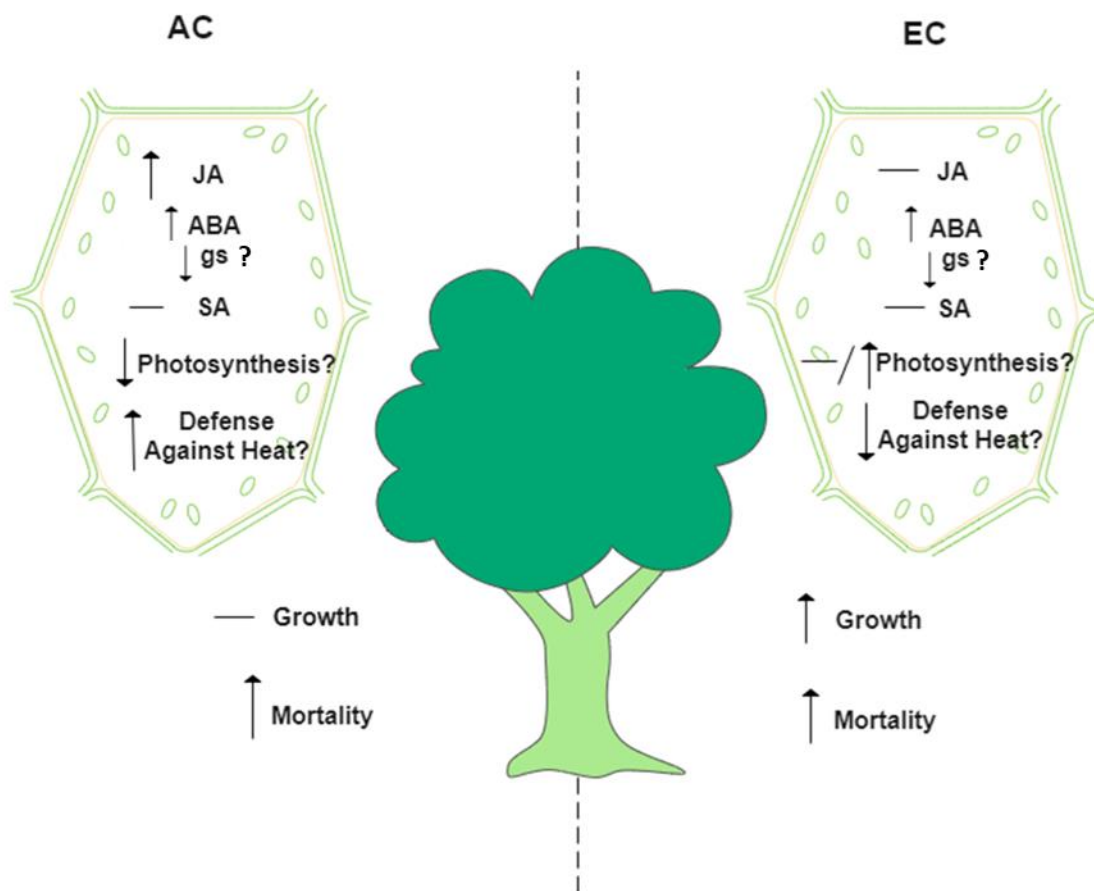


Figure 5.1. Schematic representation of the impact of warming on poplar growth, mortality, and stress hormone concentrations with their proposed effects on photosynthesis and defence against heat stress in mesophyll cells under different CO₂ conditions (AC= ambient CO₂ and EC= elevated CO₂). Up arrows indicate an increase in a given parameter, down arrows indicate a decrease in a given parameter, a straight horizontal line shows no change in a given parameter, and a question mark indicates results seen in the literature but not measured in my work. JA- jasmonic acid, SA-salicylic acid, ABA-abscisic acid; g_s-stomatal conductance.

5.4. Impacts of Mycorrhiza on Poplar Growth and Survival Under Climate Change

Given that most terrestrial plant species form mycorrhizal associations (Brundrett et al., 1996; Bonfante & Genre, 2010), understanding the implications that climate change will have on mycorrhizal fungal abundance and their ability to promote plant growth is imperative.

Mycorrhizal inoculation positively influenced hybrid poplar growth and leaf health indices under warming and elevated CO₂ conditions, supporting results from other plant species (Landhäusser et al., 2002; Kreuzwieser & Gessler, 2010; Alberton et al., 2010; Leppälampi-Kujansuu et al., 2013; Liu et al., 2014). This growth promotion can be attributed to several mycorrhizal-mediated processes, including induction of heat shock proteins, increased access to nutrients and water, and dampening of the plant's physiological heat stress response pathways (Landhäusser et al., 2002; Huang et al., 2014; Pozo, 2015). The alteration of stress hormones under stress conditions, such as the reduction in ABA concentrations under stress conditions, further supports the plant's ability to maintain or even enhance growth by mitigating the physiological constraints typically imposed by abiotic stresses. The decrease in ABA concentrations observed in my inoculated trees, for example, suggests a reduction in water stress, which may allow trees to allocate more resources to aboveground growth under AC and warming conditions, as documented in Chapter 3. Elevated CO₂ levels increase carbon fixation, leading to increased root exudation and potentially fostering greater mycorrhizal symbiosis (Bowes, 1991; Dusenge et al., 2019). This mechanism may account for the augmented mycorrhizal colonization and plant growth under EC conditions, as detailed in Chapter 3. However, under EC, greater mycorrhizal colonization of tree roots seems to increase tree mortality even though biomass may increase. Moreover, similar patterns between the three mycorrhizal fungi regarding their impacts on foliar stress hormone concentrations and biomass or mortality were determined. Inoculation with any of the three

mycorrhizal fungi resulted in a positive correlation between JA and ABA as biomass increased, especially under +8°C warming conditions. This may suggest that the mycorrhizal fungi can augment tree stress hormone pathways, protecting photosynthetic machinery, reducing heat damage and improving plant resiliency to other potential biotic or abiotic stresses.

Soil fungi are typically limited by moisture, and drought can suppress their abundance (Tedersoo et al., 2014). However, some soil fungi, including mycorrhizal fungi, may be less affected by drought due to their hyphal structures, which can store water and explore surrounding areas for moisture (Meisner et al., 2018). Mycorrhizal fungi can also synthesize or stimulate plant production of ABA (Martín-Rodríguez et al., 2016), a stress hormone that regulates stomatal conductance. If mycorrhizal fungi increase ABA levels, stomatal closure occurs, leading to water conservation, which benefits plant growth (Ludwig-Müller, 2010; Begum et al., 2019), but also soil fungi. Based on my work, the interactive effects of elevated temperature and CO₂ on mycorrhizal plant growth promotion seem to be synergistic, with the impacts of EC on growth promotion being masked by the growth-promoting effects of moderate temperature.

While considerable research has focused on the direct hormonal responses of plants to abiotic stresses, there has been relatively little investigation into the association between plants, their fungal symbionts and the plant hormonal response to stress (Singh et al., 2011; Dastogeer, 2018). These fungi, both aboveground and belowground, play a significant role in modifying plant responses to stress and can be used to improve plant stress tolerance and resiliency, thus increasing growth and productivity. In leaves of *Quercus robur* trees inoculated with

Scleroderma citrinum, no changes were observed in foliar JA levels, but SA levels increased by 131%, and ABA levels increased by 200% compared to non-inoculated trees (Kebert et al., 2023). Furthermore, in the hybrid poplar species *Populus x canescens*, inoculation with mycorrhizal fungi resulted in a 37% increase in SA concentrations and a 92% increase in ABA concentrations, while JA concentrations decreased by 47% (Luo et al., 2009). These studies indicate that inoculation with mycorrhizal fungi can increase SA levels, similar to my results with hybrid poplars inoculated with *H. finlandica*. Conversely, the impact of fungal inoculation on JA levels demonstrates variability, with some studies aligning with my findings of an elevation in JA levels (Lopez-Raez et al., 2010), whereas others observe no significant alteration (Kebert et al., 2023) or a reduction (Luo et al., 2009), highlighting the dynamic nature of JA response pathway. Moreover, some studies reveal contrasting effects of fungal inoculation on ABA levels. In some cases, inoculation increases ABA concentrations (Luo et al., 2009; Kebert et al., 2023), while in others, no significant change is observed (Lopez-Raez et al., 2010). A different trend was observed in my study, which shows that inoculation with mycorrhizal fungi reduces endogenous ABA in poplars. The variation in ABA responses may be influenced by specific fungal species, plant species, and even stress conditions.

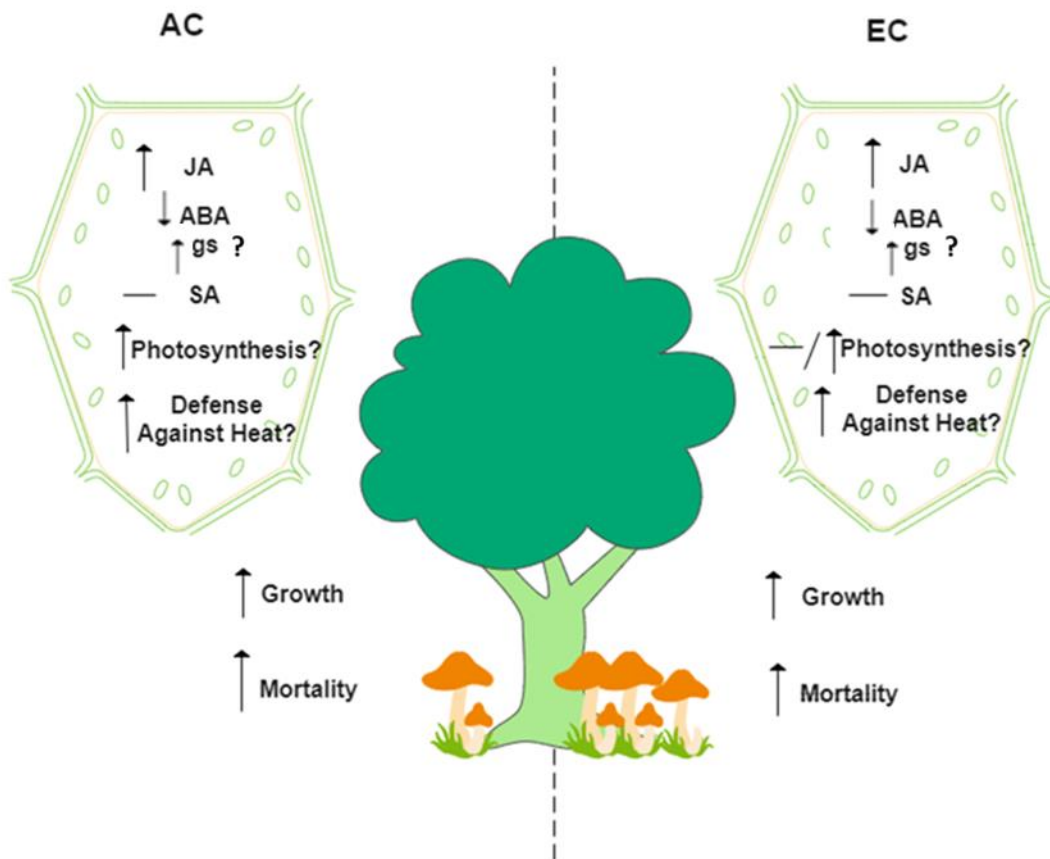


Figure 5.2. Schematic representation of the impact of warming on mycorrhizal poplar growth, mortality, and stress hormone concentrations with their proposed effects on photosynthesis and defence against heat stress in mesophyll cells under different CO₂ conditions (AC= ambient CO₂ and EC= elevated CO₂). Up arrows indicate an increase in a given parameter, down arrows indicate a decrease in a given parameter, a straight horizontal line shows no change in a given parameter compared to the non-inoculated trees, and a question mark indicates results seen in the literature but not measured in my work. JA- jasmonic acid, SA-salicylic acid, ABA-abscisic acid; g_s-stomatal conductance.

5.5 Stress Hormone Crosstalk

Although not directly assessed, my work highlights the complex interplay and potential crosstalk between the SA, JA, and ABA pathways under elevated temperature and CO₂. Optimizing and harmonizing defence mechanisms are essential for the well-being of plants. Hormone crosstalk, which refers to the interaction between distinct hormone networks, is crucial in maintaining this equilibrium (Aerts et al., 2021). It has been well-established that JA and SA often are antagonistic (Kazan & Manners, 2008; Yang et al., 2019; Nguyen et al., 2022; Li & Ahammed, 2023). Moreover, under EC conditions, there is an increased antagonistic interaction between the SA and JA pathways, leading to the suppression of JA signalling (Casteel et al., 2012; Huang et al., 2012; Kazan, 2018). However, this idea has weakened with evidence suggesting that JA and SA concentrations can be synergic when faced with physiological stress (Ullah et al., 2022), which my results support in both inoculated and non-inoculated trees. Moreover, it is clear from my experiments that ABA and JA positively correlate, indicating a potential synergistic effect under warming for both AC and EC conditions. This suggests that ABA and JA work together through a common stress response pathway, potentially increasing thermal tolerance and reducing the effects of climatic stresses (Kazan & Manners, 2008; Yang et al., 2019; Nguyen et al., 2022; Li & Ahammed, 2023).

The hormones ABA and SA are antagonistic both in terms of plant stress and growth responses, with the exception of viral resistance, in which they may act synergistically (Moeder et al., 2010; Alazem et al., 2019; Aerts et al., 2021). In my work, non-inoculated poplars grown under AC conditions showed an ABA-SA additive response to warming, while following the expected

antagonistic response under EC conditions. Moreover, inoculated poplars showed a positive correlation between ABA and SA levels under warming for both CO₂ conditions. It has been suggested that SA and ABA stress response pathways can integrate using the Ca²⁺-dependent protein kinases to regulate stomata (Alazem et al., 2019). It is possible that elevated temperature and inoculation with mycorrhizal fungi encourage the integration of the SA and ABA pathways to improve stomal efficiency to help conserve water under high VPD conditions or increase gas exchange to maintain carbon assimilation rates for plant growth and maintain mycorrhizae. In their entirety, these interactions highlight that the modulation of SA, JA, and ABA levels is part of a complex hormonal network that influences plant defence responses and stress tolerance. Elucidating these interactions between hormones at both the molecular and systems levels can improve our ability to mitigate and improve the stress response pathways for great plant growth and development.

5.6. Significance and Implications of My Results

Enhancing our knowledge of soil fungi in boreal ecosystems is becoming increasingly important as this habitat faces rising temperatures due to climate change. Increased warming in these regions can increase microbial and fungal activity, leading to altered nutrient cycling and increased soil respiration, increasing atmospheric CO₂ levels (Yan et al., 2022; Nissan et al., 2023). Additionally, studying how mycorrhizal fungi assist plants in coping with environmental stresses can help researchers understand ways to incorporate these mechanisms and fungi into agricultural and forestry practices to mitigate the effects of climate change on plants. My thesis addressed a significant gap in climate change studies by investigating the combined impacts of

elevated CO₂ concentrations and temperature on mycorrhizal-tree interactions. This approach uses realistic environmental conditions that could be experienced by mycorrhizal-poplar trees within the boreal forest by 2100. Furthermore, my investigation into what fungal root isolates from the southern boreal region exude into the rhizosphere and the effects of these exudates on plant growth adds depth to our understanding of mycorrhizal diversity. My research into identifying three specific mycorrhizal partners that can, under the right conditions, enhance tree growth and stress tolerance under future climate scenarios offers insights for forest management and climate change mitigation strategies. Moreover, examining stress hormone concentrations in mycorrhizal-inoculated trees can provide valuable insights into how these symbiotic associations influence plant responses to environmental stressors and the impacts this could have on plant growth. My research also adds to the scientific discourse by helping us understand the intricate relationships between mycorrhizal fungi and trees (Figure 5.2) within the context of climate change. Lastly, my work on fungal exudates can also have applications in agriculture and biotechnology, such as helping identify compounds to improve plant growth, suppress plant pathogens, and produce enzymes and other compounds with industrial or medicinal value. However, the complexity and diversity of exudates make it challenging to understand the full range of their functions and roles in an ecosystem.

5.7. Limitations, Future Studies, and Improvements

While I planned and conducted my experiments with care to minimize confounding variables, I encountered some limitations. My research findings may not be generalizable to other plant species as I focused on assessing the growth, leaf health, and foliar stress hormone

concentrations in a single tree hybrid species, *Populus x canadensis*, under diverse climatic conditions. To address this, future research should encompass a broader array of plant species, including both conifer and broadleaf species. Additionally, examining the stress hormone response in various plant tissues under different climate change and mycorrhizal inoculation scenarios would also help alleviate this limitation. Furthermore, my research took place in controlled laboratory settings, which may not fully replicate the dynamics found in field or forest environments. Therefore, caution is warranted when extrapolating my results to real-world scenarios or forest management practices. Unexpected challenges with the experimental growing spaces led to data collection in Chapters 3 and 4 occurring only once rather than twice in two years as planned initially. This necessitates repeating the experiments within Chapters 3 and 4 to ensure consistent results. While my study highlights the importance of temperature and CO₂ levels in influencing plant growth and stress hormone levels under future climate scenarios, it is essential to consider other environmental factors that could play significant roles as well. Factors such as light intensity, drought, nutrient availability, and biotic interactions could all have a profound impact on how plants and their stress hormones respond to mycorrhizal interactions under warming and elevated CO₂ levels. Thus, despite employing a multi-factorial approach within my research, I suggest incorporating additional factors, such as drought, to enhance my study's relevance to what may happen within the boreal forest under climate change.

For future research, I suggest exploring potential taxonomic patterns in stress hormone production among various plant species and their mycorrhizal partners, as this could provide valuable insights into how mycorrhizal fungi (especially ectomycorrhizal fungi) initiate and maintain symbiosis with the host plant and may aid in the understanding in the evolution of

phytohormone synthesis in mycorrhizal fungi. I also suggest broadening the pool of plant hormones screened to assess the impacts of elevated temperature and CO₂ on trees to include gibberellic acid, indole-3-acetic acid, cytokinins or strigolactones, which would further enrich our understanding of plant growth regulation and stress adaptation mechanisms. I also think that investigating the impact of mycorrhizal inoculation on photosynthetic parameters under climate change conditions would contribute to our knowledge of the role that mycorrhizal fungi have on the maximum rate of electron transport and carbon assimilation in the chloroplast under future warming conditions. The examination of heat shock proteins, antioxidant enzymes, and fungal exudates within an experimental system such as mine could offer a more comprehensive understanding of the complex interactions between plants and mycorrhizal fungi under environmental stress. My HPLC-MS analysis of fungal exudates revealed unidentified peaks, prompting a future targeted analysis of compounds such as phenolics or lipids. Exploring potential synergistic effects by testing multiple fungal species on one host plant and considering the inclusion of growth-promoting rhizobacteria could add depth to my work and allow for a multi-tiered symbiosis investigation. This holistic approach aligns with my fascination and speculation on how future climatic stresses may impact such symbiotic relationships, building upon the results obtained within this thesis.

5.8. Conclusion

The future of studying the influence of climate change on soil fungi and their plant growth-promoting properties is bright. I am confident that through improved techniques, data collection, and innovation within mycology, we can further elucidate the underpinnings of

mycorrhizal-plant interactions, improve soil health, and ensure the sustainability of global soil ecology. My research has shown that the impacts of moderate climate change may have a net benefit on poplar growth in the boreal biome and that many mycorrhizal fungi do provide growth promotion and amelioration of stress under climate change. More multifactorial study designs with standardized experimental procedures, larger sample sizes, and including more fungal and plant species within our studies will help increase our understanding and help mitigate the impacts of climate change on boreal trees and their mycorrhizal partners. These strategies include increasing protected areas, promoting sustainable logging practices, and implementing policies to reduce greenhouse gas emissions. By adopting these measures, we can safeguard the integrity of the boreal forest, conserve biodiversity, and secure the livelihoods and cultural practices of local communities for generations to come.

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Appendices

Appendix 2.1. Modified Melin Norkrans Medium used for fungal cultures for 1 L solution to a pH of 5.8 (Marx, DH. (1969). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*. 59(2), 153–163.)

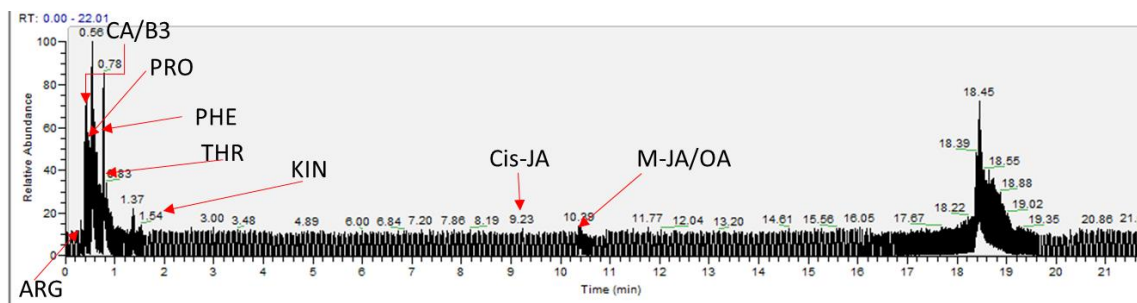
1.	2.0 g malt extract
2.	5.0 g glucose
3.	1.0 g yeast extract
4.	0.5 g potassium phosphate monobasic
5.	0.25 g ammonium phosphate dibasic
6.	0.15 g magnesium sulphate
7.	500 μ L calcium chloride (10% solution)
8.	250 μ L sodium chloride (10% solution)
9.	120 μ L ferric chloride (10% solution)
10	100 μ L thiamine HCL (1 mg/mL solution)
11	15 g agar

Appendix 2.2. Chemical standards used for HPLC-MS targeted analysis to identify compounds from 14 root-associated fungal exudates. The retention time (RT), peak areas and associated standard concentration are reported for each compound tested. Compounds with ND listed were not detected in the analysis. The amino acids aspartic acid, glutamic acid, glycine, isoleucine, leucine, serine or valine were not detected. Phytohormone abbreviations: jasmonic acid (JA); methyl jasmonate (M-JA); cis-jasmonate (Cis-JA); salicylic acid (SA); abscisic acid (ABA); gibberellic acid (GA); kinetin (KIN); and indole-3-acetic acid (IAA).

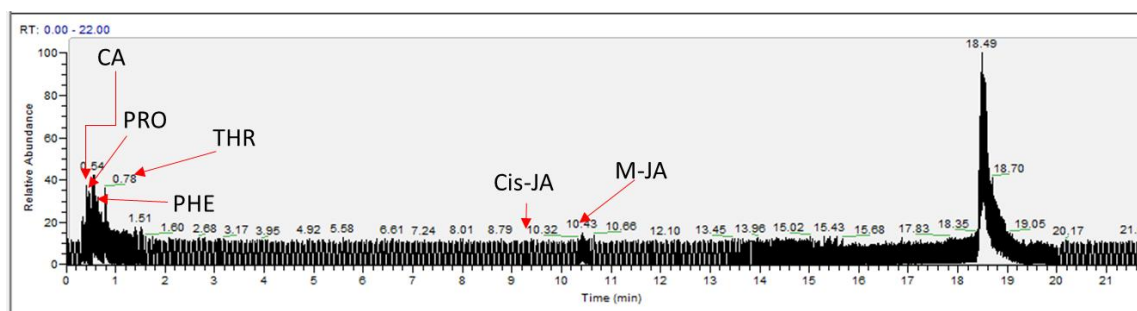
<i>Metabolite</i>	RT	Peak Area	Std Concentration (mM)
A) Phytohormones			
JA	6.49-9.00	205523126.4	4.755790175
Cis-JA	8.59-12.00	456431182.9	6.088428333
Meth-JA	2.81-4.00,9.00-11.00	526653226.3	4.458314757
SA	5.45-7.0	34039574.96	7.240028671
ABA	7.59-8.5	75478729.54	3.783292978
GA	0.47-.67, 5.00-5.51	17726633.64	2.887086064
IAA	0.4-0.74, 5.00-7.00	312605866.4	5.708283862
KIN	0.43-2, 2.46-3.00	255346617.4	4.646624227
B) Amino Acids			
Alanine	3.04-3.16	124057830.2	0.0025
Arginine	0.04-0.48	435212753.5	0.0025
Aspartic acid	ND	ND	-
Cystine	13.51-13.84	6181826.569	0.0025
Glutamic acid	ND	ND	-
Glycine	ND	ND	-
Histidine	0.5	50814026.77	0.0025
Isoleucine	ND	ND	-
Leucine	ND	ND	-
Lysine	0.02-0.04	6970406.427	0.0025
Methionine	0.53-0.57	212555551.2	0.0025
Phenylalanine	0.69-0.82	118229978.8	0.0025
Proline	0.42-0.53	101628053.5	0.0025
Serine	ND	ND	-
Threonine	0.82-1.23	548138349.5	0.0025

<i>Tyrosine</i>	0.53-69	212555551.2	0.0025
<i>Valine</i>	ND	ND	-
C) Organic Acids			
<i>citric acid</i>	1.00-2.00	23810418.47	5.202372282
<i>succinic acid</i>	0.42-1.02	7757216.468	8.468117537
<i>oxalic acid</i>	2.2, 14.48	1250500.196	11.10740864

A



B



Appendix 2.4 Sample targeted fungal exudate media chromatograms of A) *C. geophilum* and B) *H. finlandica* isolate C. Compound abbreviations: methyl jasmonate (M-JA); cis-jasmonate (Cis-JA); kinetin (KIN); citric acid (CA); oxalic acid (OA); proline (PRO); phenylalanine (PHE); threonine (THR); Arginine (ARG).

Appendix 3.1. Effects of elevated CO₂ and temperature on poplar height in pooled inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA), showing DF, SS, F values and P values for the main effects of the fungal species treatments. Bolded numbers represent P-values less than 0.05 ($p < 0.05$).

	DF	SS	F	p-value
AC0T	3	114.326	0.464	0.710
AC4T	3	77.694	0.308	0.820
AC8T	3	513.344	2.148	0.130
EC0T	3	25.063	0.0854	0.967
EC4T	3	595.250	1.569	0.231
EC8T	3	100.308	0.552	0.653

Appendix 3.2. Effects of elevated CO₂ and temperature on poplar biomass in pooled inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA), showing DF, SS, F values and P values for the main effects of the fungal species treatments. Bolded numbers represent P-values less than 0.05 ($p < 0.05$).

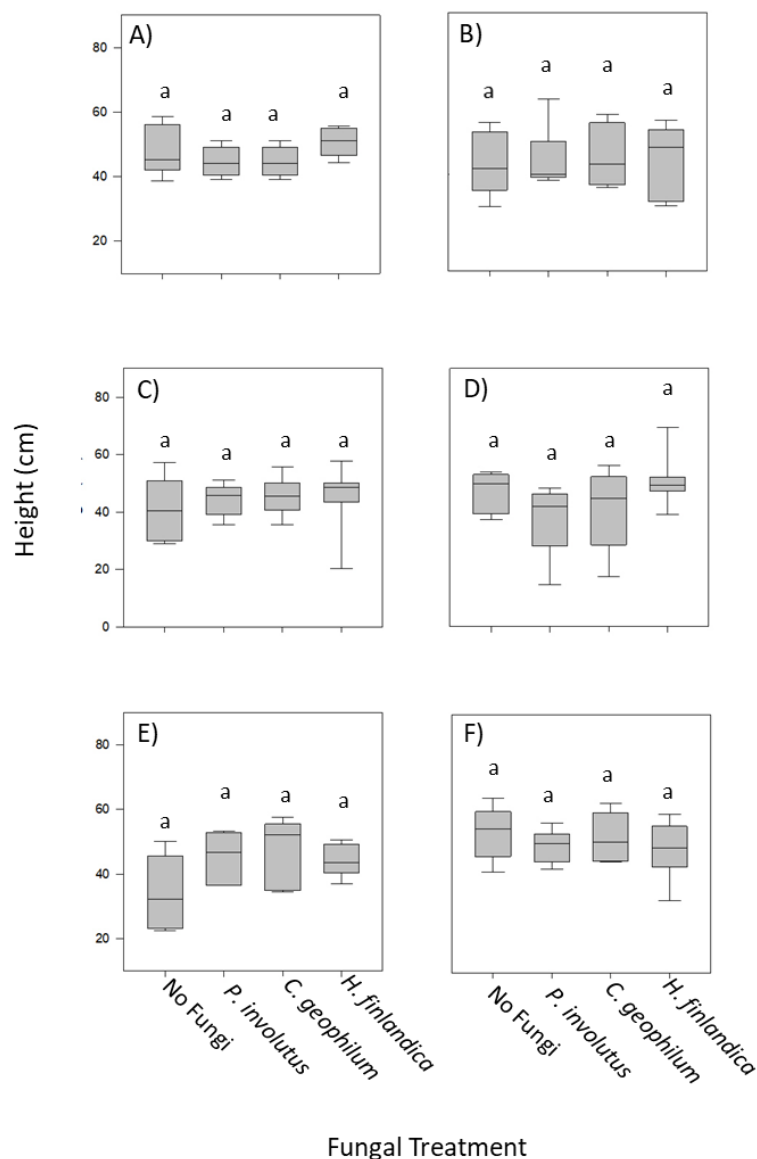
	DF	SS	F	p-value
AC0T	3	30.930	2.464	0.105
AC4T	3	11.032	1.285	0.307
AC8T	3	319.449	8.250	<0.001
EC0T	3	87.700	12.379	<0.001
EC4T	3	258.746	0.527	0.670
EC8T	3	0.0134	5.682	0.006

Appendix 3.3. Effects of elevated CO₂ and temperature on poplar root: shoot ratio in pooled inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA), showing DF, SS, F values and P values for the main effects of the fungal species treatments. Bolded numbers represent P-values less than 0.05 ($p < 0.05$).

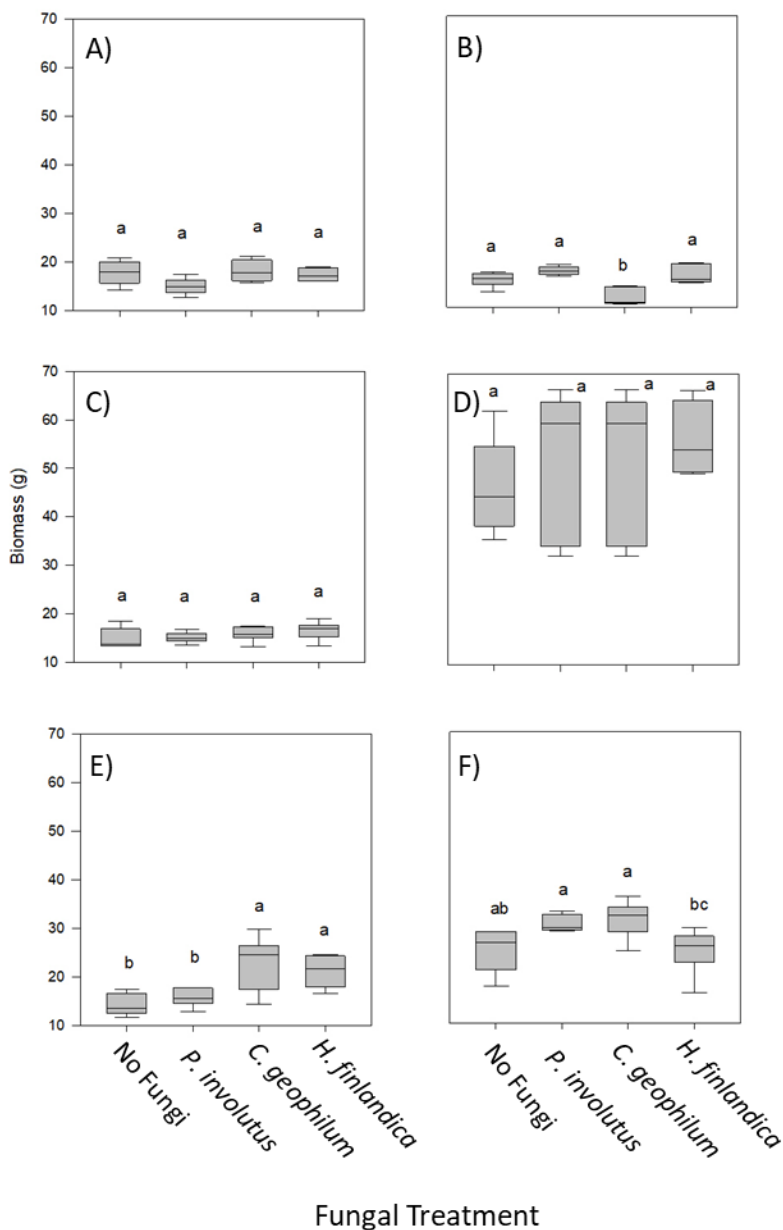
	DF	SS	F	p-value
AC0T	3	0.134	0.768	0.531
AC4T	3	0.0152	0.935	0.442
AC8T	3	1.177	4.443	0.015
EC0T	3	0.467	5.373	0.007
EC4T	3	0.00462	0.0431	0.988
EC8T	3	0.639	8.642	<0.001

Appendix 3.4. Effects of elevated CO₂ and temperature on poplar leaf greenness in pooled inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA) on ranks for the main effects of the fungal species treatments, indicating H-values, degrees of freedom (DF), and p-values; Bolded numbers represent $p < 0.05$.

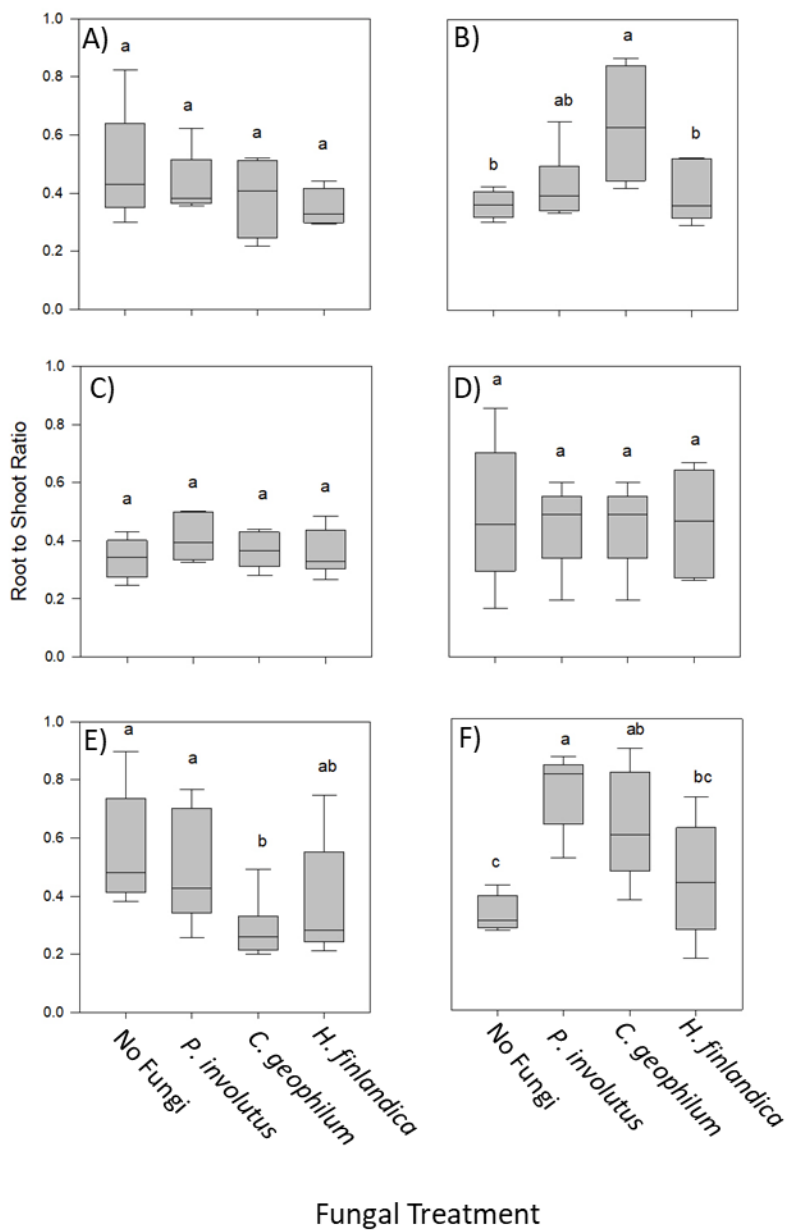
	DF	H	p-value
AC0T	3	4.539	0.209
AC4T	3	2.997	0.392
AC8T	3	3.539	0.316
EC0T	3	1.897	0.594
EC4T	3	2.816	0.421
EC8T	3	3.748	0.290



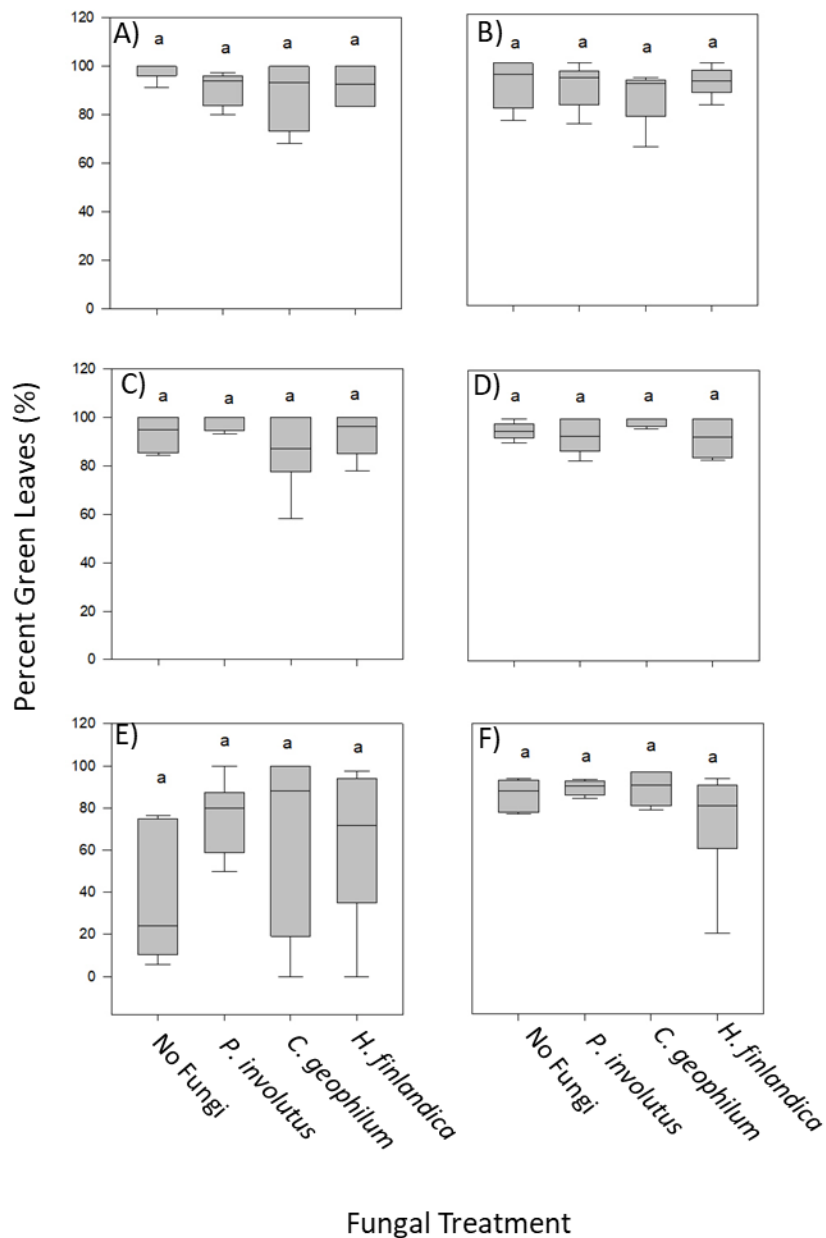
Appendix 3.5. Poplar height in response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n \geq 4$.



Appendix 3.6. Poplar dry biomass in response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n \geq 4$.



Appendix 3.7. Poplar root: shoot ratio in response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n \geq 4$.



Appendix 3.8. Percent green leaves in response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8 °C, $n \geq 4$.

Appendix 4.1. Effects of elevated CO₂ and temperature on jasmonic acid (JA) leaf levels stress and growth hormones in inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA), showing DF, SS, F values and P values for the main effects of the fungal species treatments. Bolded numbers represent P-values less than 0.05 ($p < 0.05$).

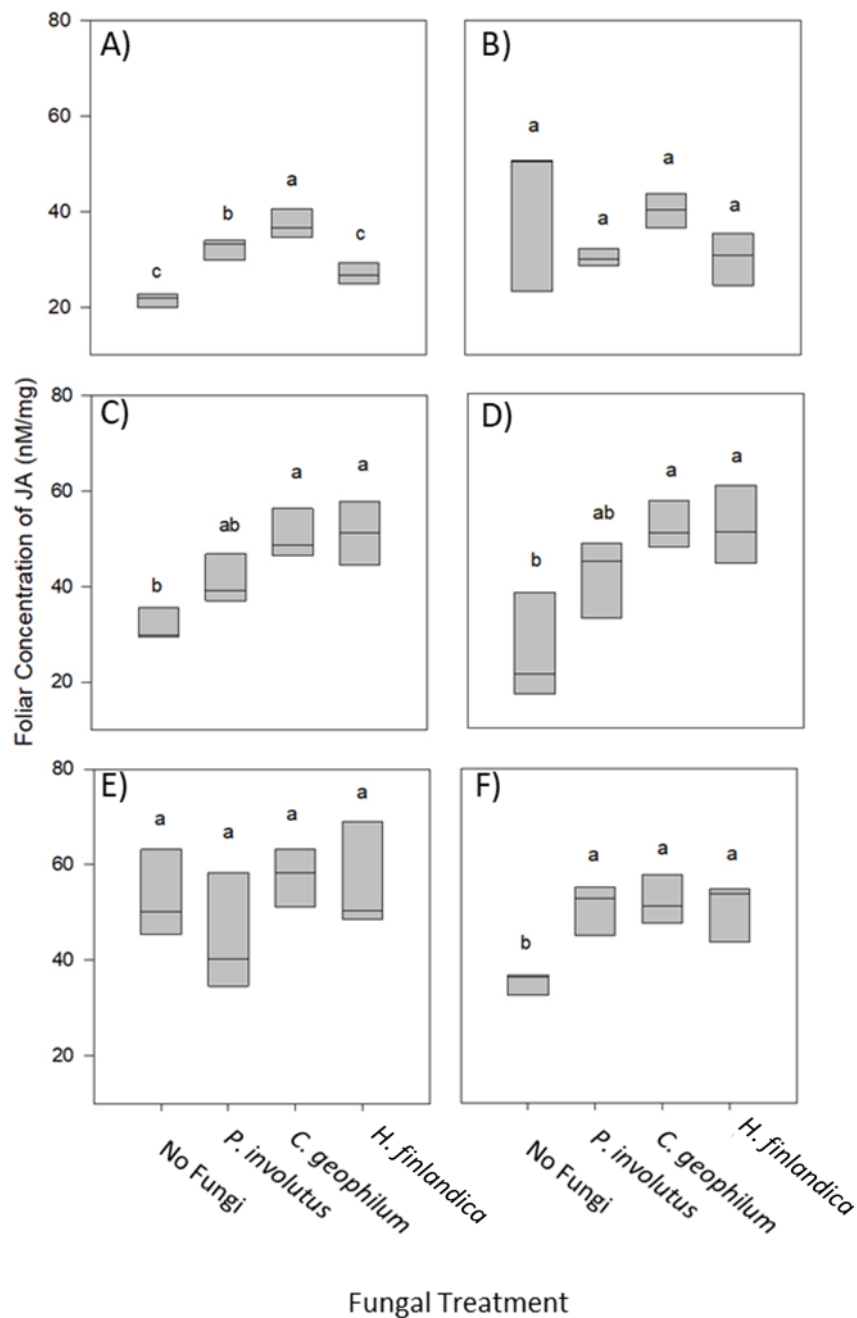
	DF	SS	F	p-value
AC0T	3	412.638	25.762	<0.001
AC4T	3	766.245	9.314	0.005
AC8T	3	310.852	1.028	0.430
EC0T	3	334.952	1.515	0.283
EC4T	3	1406.591	6.549	0.015
EC8T	3	581.872	7.908	0.009

Appendix 4.2. Effects of elevated CO₂ and temperature on salicylic acid (SA) leaf levels stress and growth hormones in inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA), showing DF, SS, F values and P values for the main effects of the fungal species treatments. Bolded numbers represent P-values less than 0.05 ($p < 0.05$).

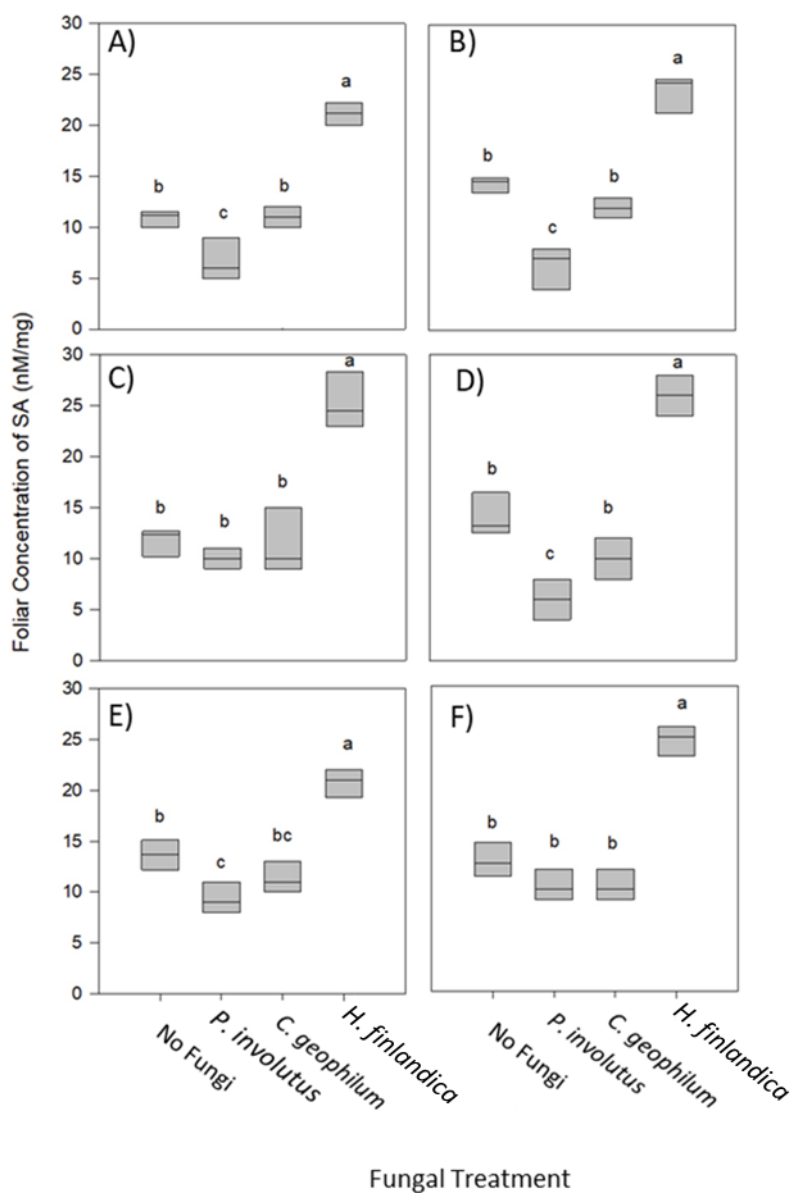
	DF	SS	F	p-value
AC0T	3	340.049	63.177	<0.001
AC4T	3	460.909	29.746	<0.001
AC8T	3	223.756	34.557	<0.001
EC0T	3	453.743	65.712	<0.001
EC4T	3	672.010	54.096	<0.001
EC8T	3	424.590	58.971	<0.001

Appendix 4.3. Effects of elevated CO₂ and temperature on abscisic acid (ABA) leaf levels stress and growth hormones in inoculated and non-inoculated poplars. Summary report of a one-way analysis of variance (ANOVA), showing DF, SS, F values and P values for the main effects of the fungal species treatments. Bolded numbers represent P-values less than 0.05 ($p < 0.05$).

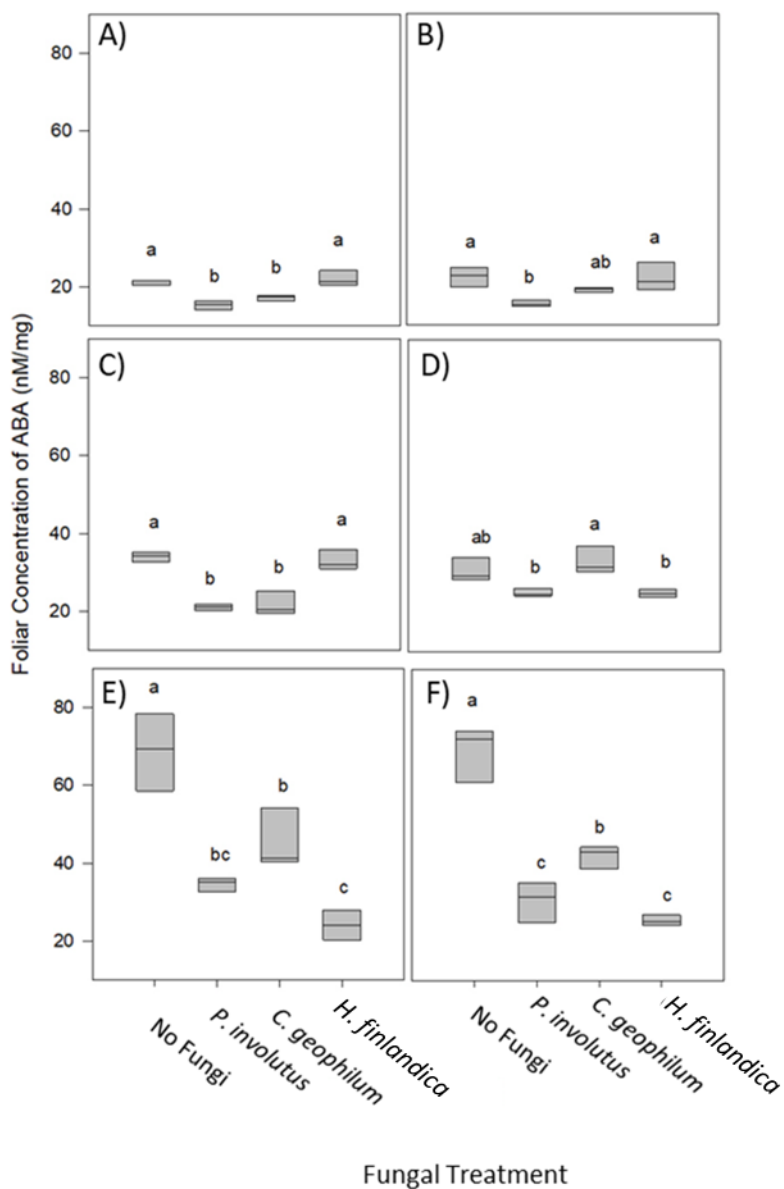
	DF	SS	F	p-value
AC0T	3	86.909	19.345	<0.001
AC4T	3	440.429	31.663	<0.001
AC8T	3	3295.620	24.775	<0.001
EC0T	3	92.829	6.218	0.017
EC4T	3	149.196	8.395	0.007
EC8T	3	4316.083	51.832	<0.001



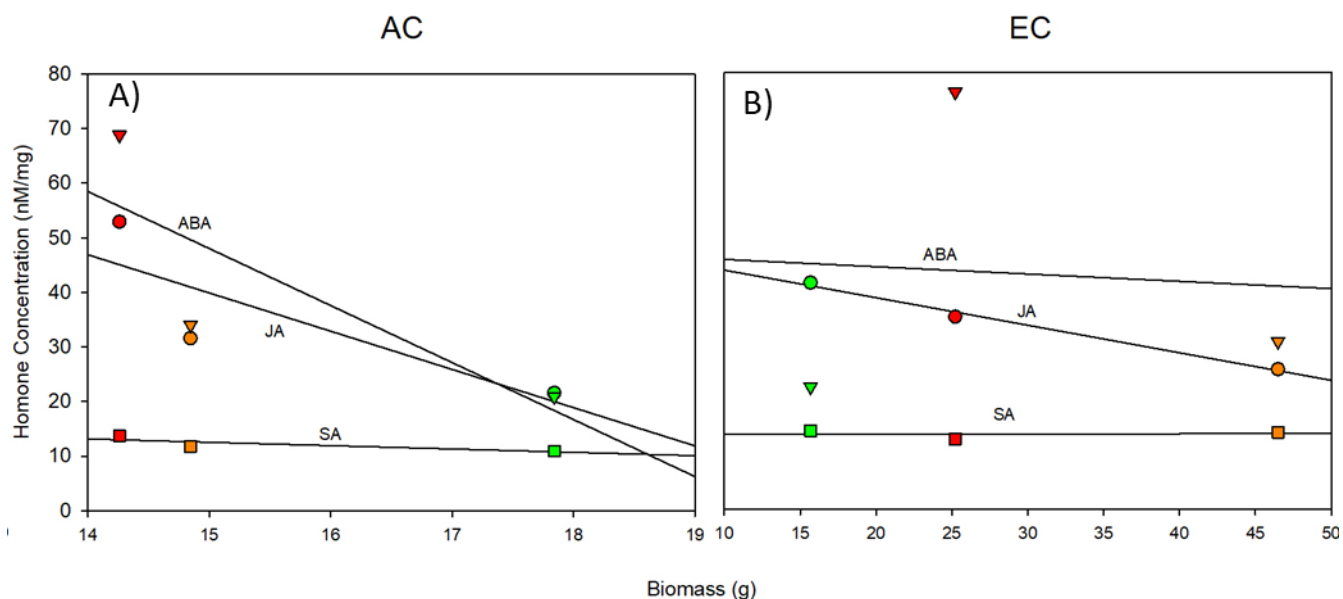
Appendix 4.4. Foliar jasmonic acid (JA) concentrations response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.



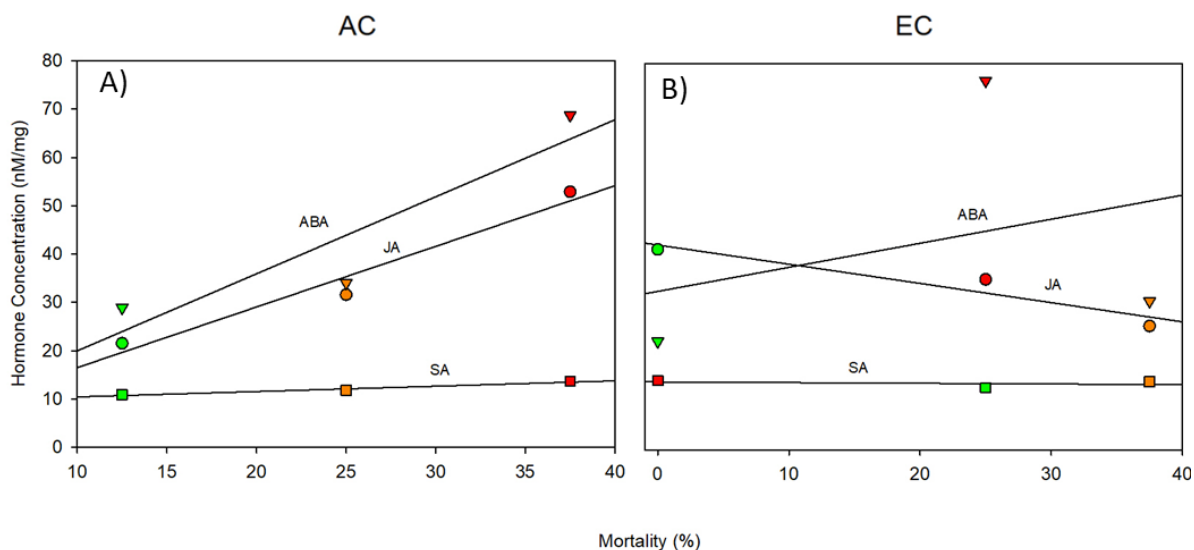
Appendix 4.5. Foliar salicylic acid (SA) concentrations response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.



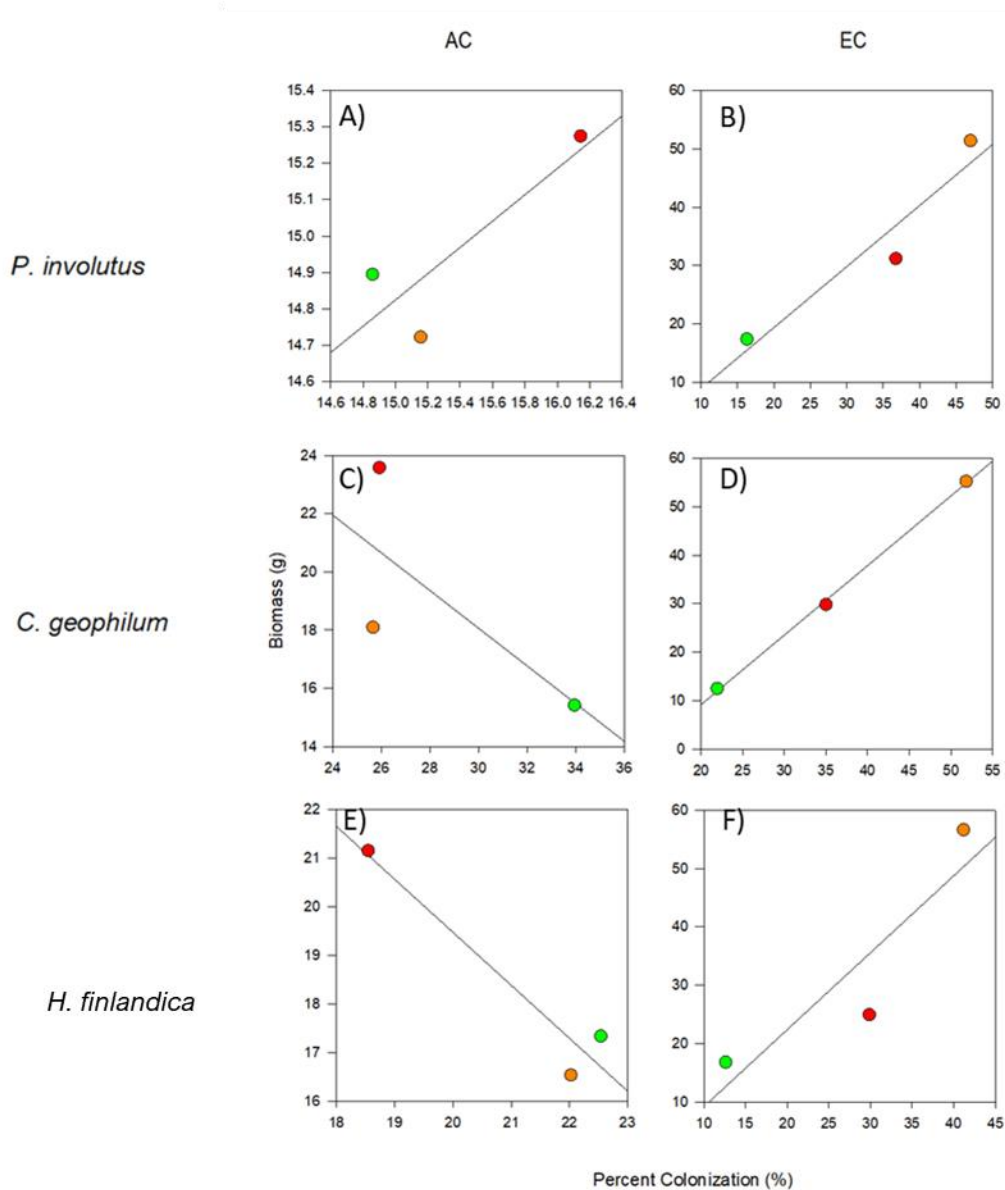
Appendix 4.6. Foliar abscisic acid (ABA) concentrations response to ambient CO₂ (A, C, E) and elevated CO₂ (B, D, F) across temperature treatments 0T (A, B), 4T (C, D), or 8T (E, F) in poplars inoculated with either No fungus, *Paxillus involutus*, *Cenococcum geophilum*, or *Hyaloscypha finlandica*. The box plot spans the first to the third quartile segmented by the median; the whisker below the box is the minimum, and the whisker above the box, is the maximum. Bars with different letters represent statistically significant differences across the six treatments (Holm-Sidak post hoc test, $p < 0.05$). 0T, ambient temperature; 4T, ambient temperature +4 °C; 8T ambient temperature +8°C, $n = 3$.



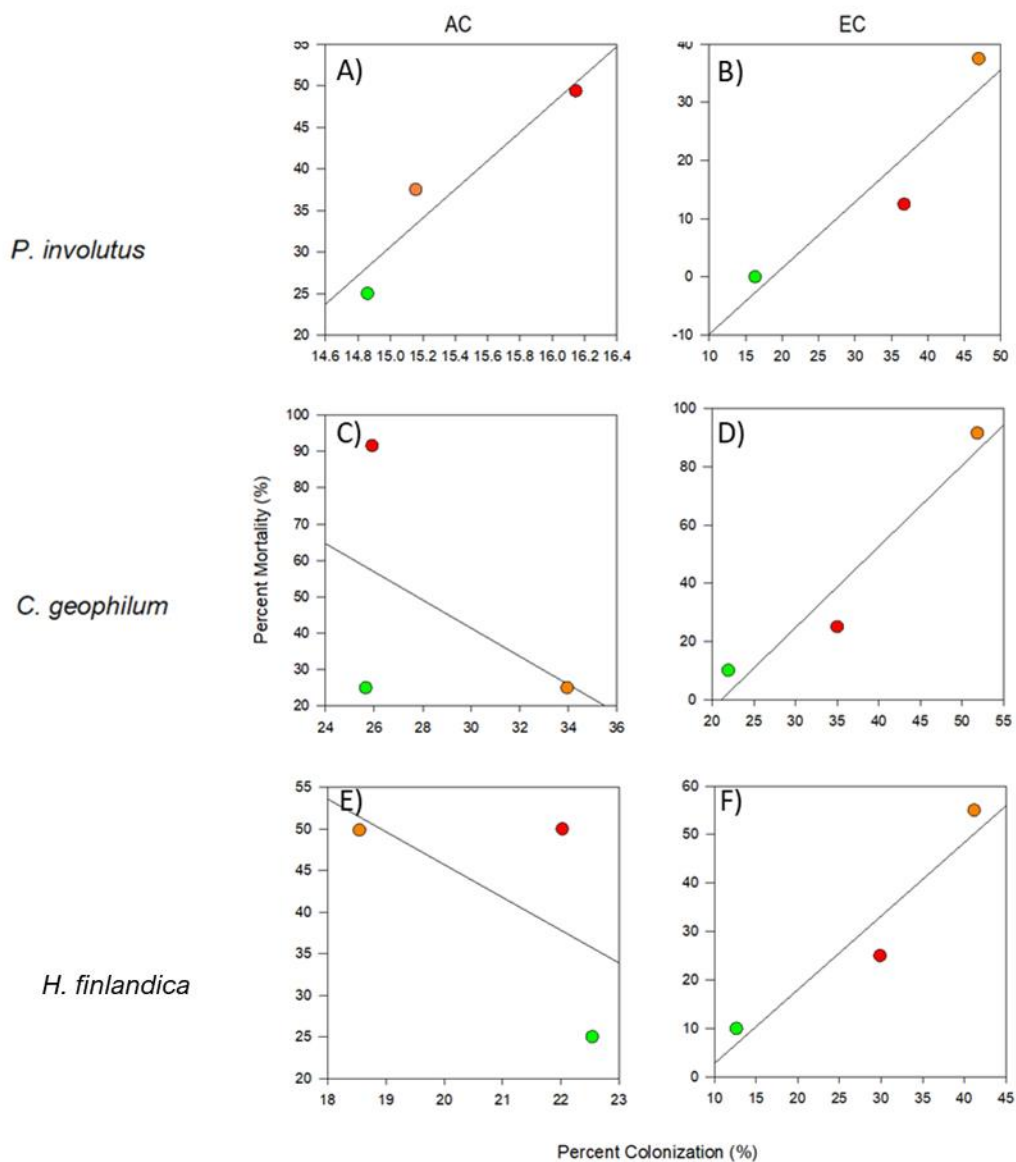
Appendix 5.1. The hormone concentration (nM/mg) as a function of the biomass (g) of poplars under AC (A) or EC (B). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.



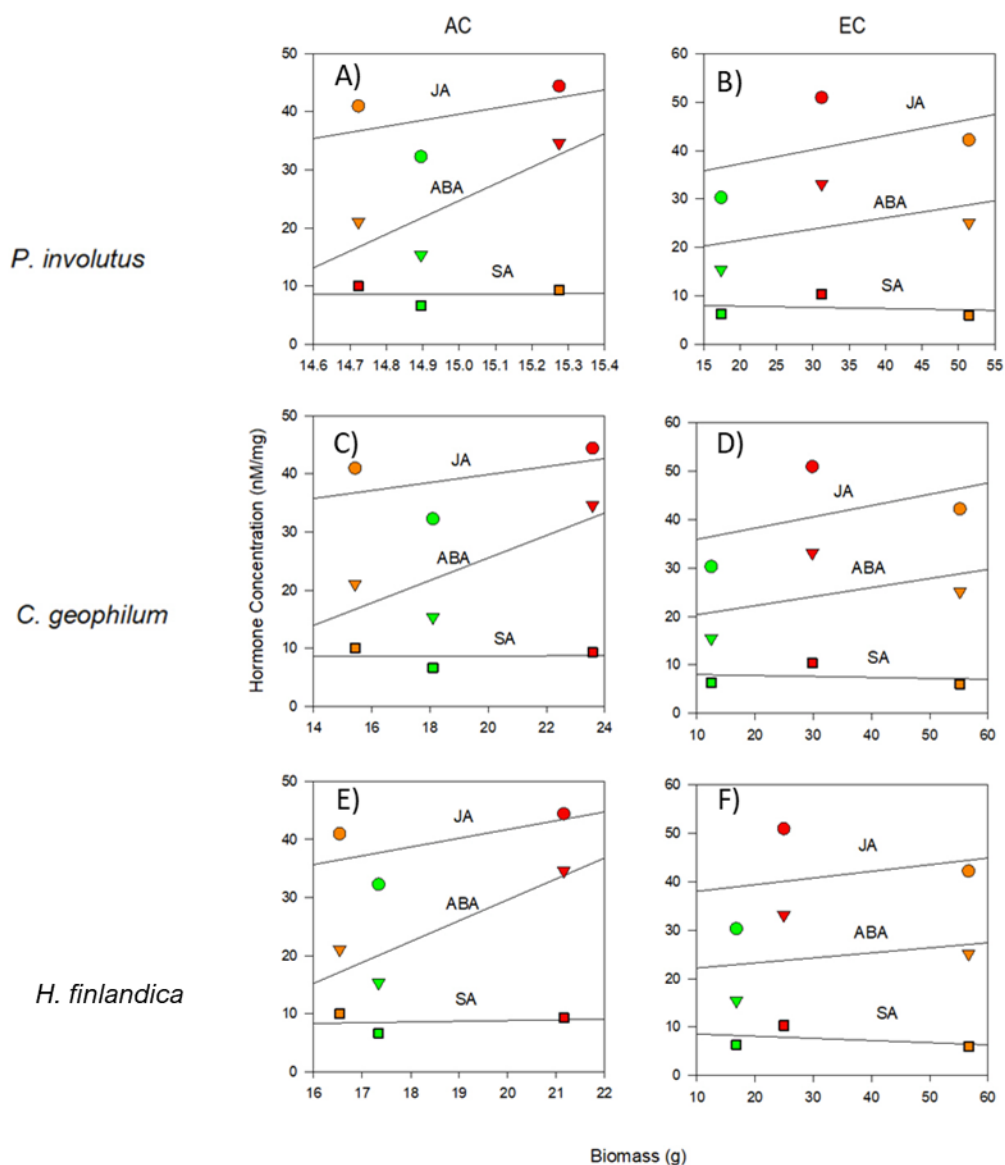
Appendix 5.2. The hormone concentration (nM/mg) as a function of the mortality (%) of poplars under AC (A) or EC (B). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.



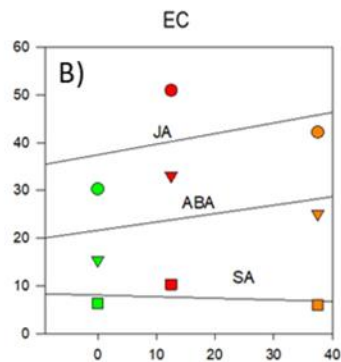
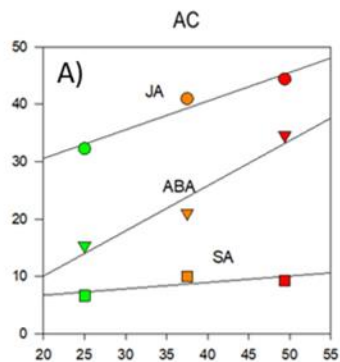
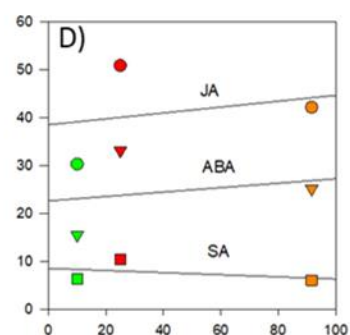
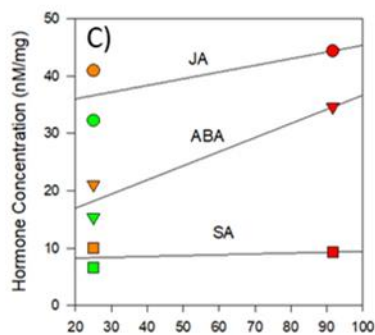
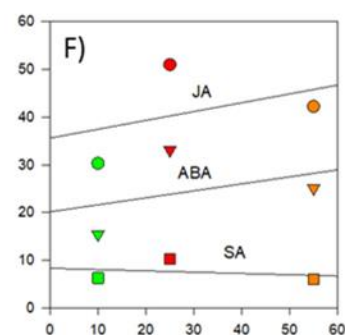
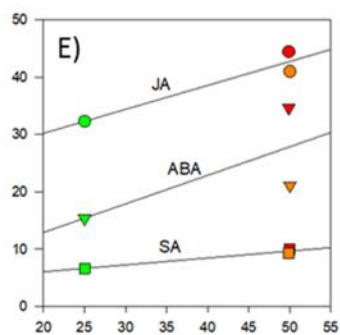
Appendix 5.3. Poplar biomass (g) as a function of the percent colonization (%) of mycorrhizae under AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.



Appendix 5.4. Poplar mortality (%) as a function of the percent colonization (%) of mycorrhizae AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.

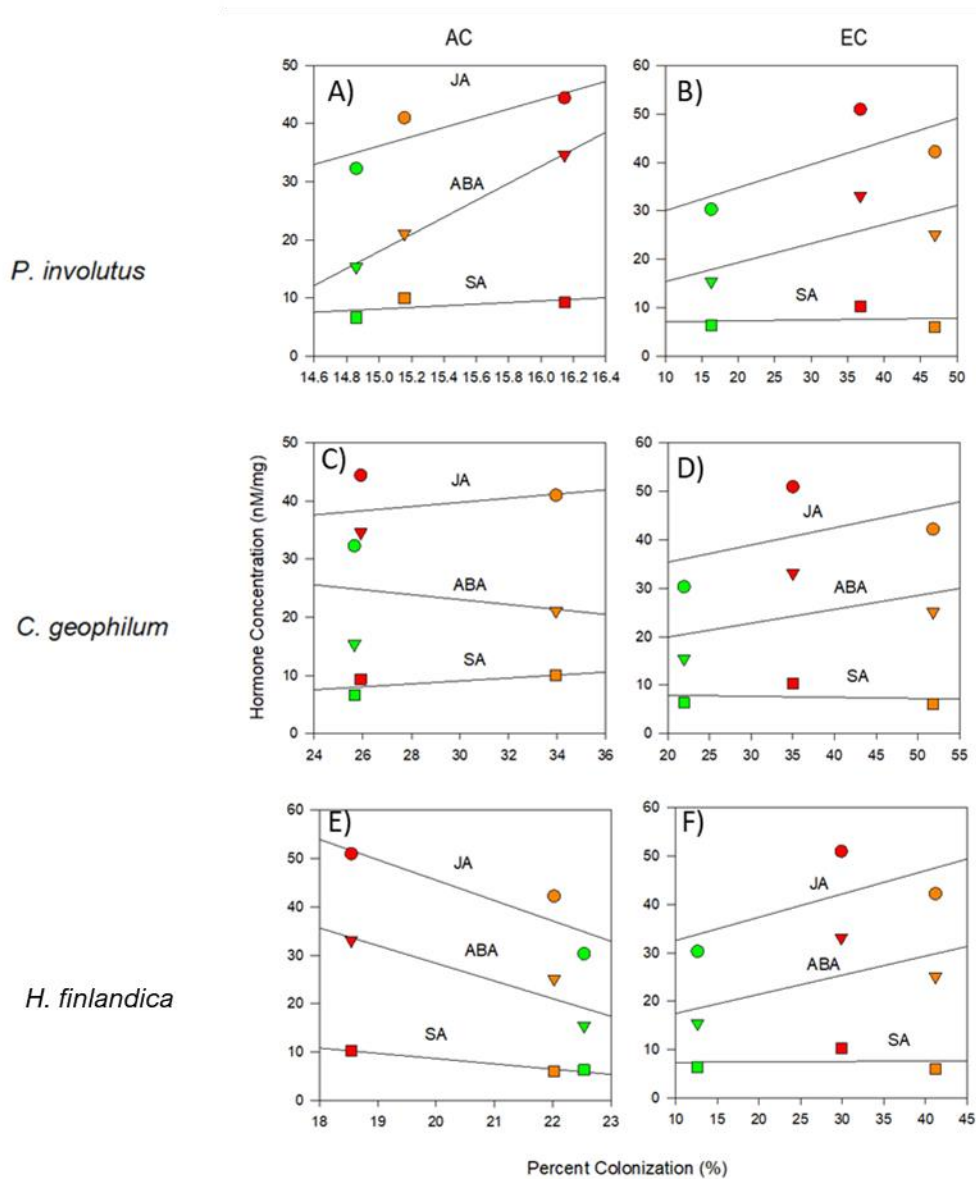


Appendix 5.5. The hormone concentration (nM/mg) as a function of the biomass (g) of mycorrhizal poplars AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.

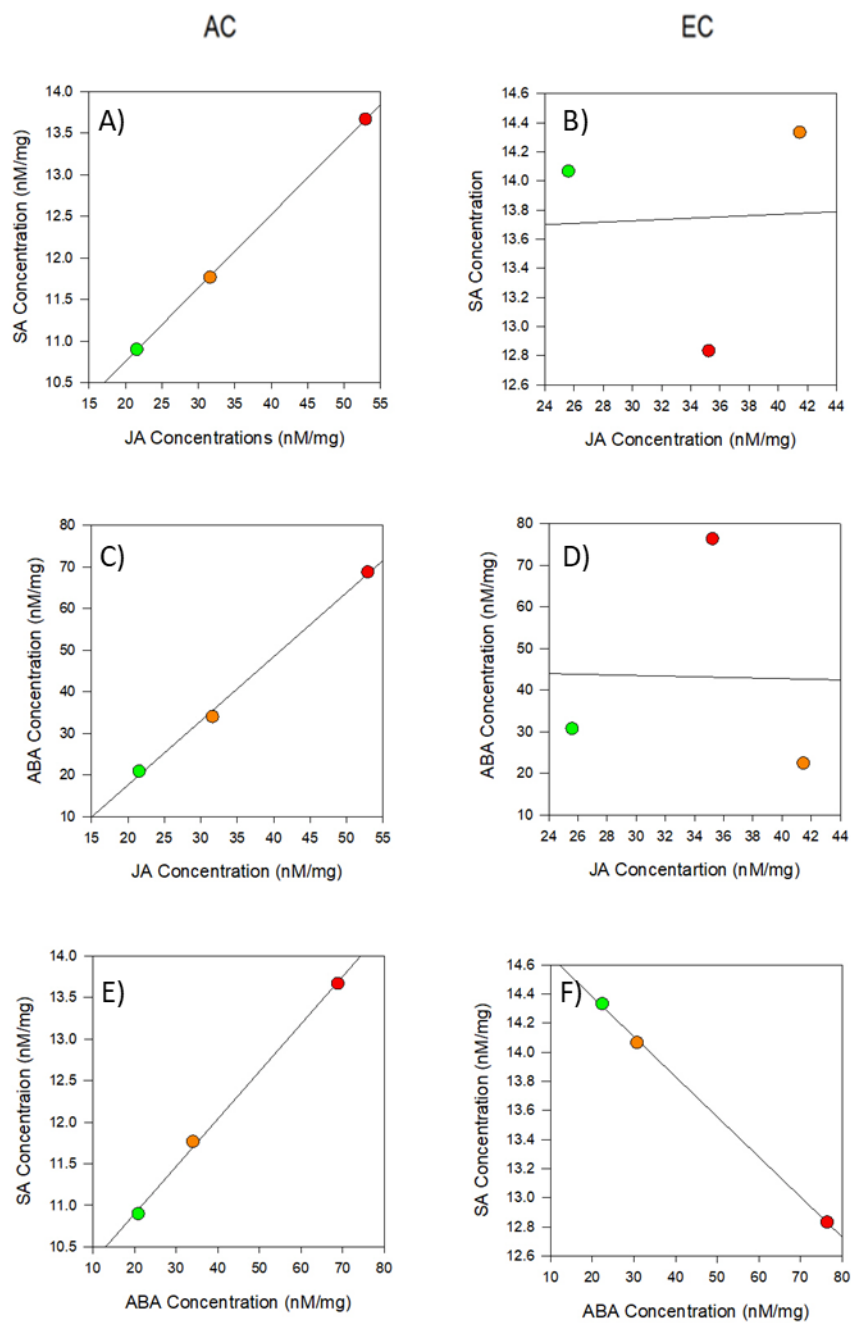
P. involutus*C. geophilum**H. finlandica*

Percent Mortality (%)

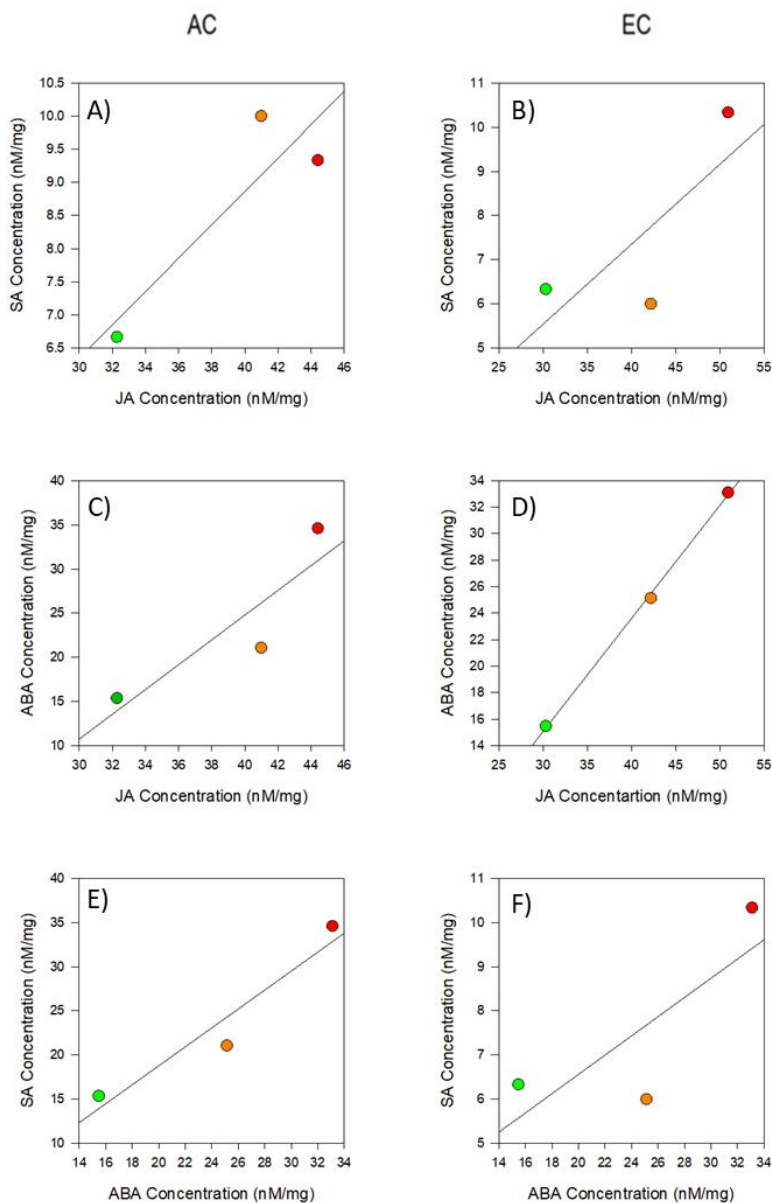
Appendix 5.6. The hormone concentration (nM/mg) as a function of the mortality (%) of mycorrhizal poplars AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.



Appendix 5.7. The hormone concentration (nM/mg) as a function of percent mycorrhizal colonization (%) of poplars AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.



Appendix 5.8. Non-inoculated poplar phytohormone (nM/mg) association study AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.



Appendix 5.9. Mycorrhizal Inoculated poplar phytohormone (nM/mg) association study AC (A, C, E) or EC (B,D,F). Lines represent regression lines for each stress hormone, jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA). Symbols represent stress hormones (JA = circle; ABA = triangle; SA = square). Colors represent growth temperature (0T = green; 4T = orange; 8T = red). Each data point represents the average value for that treatment.

Curriculum Vitae

Joshua J. R. Frank-Webb

Education

2016- April 2024 Doctor of Philosophy, Biology Candidate, Western University, London, ON
Supervisor: Dr. Danielle Way and Dr. Tod Ramsfield

2014-2016 Masters of Science, Biology, Western University, London, ON Supervisor: Dr. Sheila Macfie

2010-2014 Bachelors of Science, Honors Biology, Western University, London, ON B.Sc.
Thesis Supervisor: Dr. Sheila Macfie

Appointments

January 2023-Present Part-Time Professor, School of Applied Science and Technology,
Fanshawe College

Published Materials

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2024). Characterizing root-associated fungal exudate profiles and investigating their impacts on plant growth. (In progress)

Frank, J.J.R., Ramsfield, T. & Way, D. (2024). Complex Effects of Root-Associated Fungi on Poplars Under Elevated Temperature and CO₂. (In progress)

Select Oral and Poster Presentations

Frank, J.J.R. (2022). Impacts of elevated temperature and carbon dioxide on poplar-fungal interactions. Canadian Fungal Network Seminar Series. Online [Oral]

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2021). Root-associated fungi alter tree growth and phytohormone concentrations under elevated temperature and CO₂ level. Canadian Society of Plant Biologists Eastern Regional Meeting. Online [Poster]

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2019). Impacts of elevated temperature and carbon dioxide on popular fungal interactions. Canadian Society of Plant Biologists Eastern Regional Meeting. St. Catherines, ON, Canada [Oral]

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2019) Impacts of root-associated fungi on tree growth under elevated temperature and CO₂. Plant Canada. Guelph, ON, Canada. [Poster]

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2018) Impacts of root-associated fungi on tree growth under elevated temperature and CO₂. Canadian Society of Plant Biologists Eastern Regional Meeting. London, ON, Canada. [Poster]

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2017) Characterizing root-associated fungal exudate profiles and their impacts on plant growth. Plants from Sea to Sky; joint meeting of The Canadian Society of Plant Biologists and the Canadian Society for Horticultural Science annual meeting. Vancouver, BC, Canada. [Poster]

Frank, J.J.R., Abou-Zaid, M., Ramsfield, T. & Way, D. (2017) Rooting out the role of mycorrhizae in modifying tree responses to climate change. Fallona Family Interdisciplinary Showcase. London, ON, Canada. [Poster]

Educational Outreach

2020 Media Coverage: Biology Research Digest (winter 2020, issue 3), providing a quick overview of my Ph.D. research.

2019 March Break Open House Cell Biology Communicator and Microscopist Demonstrating cell culturing techniques, visualizing mammalian and fungal cells under the microscope, and providing information about Western's biology program, cell biology courses, and advice to succeed in undergraduate studies to prospective undergraduate students and their parents. (Western University, Biology Department, Cell Biology Station)

2018 March Break Open House Cell Biology Communicator and Microscopist Demonstrating cell culturing techniques, visualizing mammalian and fungal cells under the microscope, and providing information about Western's biology program, cell biology courses, and advice to succeed in undergraduate studies to prospective undergraduate students and their parents. (Western University, Biology Department, Cell Biology Station)

2017 Pint of Science Speaker: Presented a talk entitled 'A warming world: The good, the bad, and the ugly', at the Poachers Arms London, ON May 16, 2017.

2017 Climate change outreach/NatureWatch outreach: Gave a lecture to an elementary school about climate change and how they can get involved in tracking climate change through plant phenology, April 24, 2017.

2017 Media Coverage: Western Science Speaks Researcher Spot Light Interview entitled, "Plants in the Age of Climate Change".

2016 March Break Open House Cell Biology Communicator and Microscopist Demonstrating cell culturing techniques, visualizing mammalian and fungal cells under the microscope, and providing information about Western's biology program, cell biology courses, and advice to succeed in undergraduate studies to prospective undergraduate students and their parents. (Western University, Biology Department, Cell Biology Station)

2016 Science Fair Judge: A judge for a grade 5-8 science fair held at Western University where I listen to student presentation and demonstrations, as well as evaluated their work for the propose of recommending a winner to the event coordinator.

University Services

2015 Complex Systems Modeling and Analysis Hiring Committee

Graduate student representative that evaluated and met with prospective candidates for the professorship position and submitted feedback to the educational hiring committee (Western University)

Awards and Scholarships

- 2019 Dr. Irene Uchida Fellowship in Life Sciences, Western University (\$3,000 CAD)
- 2017 Ruth Horner Arnold Fellowship, Western University (\$3,000 CAD)
- 2017 Biology Graduate Student Travel Award, Western University (\$500 CAD)
- 2016 George H. Duff Student Travel Bursary, Canadian Society of Plant Biologists
(\$500 CAD)
- 2010-14 Green Shield Canada Scholarship, CAMI Automotive, Ingersoll, ON (2500 CAD/yr)