Herbivory resistance and leaf nutritional quality of hybrid poplar (*Populus deltoides* × *petrowskyana)* × *petrowskyana* exposed to salinity and *Orgyia leucostigma*

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ABSTRACT

When plants encounter salinity and herbivory simultaneously, these stress factors may interact synergistically or antagonistically due, among other things, to crosstalk between signaling pathways involved in the response to each factor. Although the effects of salinity and herbivory have been widely examined independently, their interactive or sequential impacts remain poorly understood, particularly in woody species. This study investigated 1) how salinity affects nutritional quality of hybrid poplar (*Populus* 'Okanese') leaves and resistance to a generalist herbivore *Orgyia leucostigma*, and 2) how prior herbivory exposure modifies plant responses to subsequent salinity stress and insect feeding. Hybrid poplar cuttings were grown in soil under greenhouse conditions. In the first experiment, four-week-old cuttings were exposed to either 0 or 100 mM NaCl. After four weeks of salt treatment, total phenolic content was higher in salt-treated plants, yet it was not affected by herbivory. Salinity led to higher concentrations of Na and Cl in the leaves, while macronutrients such as K, P, and S showed lower levels, changing leaf nutritional quality. However, constitutive and induced resistance to *O. leucostigma* were both unaffected by salinity or its interaction with herbivory, and overall resistance remained consistently high across all treatments. After two additional weeks of salt treatments, leaf Na and Cl levels increased by seven- and two-fold respectively. In addition, induced resistance was greater in salt-grown plants. In the second experiment, plants were first subjected to herbivory and then to salinity for four weeks. Plants previously exposed to herbivory had lower level of leaf phenolics compared to non-herbivory controls and salinity did not alter this response. However, plants grown in salinity had lower total soluble protein concentrations. Neither macronutrient nor micronutrient concentrations were significantly affected by salinity, herbivory, or their interaction and induced resistance to *O. leucostigma* was not affected by previous

herbivory or subsequent salinity. Overall, a longer exposure (six weeks) to salinity enhanced induced resistance to the herbivore, but previous herbivory did not improve the responses of hybrid poplar to salinity and no synergistic effects between the two stresses were observed.

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CHAPTER 1 - GENERAL INTRODUCTION

Plants are frequently exposed to multiple environmental stressors in their natural and agricultural ecosystems (Sewelam et al., 2014). Among these, the combined effects of abiotic and biotic factors, particularly salinity and insect herbivory, pose a significant challenge to plant productivity, survival, and fitness. Although plant responses to salinity and herbivory have been studied individually, much less is known about how these factors affect plants when they occur together, especially in long-lived woody plants. Addressing this knowledge gap is important because the simultaneous or sequential occurrence of these stresses can produce stronger or weaker effects than expected, resulting in complex changes in plant growth and defense against future detrimental conditions (Atkinson & Urwin, 2012; Mittler, 2006).

Globally, soil salinization represents a major abiotic constraint on plant growth, impacting approximately 7 % of the terrestrial area and approximately one-third of irrigated lands (Chele et al., 2021; Shrivastava & Kumar, 2015). Soil salinity alters plant water relations, causes ionic toxicity, and disrupts nutrient balances (Hasegawa et al., 2000; Munns & Tester, 2008). These disruptions can affect plant-insect interactions by modifying leaf chemistry, nutrient availability, and physical and chemical defenses. Likewise, through tissue damage and contact with oral secretions of herbivores, herbivory triggers signaling cascades that alter plant physiology and potentially affect the ability of plants to tolerate subsequent abiotic stresses (Howe & Jander, 2008). Recent studies suggest that herbivory may act as a priming agent, triggering physiological and molecular changes that enhance the capacity of plants to respond more rapidly and effectively to future stressors (Conrath et al., 2015). Similarly, salinity can also function as a priming stimulus. In this context, prior salt exposure may not elicit an immediate defensive response, but it can enhance tolerance to later stress by activating shared signaling

pathways mediated by jasmonic acid (JA), salicylic acid (SA), and ethylene (ET) (Fujita et al., 2006; Walters & Heil, 2007).

While some studies have explored how salinity alters plant resistance to herbivores, with outcomes that vary depending on the plant and herbivore species, comparatively few studies have examined how herbivory may prime plants responses to salinity. In temperate and boreal regions, insect herbivory typically occurs early in the growing season, coinciding with leaf emergence and peak foliar nutrient availability (Gandhi & Herms, 2010). During this period, deciduous species such as those in the genus *Populus* are especially susceptible to defoliating insects, including lepidopteran larvae and chrysomelid beetles (De Tillesse et al., 2007). These early-season herbivore attacks can induce substantial biochemical and physiological changes in leaves, such as the accumulation of phenolic compounds (Bernards & Båstrup-Spohr, 2008), changes in the level of protein content (Urbaneja-Bernat et al., 2024), and redistribution of nutrients (Zhou et al., 2015). Such changes may affect how the plant copes with later environmental stressors.

De-icing salts applied during winter often leach into nearby soils as snowmelt and rain transport them downward (Cunningham et al., 2008). These salts can accumulate in the root zone and persist throughout the growing season (Equiza et al., 2017). Similarly, in irrigated systems, salinity may gradually increase due to evapotranspiration, the use of saline water sources, and the intensive application of fertilizers (Qadir et al., 2007; Rengasamy, 2010). As a result, many ecosystems including urban roadside environments, and riparian zones are exposed to salinity during key growth periods. In these environments, trees such as *Populus* species often experience salinity stress alongside natural biotic pressures like insect herbivory, highlighting the ecological relevance of studying these stresses in combination. Poplars are especially well suited

for investigating these interactions due to their ecological importance, genetic tractability, and responses to both herbivore attack and salt stress (Bradshaw et al., 2000; Philippe & Bohlmann, 2007). Their rapid growth, ease of clonal propagation, broad genetic diversity, and wide geographic distribution make them ideal for controlled experimentation (Stanton et al., 2009). Ecologically, poplars are among the most productive trees in northern riparian ecosystems, while economically they are valued for timber, bioenergy, and environmental applications such as shelterbelts (Chen & Polle, 2010; Richardson et al., 2014). The large diversity within the genus *Populus* includes species that vary widely in salinity tolerance, ranging from highly sensitive (*P. popularis*) to strongly tolerant (*P. euphratica*) (Chen et al., 2002; Chen & Polle, 2010; Ottow et al., 2005). Hybrid poplars are generally moderately sensitive to salinity, though their responses vary among genotypes (Moran, 2023). Importantly, poplars are naturally susceptible to a wide array of insect herbivores, making them a powerful model for studying how abiotic stresses such as salinity shape plant-herbivore interactions (Philippe & Bohlmann, 2007).

In this study, the lepidopteran, white-marked tussock moth (*Orgyia leucostigma*) was selected because it is a generalist herbivore native to North America that feeds on numerous deciduous trees, including poplars, birches (*Betula* spp.), maples(*Acer* spp.), and oaks (*Quercus* spp.), and is frequently encountered in both forests and urban habitats (Schowalter et al., 2018). Outbreaks of this species can cause substantial defoliation, reducing tree growth and vigor (MacGillivray, 1950; Schowalter, 2018). Since hybrid poplars are widely used in forestry, shelterbelts, and urban plantings, their susceptibility to a common, locally abundant herbivore such as *O. leucostigma* makes it a suitable model to determine how salinity affects plant resistance and herbivore performance. Therefore, the main goal of this study was to determine the interactive effects of salinity and insect herbivory in hybrid poplar (*Populus*) cultivar

‘Okanese’, focusing on how these factors affect leaf nutritional quality and resistance to the white-marked tussock moth (*Orgyia leucostigma*).

The first specific objective of this study was to determine the effect of salinity on herbivory resistance in hybrid poplar. That included determining the effect of salinity on leaf nutritional quality known to affect herbivory resistance and the association between the changes in leaf nutritional quality and herbivory resistance. I hypothesized that salinity would increase resistance of hybrid poplar to *O. leucostigma* by reducing the nutritional quality and enhancing defensive chemistry. While salinity can elevate N levels, potentially increasing herbivore attractiveness (Mattson, 1980), it simultaneously promotes changes in ion balance (K^+, Ca^{2+}, and Na^+; Munns and Tester, 2008) and stimulates the production of phenolic compounds (Petridis et al., 2012) which are known to reduce the palatability of the leaf tissues and interfere with herbivore digestion (Duffey & Stout, 1996).

The second specific objective was to determine the effect of herbivory on the responses of plants to salinity and to further herbivory. It was hypothesized that prior herbivory could prime responses to salinity by inducing biochemical changes such as phenolic accumulation and shifts in elemental and protein content that would enhance subsequent responses to salt stress. Such priming is hypothesized based on evidence that herbivory activates signaling processes involving Ca^{2+} fluxes, reactive oxygen species (ROS) production, and JA- and ABA- mediated signaling, which overlap with mechanisms underlying abiotic stress tolerance (Erb & Reymond, 2019; Frost et al., 2008). Through these shared pathways, prior herbivory may induce physiological adjustments that enhance tolerance to subsequent salinity exposure. Furthermore, it was expected that this priming would also increase resistance to later herbivory by maintaining elevated concentrations of defense metabolites in leaves.

CHAPTER 2 - LITERATURE REVIEW

2.1. Salinity stress

2.1.1. Introduction

Soil salinity is one of the most critical environmental stress factors affecting global agriculture, significantly impacting crop productivity and ecosystem stability. According to the Food and Agriculture Organization (FAO), over 800 million hectares of land worldwide are currently affected by salinity, and this is predicted to worsen under climate change due to rising temperatures and reduced rainfall (Cortés, 2019). Primary salinization, which leads to most salt-affected lands in arid and semi-arid regions, is consequence of naturally occurring processes such as weathering and deposition of oceanic salts (Munns & Tester, 2008). In addition, a significant proportion of arable land is currently affected by secondary salinization caused by land clearing, irrigation, and fertilizer use (Cuevas, 2019). In Canada, anthropogenic sources such as road de-icing salts and mining operations have also contributed to increasing soil salinity in the plant root zone in the soil of forests and urban landscapes (Environment Canada, 2011).

Electrical conductivity (EC) is a key measurement for assessing soil salinity and quantifying the concentration of soluble salts. The FAO defines saline soils as having an EC ≥ 4 dS/m, equivalent to a 40 mM NaCl concentration or an osmotic pressure of approximately 0.2 MPa (Munns & Tester, 2008), whereas values below 1 dS/m indicate non-saline conditions with minimal impact on plants and soil microbiota (Rusydi, 2018). The intermediate range between 1 and 4 dS/m is often described as slightly saline. Within this range, some salt-sensitive plant species may already exhibit reduced growth, as even mild salinity can impair nutrient uptake and water absorption (Ehtaiwesh, 2022). Salinity arises primarily from the accumulation of ions such

as sodium (Na^+), magnesium (Mg^{2+}), calcium (Ca^{2+}), sulfate (SO_4^{2-}), chloride (Cl^-), and bicarbonate (HCO_3^-). NaCl constitutes 50-80 % of all soluble salts formed. It is particularly harmful because it contributes to deteriorating soil structure and impairing root function (Safdar et al., 2019).

Plant tolerance to soil salinity varies widely, and species are generally classified as either halophytes or glycophytes. Halophytes, which account for only about 0.25 % of all angiosperms, are naturally adapted to saline habitats such as salt marshes and coastal zones. Halophytic plants can complete their life cycles at NaCl concentrations ≥ 200 mM (20 dS/m), with some species being able to tolerate over 1M (Flowers et al., 2010; Shabala, 2013). This tolerance is conferred by specialized physiological and biochemical mechanisms, including ion compartmentalization, salt exclusion, salt secretion, and osmotic adjustment (Munns & Tester, 2008; Parida & Das, 2005). In contrast, most terrestrial plants are glycophytes, a diverse group that includes many crops and forest species which generally exhibit growth reductions at NaCl concentrations between 50 and 150 mM (Munns & Tester, 2008; Zhu, 2001). However, salt sensitivity among glycophytes is highly variable. While some salt-tolerant glycophytes can endure moderate salinity (EC 4-8 dS/m), salt-sensitive glycophytes exhibit growth inhibition at very low salinity, often below 1-2 dS/m (Flowers & Colmer, 2008), because they evolved in low-sodium environments (Cheeseman, 2015).

2.1.2. The effects of salinity on plants

2.1.2.1. Initial phase: osmotic and ion-independent responses

Plant responses to salinity can generally be categorized into two sequential phases (Munns & Tester, 2008). The initial phase, known as the ion-independent response, takes place within minutes to a few days and primarily involves signal perception and transmission related to Na^+

presence (Negrão et al., 2017). During this phase, plants experience osmotic stress, resulting from a decrease in soil water potential due to the presence of salts, making it more difficult for plant roots to absorb water (Eraslan et al., 2007). This immediate stress can disrupt various physiological functions, including membrane integrity, nutrient uptake, gas exchange and the efficiency of photosynthesis (Gupta & Huang, 2014; Munns & Termaat, 1986). One of the primary plant responses to salinity stress is stomatal closure, a mechanism that minimizes water loss by reducing transpiration to conserve water in the plant. In response to osmotic stress, ABA biosynthesis is induced primarily in vascular and mesophyll tissues and subsequently transported to guard cells, where it serves as a central regulator of stomatal closure (Kuromori et al., 2018). ABA triggers signaling cascades that activate ion channels, leading to K^+ and anion efflux, turgor loss, and reduced stomatal aperture (Munemasa et al., 2015). However, stomatal closure also limits the uptake of carbon dioxide (CO_2; Chaves et al., 2009), which is essential for the Calvin cycle of photosynthesis (Hossain & Dietz, 2016). The reduction in CO_2 availability slows carbon fixation, leading to an energy surplus in the chloroplast as the absorbed light cannot be efficiently used. This imbalance disrupts the photosynthetic electron transport chain and causes the overproduction of ROS. The accumulation of ROS leads to an oxidative stress, harming cellular components such as membranes, proteins, and DNA (Azeem et al., 2023; Bose et al., 2014; Munns & Tester, 2008).

2.1.2.2. Second phase: ionic and ion-dependent responses

In the second phase of salt stress, known as the ion-dependent phase, plants begin to accumulate toxic levels of ions particularly Na^+ and Cl^- primarily in older leaves (Joshi et al., 2022). As these ions build up in the cytosol and organelles, their concentrations exceed the levels compatible

with normal cellular metabolism, disrupting intracellular ion homeostasis and causing toxicity (Munns & Tester, 2008). This interferes with essential metabolic processes, including enzyme activity, protein synthesis, and cellular signaling. One major consequence is a nutritional imbalance, as Na^+ ions compete with K^+ ions, thereby reducing K^+ uptake and impairing processes such as enzyme activation and osmoregulation (Shabala & Mackay, 2011). In addition, excessive internal Na^+ and Cl^- levels impair the uptake and translocation of other essential macro- and micronutrients such as Ca^{2+}, magnesium (Mg^{2+}), nitrate (NO_3^-), zinc (Zn), and iron (Fe), further exacerbating physiological dysfunction (Kumar et al., 2021; Marschner, 2011). Moreover, ionic stress impairs organelle function and disturbs electron transport chains in both chloroplasts and mitochondria, resulting in excessive ROS production (Apel & Hirt, 2004; Gill & Tuteja, 2010). The ensuing oxidative stress damages membranes and photosynthetic structures, accelerating chlorophyll degradation. This is visually evident as chlorosis and necrosis, predominantly in older leaves that act as sinks for ions (Wang et al., 2024). The cumulative effects of ion toxicity, oxidative damage, and nutrient deficiency ultimately impair photosynthesis and cellular metabolism (Marschner, 2011).

2.1.3. Salt tolerance in plants

Plants exposed to salinity activate a range of physiological and biochemical mechanisms, including ion compartmentalization, osmotic adjustment, selective ion transport, and antioxidant defenses. These mechanisms are widespread across plant species. For example, halophytes possess specialized structures such as salt glands on their leaves and stems (Thomson, 1975) and salt bladders that sequester or secrete excess salts, along with highly selective ion transport systems that regulate salt entry at its source (Shabala et al., 2014). In contrast, glycophytes

depend on comparatively less effective mechanisms to maintain ionic balance (Polle & Chen 2015; Shabala et al., 2014). Salt-tolerant species rarely rely on a single mechanism to survive under salinity; instead, they have multiple interacting pathways that allow them to tolerate salinity (Niknam & McComb, 2000). Key adaptive responses, briefly explained below include, osmotic adjustment, ion homeostasis, antioxidant induction, and signaling and hormonal crosstalk (Cuin & Shabala, 2008; Hossain & Dietz, 2016; Liang et al., 2018; Parida & Das, 2005).

2.1.3.1. Osmotic adjustment via compatible solutes

Plants exposed to salinity often regulate their internal osmotic environment by accumulating either inorganic ions or low molecular weight organic compounds that decrease the osmotic potential and water potential thus restoring cellular turgor pressure (Ashraf & Harris, 2004; Singh et al., 2015). These solutes are termed "compatible osmolytes" because they do not disrupt normal biochemical processes, even at high intracellular levels (Brown & Simpson, 1972). These osmolytes include proline, glycine betaine, polyols (e.g., mannitol, sorbitol), and soluble sugars (e.g., glucose, sucrose, and trehalose), which are synthesized or mobilized to increase the solute concentration of the cytoplasm thereby lowering osmotic potential and helping cells retain water under stress. In addition to maintaining osmotic balance, they stabilize membranes and thus mitigate the toxic effects of excess ions (Ozturk et al., 2021). The accumulation of proline under salt stress not only contributes to osmotic adjustment but also act as a potent antioxidant (Smirnoff & Cumbes, 1989). Therefore, elevated proline levels are widely used as a physiological indicator of salt stress tolerance (Abdelhamid et al., 2013; Balasubramaniam et al., 2023). For instance in *Populus euphratica* accumulates proline under salinity (Watanabe et al.,

2000). In addition, glycine betaine application in *Phaseolus vulgaris* plants exposed to salinity improves osmoregulation maintaining a higher K^+/Na^+ ratio and antioxidant activity (Sofy et al., 2020). Similarly, soluble sugars protect cellular integrity by stabilizing enzymes and lipid bilayers, which helps maintain membrane structure and prevent ion leakage or lipid peroxidation (Keunen et al., 2013; Singh et al., 2022). Evidence from transgenic *Arabidopsis thaliana* plants overexpressing the *Triticum aestivum* salt stress tolerance gene *TaSST*, which encodes a cytomembrane-localized protein strongly induced under salinity stress, demonstrated significantly higher accumulation of soluble sugars and proline and markedly enhanced salt tolerance compared with wild-type plants (Li et al., 2016). Notably, trehalose also plays a distinctive role due to its reversible water-binding capacity, which aids in protecting cells from osmotic damage. In addition to sugars, polyols act as both osmoprotectants and scavengers of ROS. For example, mannitol, an acyclic sugar alcohol, contributes substantially to osmotic adjustment and enhances salt tolerance in higher plants (Pujni et al., 2007).

2.1.3.2. Ion homeostasis and membrane level adaptations

Salinity disrupts ionic equilibrium in plants primarily through excessive Na^+ influx, which triggers cytosolic Na^+ accumulation and membrane depolarization, resulting in K^+ efflux. The ensuing loss of K^+ compromises enzyme activity, osmotic balance, and antioxidant regulation, ultimately impairing cellular metabolism (Arif et al., 2020). A critical determinant of salt tolerance is the regulation of the cytosolic Na^+/K^+ ratio, since high ionic ratios (e.g., Na^+/K^+, Na^+/Ca^{2+}) interfere with nutrient transport and signaling. To mitigate these effects, some plants can restrict Na^+ entry, enhance its extrusion, and/ or compartmentalize excess Na^+ into vacuoles (Zhu, 2003). The plasma membrane Na^+/H^+ antiporter plays a central role in extruding Na^+,

while tonoplast-localized NHX transporters compartmentalize Na^+ into vacuoles. These processes are powered by proton gradients established by plasma membrane H^+-ATPases and vacuolar H^+-pyrophosphatases (Blumwald et al., 2000; Brini & Masmoudi, 2012). The Salt Overly Sensitive (SOS) pathway is especially important in this regulation: SOS2 kinase, activated by the calcium sensor SOS3, phosphorylates SOS1 to promote Na^+ efflux and indirectly stimulates H^+-ATPase activity, thereby reinforcing vacuolar sequestration (Qiu et al., 2004; Yang & Guo, 2018; Zhu, 2000). Together, these mechanisms maintain ion homeostasis and protect cellular metabolism under saline conditions. Such mechanisms help prevent high ratios between Na^+ and K^+, Na^+ and Ca^{2+} (Horie et al., 2012).

However, Na^+ accumulation under salinity is often accompanied by a decline in cytosolic K^+, due to ionic competition making it difficult to maintain low cytosolic Na^+/K^+ ratios which is crucial for cellular integrity (Pottosin & Shabala, 2014). In response to this ionic imbalance, Ca^{2+} plays a critical dual role, it stabilizes cellular redox potential by activating antioxidant enzymes and acts as a secondary messenger in salt stress signaling. Salt stress triggers cytosolic Ca^{2+} spikes, initiating adaptive responses through pathways involving calmodulin and Ca^{2+}-dependent protein kinases (Plasencia et al., 2021; Safdar et al., 2019).

Under salinity, oxidative stress induces the production of nitric oxide (NO), which enhances the activity of H^+-ATPases and H^+-pyrophosphatases in both the plasma membrane and tonoplast, thereby supporting Na^+/H^+ antiport activity and promoting the sequestration of Na^+ into vacuoles. This process helps reduce cytosolic Na^+ toxicity and supports ionic homeostasis. In addition, NO has been shown to affect Ca^{2+} signaling by regulating calmodulin and Ca^{2+}-dependent pathways, contributing to the activation of osmotic stress tolerance responses (Ali et al., 2019: Joshi et al., 2022).

2.1.3.3. Antioxidant defense against oxidative stress

Plants deploy both enzymatic and non-enzymatic elements of the antioxidant defense system that work to neutralize or eliminate excess ROS, thereby reducing the harmful impacts of oxidative stress (Sachdev et al., 2021). Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase (GPX), polyphenol oxidase (PPO), peroxiredoxin (PRX), thioredoxin (TRX), and glutathione S-transferase (GST; Apel & Hirt, 2004; Gill & Tuteja, 2010). Among these, SOD acts as the first line of defense by converting $O_2\bullet^-$ into H_2O_2, which is subsequently detoxified by CAT, APX and associated peroxidases. Non-enzymatic antioxidants such as ascorbate (AsA), glutathione (GSH), and flavonoids complement these enzymes by directly scavenging ROS and maintaining redox homeostasis. A central role is played by the ascorbate-glutathione (AsA-GSH) cycle, which integrates APX, GR, dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), together with AsA and GSH, to regulate H_2O_2 levels and sustain cellular redox balance (del Río et al., 2018; Hasanuzzaman et al., 2021).

The effectiveness of this antioxidant system varies depending on species, stress intensity, and developmental stage. In salt-sensitive poplar species such as *Populus popularis*, the antioxidant defense system is insufficient to fully counteract ROS accumulation under prolonged salinity. Although antioxidant enzyme activities (SOD, APX, GR) increased 10- to 18-fold in the xylem sap, ROS continued to accumulate in both leaves and xylem, indicating that production of ROS outpaced scavenging capacity (Wang et al., 2008). This imbalance led to oxidative damage in leaf tissues, as evidenced by visible symptoms such as chlorosis and necrosis, which result from ROS-induced disruption of cellular structures and functions. This is likely impaired by high Na^+ and Cl^- accumulation in chloroplasts, which disrupt the photosynthetic function (Wang et al.,

2007). Similarly, in *Populus* × *canescens*, salinity stress induced SOD activity, but not CAT, suggesting an uncoordinated antioxidant response and differential regulation of enzymatic components (Bolu & Polle, 2004).

Moreover, exogenous treatments such as gibberellic acid (GA), SA, melatonin (MT), silicon, and selenium have been shown to enhance antioxidant responses and reduce oxidative damage under salinity in species such as *Vicia faba* and *Beta vulgaris* (Dawood et al., 2022; Zhang et al., 2021). These compounds promote the activities of antioxidant enzymes such as SOD, APX, GR, and CAT while lowering levels of ROS indicators and malondialdehyde (MDA) by modulating redox sensitive signaling networks, that upregulate the expression of antioxidant genes and enhance enzymatic ROS scavenging capacity.

2.1.3.4. Signaling and hormonal crosstalk

The exposure of plants to salt activates a cascade of signaling pathways. The Salt Overly Sensitive (SOS) pathway is particularly important in ionic homeostasis (Qiu et al., 2002). This pathway is regulated by the interaction of three key proteins: SOS3, a calcium-binding sensor protein; SOS2, a serine/threonine kinase; and SOS1, a Na^+/H^+ antiporter located on the plasma membrane (Yang & Guo, 2018). When plants are exposed to salinity, the level of cytosolic Ca^{2+} rises, which is identified by SOS3 (Quintero et al., 2002). This sensor then associates with SOS2, triggering its kinase activity. The resulting SOS2-SOS3 complex phosphorylates SOS1, thereby enhancing its ability to efflux Na^+ from the cytoplasm (Quintero et al., 2011). This mechanism helps reduce Na toxicity and contributes significantly to salt tolerance in plants (Figure 2.1).

In addition to calcium-based signaling, multiple phytohormones such as ABA, JA, SA and ethylene are essential to plant responses under salinity (Fahad et al., 2015). These hormones coordinate complex signaling networks that enable plants to grow in high-salinity environments.

For example, ABA biosynthesis is typically upregulated under salt stress, leading to stomatal closure and the induction of stress-responsive gene expression (Bharath et al., 2021). JA and SA contribute to salinity tolerance by enhancing antioxidant defense mechanisms and modulating ion uptake and transport, thus helping to preserve ionic equilibrium (Karimi et al., 2025; Zhu et al., 2022). Ethylene also supports salt stress adaptation by regulating Na^+/K^+ homeostasis, nutrient acquisition, and ROS detoxification, primarily through the activation of antioxidant pathways (Riyazuddin et al., 2020). Melatonin, an indole-based signaling molecule naturally present in plants, also participates in these hormonal crosstalk networks. It enhances salinity tolerance by stimulating antioxidant enzyme activity that maintain redox balance and mitigate oxidative stress (Li et al., 2012).

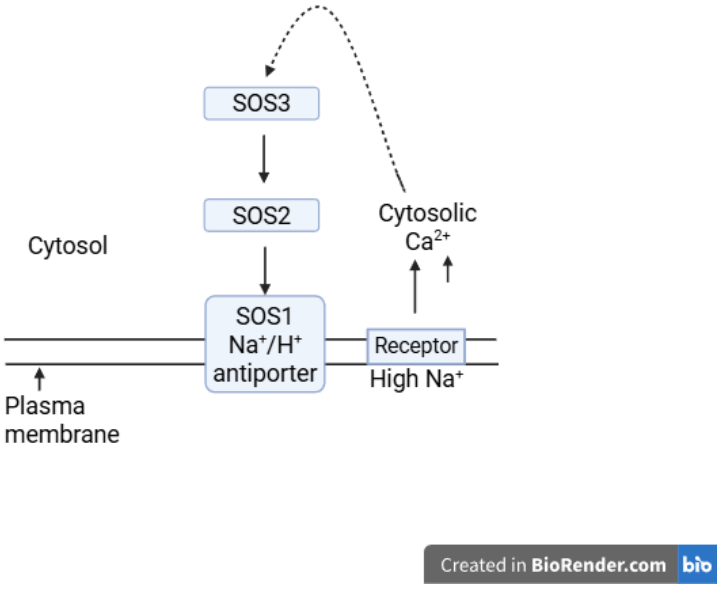

Figure 2.1. Schematic diagram of the Salt Overly Sensitive (SOS) signaling pathway involved in plant salt tolerance. High external Na^+ levels induce an increase in cytosolic Ca^{2+}, which is detected by the calcium-binding protein SOS3. SOS3 then forms a complex with the protein kinase SOS2 which becomes activated. The activated SOS3-SOS2 complex enhances the transcription and possibly post-translational regulation of the plasma membrane Na^+/H^+ antiporter SOS1, promoting Na^+ extrusion from the cytosol. This pathway plays a central role in maintaining ionic homeostasis, particularly Na^+/K^+ balance, thereby contributing to plant salt tolerance (modified from Cheong and Yun, 2007; Zhu, 2003). The figure was produced with BioRender.com.

2.2. Insect herbivory

2.2.1. Introduction

Insect herbivory, the consumption of plant tissues by insect feeders, is ubiquitous ecological interaction that affects plant survival, growth, and productivity (Schoonhoven et al., 2005). Collectively with fungal infections and other pathogens, herbivores account for over 50 % of global plant damage, surpassing the impact of abiotic stressors (Faiola & Taipal, 2020; Kautz et al., 2017). Insect herbivores are categorized into feeding guilds based on their dietary strategies and plant tissue preferences. Leaf-chewing generalists (e.g., caterpillars and grasshoppers) take bites at foliage to consume it, while highly specialized leaf-mining larvae tunnel internally, leaving visible trails. Other guilds, including sapsuckers, gall-inducers, and root feeders, occupy distinct niches but are less prevalent (Adu-Acheampong & Samways, 2019; Novotny et al., 2010).

Beyond feeding guild distinctions, the nutrient content of plant tissues also strongly shapes herbivore preferences. Herbivorous insects preferentially feed on plants with higher levels of leaf N and P, both of which are crucial for plant growth and development (Gu et al., 2022; Rode et al., 2017). Conversely, defensive compounds such as polyphenols (e.g., condensed tannins) and physical traits such as increased leaf mass per area (LMA) or high cellulose and lignin content deter herbivory by reducing digestibility and increasing leaf toughness, often through lignification or cellulose accumulation (Kitajima et al., 2012; Singh & Kariyat 2020; Singh et al., 2021).

2.2.2. Defensive mechanisms in plants against herbivory

Plants deploy chemical defenses that are often toxic or unpalatable to generalist herbivores, thereby reducing feeding. These include phenolic compounds (e.g., tannins and flavonoids), terpenoids (e.g., monoterpenes and diterpenes), and N-containing compounds (e.g., alkaloids, glucosinolates, and cyanogenic glycosides; Mithöfer & Boland, 2012). Many of these compounds alter the taste or odor of plant tissues, making them less attractive. Other compounds are volatile and function indirectly by attracting natural enemies of herbivores through the release of volatile organic compounds (Agrawal, 2005). Phenolic compounds deter herbivory through several mechanisms: upon oxidation, they form quinones that bind to dietary proteins and reduce digestibility, lowering the effective nutritional value of foliage (Bhonwong et al., 2009; Duffey & Stout, 1996). They can also directly inhibit digestive enzymes or act as toxins, thereby slowing insect growth and performance (Bhonwong et al., 2009). Additionally, phenolics contribute to redox cycling by modulating ROS, which activate defense-related signaling pathways and further strengthen plant resistance (Maffei et al., 2007). Structural traits such as spines, thorns, and lignified or thickened cell walls similarly reduce leaf palatability and accessibility, thereby limiting tissue loss to herbivores (He et al., 2011). Collectively, these physical defenses form a critical first barrier against insect feeding and often complement chemical deterrents by providing mechanical resistance (Denno et al., 1990; Dimarco et al., 2012). Plant nutrient content, particularly N, P, and K, significantly affect herbivore interactions with host plants by enhancing plant palatability and nutritional quality, which promotes herbivore growth, survival, and reproduction (Zeng, 2024). Additionally, nutrient uptake facilitated by beneficial microbes such as mycorrhizal fungi and rhizobacteria can alter plant

secondary metabolism, indirectly impacting plant resistance or tolerance to herbivore attacks (Zeng, 2024).

Nitrogen is a key nutrient for host preference in herbivores. Specialist herbivores that can handle plant toxins such as phenolic glycosides, tannins, and alkaloids may still face reduced feeding efficiency, but high N levels in the leaves can help make up for this by providing the nutrients they need to grow and survive (Volf et al., 2015). In contrast, generalist herbivores often lack the specialized detoxification mechanisms needed to tolerate plant toxins, so even if a plant has high N content, they may be unable to benefit from it if defensive compounds interfere with feeding or digestion (Ali and Agrawal, 2012). Moreover, limiting N availability may enhance resistance to generalists; for instance, in *Solanum lycopersicum*, reduced N supplies increased resistance to the generalist whitefly *Bemisia tabaci* without compromising fruit yield (Ramachandran et al., 2020).

In addition to localized responses to herbivore damage, plants have systemic signaling networks that initiate defense-related gene expression and metabolic changes in distal, undamaged tissues. Herbivore-induced JA and its conjugate JA-Ile rapidly accumulate in both wounded and distal leaves, where their presence is associated with the activation of systemic defense responses (Glauser et al., 2008; Mousavi et al., 2013). For instance, *Mythimna separata* feeding on *Zea mays* induces defense-related responses in both damaged and adjacent leaves, mediated by JA signaling (Malook et al., 2019). Similarly, *Arabidopsis thaliana* exhibits rapid jasmonate upregulation within minutes of herbivory by *Helicoverpa armigera* and *Plutella xylostella*, with systemic changes observed across the entire plant (Glauser et al., 2008). These systemic signals, involving hormones like JA, enable plants to activate defense responses that protect undamaged tissues against subsequent attacks (Wasternack & Song, 2019). However, the

mechanisms coordinating long-distance communication between damaged and intact leaves are still being unravelled (Liu et al., 2022).

2.2.3. Constitutive and induced resistance

Plant defenses against herbivory have been categorized into three fundamental strategies: deterrence (antixenosis), resistance (antibiosis), and tolerance (compensatory mechanisms that mitigate fitness consequences of damage; Painter 1951). Deterrence involves morphological and chemical traits, such as coloration, odors, or physical textures (e.g., trichomes), that discourage herbivores from feeding (Hanley et al., 2007).

Resistance includes structural and biochemical traits that impair the performance of herbivores directly. These traits can be constitutive, such as thick cuticles, lignified tissues, or preformed compounds (e.g., tannins, alkaloids), or induced, activated after herbivore attack through signaling pathways involving JA, SA, and ethylene (Karban & Baldwin, 1997; Tian et al., 2014). Induced resistance often involves the production of defensive proteins (e.g., protease inhibitors, polyphenol oxidases, chitinases) and secondary metabolites (e.g., phenolics, terpenoids, alkaloids) that reduce palatability, disrupt digestion, or increase toxicity (Constabel et al., 2000; Fürstenberg-Hägg et al., 2013; Dixit et al., 2017). These defenses may occur locally or be systemically induced in undamaged leaves (Howe & Jander, 2008). Some compounds, such as herbivore-induced volatiles, also function indirectly by attracting predators or parasitoids of herbivores (Turlings & Erb, 2018).

Tolerance is an induced response, involves compensatory physiological and morphological adjustments such as enhanced photosynthesis, leaf regrowth, or altered resource allocation following damage (Koch et al., 2016; Strauss & Agrawal 1999). While plants may

combine deterrence, resistance, and tolerance, trade-offs between constitutive and induced resistance or between resistance and tolerance may occur (Kalske & Kessler, 2023; Kempel et al., 2011; Núñez-Farfán et al., 2007).

2.3.Interactions among stress factors in plants

2.3.1. Introduction

In natural and agricultural environments, plants are rarely exposed to single stressors. Instead, they frequently face multiple, concurrent stressors, both abiotic (e.g., drought, salinity, heat, nutrient limitation) and biotic (e.g., insect herbivory, pathogen infection). Plant responses to single stress factors have been widely examined and are relatively well understood. When multiple stress factors occur simultaneously, their interactions are often complex, yet knowledge of how they affect plant growth, productivity, and defense is still limited (Nawaz et al., 2023).

The interaction between multiple stress factors can result in synergistic effects, where damage is greater than the sum of individual stress responses; antagonistic effects, where one stress factor mitigates the impact of the other one(s) or neutral effects, where the combined impact is similar to that of a single stress (e.g., lack of interaction; Atkinson & Urwin, 2012; Suzuki et al., 2014). These outcomes are shaped by the type, intensity, duration and timing of exposure, as well as plant species, developmental stage, and ecological context (Rejeb et al., 2014).

Several studies illustrate the complexity of plant responses to combined abiotic and biotic stressors. In some cases, abiotic stress can exacerbate disease or herbivory. For example, drought increased the severity of *Sclerotium rolfsii* infection in chickpea (*Cicer arietinum*) and reduced parasitoid attraction in sugar beet (*Beta vulgaris*), weakening tritrophic defenses (Sinha et al.,

2019; Rahman et al., 2025). Similarly, salinity disrupted SA signaling in cucumber (*Cucumis sativus*), increasing susceptibility to *Pseudomonas syringae* pv. *lachrymans* (Chojak-Koźniewska et al., 2017). In other cases, stress-induced metabolic changes cause variable or contrasting effects on plant resistance depending on the type or intensity of the stress encountered. Apple (*Malus domestica*), for instance, showed enhanced resistance to insect herbivory under moderate drought but reduced resistance under severe stress, likely due to fructose accumulation that increased palatability to *Spodoptera littoralis* (Gutbrodt et al., 2012). By contrast, heavy metal (low concentration of cadmium) exposure in *Populus yunnanensis* enhanced resistance to both herbivores and pathogens (Lin et al., 2020). Positive outcomes have also been reported in tomato (*Solanum lycopersicum*), where drought induced greater trichome density and enhanced resistance to the whitefly *Trialeurodes vaporariorum* (González-Klenner et al., 2022), and in milkweed (*Asclepias* spp.), where drought-driven cardenolide accumulation reduced the performance of monarch caterpillars (*Danaus plexippus*) and aphids (*Aphis nerii*; Carvajal Acosta et al., 2022). However, not all interactions are beneficial: in potato (*Solanum tuberosum*), sequential heat stress and herbivory suppressed jasmonate-mediated defenses, ultimately promoting the growth of larvae of the potato tuber moth (*Phthorimaea operculella*; Zhong et al., 2024). These examples underscore that stress interactions are not universally synergic; instead, they reflect complex physiological trade-offs and crosstalk among hormonal signaling pathways particularly involving ABA, JA, SA, and ethylene. Understanding these interactions is crucial for predicting plant performance under climate change and for developing resilient cropping and forestry systems.

2.3.2. Interaction between salinity and resistance to herbivores

Salinity and herbivory are two widespread stressors that frequently co-occur, particularly in irrigated agroecosystems, roadside habitats, and coastal or arid environments where salt accumulation in the soil coincides with insect activity. Their combined impact on plants is especially relevant under climate change, as both soil salinization and insect herbivore pressure are projected to intensify in many regions (Deutsch et al., 2018; Zörb et al., 2019). As with drought, the effects of salinity on herbivory are variable, ranging from increased resistance (Han et al., 2016; Hemminga & van Soelen, 1988) to decreased resistance (Nabity et al., 2006; Quais et al., 2019), or no significant change at all (Hemminga & van Soelen, 1988; Munck et al., 2010). These inconsistencies in both halophytes and glycophytes (Table 2.1 and Table 2.2) highlight the complex and species-specific nature of salinity-herbivory interactions and the complexity of the plant response.

A study conducted on dixie iris (*Iris hexagona*), a moderately salt-tolerant wetland species, showed that plants exposed to salt had a lower density of leaf miner (*Cerodontha iridophora*) compared to plants that were not exposed to salt (Schile and Mopper, 2006). In addition, salinity stress enhanced herbivore resistance in Indian mustard (*Brassica juncea*) plants, as indicated by increased Na and proline content and decreased N content in leaf tissues (Renault et al., 2016). On the contrary, a study on soybean (*Glycine max*) showed that exposure to salinity resulted in lower resistance to cabbage looper (*Trichoplusia ni*), both constitutive and induced (Avila-Sakar et al., 2018). This decline was associated with reduced plant biomass, lower chlorophyll content, decreased trichome density, and disrupted N fixation under salt stress, along with altered shoot nutrient profiles (elevated Na, K, Ca, Mn, B, and Cl levels). These physiological changes likely impaired the ability of plants to mount effective defenses against

herbivory (Avila-Sakar et al., 2018). A study examining the interaction of salinity and herbivory in rice (*Oryza sativa*) showed that salinity stress strongly affected brown planthopper (*Nilaparvata lugens*) via bottom-up effects. Specifically, increased salinity levels interfered with egg hatching, extended nymphal development periods, reduced adult longevity, and decreased oviposition rates, highlighting the bottom-up effects of salinity stress, where changes in plant quality due to salinity affect herbivore populations. (Quais et al. 2019). On the other hand, urban trees exposed to de-icing salts showed no significant change in herbivory resistance compared to trees not exposed to salt, suggesting that, while de-icing salts may have negative effects on urban trees (mostly conifers and shrubs), they do not necessarily affect the ability of plants to resist herbivory (Munck et al. 2010). While the effects of salinity on herbivore resistance have been explored in various species, relatively little is known about how herbivory might shape a plant's response to subsequent abiotic stress such as salinity, leaving a significant gap in our understanding of stress interaction dynamics in plants.

Emerging evidence suggests that biotic stress, such as herbivory, can "prime" plants through shared signaling pathways and physiological adjustments and, thus, enhance tolerance to abiotic challenges (Nguyen et al., 2016; Walters and Heil, 2007). For instance, herbivory triggers signaling cascades involving JA, SA, and ethylene, which are also involved in responses to abiotic stress, including salinity (Fujita et al., 2006; Kissoudis et al., 2014; Pieterse et al., 2012). It has been shown that herbivore elicitors can enhance the ability of the desert evergreen shrub, *Ammopiptanthus nanus* to withstand salt stress by activating the JA-signaling pathway, increasing plasma membrane H^+-ATPase activity, promoting cytosolic Ca^{2+} accumulation, and subsequently limiting K^+ leakage and Na^+ accumulation in the cytosol (Chen et al., 2020). These

findings suggest that herbivore-induced resistance may not only affect insect performance but also induce the initial steps of the plant physiological responses to abiotic stressors like salinity.

Although the interactive effects of salinity and herbivory have been increasingly explored, significant gaps remain in our understanding, particularly those concerning the mechanisms underpinning these interactions. In addition, there is limited number of studies on long-lived woody species. However as previously explained. in both natural and managed systems, plants commonly encounter abiotic (e.g., salinity) and biotic (e.g., herbivory) stress in combination or sequentially. These overlapping stress factors may trigger complex factors physiological and biochemical responses within the plant, particularly through signaling pathways that may share common elements, interact synergistically, or inhibit one another (Atkinson & Urwin, 2012; Mittler, 2006). Investigating the crosstalk between salinity and herbivory-induced pathways is thus important for understanding the broader regulatory networks governing plant defense and adaptation. This priming may involve the accumulation of secondary metabolites, such as phenolics and terpenoids which play dual roles in direct defense against herbivores and in mitigating oxidative stress caused by salinity. Herbivory-induced activation of these compounds can precondition plants to cope more effectively with subsequent abiotic stress by enhancing antioxidant capacity, stabilizing cellular structures, and regulating ion transport (Mithöfer & Boland, 2012; Sharma et al., 2019). This suggests that exposure to one type of stress, such as herbivory, can help plants to handle another stressor like salinity, highlighting how interconnected their defense strategies.

Table 2.1. Effects of salinity stress on herbivory resistance in non-halophytic plant species. The table summarizes documented changes in plant resistance (increased, decreased, or no change) to various herbivores under salinity stress conditions.

Resistance change	Plant species	Herbivore	Reference
Decreased	*Gossypium hirsutum* (cotton)	*Agrotis segetum* (turnip moth) and *Aphis gossypii* (cotton aphid)	Zhang et al., 2024
	Gossypium hirsutum	Simulated herbivory/ *Spodoptera frugiperda* (fall armyworm)	Quijano-Medina et al., 2021
	Glycine max (soybean)	*Trichoplusia ni* (cabbage looper)	Avila-Sakar et al., 2018
	Glycine max and *Zea mays* (maize)	*Tetranychus urticae* (two-spotted spider mite)	Eichele-Nelson *et al*., 2017
	Fragaria ananassa (strawberry)	*Tetranychus cinnabarinus* (carmine spider mite)	Cakmak & Demiral, 2007
	Citrus reticulata (mandarin)	*Tetranychus urticae* (spider mites)	Aucejo-Romero et al., 2004
	Capsicum annuum (sweet pepper)	*Myzus persicae* (green peach aphid)	Polack et al., 2004
Increased	*Solanum lycopersicum* (tomato)	*Helicoverpa zea* (tomato fruit worm caterpillar)	Pawar et al., 2025
	Solanum lycopersicum	*Spodoptera exigua* (beet armyworm)	Marsack & Connolly, 2022
	Triticum aestivum (bread wheat)	*Rhopalosiphum padi* (bird cherry-oat aphid)	Ghodoum et al., 2021

Table 2.1. continued

Resistance change	Plant species	Herbivore	Reference
Increased	*Oryza sativa* (rice)	*Nilaparvata lugens* (brown planthopper)	Quais et al., 2019
	Glycine max	*Pseudoplusia includens* (soybean looper)	Najjar et al., 2018
	Solanum lycopersicum	*Tuta absoluta* (leaf miner)	Han et al., 2016
	Brassica juncea (Indian mustard)	*Trichoplusia ni*	Renault et al., 2016
	Iris hexagona (dixie iris)	*Cerodontha iridiphora* (leaf miner)	Schile & Mopper, 2006
	Solidago altissima (tall goldenrod)	*Trirhabda borealis* (leaf beetles)	Martel, 1998
No change	*Poplar* spp.	*Orgyia leucostigma*	Moran, 2023
	Solanum lycopersicum	*Trichoplusia ni*	Thaler & Bostock, 2004

Table 2.2. Effects of salinity stress on herbivory resistance in halophytic plant species. The table summarizes documented changes in plant resistance (increased, decreased, or no change) to various herbivores under salinity stress conditions.

Resistance change	Plant species	Herbivore	Reference
Decreased	*Atriplex subspicata* (saline saltbush)	*Spilosoma virginica* (yellow bear caterpillar)	Nabity et al., 2006
Increased	*Spartina alterniflora* (saltmarsh cordgrass)	Simulated herbivory	Wittyngham, 2021
	Aster tripolium (sea aster)	*Agapanthia villosoviridescens* (golden-bloomed grey longhorn beetle)	Hemminga and Soelen, 1988
No change	*Spartina foliosa* (california cordgrass)	*Haliaspis spartinae* (armored scale)	Long and Porturas, 2014

CHAPTER 3 – MATERIALS AND METHODS

This study examined how salinity and insect herbivory interact and affect resistance of hybrid poplar (*Populus* 'Okanese') to the white-marked tussock moth (*Orgyia leucostigma*) which is a native defoliator of poplar. *Populus* was chosen as it is an ecologically and economically important tree species in North America and it is naturally exposed to both salinity and insect herbivory. Two experiments were conducted to address different aspects of this interaction: the first tested how salinity affects resistance to herbivory (experiment 1), and the second tested whether prior herbivory alters resistance to herbivory and plant responses to later salinity stress (experiment 2).

3.1. Plant material and growth conditions

Unrooted hardwood hybrid poplar cultivar Okanese [(*Populus deltoides* x *P. petrowskyana*) x *P. petrowskyana*] cuttings (about 15 cm length) were provided in February 2023 (experiment 1) and April 2024 (experiment 2) by Dr. Raju Soolanakayahally (Agriculture and Agri-Food Canada) from the PFRA Shelterbelt Centre, Indian Head, Saskatchewan. This cultivar is a male clone, and all cuttings were obtained from the same stool bed (a nursery bed used to clonally propagate woody plants), ensuring a fixed genetic background and uniform clonal material.

One day before planting, cuttings were soaked in distilled water and stored overnight at 4 °C to rehydrate. Cuttings, sectioned to keep only two axillary buds were planted (Figure 3.1A, B), one each, in 6-L plastic pots filled with Sunshine Mix #4 (Sun Gro Horticulture, USA).

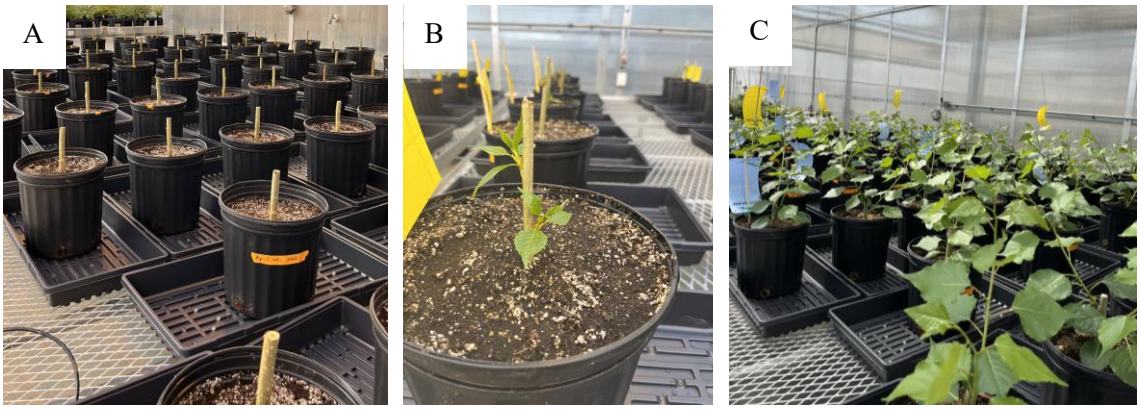

Figure 3.1. Hybrid poplar (*Populus* 'Okanese') (A) Greenhouse setup showing soil-filled pots arranged for rooting of stem cuttings. (B) Close-up of a newly sprouted poplar cutting with initial leaf development. (C) Established plants after four weeks of growth, before the initiation of salinity and herbivory treatments.

The experiments were conducted in a climate-controlled greenhouse with daytime temperatures of 20 - 24 °C and nighttime temperatures of 15 - 17 °C. A photo period of 16 h supplemented with high pressure sodium light was maintained throughout the experiment. Plants were fertilized with a half-strength Hoagland solution (Appendix 1; Table A1) every three weeks. The soil moisture was monitored every other day to maintain consistent moisture levels (40 - 50 %) using an ML3 ThetaProbe Soil Moisture Sensor throughout the experiments.

3.2. Insect material and insect rearing

Eggs of *O. leucostigma* (white-marked tussock moth) were obtained from the Natural Resources Canada Insect Production and Quarantine Laboratories (Great Lakes Forestry Centre, Ontario; Roe et al. 2018) and reared on an artificial Bell diet (Figure 3. 2A) in a CMP 6050 growth chamber (Conviron, Canada) at a temperature of 22°C and 50 % relative humidity with a 12-h light/dark photoperiod. The larvae were reared until they reached the third instar stage (1.5-2.0 cm), which took approximately one month (Figure 3.2B).

Figure 3.2. (A) Eggs of *Orgyia leucostigma* (white-marked tussock moth) (B) Third-instar larva of *O. leucostigma* feeding on a hybrid poplar leaf.

3.3. Experiment 1

The first experiment was conducted to determine the effects of salinity on the nutritional composition of leaves and on plant resistance to herbivores (objective 1; Figure 3.4). After four weeks of growth (Figure 3.1C), 60 poplar cuttings were divided equally between two treatments: 30 were exposed to 0 mM NaCl (control) and 30 to 100 mM NaCl (salt treatment). The selection of 100 mM NaCl as the treatment concentration was based on findings from a preliminary study, which showed that this level of salinity was sufficient to induce reductions in stomatal conductance, photosynthetic rate, and chlorophyll fluorescence without causing visible tissue damage or plant mortality. To prevent osmotic shock, plants assigned to the 100 mM NaCl, were initially irrigated twice with 50 mM NaCl, and after eight days of initial salt treatment, the concentration was increased to 100 mM NaCl. Salinity treatment was applied by irrigating all salt-treated plants with the same volume of 100 mM NaCl solution at regular intervals, ensuring soil moisture remained consistently between 40 -50 %. Control plants received distilled water to maintain comparable soil moisture conditions. After one, three, and five weeks of exposure to salt, photosynthesis, stomatal conductance, transpiration and chlorophyll fluorescence were

measured. After four weeks of salinity treatment, half of the plants from each treatment (15 per group) were subjected to herbivory by third-instar *O. leucostigma* larvae as part of an *in vivo* feeding assay and *in vitro* leaf disc assay was also conducted to assess constitutive and induced resistance. Salinity treatments continued for an additional two weeks (for a total of six weeks), after which a second *in vivo* feeding assay was performed to assess induced resistance. This assay was carried out on a subset of plants, including both a group previously exposed to herbivory and one not previously exposed to herbivory, resulting in four treatment combinations: control-NH (non-herbivory), control-H (herbivory), salt-NH and salt-H. Leaf and soil samples were collected for biochemical analyses including elemental composition, and phenolic content to identify putative mechanisms underlying treatment effects on herbivore performance and plant defense.

3.3.1. Assessment of constitutive resistance

Choice leaf disc assay 1: a choice assay was conducted using leaf discs to measure constitutive resistance after four weeks of NaCl exposure. Two mature leaves that had been developed during salt treatment were excised from each plant, and two leaf discs (1.77 cm^2 each) were collected from each leaf using a cork borer. One disc from each salt treatment was placed on a Petri plate (6 cm diameter) with a third-instar *O. leucostigma* larva (one larva per plate), and the larvae were allowed to feed for two hours. Prior to the assay, larvae were starved for 24 h following standard protocols commonly used to standardize hunger levels in insect herbivory experiments (Renault et al., 2016; Rapo et al., 2019). Preliminary observations also indicated that starvation beyond 24 h reduced larval activity upon exposure to leaf tissue, suggesting that longer starvation periods may decrease the activity in this species under the experimental conditions. To assess any

changes in leaf area due to desiccation over the course of the assay, a Petri plate with two leaf discs from the same leaves with no larvae was used. At the end of the feeding period, the larvae were removed, and a photograph of the remaining area of each leaf disc was taken. It was then quantified using ImageJ software (Version 1.53t) and compared to the initial area measured from the corresponding control disc. Resistance was calculated using the formula $R = A_f / A_i$ (Ávila-Sakar et al., 2018), where A_f is the final leaf area after herbivory, corrected for desiccation, and A_i is the initial area of the leaf disc.

In parallel, an *in vivo* feeding assay (*in vivo* 1) was conducted to assess constitutive resistance by measuring leaf area before and after exposure to herbivory, and to induce resistance in plants. To prevent potential induction of resistance in control plants through volatile organic compounds emitted by herbivore-damaged plants, the two groups (control vs. herbivory-exposed) were kept physically separated during the assay. A single mature leaf (developed during salt treatment) from each plant was selected. Two third-instar *O. leucostigma* larvae (starved for 24 h) were confined on the leaf using a white tulle mesh bag to allow continuous feeding while preventing escape (Figure 3.3). The estimation of the initial leaf area was recorded using a transparent paper and a LI-3100 area meter (LI-COR, USA). After 24 h of feeding, larvae were removed, leaves were harvested, and the remaining leaf area was measured.

3.3.2. Assessment of induced resistance

Choice leaf disc assay 2: to assess induced resistance, a choice disc assay was conducted 48 h after the initial herbivory exposure (24 h following the insect removal), allowing sufficient time for the activation of plant defense responses (Vos et al. 2013). This approach would enable the evaluation of changes in leaf palatability/ resistance resulting from the earlier herbivory event

(Broekgaarden et al., 2007; Karban, 2020). Leaf discs were collected from mature leaves that developed following salt exposure; these were the leaves positioned directly above and below the leaf used in the choice leaf disc assay 1.

3.3.3. Long term induction

After two additional weeks of salt treatment, both *in vivo* feeding (*in vivo* 2) and choice assay using leaf discs were conducted using the same methods described previously. This was done to assess induced resistance after six weeks of salinity exposure and two weeks following the initial herbivory treatment, thereby allowing the evaluation of longer-lasting changes in plant tissue palatability due to herbivory under continued salt stress. The remaining portion of each leaf, after obtaining leaf discs in choice assays conducted after 4 weeks and 6 weeks of salt exposure, was freeze-dried for total phenolic content analysis.

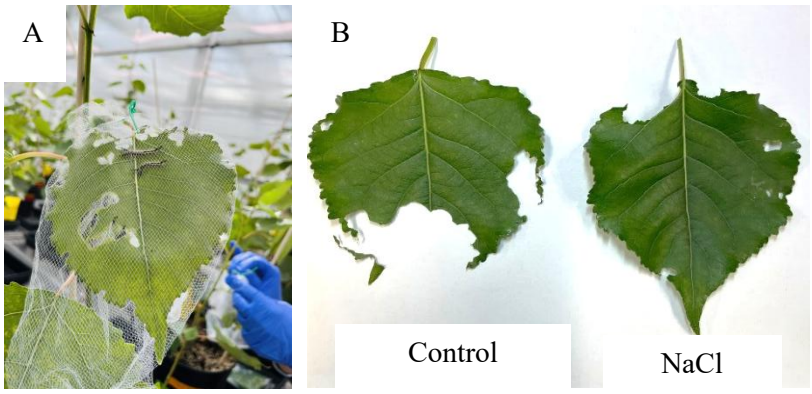

Figure 3.3. *in vivo* feed assay (A) A hybrid poplar leaf enclosed in a tulle mesh bag with two *O. leucostigma* larvae (B) Herbivory damage after 24 h on leaves from control (left) and NaCl-treated (right) plants

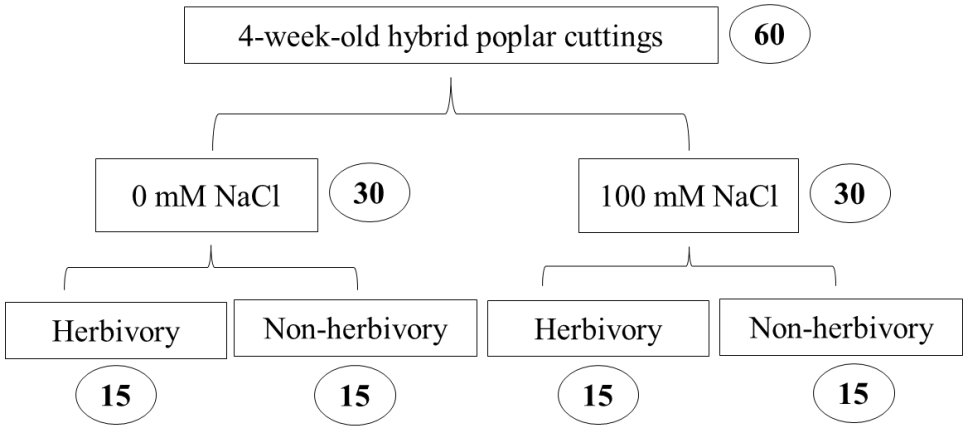

Figure 3.4. Experimental design for experiment 1. A total of 60 four-week-old hybrid poplar cuttings were randomly assigned to two salinity treatments: 0 mM NaCl or 100 mM NaCl (30 plants per treatment). After four weeks of salinity exposure, each salinity group was further divided into two herbivory treatments (herbivory or non-herbivory), resulting in four treatment combinations with 15 plants per group. A choice leaf-disc assay and in vivo feeding assay was conducted after four weeks to assess resistance. Although not shown in the diagram, plants remained under their respective salinity treatments for an additional two weeks, after which a second-choice assay was performed using newly developed mature leaves to evaluate longer-term salinity-induced resistance.

3.5. Experiment 2

A second experiment was conducted to determine whether plants exposure to herbivore feeding changes their responses to salinity and to subsequent herbivory (objective 2; Figure 3.5). Forty hybrid poplar cuttings were planted in the greenhouse as previously described in section 3.1. After four weeks of growth, the cuttings were divided into two herbivory treatments: 20 plants were assigned to the herbivory group and 20 to the non-herbivory control group.

To induce resistance in plants, two starved third-instar *O. leucostigma* larvae were placed on a single mature leaf on two lateral shoots per plant (one leaf per shoot) ensuring sufficient and localized herbivory without causing extensive damage to the plant. For the *in vivo* feeding assays, 3rd instar *O. leucostigma* larvae were allowed to feed on the plant, as in experiment 1, except that the feeding period was extended to 48 h (instead of 24) to promote a stronger induction of plant defense responses. Longer feeding durations are known to enhance the induction of defense-related enzymes such as polyphenol oxidase (Constabel et al., 2000).

After feeding for 48h, the larvae were removed, and half of the leaves from each lateral shoot were excised to standardize tissue removal across all plants in the herbivory treatment. Salt treatments (0 or 100 mM NaCl) were initiated 24 h after insect removal and maintained for four weeks. Ten plants from each herbivory group (herbivory / non-herbivory) were randomly assigned to each salt treatment (as described in experiment 1), resulting in four experimental groups: 1) Control (0 mM NaCl, no herbivory), 2) 0 mM NaCl, herbivory, 3) 100 mM NaCl, no herbivory and 4)100 mM NaCl, herbivory, with 10 plants per treatments (10 replicates).

After four weeks of salinity treatment, herbivory resistance was re-evaluated in all plants using both *in vivo* feeding assays and *in vitro*, choice-leaf disc assays using two mature leaves per plant (one from each lateral shoot) that had developed after the initial herbivory period. The

assay followed the same protocol as in experiment 1. Two third-instar *O. leucostigma* larvae (starved for 24 h) were placed on each designated leaf and allowed to feed for 48 h. Leaf area consumed was quantified to evaluate resistance. For the leaf disc assay, one mature leaf from each lateral shoot that had developed after the initial herbivory exposure was collected. Leaf discs (1.77 cm^2) were obtained from those leaves (two per leaf). Each assay plate contained eight discs, representing all combinations of salt-by-herbivory treatment levels from each lateral shoot. Leaf discs were then randomly arranged on a filter paper inside a Petri plate (15 cm in diameter). Four third-instar *O. leucostigma* larvae (starved for 24 h) were placed on each plate and allowed to feed for two hours (Figure 3.6). Resistance was later determined using the same methodology as described in experiment 1. A plate containing leaf discs obtained from the same leaves used in the above leaf disc assay but without larvae was used for the same purpose described in the section 3.3.1. The remaining leaf part after obtaining leaf discs was freeze dried for analysis of total phenolic compounds. These tests aimed to assess whether prior herbivory and/or salinity affected the resistance of hybrid poplar to insect herbivory.

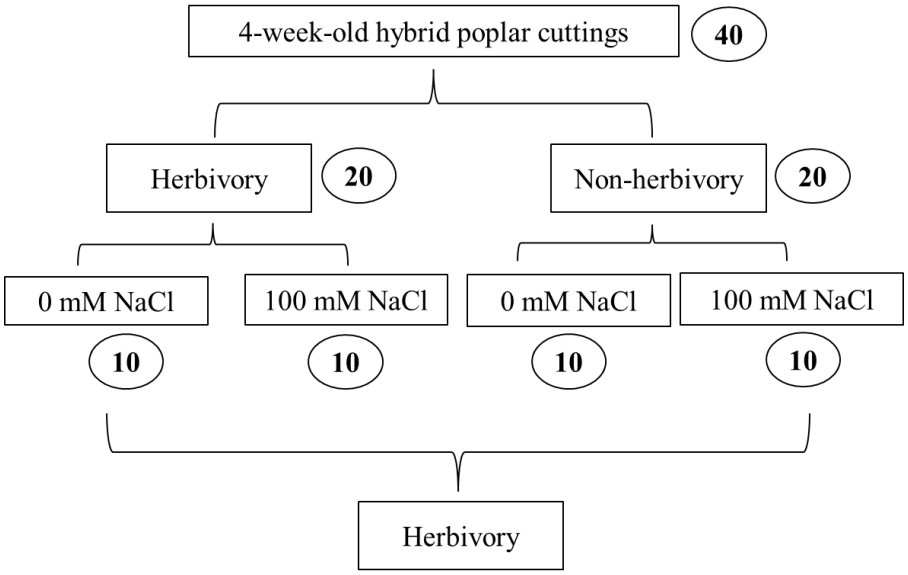

Figure 3.5. Experimental design for experiment 2. A total of 40 four-week-old hybrid poplar cuttings were randomly assigned to two initial treatments: herbivory (20 plants) or non-herbivory (20 plants). After the first herbivory phase, each group was further divided into two salinity treatments: 0 mM NaCl or 100 mM NaCl, resulting in four treatment combinations with 10 plants per group. Following four weeks of salinity exposure, all plants were used for a final herbivory assay to evaluate induced resistance.

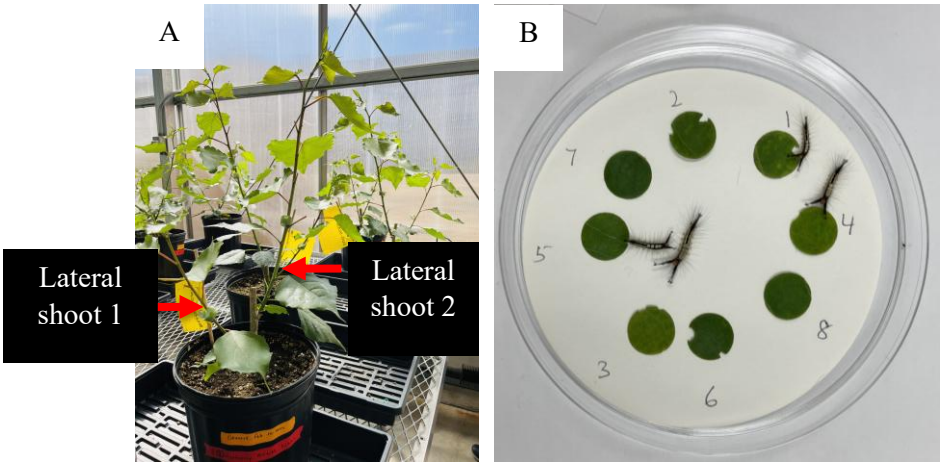

Figure 3.6. Plant material and leaf disc assay setup used to assess herbivory resistance. (A) A hybrid poplar plant showing two lateral shoots from which mature leaves were collected for both feeding assays. (B) Leaf disc assay setup with third-instar *Orgyia leucostigma* larvae feeding on eight leaf discs, representing two lateral shoots × four treatment combinations.

3.6. Physiological measurements

3.6.1. Gas exchange

A portable infrared gas analyzer (Li-6400, LI-COR, USA) was used to record leaf photosynthetic rate, transpiration, and stomatal conductance in experiment 1. The reference CO_2 concentration in the gas analyser was set to 400 μmol mol^{-1} with a controlled air flow rate of 400 μmol s^{-1}. Photosynthetically active radiation (PAR) was adjusted to 700 μmol m^{-2} s^{-1}. Measurements were taken on a fully expanded leaf after one, three and five weeks of salinity treatments, between 10:00 AM and 2:00 PM when stomatal conductance is typically stable and near their daily maximum (Bryant et al., 2023).

3.6.2. Chlorophyll florescence

In experiment 1, leaf chlorophyll fluorescence (F_v/F_m) was measured with a portable fluorometer (OS-30P, Opti-Sciences, USA) one, three, and five weeks after the onset of salinity treatments to evaluate the impact of salinity on the efficiency of photosystem II as it is the most sensitive component of the photosynthetic apparatus to environmental stress, and its efficiency provides an early indicator of photosynthetic performance under abiotic stressors (Gururani et al., 2015). A fully expanded leaf from the central portion of the dominant shoot was selected and covered using a clip for 30 minutes for dark adaptation before measuring the chlorophyll fluorescence. The readings were taken immediately following exposure to a saturating light pulse of 1800 μmol m^{-2} s^{-1} also between 10:00 AM and 2:00 PM when light intensity and photosynthetic activity are relatively stable. Chlorophyll fluorescence, expressed as F_v/F_m, is widely used to estimate the maximum photochemical efficiency of PSII (Maxwell & Johnson, 2000), with values below 0.75 typically signaling stress-induced photoinhibition.

3.7. Growth measurements

Non-destructive growth measurements were performed in experiment 1. Leaf area was assessed at four and six weeks following the initiation of NaCl treatments. Fully expanded leaves formed after salt exposure were selected for measurement. Leaf area was determined by tracing the leaf onto transparent paper and measuring it using a LI-3100 Area Meter (LI-COR, USA). This approach allowed for the accurate quantification of leaf surface area without wounding the plant.

Shoot height of the two laterals was measured before the onset of salinity treatment and again after six weeks of salinity exposure. Shoot height was determined by measuring from the base to the apex. The height-based relative growth rate (RGR) was calculated using the formula:

$RGR = (H_t - H_{(t-1)})/ (H_{(t-1)} \times \Delta t$

where are H_t and $H_{(t-1)}$ are heights at consecutive times t and t-1, and $\Delta t = t - t$-1 is the duration of the treatment in days (Hastwell and Facelli, 2003). This method provides an estimate of the average daily increase in height over the treatment period, allowing comparisons between control and salt-treated plants, without damaging the plants, making it suitable for growth studies in woody species.

Leaf water content was measured after six weeks of salt treatment. A fully expanded leaf was excised, and its fresh weight (FW) was recorded. The leaf was then frozen and then freeze-dried for 48 h to obtain the dry weight (DW). Leaf water content was calculated using the formula:

$\text{Leaf water content (\%)} = [(FW - DW) / FW] \times 100$

3.8. Soil parameters

To assess the impact of salinity on soil chemistry, both soil EC and pH were measured in experiment 1. At the end of the experiment after six weeks of salt treatments, soil samples were collected from 10 pots from each salinity treatment, oven-dried at 60 °C for one week. After that 5 g of each soil sample were mixed with 35 ml of deionized water to form a saturated paste that was filtered (Whatman No. 1 filter paper) under vacuum. Measurements of EC and pH were taken from the filtrate using a Thermo Scientific Orion 3-Star conductivity meter and a Fisher Scientific AR25 dual-channel pH/Ion meter.

3.9. Biochemical measurements

3.9.1. Elemental analysis

To evaluate elemental composition, freeze-dried leaf samples from experiment 1 (remaining leaf tissue from leaf disc assay) and experiment 2 (two leaves positioned above the leaf used for the leaf disc assay) were finely ground in liquid nitrogen using a mortar and pestle. A 0.5 g portion of leaf sample (combined leaf tissues from three plants) was submitted to Stratford Agri Analysis (Stratford, ON) for nutrient profiling, following the protocol outlined by Shao et al. (2020). N content was determined using a Leco TruSpec N analyzer, while concentrations of macronutrients and micronutrients including Na, Ca, P, K, Mg, Fe, Cu, Mn, Zn, and B were quantified using direct current plasma emission spectroscopy. For chloride measurement, 0.05 g of powdered dry tissue were mixed with 10 ml of 0.5 M nitric acid, agitated on a shaker for 30 min, and then treated with 200 µl of 5 M sodium nitrate to stabilize ionic strength. The chloride

content was immediately measured with a chloride ion-selective electrode (Accumet, USA) and calculated based on a sodium chloride standard curve.

3.9.2. Total phenolic content

Total phenolics were measured because they are one of the most abundant and inducible classes of defensive metabolites in *Populus* (Movahedi et al., 2021), and they play a role in herbivore deterrence (Dixit et al., 2017) and oxidative stress mitigation under abiotic stress (Bistgani et al., 2019). Total phenolic content was determined in both experiments by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007; Chen & Markham.2021). Leaf samples (the remaining leaves after obtaining leaf discs for resistance assays) were first freeze-dried and finely ground using liquid nitrogen. A 0.05 g subsample of the powdered tissue was then extracted with 10 mL of 40 % ethanol and incubated in the dark at room temperature for 24 h on the shaker. Following extraction, samples were vortexed and centrifuged at 4900 g for 10 minutes. From the resulting supernatant, 1 mL was mixed with 0.5 mL of 50 % Folin and Ciocalteu's phenol reagent (MP Biomedicals, UK). After a 3-minute reaction time, 1 mL of 5 % sodium carbonate (Na_2CO_3) was added. The mixture was incubated in the dark for 30 minutes, and absorbance was read at 750 nm using an Ultrospec 2100pro spectrophotometer. Total phenolic concentration was quantified using a gallic acid standard curve prepared in 40 % ethanol, with standard concentrations of 0, 10, 20, 30, 40, and 50 mg/L. A blank was prepared using 1 mL of 40 % ethanol, 0.5 mL of Folin-Ciocalteu reagent, and 1 mL of 5 % Na_2CO_3.

3.9.3. Total protein content

In experiment 2, total soluble protein content of hybrid poplar tissues was quantified by the Bradford method (Bradford, 1976) using the leaf above the one used for the disc assay. One gram of frozen leaf tissue was ground to a fine powder using liquid nitrogen, and the homogenized tissue was immediately extracted in 10 mL of cold 100 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1 mM ascorbic acid. To reduce interference from phenolic compounds, 1 % polyvinylpolypyrrolidone was included in the extraction buffer. The samples were vortexed for 1 minute, shaken on ice for 20 minutes, and centrifuged at 19,000 g for 20 minutes at 4 °C. The resulting supernatant (200 µL) was mixed with 5 mL of diluted Bio-Rad protein assay dye reagent (Coomassie Brilliant Blue G-250 dye along with phosphoric acid and methanol; Bio-Rad Laboratories, USA) in triplicate, vortexed, and incubated for 5 minutes at room temperature. A blank was prepared using 200 µL of buffer instead of extract. Absorbance was measured at 595 nm using an Ultrospec 2100pro spectrophotometer. Protein concentrations were calculated based on a standard curve generated using BSA solutions ranging from 0 to 80 µg.

3.9. Statistical analysis

All statistical analyses were performed using R software (R version 4.4.3). For comparisons between control and salinity treatments, one-way ANOVA was used for most physiological and growth parameters (photosynthesis, stomatal conductance, transpiration, and relative growth rate). Normality was assessed using the Shapiro-Wilk test, and Levene's test was used to evaluate homogeneity of variance. For multi-factor analyses involving salinity and herbivory, a two-way ANOVA was conducted to assess main effects and interactions. Paired t-tests were

performed for pairwise comparisons between two related measurements (e.g., before and after treatment within the same group/treatment). All tests used a significant threshold of $P = 0.05$. Tukey's Honest Significant Difference (HSD) post hoc test was performed to test pairwise differences among treatment means. Results were reported as means ± standard error (SE), and percentage changes were calculated relative to control means to indicate treatment effects.

To control the increased risk of Type I error associated with multiple dependent variables, a Bonferroni correction was applied to the elemental datasets. As Na^+ and Cl^- concentrations were directly influenced by the NaCl treatment and therefore, they were excluded from the correction. Consequently, only comparisons with $P < 0.004$ (0.05/12) were considered statistically significant.

CHAPTER 4 - RESULTS

4.1. Experiment 1

4.1.1. Physiological measurements

4.1.1.1. Gas exchange

After one week of treatment, salinity had no significant effect on leaf photosynthesis, stomatal conductance, or transpiration ($P = 0.15$, $P = 0.12$, and $P = 0.072$, respectively; Table 4.1; Appendix 2). After three weeks of salinity, plants grown under salinity had significantly reduced stomatal conductance (69.2 % decrease, $P = 0.00091$) and transpiration (54.1 % decrease, $P = 0.00063$), while photosynthesis remained unaffected ($P = 0.25$) compared to the control plants. At week five, all three parameters were significantly lower in plants in the salinity treatment: photosynthesis (62.5 % decrease, $P = 0.049$), stomatal conductance (95.4 % decrease, $P < 0.001$), and transpiration (94.6 % decrease, $P < 0.001$) compared to the control plants (Table 4.1).

4.1.1.2. Leaf chlorophyll fluorescence

Leaf chlorophyll fluorescence (F_v/F_m) was used to assess maximum photochemical efficiency of photosystem II. After one week of treatment, F_v/F_m was slightly but significantly higher in salt-treated plants compared with control plants ($P = 0.026$; Table 4.1). After three and five weeks there were no significant differences between the treatments ($P = 0.37$ and $P = 0.077$, respectively; Table 4.1).

4.1.1.3. Leaf water content

Leaf water content showed a transient response to salinity. While after four weeks, salinity significantly reduced leaf water content ($P = 0.0391$), no difference between treatments was observed ($P = 0.516$; Table 4.2) after six weeks.

4.1.2. Growth measurements

Leaf area was not significantly affected by salinity at either four weeks or six weeks of salt treatments (Table 4.3). In contrast, height-based RGR was strongly decreased by salinity ($P < 0.001$). On average, salinity reduced RGR by approximately 21%, and this decline was consistent across both lateral branches (Table 4.3).

4.1.3. Biochemical measurements

4.1.3.1. Total phenolic content

After four weeks of salinity exposure, hybrid poplar leaves accumulated ~21 % more total phenolic compounds (constitutive level of phenolics) than the controls ($P = 0.002$; Figure 4.1). Following 48 h herbivory treatment, there were no significant effects of salinity, herbivory, or their interaction on phenolic content ($P = 0.072$, $P= 0.381$ and $P = 0.552$ respectively; Figure 4.2). Although phenolics tended to be slightly higher in salt-treated plants, particularly under herbivory, none of the means differed significantly from each other, according to a Tukey HSD multiple-comparisons test.

Paired *t*-tests were conducted separately for control (0 mM NaCl) and salt-treated (100 mM NaCl) plants to compare total phenolic content before and after herbivory within the same

individuals. In both treatments, phenolic contents measured 48 h after herbivory were significantly lower than the constitutive levels (prior to herbivory) (11.9 % decrease in control, $P = 0.0064$; 5.5 % decrease in salt-treated plants, $P < 0.001$; Figure 4.3). However, phenolic levels after 48 h herbivory (9.09 ± 0.23 mg g^{-1} DW for control;10.23 ± 0.53 mg g^{-1} DW for salt-treated plants) were not different from those measured in non-herbivory plants sampled at the same time point (9.78 ± 0.32 mg g^{-1} DW in control;10.36 ± 0.61 mg g^{-1} DW in NaCl).

After six weeks of salt treatments, a two-way ANOVA revealed no significant effects of salinity, herbivory, or their interaction ($P = 0.513$, $P = 0.665$ and $P = 0.784$ respectively; Figure 4.4) on leaf phenolic levels.

4.1.3.2. Elemental analysis

After four weeks of salt exposure and 48 h after herbivory, leaf Na concentrations were ~15-fold higher than in controls ($P = 0.0016$; Table 4.4). Moreover, leaf Cl concentrations were also affected under salinity ($P < 0.001$; Table 4.4), reaching values more than 60-fold higher than controls, regardless of herbivory treatment. Neither herbivory nor the salinity × herbivory interaction significantly affected leaf Na or Cl levels. In addition to Na and Cl, several macronutrients were affected by salinity. Leaf P, K and S showed lower levels under salinity ($P = 0.0013$, $P = 0.0004$ and $P < 0.001$ respectively) whereas leaf N, Ca, and Mg were not significantly affected. None of the quantified macronutrients were significantly affected by herbivory, and no salinity × herbivory interactions were detected (Table 4.4).

For micronutrients, according to two-way ANOVA, salinity led to higher Mn levels but lower levels of Fe, Mo, and B (Table 4.4). In addition, plants experienced herbivory had lower levels of Fe ($P = 0.0018$) in leaves. Only leaf Fe was affected by a salinity × herbivory

interaction, with the lowest level being that of plants that experienced both herbivory and salinity ($P < 0.001$; Table 4.4). A two-way ANOVA also showed that salinity significantly increased Na/K, Na/Ca, Na/Mg ratios ($P < 0.001$; Table 4.5), while herbivory and the salinity × herbivory interaction had no significant effects. Tukey's HSD showed that these ratios were significantly higher in both salinity groups (herbivory and non-herbivory plants) than in the two control groups (herbivory and non-herbivory), whereas non-herbivory and herbivory plants did not differ within each salinity level. On average, the ratios increased on the order of 15 to 20 times under salinity relative to controls, indicating that salinity, and not herbivory, drove the marked shifts in ionic balance (Table 4.5).

Six weeks after salinity treatments and two weeks after the first herbivory treatment leaf Na concentrations were affected by salinity ($P < 0.001$; Table 4.6). Salt-treated plants contained higher leaf Na levels than controls, with values approximately 20-fold higher under salinity, regardless of herbivory treatment. Herbivory and the salinity × herbivory interaction had no significant effect on leaf Na. Leaf Cl concentrations were also significantly elevated under salinity ($P < 0.001$; Table 4.6), with levels more than 100-fold higher in salt-treated plants compared to controls. Herbivory and the salinity × herbivory interaction showed no significant effects on leaf Na and Cl concentration. Among macronutrients, the leaf Ca levels were slightly (~22%) higher in salt-treated plants compared to controls ($P = 0.037$). Moreover, N, P, K, Mg, and S in leaves showed no significant differences between control and salinity treatments (Table 4.6). Regarding micronutrients, plants exposed to salinity had higher levels of Mn (100%) (Table 4.6) Furthermore, leaf Fe, Cu, Mo, and B were not affected by salinity. Neither herbivory nor its interaction with salinity had significant effects on leaf elemental concentrations (Table 4.6). Na/K, Na/Ca, and Na/Mg were also assessed, using two-way ANOVAs across the four treatment

groups which showed Na/K, Na/Ca and Na/Mg were affected by salinity ($P = 0.0015$, $P = 0.0061$, and $P = 0.0034$ respectively; Table 4.7). Neither herbivory nor its interaction with salinity had significant effects on these ratios after six weeks of salinity treatments. Tukey's HSD showed that plants exposed to salinity had higher values for all three ratios than control plants ($P < 0.05$).

4.1.4. Resistance to herbivores

4.1.4.1. Resistance after four weeks of salt treatments

In vitro constitutive resistance, assessed using the leaf disc assay, after four weeks of salinity treatments, was not significantly affected by salinity ($P= 0.123$; Figure 4.5A). Similarly, *in vivo* measurements of constitutive resistance revealed no significant difference between salinity treatments ($P = 0.871$; Figure 4.5B).

Induced resistance, measured through the disc assay, was not affected by salinity ($P = 0.891$; Figure 4.5C). Across all assays, hybrid poplar exhibited consistently high levels of resistance to *O. leucostigma*, with average constitutive resistance values of 0.86 ± 0.01 (disc assay) and 0.87 ± 0.01 (*in vivo*), and induced resistance averaging 0.92 ± 0. 02 where 1 represents maximum resistance.

4.1.4.2. Resistance after six weeks of salt treatment and two weeks after first herbivory treatments

After six weeks of salt treatment and two weeks after herbivory exposure, induced resistance increased significantly in salt-treated plants compared to controls ($P = 0.048$) but was not

affected by herbivory ($P = 0.397$), and there was no significant salinity × herbivory interaction ($P = 0.172$; Figure 4.6).

4.1.5. Soil parameters

Soil EC was approximately ten-fold higher in soil treated with salt than in the control soil ($P < 0.001$; Table 4.8). Soil pH was significantly higher under salt treatments compared to controls ($P = 0.0099$; Table 4.8)

Table 4.1. Gas exchange parameters: photosynthesis (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (mmol H_2O m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), and chlorophyll fluorescence: F_v/F_m of hybrid poplar (*Populus* 'Okanese') leaves measured one, three, and five weeks after initiating NaCl treatments (0 and 100 mM). Values are means ± SE (n = 6-8).

Treatment duration (week)	NaCl (mM)	Photosynthesis (µmol CO_2 m^{-2} s^{-1})	Stomatal conductance (mmol H_2O m^{-2} s^{-1})	Transpiration (mmol H_2O m^{-2} s^{-1})	Leaf chlorophyll fluorescence F_v/F_m
1	0	9.39 ± 0.63	0.0711 ± 0.0060	1.92 ± 0.15	0.779 ± 0.0039
1	100	10.80 ± 0.71	0.0588 ± 0.0048	1.53 ± 0.15	0.791 ± 0.0034*
3	0	5.66 ± 1.24	0.2870 ± 0.0487	3.69 ± 0.45	0.641 ± 0.0308
3	100	7.64 ± 1.14	0.0884 ± 0.0178**	1.69 ± 0.22**	0.690 ± 0.0432
5	0	0.614 ± 0.176	0.0744 ± 0.0121	1.72 ± 0.25	0.509 ± 0.0439
5	100	0.230 ± 0.0621*	0.00343 ± 0.00037**	0.09 ± 0.01**	0.596 ± 0.0179

**$P < 0.001$; * $P < 0.05$

Table 4.2. Leaf water content (%) and leaf area (cm^2) of hybrid poplar measured after four and six weeks of NaCl treatment 0 and 100 mM). Values are means ± SE (n = 6-12).

	Leaf water content (%)		Leaf area (cm^2)	
Treatment duration (week)	0 mM NaCl	100 mM NaCl	0 mM NaCl	100 mM NaCl
4	76.1 ± 0.35	72.6 ± 1.57 *	121.9 ± 6.2	127.6 ± 15.1
6	75.7 ± 0.72	76.4 ± 0.69	90.4 ± 7.9	73.3 ± 9.5

* $P < 0.05$

Table 4.3. Effect of salinity on the height-based RGR of hybrid poplar. RGR values were calculated from the growth of two lateral shoots (lateral 1 and lateral 2) using plant height measurements (cm). Values are means ± SE, n=10

Lateral shoot	NaCl (mM)	RGR (cm cm^{-1} day^{-1}) ($\times 10^{-3}$)
Lateral 1	0	27.3 ± 0.3
	100	20.9 ± 0.6*
Lateral 2	0	25.5 ± 0.4
	100	20.9 ± 0.7*

* $P < 0.05$

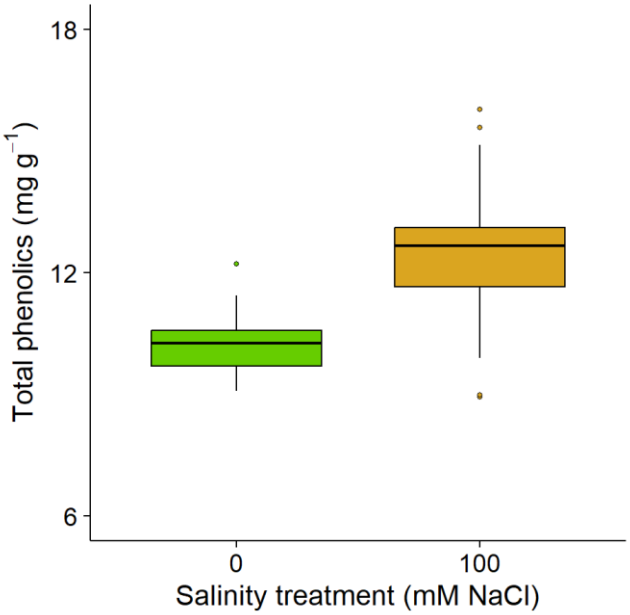

Figure 4.1. Phenolic content (mg g^{-1} DW) measured in hybrid poplar leaves before exposure to herbivory (constitutive levels) after four weeks under 0 and 100 mM NaCl. Boxplots show median and interquartile range (IQR); whiskers = 1.5×IQR; outliers shown as points; n = 15

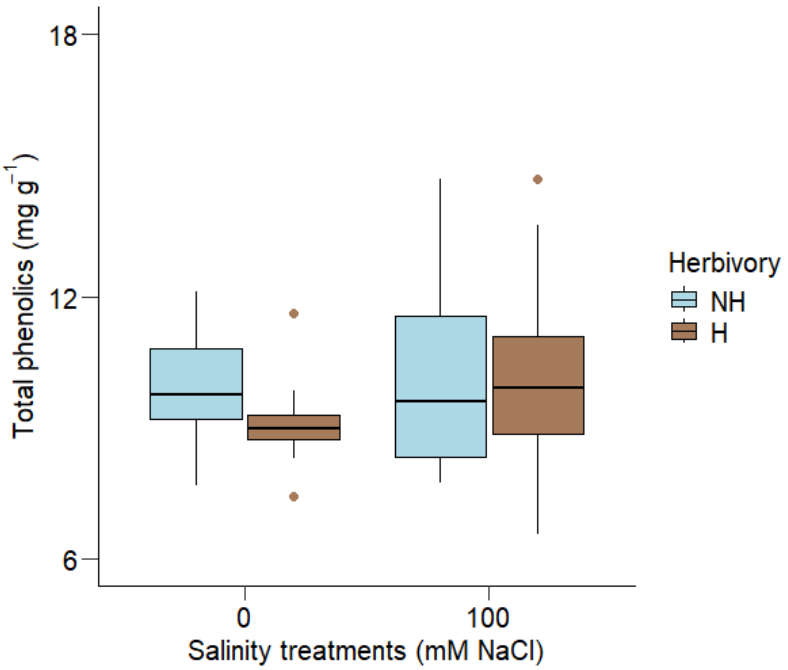

Figure 4.2. Phenolic content (mg g^{-1} DW) measured in hybrid poplar leaves 48h after exposure to herbivory (induced levels) after four weeks under 0 and 100 mM NaCl) (NH = no herbivory; H = herbivory). Boxplots as in Figure. 4.1; n =15.

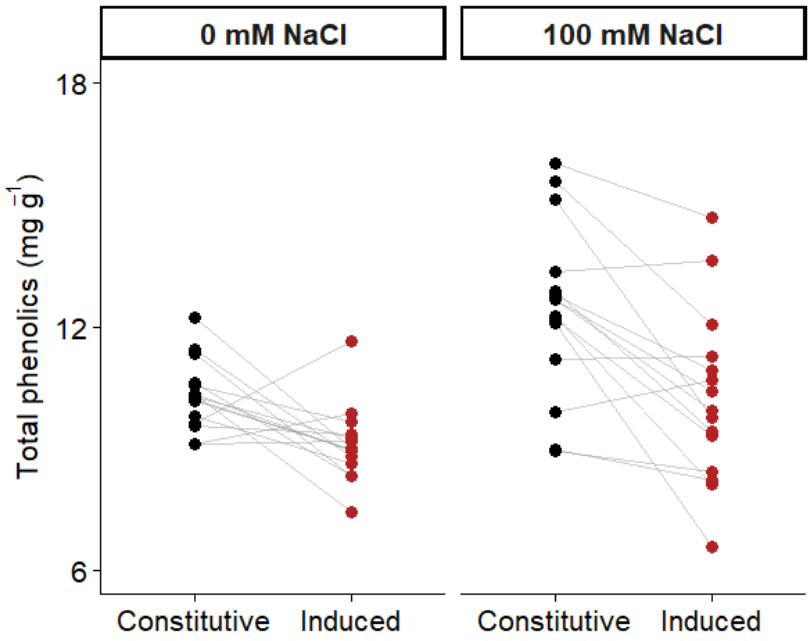

Figure 4.1. Phenolic content (mg g^{-1} DW) in hybrid poplar leaves after four weeks of exposure to 0 mM or 100 mM NaCl. Constitutive levels were measured before herbivory, and induced levels were measured 48 h after herbivory. Lines connect paired measurements from the same replicate (n = 15 paired individuals per treatment).

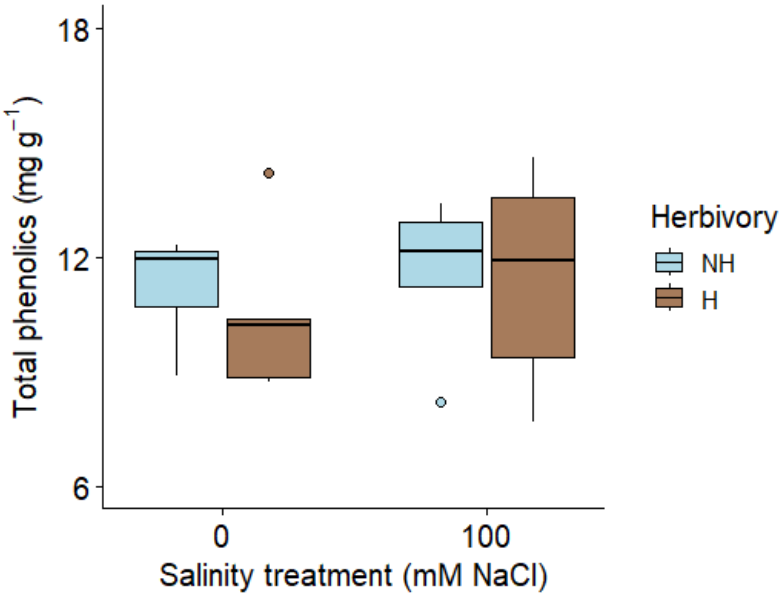

Figure 4.2. Phenolic content (mg g^{-1} DW) hybrid poplar leaves six weeks after initiation of 0 and 100 mM NaCl treatments and two weeks after first herbivory treatments (NH = no herbivory; H = herbivory). Boxplots as in Figure. 4.1; n = 5.

Table 4.4. Mean (± SE) concentrations of elements (mg kg^{-1} DW) in hybrid poplar leaves exposed NaCl 0 or 100 mM for four weeks and 48 h after insect herbivory (NH = no herbivory; H = herbivory) treatments. Reported *P*-values indicate the main effects of salinity (S) and herbivory (I), as well as their interaction (S × I) based on two-way ANOVA (n = 5).

Elements	Treatments				*P* value		
	S (0), I (NH)	S (0) I(H)	S (100), I(NH)	(S)100, (I)H	S	I	S*I
Na	62.9 ± 7.29	79.0± 38.8	856.5± 357.0	1262.0 ± 379.0	**0.0016**	0.432	0.467
Cl	408.0 ± 31.1	480.0 ± 50.2	29,091.0 ± 3,531.0	27,677.0 ± 4,430.0	**< 0.001**	0.816	0.796
N	38,010 ± 1,300	37,240 ± 1,830	35,330 ± 1,116	36,302 ± 464	0.175	0.938	0.504
P	3,489 ± 190	3,444± 217	2,562 ± 133	2,816 ± 241	**0.0013**	0.604	0.468
K	37,509 ± 2,425	37,845 ± 1,582	24,871 ± 2,320	30,462 ± 2,508	**0.0004**	0.204	0.258
Ca	16,507 ± 645	15,984 ± 444	15,785 ± 1,148	16,496 ± 758	0.896	0.907	0.447
Mg	4,309 ± 156	3,806 ± 129	3,648 ± 205	3,759 ± 177	0.052	0.264	0.087
S	7,338± 250	7,267 ± 225	5,574 ± 292	6,004 ± 52	**< 0.001**	0.435	0.280
Fe	146.6 ± 7.57	102.7 ± 6.44	101.1 ± 8.16	95.9 ± 2.75	**0.0011**	**0.0018**	**< 0.001**
Mn	27.6 ± 2.13	28.6 ± 1.49	54.3 ± 2.74	55.6 ± 3.14	**< 0.001**	0.641	0.965
Cu	0.63 ± 0.27	1.34 ± 0.28	0.048 ± 0.041	1.14 ± 0.39	0.176	**0.0049***	0.498
Zn	95.8 ± 4.67	98.0 ± 6.34	82.7 ± 5.13	80.1 ± 3.54	**0.0071***	0.972	0.639
Mo	0.938 ± 0.068	0.800 ± 0.019	0.434 ± 0.064	0.482 ± 0.039	**< 0.001**	0.396	0.091
B	43.6 ± 3.36	57.3 ± 1.41	38.0 ± 2.88	58.8 ± 2.54	**< 0.001**	0.456	0.199

* Not considered statistically significant under the Bonferroni-corrected alpha level of 0.05 / 12 ≈ 0.004, Na and Cl were excluded from Bonferroni interpretation as they were treatment ions.

Table 4.5. Mean (± SE) ion ratios (Na/K, Na/Ca, Na/Mg) in hybrid poplar leaves under combinations of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) measured after four weeks of salinity treatment and 48 h following insect herbivory. Different superscript letters within a row indicate significant differences among treatment combinations according to Tukey's HSD test on log10 -transformed data ($P <$ 0.05). Means and SE are on the original scale (n = 5).

Ratio	0-NH	0-H	100-NH	100-H
Na/K	0.002 ± 0.000^{b}	0.002 ± 0.001^{b}	0.032 ± 0.011^{a}	0.039 ± 0.009^{a}
Na/Ca	0.004 ± 0.001^{b}	0.005 ± 0.002^{b}	0.050 ± 0.018^{a}	0.073 ± 0.021^{a}
Na/Mg	0.015 ± 0.002^{b}	0.020 ± 0.009^{b}	0.22 ± 0.09^{a}	0.32 ± 0.09^{a}

Table 4.6. Mean (± SE) concentrations of elements (mg kg^{-1} DW) in hybrid poplar leaves exposed NaCl 0 or 100 mM for six weeks and two weeks after first insect herbivory (NH = no herbivory; H = herbivory) treatments. Reported *P*-values indicate the main effects of salinity (S) and herbivory (I), as well as their interaction (S × I) based on two-way ANOVA (n=3)

Elements	Treatments				*P* value		
	S (0), I (NH)	S (0) I (H)	S (100), I (NH)	S (100), I (H)	S	I	S * I
Na	344.3 ± 280.8	500.1 ± 387.0	6957.9 ± 1108.6	8269.7 ± 1705.2	**< 0.001**	0.502	0.595
Cl	428 ± 26.1	451 ± 78.4	59,303 ± 143	51,861± 3,426	**< 0.001**	0.0625	0.0612
N	31,490 ± 5,685	30,380 ± 1,080	33,620 ± 1,962	33,223± 749	0.443	0.813	0.911
P	3038 ± 486	2613 ± 203	2336 ± 272	2490 ± 227	0.229	0.681	0.389
K	34,143 ± 4,368	30,753 ± 794	29,977 ± 1,705	32,747 ± 2,418	0.695	0.910	0.282
Ca	18701 ±1254	16686 ±2116	21745 ±1097	21419 ±1550	**0.037***	0.473	0.602
Mg	4780 ± 98	4013 ± 493	4876 ± 663	4991 ± 476	0.295	0.384	0.463
S	6811 ± 562	6154 ± 562	5737 ± 562	6528 ± 562	0.550	0.908	0.233
Fe	433.7 ± 317.9	298.8 ± 150.2	1277.7 ± 445.3	555.6 ± 252.8	0.114	0.205	0.372
Mn	21.12 ± 1.90	21.57± 1.78	42.08 ± 9.12	44.40 ± 8.76	**< 0.001**	0.774	0.921
Cu	0.197 ± 0.197	0.433 ± 0.413	0.000 ± 0.000	0.910 ± 0.473	0.120	0.682	0.336
Zn	145.72 ± 25.5	147.01 ± 32.5	263.49 ± 3.35	253.62 ± 71.6	**0.0267***	0.917	0.893
Mo	2.237 ± 1.367	0.977 ± 0.018	0.900 ± 0.511	1.247 ± 0.543	0.512	0.573	0.332
B	81.1 ± 9.40	73.2 ± 9.79	74.3 ± 12.7	60.6 ± 8.22	0.369	0.318	0.782

*Not considered statistically significant under the Bonferroni-corrected alpha level of 0.05 / 12 ≈ 0.004, Na and Cl were excluded from Bonferroni interpretation as they were treatment ions.

Table 4.7. Mean ± SE ion ratios (Na/K, Na/Ca, Na/Mg) in hybrid poplar leaves six weeks after salinity (0 or 100 mM NaCl) and two weeks after herbivory (NH = no herbivory, H = herbivory; n = 3). Different superscript letters within a row indicate significant differences among treatment combinations according to Tukey's HSD test on $\log_{10}$-transformed data ($P < 0.05$). Means and SE are on the original scale.

Ratio	0-NH	0-H	100-NH	100-H
Na/K	0.009 ± 0.007^{b}	0.016 ± 0.012^{b}	0.23 ± 0.02^{a}	0.25 ± 0.04^{a}
Na/Ca	0.020 ± 0.016^{b}	0.036 ± 0.029^{b}	0.32 ± 0.04^{a}	0.39 ± 0.08^{a}
Na/Mg	0.074 ± 0.061^{b}	0.12 ± 0.09^{b}	1.38 ± 0.11^{a}	1.80 ± 0.47^{a}

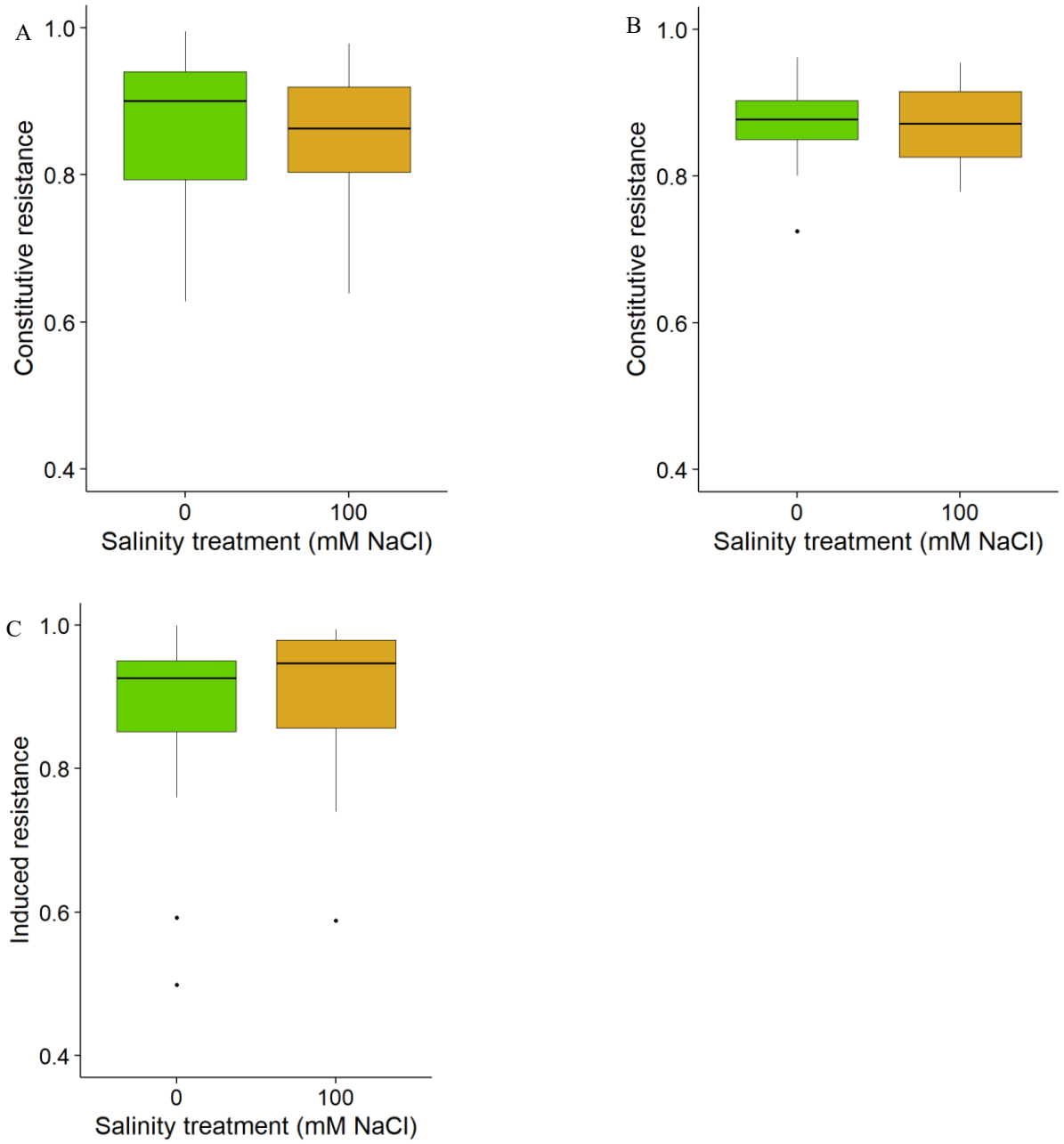

Figure 4.3. Constitutive resistance (A) Leaf disc assay, (B) *in vivo* feeding assay and induced resistance to *O. leucostigma* after 48h (C) Leaf disc assay, measured in hybrid poplar leaves after four weeks of salt treatment. Boxplots as in figure 4.; n = 15

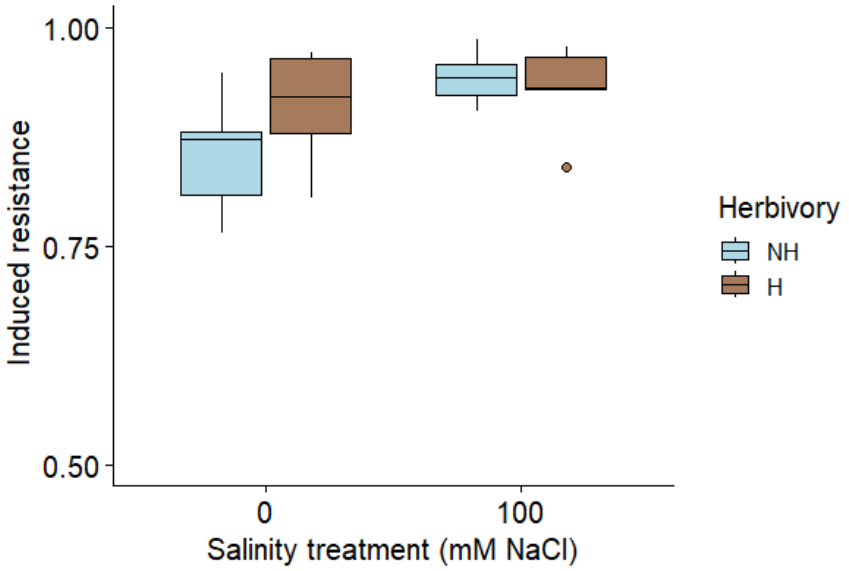

Figure 4.4. Induced resistance to *O. leucostigma* in hybrid poplar (leaf-disc assay), after six weeks of salt treatment. Plants were exposed to two salinity levels (0 or 100 mM NaCl) and either had a history of herbivory or not (H = herbivory, NH = no herbivory). Boxplots as in Figure. 4.1; n = 5

Table 4.8. Soil conductivity (dS m^{-1}) and soil pH under 0 vs 100 mM NaCl (mean ± SE) after six weeks of salinity treatments

NaCl (mM)	Soil conductivity (dS m^{-1})	Soil pH
0	0.957 ± 0.039	5.76 ± 0.05
100	9.859 ± 0.363**	5.94 ± 0.04*

**$P < 0.001$; * $P < 0.05$

4.2. Experiment 2

4.2.1. Biochemical measurements

4.2.1.1. Total phenolic content

In the second experiment, phenolic compounds were determined in hybrid poplar leaves that were first exposed to herbivory for 48 h and then subjected to salinity stress (0 or 100 mM NaCl) for four weeks. Total phenolic content in hybrid poplar leaves was significantly affected by prior herbivory ($P = 0.026$; Figure 4.7; Appendix 3), with herbivory-exposed plants showing lower levels than unexposed plants. In contrast, neither salinity ($P = 0.426$) nor the salinity × herbivory interaction ($P = 0.6211$) had a significant effect on phenolics

4.2.1.2. Total protein content

Total protein content in hybrid poplar leaves was significantly affected by four weeks of salt treatment, but not by prior herbivory or their interaction ($P = 0.012$, 0.8734 and 0.928 respectively; Figure 4.8). In the absence of herbivory, protein content was 3.79 ± 0.54 mg g^{-1} FW in controls and 2.82 ± 0.33 mg g^{-1} FW under salinity, corresponding to ~25 % lower levels. Similarly, in herbivory-exposed plants, protein content was 3.68 ± 0.29 mg g^{-1} FW in controls compared to 2.77 ± 0.21 mg g^{-1} FW under salinity (~25 % lower).

4.2.1.3. Elemental analysis

Leaf Na and Cl levels were significantly higher under salinity ($P = 0.0002$ and $P = 0.0001$ respectively; Table 4.9) according to the two-way ANOVA analysis. Interestingly, despite the accumulation of salt, leaf K, Ca, and Mg concentrations were not affected by salinity. Leaf P and S concentrations were not affected under salinity ($P = 0.032$ and $P = 0.015$ respectively; Table

4.9). However, leaf N content tended to be slightly higher under salinity compared to the control ($P = 0.026$; Table 4.9) but this effect was not significant. Similarly, in the absence of salinity, leaf Mg and Fe levels were slightly higher in herbivory-exposed plants ($P = 0.043$ and $P = 0.041$, respectively; Table 4.9) compared to plants not exposed to herbivory. No significant salinity × herbivory interaction effects were detected for any of the macronutrients and micronutrients quantified (Table 4.11).

Salinity had a strong and highly significant effect on ion ratios in hybrid poplar leaves. The Na/K ratio increased more than 40-fold under salinity compared to control conditions ($P < 0.001$). Similar trends were observed for Na/Ca ($P < 0.001$) and Na/Mg ($P < 0.001$). In contrast, herbivory did not significantly affect any of the ratios, and no significant salinity× herbivory interactions were detected (Table 4.10). Tukey's HSD showed that ion ratios differed only between the two salinity levels, while herbivory had no effect within each level.

4.2.2. Resistance to herbivores

Induced resistance was determined in hybrid poplar leaves that were first exposed to herbivory for 48 h and then subjected to salinity for four weeks. A two-way ANOVA revealed no statistically significant effects of previous herbivory ($P = 0.770$) salinity ($P = 0.339$), or their interaction ($P= 0.763$) on induced resistance assessed using a leaf disc assay (Figure 4.9A). An *in vivo* herbivory assay was also performed using *O. leucostigma* larvae to assess induced resistance; similarly, neither salinity ($P = 0.340$), herbivory ($P= 0.525$), nor their interaction ($P = 0.378$) had a statistically significant effect on induced resistance (Figure 4.9B).

A paired *t*-test was conducted to compare the constitutive resistance of the same set of control plants measured at 4 and 8 weeks of age in experiment 2. The four-week measurement

corresponded to the time when these plants were first exposed to herbivory, before any salinity treatment was applied, whereas the eight-week measurement represented the same plants maintained under control (non-saline) conditions for an additional four weeks. Although the difference was not statistically significant ($P = 0.061$), resistance tended to be lower at eight weeks (Figure 4.10).

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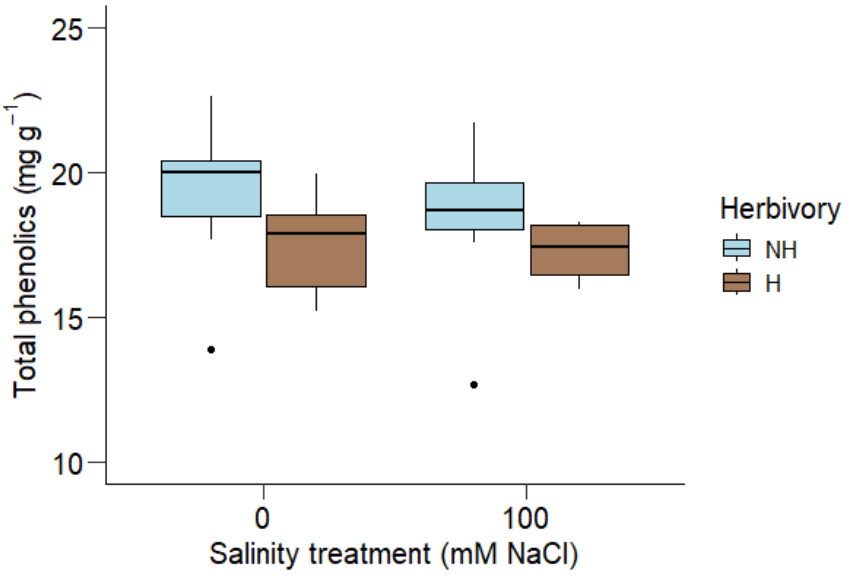

Figure 4.5. Leaf total phenolic content (mg g^{-1} DW) in hybrid poplar after four weeks of salinity treatment (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory). Boxplots as in Figure. 4.1; n = 10

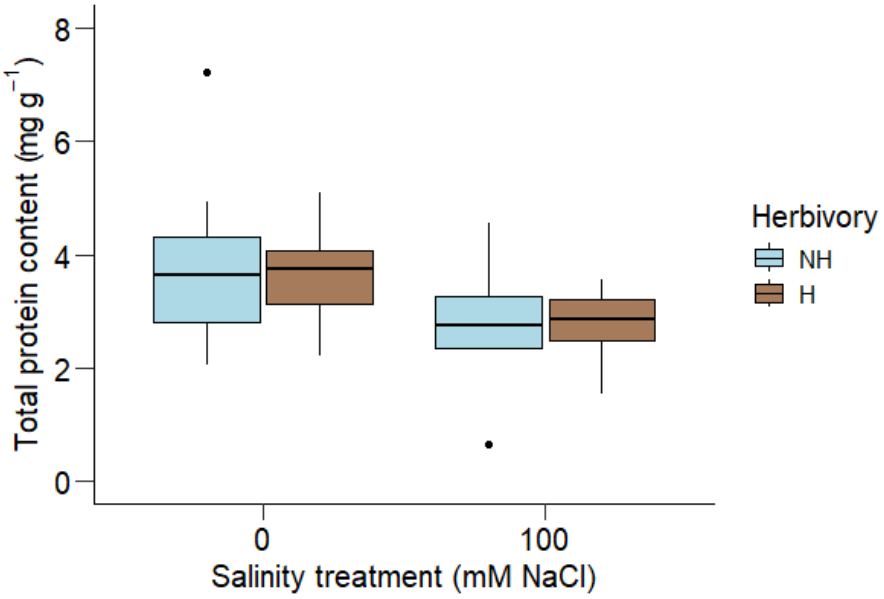

Figure 4.6. Protein content (mg g^{-1} FW) in hybrid poplar leaves after four weeks under two salinity treatments (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory). Boxplots as in Figure. 4.1; n = 10

Table 4.9. Mean ± SE concentrations mineral elements (mg kg^{-1} DW) in hybrid poplar leaves after four weeks of exposure to 0 or 100 mM NaCl and prior insect herbivory (NH = no herbivory, H = herbivory). Reported *P*-values correspond to the main effects of salinity (S), herbivory (I), and their interaction (S × I), based on two-way ANOVA (n = 3).

Elements	Treatments				P value		
	S (0), I (NH)	S (0) I (H)	S (100), I (NH)	S (100), I (H)	S	I	S *I
Na	60.2 ± 1.95	60.7 ± 3.59	2815.34 ± 1515.20	2101.38 ± 636.34	**0.0002**	0.1238	0.1244
Cl^-	456.47 ± 109.01	392.25 ± 41.25	26668.83 ± 6844.91	28693.85 ± 1756.14	**0.0001**	0.7885	0.7751
N	27403.33 ± 914.36	30583.33 ± 1269.23	31103.33 ± 1465.99	32796.67 ± 364.25	**0.0263***	0.0553	0.5135
P	3013.02 ± 160.83	3257.18 ± 160.51	2833.49 ± 202.46	2620.33 ± 83.57	**0.0323***	0.9242	0.1854
K	29596.31 ± 160.24	30748.53 ± 1192.82	27804.87 ± 5566.82	26144.68 ± 2110.70	0.3231	0.9354	0.6557
Ca	12214.94 ± 903.44	13732.37 ± 473.79	13550.85 ± 1379.30	14690.36 ± 516.14	0.2363	0.1763	0.8382
Mg	3326.25 ± 172.14	3531.48 ± 136.77	3010.85 ± 139.72	3449.83 ± 62.49	0.1766	**0.0429***	0.4083
S	4614.65 ± 157.46	5506.43 ± 385.12	4130.70 ± 282.58	4401.51 ± 116.31	**0.0152***	0.0544	0.2634
Fe	81.96 ± 2.83	91.63 ± 3.62	64.63 ± 5.15	72.06 ± 1.27	**0.0008**	**0.0408***	0.7570
Mn	40.54 ± 3.90	22.72 ± 2.30	49.07 ± 13.70	45.88 ± 3.73	0.0662	0.1960	0.3547
Cu	0.68 ± 0.26	0.37 ± 0.05	0.37 ± 0.13	0.45 ± 0.12	0.4767	0.4854	0.2504
Zn	91.84 ± 4.31	90.70 ± 9.35	80.09 ± 8.16	88.92 ± 7.50	0.3970	0.6245	0.5283
Mo	1.17 ± 0.25	0.85 ± 0.05	0.35 ± 0.03	0.34 ± 0.05	**0.0010**	0.2484	0.2573
B	45.95 ± 2.90	50.01 ± 1.52	21.82 ± 2.62	23.08 ± 1.82	**0.0000**	0.2410	0.6650

* Not considered statistically significant under the Bonferroni-corrected alpha level of 0.05 / 12 ≈ 0.004, Na and Cl were excluded from Bonferroni interpretation as they were treatment ions.

Table 4.10. Mean ± SE ion ratios (Na/K. Na/Ca, Na/Mg) in hybrid poplar leaves under combinations of NaCl (0 or 100 mM) and prior herbivory (H = herbivory, NH = no herbivory) treatments. Different superscript letters within a row indicate significant differences among treatment combinations according to Tukey's HSD test on $\log_{10}$-transformed data ($P < 0.05$). Means and SE are on the original scale; n=3

Ratio	0-NH	0-H	100-NH	100-H
Na/K	0.0019 ± 0.00006^{b}	0.0021 ± 0.0002^{b}	0.088 ± 0.037^{a}	0.080 ± 0.024^{a}
Na/Ca	0.0047 ± 0.0003^{b}	0.0048 ± 0.0060^{b}	0.189 ± 0.093^{a}	0.144 ± 0.044^{a}
Na/Mg	0.017 ± 0.0003^{b}	0.0185 ± 0.0013^{b}	0.893 ± 0.460^{a}	0.614 ± 0.189^{a}

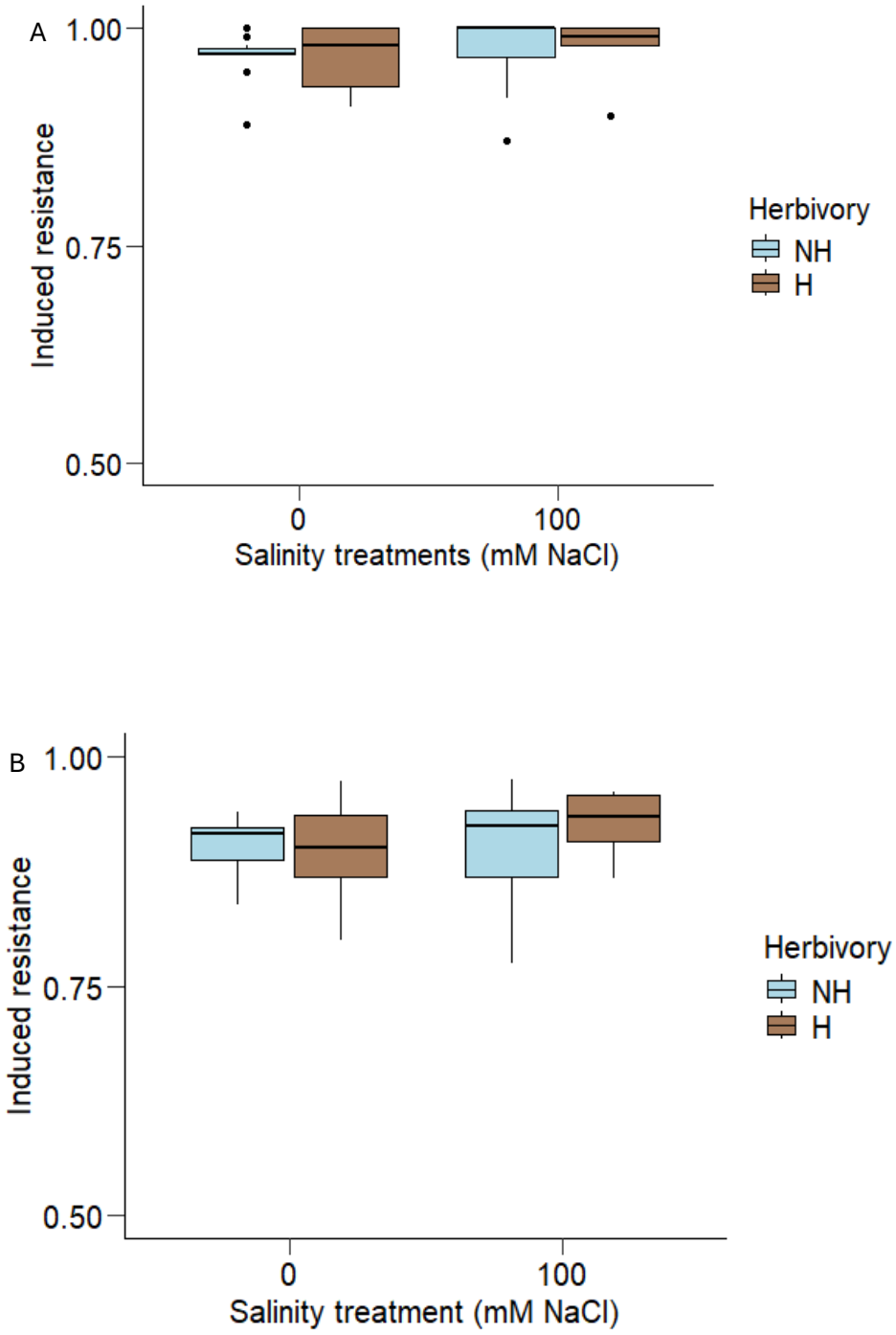

Figure 4.7. Induced resistance to *O. leucostigma* in hybrid poplar leaves (A) Leaf disc assay and (B) *in vivo* feeding assay, after four weeks two salinity treatments (0 or 100 mM NaCl) with and without prior herbivory (NH = no herbivory, H = herbivory). Boxplots as in Figure. 4.1; n = 9-10

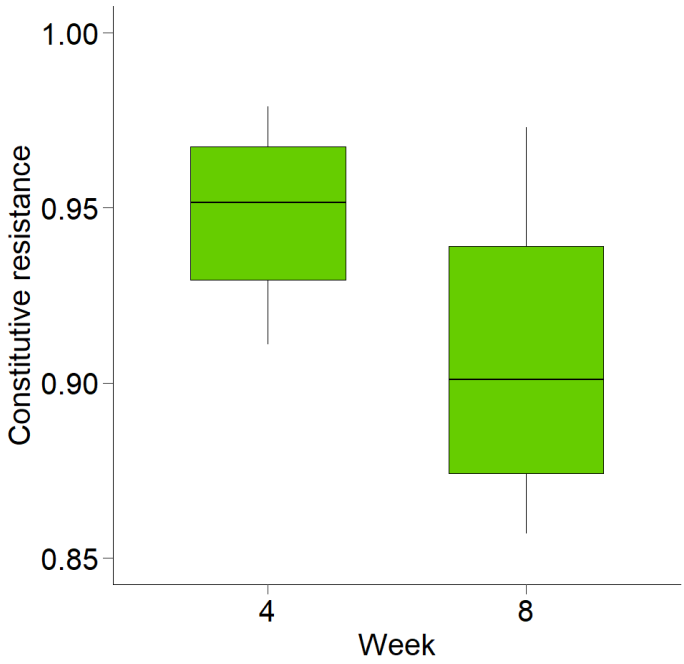

Figure 4.8. Constitutive resistance of hybrid poplar leaves at 4 and 8 weeks. Boxplots as in figure 4.1. Values are the average of two subsamples per plant (two lateral shoots). No significant difference between weeks (paired t-test, $P > 0.05$; n = 9 paired individuals).

CHAPTER 5 -DISCUSSION

5.1. Experiment 1

5.1.1. The effect of salinity on physiological measurements

Physiological parameters were measured to check whether the salt treatment applied caused a physiological stress on the plants. Leaf gas exchange was measured over time to determine how salinity affected photosynthesis, stomatal conductance and transpiration in hybrid poplar. After one week of salt exposure, salinity had no significant effect on photosynthetic rates, stomatal conductance, or transpiration in hybrid poplar which indicates that the initial osmotic stress during this early phase did not impair gas exchange parameters. In addition, chlorophyll fluorescence (F_v/F_m) was slightly higher in salt-treated plants (0.791) compared to controls (0.779) yet both remained within the optimal range of 0.75-0.85 (Kitajima & Butler, 1975). Declines in this ratio often occur when excessive ROS directly damage PSII reaction centers or indirectly impair their repair by limiting synthesis of the D1 protein, a core component of PSII (Wang et al., 2016). The absence of such declines in my study indicates that the Okanese cultivar of *Populus* maintained normal PSII function after one week of salt exposure.

By week three, salinity caused large reductions in stomatal conductance and transpiration in hybrid poplar, while photosynthesis remained unaffected. A similar pattern of reduced stomatal conductance and transpiration has been reported in several hybrid poplar cultivars (Okanese, Assiniboine, CanAM) grown in hydroponics (Moran, 2023), although in that specific case, photosynthesis also declined under salinity in Okanese. Thus, both studies indicate a strong stomatal response to salinity after similar number of weeks of salt treatments, but the different medium used (soil vs. hydroponic solution) could explain the contrasting effects on photosynthesis. In hydroponics, roots are exposed directly to uniform salt concentrations, which

may intensify ionic stress, whereas in soil, salt accumulation is slower and more heterogeneous due to binding with soil particles and leaching which could delay the onset of ionic stress. Therefore, under soil conditions, the early phase of salinity is dominated by osmotic effects, expressed as reduced stomatal conductance and transpiration, while photosynthesis can be maintained until Na^+ and Cl^- ions accumulate to greater concentrations in leaf tissues (Flowers & Colmer, 2015; Munns & Tester, 2008). The observed reduction in stomatal conductance indicates stomatal closure, a typical early osmotic response to salinity that helps limit water loss through transpiration (Munns & Tester, 2008). Initially, this response is triggered by the osmotic effects of NaCl, which lower leaf and guard cell water potential, reducing guard cell turgor a hydropassive response that occurs within the first few hours of exposure. As stress persists, ABA accumulation reinforces stomatal closure through hormonal signaling, enhancing water conservation under salinity (Karimi et al., 2021). Such closure reduces transpiration and CO_2 uptake without immediately impairing C fixation (Flexas et al., 2004; Lawlor & Cornic, 2002). With continued salt exposure, other non-stomatal factors such as reduced mesophyll conductance (the diffusion of CO_2 from substomatal cavities to chloroplasts (Flexas et al., 2004, 2007) and ionic toxicity (Parida & Das, 2005) likely contributed to the pronounced decline in photosynthetic rates observed at week five. In plants not exposed to salt, stomatal conductance increased at week three compared to week one, reflecting peak stomatal activity in fully expanded leaves. Following establishment, the poplar cutting in my study likely allocated resources to rapid leaf expansion and shoot elongation, resulting in high photosynthetic rates in the first week (Hieke et al., 2002). However, as the plants mature, carbon is increasingly diverted toward secondary growth (e.g., lignification) and carbohydrate storage in woody tissues which utilize assimilates more slowly than young, actively growing tissues (Paul & Foyer, 2001). This

gradual change in allocation likely reduced the relative sink demand for photosynthesize in developing leaves, creating sink limitation feedback that downregulates photosynthesis (Paul & Foyer, 2001). Thus, the decline in photosynthetic rate seen in plants not exposed to salt may reflect a combination of leaf age-related decreases in photosynthetic capacity and developmental reallocation of resources rather than external stress. Insect feeding can also impair photosynthetic efficiency in remaining leaf area through wounding, induction of defense pathways, and elevated ROS production, thereby diverting carbon and energy away from carbon assimilation (Schultz et al., 2013). Previous studies have shown that herbivory can indirectly reduce carbon assimilation rates following leaf damage by inducing defense signaling, oxidative stress, and downregulation of photosynthetic genes (Visakorpi et al., 2018).

These results also showed a decline in leaf water content under salinity after four weeks of treatment, which suggests osmotic stress. However, by week six, the water content of salt-treated plants was no longer different from controls, potentially due to acclimation through osmotic adjustment (e.g., accumulation of compatible solutes) and improved ion homeostasis (Chen et al., 2009). Furthermore, the fact that the area of a fully expanded leaf (taken from the middle portion of the main shoot) was not affected by salinity after four or six weeks of treatment suggests a degree of salt tolerance, likely through osmotic adjustment that maintained turgor for leaf expansion and ion regulation that reduced ionic toxicity in leaves, thereby maintaining growth under salinity stress. In the literature, salinity often reduces leaf area in salt-sensitive woody species by inhibiting leaf expansion and accelerating leaf senescence or abscission (Paganová et al., 2022; Silveira et al., 2001) but in salt-tolerant species leaf area is often maintained under moderate salinity, as observed in our hybrid poplar.

Consistent with these stress effects, height-based RGR was lower by about 21 % under salinity after six weeks of treatments, reflecting the growth-limiting impact of salt stress. While we did not measure LAI (Leaf Area Index) or SLA (Specific Leaf Area) directly, osmotic adjustment likely contributed leaf function thereby mitigating the reduction in total biomass. Height growth is more sensitive to salinity possibly because osmotic stress restricts cell expansion, leading to reduced elongation and adaptive growth restraint.

5.1.2. The effect of salinity and herbivory on nutritional quality of hybrid poplar leaves

Nutritional quality in this study refers to the nutrients and compounds in leaves that influence their palatability and the ability of herbivores to use them for growth. It depends on both essential nutrients (e.g., N, P, K, Ca, Mg) and secondary compounds like phenolics, which can lower palatability and reduce insect protein digestion (Amtmann et al., 2008; Li et al., 2024).

After four weeks of salt exposure, hybrid poplar leaves used for herbivory assays showed a higher level of leaf total phenolics than control plants. Phenolic compounds are recognized for their antioxidant function in scavenging ROS generated under salinity stress (Valifard et al., 2014; Waśkiewicz et al., 2012). Increased phenolics content is typical in woody plants under salinity, where the protective role of such compounds may be more important given the slower growth and longer-lived tissues compared to herbaceous species (Cipollini et al., 2017). Indeed, salinity-induced activation of the phenylpropanoid pathway often enhances the biosynthesis of these metabolites (Navarro et al., 2006), and higher polyphenol levels have been reported across a range of species. Thyme (*Thymus vulgaris* and T. *daenensis*) showed higher phenolic content at 60 mM NaCl (Bistgani et al., 2019), while Romaine lettuce (*Lactuca sativa* var. longifolia) and Verte lettuce (*L. sativa* var. Verte de Cobham) cultivars showed increases in flavonoids and

phenolic acids under 100 mM NaCl (Mahmoudi et al., 2010). Moreover, hybrid poplars produce both constitutive and inducible phenolics (Tsai et al., 2006; Stevens & Lindroth, 2005; Bistgani et al., 2019). In fact, several poplar cultivars, including Okanese, can maintain high constitutive levels of phenolics (>10 mg g^{-1} DW) under salt stress (Moran, 2023).

However, following herbivory exposure (after the four weeks of salt treatments), there was no further increase in phenolic compound accumulation. Instead, within each treatment, phenolic levels measured 48 h after herbivory were significantly lower than their pre-herbivory (constitutive) levels; this level of phenolics was similar to what was found in plants not exposed to herbivory, indicating a decline over time. Rather than a typical induction, the observed decline suggests a natural temporal variation in leaf phenolic content. After six weeks growth under salinity, phenolics levels were also not significantly affected by salinity or prior herbivory. Since the complete phenolic composition of most plants, including hybrid poplar, is not fully characterized, total values reported here may not reflect the concentrations of specific compounds most directly involved in herbivore deterrence (e.g., chlorogenic acid, flavonoids, condensed tannins; English-Loeb et al., 1997). Future studies can be designed to identify and quantify individual phenolic compounds in hybrid poplar to identify which metabolites contribute most strongly to herbivore resistance to *O. leucostigma* under salinity stress.

Salt-treated plants had higher levels of Na and Cl, lower levels of K and no change in Ca and Mg in their leaves after four weeks of salt treatment and 48 h of herbivory. Comparisons with other *Populus* species highlight broader differences in salinity tolerance strategies. *Populus euphratica* tolerates salinity levels as high as 400 mM NaCl by maintaining elevated Ca and K levels to restrict Na entry and by preferentially sequestering Na in the apoplast in leaf tissues, thereby minimizing cytoplasmic toxicity (Chen et al., 2001; Ottow et al., 2005). *Populus alba*, in

contrast, confines most absorbed Na to the roots (~90%), with only a small fraction reaching the leaves, which both contributes to osmotic adjustment and protects photosynthetic tissues from ionic toxicity (Imada et al., 2009). In a previous hydroponic study, *Populus* 'Okanese' leaves accumulated approximately 20 $g \cdot kg^{-1}$ Na when exposed to 100 mM NaCl for three weeks (Moran, 2023), whereas leaves of plants in the present soil-based experiment reached a concentration of only 0.9-1.3 $g \cdot kg^{-1}$ Na after four weeks of treatment, about 19-fold lower. The substantially lower Na accumulation observed here may partly reflect the influence of the growth medium as explained above. In that earlier study conducted in our lab (Moran, 2023), Okanese plants exposed to 100 mM NaCl exhibited significant increases in both Na and Cl concentrations in the leaves but also accumulated the highest amounts of these ions in their roots compared to other cultivars tested. Root retention of Na is a tolerance mechanism in woody plants such as willow trees (*Salix* spp.), where Na can be bound to root cell walls or restricted at the xylem loading sites (Byrt et al., 2018). This pattern was also accompanied by visible root thickening in several plants, consistent with earlier observations in previous studies in our lab (Renault et al., 2005) whereby Na^+ sequestration is associated with anatomical adjustments that enhance ion storage capacity. The comparatively low Na concentrations detected in Okanese leaves in the present study suggest that a substantial portion of the absorbed Na may have been retained in the roots, thereby limiting its translocation to the shoots and contributing to salt tolerance. Although root ion levels were not measured here, this pattern is consistent with observations in other *Populus* species, such as *P. alba* (Imada et al., 2009). Furthermore, P and S concentrations were slightly lower in treated plants compared to controls in my study. Elevated levels of Cl in saline soils may reduce P availability by increasing the ionic strength of the medium and decreasing the solubility of Ca-P compounds (Balasubramaniam et al., 2023). Reductions in K, P, and S

however, likely contributed to lower protein levels, as these nutrients are critical for enzyme activation (K), energy and nucleic acid metabolism (P; Krikorian, 1988), and the synthesis of glutathione. In addition, sulfur is involved in stress signaling (Noctor et al., 2012).

To further evaluate ionic balance in hybrid poplar under salinity and prior herbivory, I calculated ratios of Na/K, Na/Ca, and Na/Mg using mean concentrations. All three ratios increased under salt stress relative to controls, which is an indication of competitive inhibition of nutrient uptake by excess Na and Cl. Such ion imbalances are typical indicators of salinity stress and are widely reported as indicators of disrupted homeostasis in plant tissues (Shahzad, 2012).

Two weeks after herbivory treatments, and following six weeks of salinity exposure, hybrid poplar leaves showed markedly higher Na and Cl concentrations compared to control plants. Between four and six weeks of salt treatment, leaf Na concentrations increased approximately seven-fold, while Cl concentrations roughly doubled, indicating progressive ion accumulation over time. By six weeks, however, leaf water content returned to levels comparable to the control, coinciding with high Cl accumulation that likely contributed to osmotic adjustment (Franco-Navarro et al., 2016). This was accompanied by selective increases in Ca, Mn, and Zn, whereas most macronutrients (N, P, K, Mg, and S) remained unaffected by salinity, in contrast to four weeks of salt treatment, where significant reductions were observed in P, K, and S, while Ca and Mg remained unchanged. The partial recovery of these nutrients by week six suggests an adjustment in ion homeostasis and nutrient transport over time, possibly reflecting improved regulation of selective ion uptake and compartmentalization as the plants acclimated to prolonged salinity (Pandolfi et al., 2016). These changes likely reflect both ionic toxicity from high Na and Cl and adaptive responses like Ca enrichment, which help stabilize membranes and regulate selective ion transport by activating Ca-dependent signaling pathways (e.g., SOS1)

under salt stress (Mahajan et al., 2008). The increased concentrations of Zn and Mn observed under salinity stress may indicate a role in strengthening antioxidant capacity (Pawar et al., 2024). Both elements serve as cofactors of superoxide dismutases (Cu/Zn-SOD and Mn-SOD), which detoxify superoxide radicals by converting them into H_2O_2. Similar enhancements in Zn and Mn availability have been linked to improved salt tolerance through reinforced ROS-scavenging systems in *Arabidopsis thaliana* (Liu et al., 2015; Wang et al., 2004). In contrast, insect herbivory, whether applied after four or six weeks of salt exposure, had minimal influence on foliar elemental concentrations, and there were no significant interactions between salinity and herbivory. Thus, the changes in elemental content of leaf tissue found in this system are due mainly to salinity.

Although phenolic compounds do not directly alter leaf nutrient value, elevated levels can influence how nutrients are utilized by herbivores. Phenolics can form complexes with proteins and digestive enzymes of herbivores, thereby lowering protein digestibility and reducing the effective nutritional value of the tissue. For example, tannins decrease protein digestion in insects through binding to proteins and digestive proteases (Barbehenn & Constabel, 2011). Thus, while salinity enhanced phenolic accumulation in hybrid poplar, this likely lowered the effective nutritional value of the leaves even though nitrogen concentrations was not changed among treatment. When considered together with the observed changes in mineral nutrition declines in K, P, and S and elevated Na/K, Na/Ca, and Na/Mg ratios, our findings indicate the fact that only some of the nutrient levels were affected by salinity after four weeks and almost none were affected by salinity after six weeks, suggests plants were able to adjust ionic imbalance over time. Thus, the reduction in nutritional quality was most pronounced during the four weeks of salt treatments.

5.1.3. Herbivory resistance of hybrid polar

The relatively similar levels of leaf area consumed by insects in control (11.7%) and salt-treated plants (15.7%) in the *in vivo* feeding assay indicate that salinity did not affect hybrid poplar resistance to *O. leucostigma*. In a previous study, in our research lab, Moran (2023) reported much lower leaf consumption (<5%) when *O. leucostigma* larvae were given a choice among several hybrid poplar cultivars (Okanese, Can Am, Walker, and Assiniboine). This may be explained by differences in the developmental stage of the *O. leucostigma* used: third-instar larvae were used in my study, whereas Moran used second instars. Younger instars generally consume less tissue overall and may be more sensitive to foliar traits, while older larvae can tolerate or detoxify defensive compounds (Barbehenn, et al., 2008), which could account for the higher levels of leaf area removed in the present study.

This study showed no effect of salinity on either constitutive or induced resistance after four weeks of treatment. Therefore, these findings do not support the hypothesis that salinity stress enhances hybrid poplar resistance to *O. leucostigma* by reducing foliar palatability after four weeks. Previous studies have reported both increased and decreased resistance under salinity in various plants. In *Brassica juncea*, a moderately salt-tolerant species, constitutive resistance to *Trichoplusia ni* was greater under salinity, likely because osmotic and ionic stress led to decreased foliar nitrogen (a nutrient sought by herbivores) despite increased proline accumulation, a known feeding stimulant (Renault et al., 2016). In contrast, in *Gossypium hirsutum* (Quijano-Medina et al., 2021) salinity prevented the induction of phenolic defences and altered trait correlations, indicating that salinity can sometimes weaken rather than strengthen herbivore resistance. Similarly, *Glycine max*, which is generally more sensitive to salt, accumulated ~40 % more leaf N under 100 mM NaCl (Ávila-Sákar et al., 2018). This elevated N

linked to osmolyte production and N-rich metabolites made leaves more palatable to herbivores (lower resistance), a well-known effect of foliar N content (Gely et al., 2020; Mattson, 1980). These contrasting results suggest that the response to herbivory of plants grown under salinity depends on how tolerant a plant is to salt and on the particular biochemical changes that occur in different tissues in response to salinity: in some species, stress responses strengthen defenses, while in others, the compounds produced for tolerance can make leaves more palatable or nutritionally attractive to herbivores. Leaf N strongly affects *O. leucostigma* performance: larvae generally grow faster on high-N foliage and tend to prefer higher-quality or mixed-age leaves rather than low-nitrogen tissues (Hale et al., 2005).Hence, when salinity elevates foliar N, feeding damage by *O. leucostigma* can increase; while when salinity lowers N or primarily boosts metabolites that deter herbivore feeding, resistance may instead be stronger suggesting that reduced feeding cannot be attributed to lower nitrogen availability. Together with previous research, these findings highlight the fact that the effect of salinity on herbivory resistance depends on multiple interacting factors, including the duration of salt exposure, the timing of measurements, the identity of the herbivore, stress intensity. In my experiment, four weeks of salt exposure did not affect resistance to *O. leucostigma* which suggests that herbivory, at least by this herbivore species is not affected by short-term salinity-induced changes in hybrid poplar.

Even though total phenolic levels were higher in salt-treated plants than in control plants after four weeks, this did not translate into enhanced herbivory resistance. This is consistent with reports that *O. leucostigma* can tolerate or detoxify phenolics at little cost (Barbehenn et al., 2008). Similarly, Kopper et al. (2002) found that moderate phenolic levels in *Betula papyrifera* reduced feeding of *O. leucostigma*, but higher concentrations had no deterrent effect and were even associated with increased consumption. Thus, while salinity induced modest increases in

leaf phenolics, these compounds may have limited defensive value against *O. leucostigma* and instead reflect a general stress-response role, potentially functioning in antioxidant activity (Kumar et al., 2023). However, high levels of phenolics in leaves could still deter other herbivores that cannot tolerate or detoxify phenolics. For instance, catechol-based phenolics in strawberry (*Fragaria ananassa*) leaves were strongly correlated with resistance to the two-spotted spider mite (*Tetranychus urticae*), where high concentrations suppressed mite development by inactivating digestive enzymes (Luczynski et al., 1990). Similarly, in oak (*Quercus robur*), high tannin levels in the leaves reduced the feeding by winter moth (*Operophtera brumata*) larvae, likely due to tannins complexing with proteins and disrupting larval digestion (Feeny et al., 1970). The contrasting responses indicate that the effectiveness of phenolic-based defense depends not only on their overall concentration, but also on the specific compounds involved and the herbivore feeding guild. Generalist leaf-chewers such as *O. leucostigma* are especially tolerant, aided by the protective peritrophic membrane that prevents high-molecular-weight tannins from damaging the gut epithelium (Barbehenn & Martin, 1992). Thus, using a single insect species may underestimate the broader defensive capacity of hybrid poplar under salinity. Future studies combining compound-specific profiling (e.g., HPLC-based metabolomics) with a wider range of herbivore guilds, such as chewing, piercing-sucking (aphids), and gall-forming species would provide a better understanding of how salinity affects constitutive and induced chemical defenses.

Compared to the 4-week exposure, where salinity had no effect on either constitutive or induced resistance, plants exposed to salt for six weeks exhibited greater induced resistance. This pattern suggests that prolonged salt exposure may prime plants to respond more effectively to herbivore attack. No significant salinity × herbivory interaction was detected, indicating that the

two stresses acted independently rather than additively or synergistically. This response highlights the complexity of multiple stress interactions, where the sequence and timing of exposure can determine whether salinity enhances or has no effect on resistance. A comparable pattern was observed in tomato (*Solanum lycoperscium*), where NaCl levels equal or greater than 50 mM deterred *Spodoptera exigua* feeding and reduced insect performance (Marsack & Connolly, 2022). Mechanistically, salinity may induce ROS accumulation (Hasanuzzaman et al., 2021), which can prime jasmonate-mediated anti-herbivore defenses and elevate resistance before insect attack. In my study, the slight increase in Ca^{2+} under salinity may have contributed to enhanced resistance, since Ca^{2+} not only acts as a stress signal but also stabilizes membranes (Tuteja & Mahajan, 2007). Even slight elevations in leaf Ca^{2+} can enhance ABA- and JA-related signaling pathways, facilitate ROS-mediated defense activation, and strengthen membrane stability. Together, these processes could reduce ion leakage and preserve metabolic resources, allowing plants to respond more efficiently to herbivory through inducible defenses. Once herbivory triggered its own defense responses, salinity could not add much further benefit, since both stresses rely on similar hormonal signals and resources. These patterns suggest that short-term salinity (4 weeks) exposure primarily promotes ion homeostasis and osmotic adjustment, while prolonged exposure (6 weeks) promotes defense priming, thereby influencing how plants respond to subsequent herbivory.

5.2. Experiment 2

5.2.1. The effects of salinity and herbivory on nutritional quality of hybrid poplar

In experiment 2, plants were exposed to herbivory prior to salinity. The lower leaf phenolic content in plants exposed to herbivory suggests that the order of stresses matters for how plants

allocate defenses. Early herbivory by this particular herbivore may have redirected resources that otherwise would have been used for phenolic production, potentially limiting the accumulation of phenolics needed for salt tolerance. This observation is consistent with previous findings in mature *Populus* species, where phenolic induction was limited or even declined after herbivory exposure. For instance, in black poplar (*Populus nigra*), the concentrations of phenolic glycosides (salicinoids) either remained unchanged or decreased following gypsy moth (*Lymantria dispar* L.) feeding (Boeckler et al., 2013). Differences in leaf age and herbivore cues can influence how plants respond to feeding (Coley, 1980; Musser et al., 2002). Since we did not separate leaves by age, younger tissues may have maintained or increased phenolics while older leaves declined after herbivory. Moreover, the saliva of some species of insect herbivores can act as an important suppressor of plant defenses. In *Helicoverpa zea*, for example, the salivary enzyme, glucose oxidase was shown to suppress induced resistance in *Nicotiana tabacum* by interfering with JA signaling and thereby preventing the induction of nicotine (Musser et al., 2002). Since glucose oxidase is widespread among caterpillar species, this mechanism likely represents a general strategy used by insects to downregulate host chemical defenses. Total phenolic content measured in experiment 2 after four weeks of salinity treatments and in absence of herbivory were consistently higher (~87%) than those observed in experiment 1. That trend was observed across all treatments, with phenolic levels in experiment 2 exceeding those in experiment 1 by ~50-90%. Both experiments were conducted under the same greenhouse conditions, but their seasonal timing likely influenced phenolic accumulation. Experiment 2 (February-May) coincided with a stronger relative increase in daylength, UV, and blue light from late winter to spring, conditions known to upregulate the phenylpropanoid pathway and stimulate phenolic metabolism (Marin et al., 2015). By contrast, experiment 1 (April-June) occurred later

in the season, when radiation levels were already high and relatively stable, possibly leading to weaker induction of phenolic production. Moreover, variation pre-collection environmental exposure of poplar cuttings used in this study, such as differing winter conditions in the stool beds, may have influenced baseline phenolic metabolism, contributing to differences between experiments.

Furthermore, hybrid poplar leaves exposed to salinity showed lower total soluble protein levels compared to controls, while overall herbivory and its interaction with salinity had no effect. Similar patterns have been reported in other woody species, where salinity stress leads to reductions in protein content. In a woody plant, *Paulownia imperialis*, salinity reduced total protein content, primarily due to osmotic and ionic stress that disrupts protein synthesis and promote degradation (Ayala-Astorga & Alcaraz-Meléndez, 2010). Similar declines in protein metabolism under saline conditions have been reported across both woody and herbaceous species. Ashraf and Waheed (1993) observed reduced soluble protein in response to salt stress in all lentils (*Lens culinaris*) lines tested, irrespective of their salt tolerance. Likewise, decreases in leaf protein concentrations under salinity have been documented in other glycophytes, including sugar beet (*Beta vulgaris*), where protein loss was accompanied by RNA degradation and altered protein profiles (Jamil et al., 2012), as well as in lentils, wheat, and other crop species (Alamgir & Ali, 1999; Gadallah, 1999; Wang & Nil, 2000). Collectively, these findings highlight that salinity stress broadly impairs protein metabolism by limiting synthesis (Ramagopal, 1987) and accelerating degradation (Zörb et al., 2010), ultimately reducing protein accumulation in plant tissues.

In experiment 2, salinity caused Na and Cl accumulation in leaves like in the case of experiment 1. However, the extent of the accumulation of Na in leaves after four weeks differed,

reaching ~857-1262 mg/kg in experiment 1, whereas in experiment 2, it was considerably higher (~2100-2800 mg/kg). By contrast, leaf Cl levels under salinity were similar between experiments (~26,000-29,000 mg/kg). This suggests that plants in experiment 2 absorbed or retained more Na in leaves, however, some elements showed contrasting responses between the two experiments. For example, K levels were lower under salinity in experiment 1 but were not significantly affected in experiment 2. Similarly, leaf N content showed no change under salinity in experiment 1 but showed slightly higher levels in experiment 2. Micronutrient responses also differed between experiments: leaf Mn reached higher levels in experiment 1 but not in experiment 2, while Zn showed lower levels only in experiment 1 under salt.

The higher levels of Mg and Fe observed under herbivory may reflect increased physiological demand. Mg is required for chlorophyll and Rubisco (Wolf et al., 2019), and its higher levels likely support compensatory photosynthesis. Fe, in turn, is a cofactor for many antioxidant and defense enzymes, and higher levels may be associated with ROS detoxification and activation of secondary metabolism (Halliwell & Gutteridge, 2015) following herbivore attack. No significant interaction effects between salinity and herbivory were detected for any of the elements quantified, which indicates that the changes in elemental concentration in the present study were primarily driven by individual stress factors rather than their combined effect.

Overall, in experiment 2, plants that experienced herbivory showed lower levels of total phenolic content in their leaves, and these levels were not affected by subsequent salinity, suggesting that early defoliation redirected metabolic resources from secondary metabolism toward repair and maintenance (Pankoke & Müller, 2013). Although overall phenolic concentrations were higher than those recorded in experiment 1, likely due to seasonal differences in light intensity and day length, the relatively lower levels in herbivory-treated

plants indicate a reduced capacity for inducible defense. Salinity caused lower total soluble protein concentrations in leaves, while N and K contents were not affected. Leaf Na concentrations were higher than in experiment 1, whereas Cl levels were comparable, indicating greater Na retention in foliage and potentially stronger ionic stress. Collectively, these findings suggest that prior herbivory weakened the protective chemical profile of the leaves and limited the plant ability to mount effective induced defenses under subsequent salinity.

5.2.2. The effects of salinity and herbivory on hybrid poplar resistance

Experiment 2 showed that prior herbivory, salinity stress, or their combination did not significantly affect the resistance of hybrid poplar. This could be explained by the timing and dynamics of plant defense responses, which are known to be rapid and short-lived in poplar species: For example, in *Populus tremula,* defense-related phenolic compounds such as catechin and procyanidin B1 sharply increased within just 5 minutes of herbivory, only to return to their baseline levels after 10 minutes (Pastierovič et al., 2024). This temporary nature of defense expression suggests that our measurement, taken four weeks after the prior herbivory treatment, would have missed such short-lived peaks. However, not all defense chemicals are formed and accumulated so rapidly. Some secondary metabolites require days to accumulate after the herbivory damage. In *P. tremuloides*, the phenolic glycosides; tremulacin and salicortin were significantly elevated only after 9 -11 days of leaf mining damage (Young et al., 2010). Thus, although herbivory presumably induced chemical defenses, their effects on larvae feeding likely diminished by the time induced resistance was assessed, explaining the absence of significant differences in resistance levels.

Furthermore, the salinity treatments applied after the herbivory treatments likely imposed ionic and osmotic stress, but not to a level severe enough to suppress or interact strongly with the poplar defense signaling pathways. *Populus* species are moderately salt-tolerant (Chen and Polle, 2010), and 'Okanese' hybrid poplar, in particular is known to exhibit adaptations to drought conditions (Barchet et al., 2014), maintaining high productivity in such environments. This physiological adaptability suggests a potential for moderate salinity tolerance, as mechanisms that confer drought resistance, such as efficient water use, osmotic adjustment through solute accumulation and activation of antioxidant defenses may also contribute to coping with saline conditions (Fang & Xiong, 2015). Furthermore, the lack of significant effects observed in induced herbivory resistance levels in hybrid poplar under salinity and herbivory in this study could partly be explained by limitations in the sensitivity of the bioassay method. Bioassays that measure herbivore feeding performance do not necessarily reflect the underlying metabolic changes in plant defense compounds. Conversely, detecting chemical alterations in plant tissues does not always translate into measurable effects on herbivore behavior (Underwood et al., 2002).

5.3. Conclusion and future research

The main goal of this study was to determine how salinity and insect herbivory, individually and sequentially affect nutritional quality, and resistance of hybrid poplar (*Populus deltoides* × *petrowskyana* × *petrowskyana* 'Okanese') leaves to the generalist lepidopteran *Orgyia leucostigma*. To address this, two complementary greenhouse experiments were conducted.

In experiment 1, I predicted that *O. leucostigma* resistance would increase under salinity stress due to changes in nutritional quality and defensive chemistry that would reduce leaf palatability. After four weeks of salt exposure, despite alterations in ion balance, and a modest

increase in total leaf phenolics content, resistance did not change. In contrast, two weeks following the first herbivory treatment (after six weeks of salinity exposure), salinity alone significantly enhanced induced resistance. Thus, hypothesis 1 was only partially supported, with resistance outcomes depending strongly on the duration of salinity stress.

In experiment 2, I predicted that prior herbivory would induce nutrient changes (greater phenolics content and Ca level, lower N and protein content) that prime the response of hybrid poplar to salinity and also increase subsequent resistance to herbivores. This prediction was not supported. Instead, leaf phenolics content declined rather than accumulated, protein levels were not altered by herbivory, and only decreased under salinity, and resistance was not enhanced under combined stress.

Overall, salinity independently enhanced resistance to *O. leucostigma*, but this effect was only detected after longer salt exposure and hybrid poplar maintained high resistance regardless of herbivory or salinity treatments. There was no salinity × herbivory interaction, indicating that herbivory neither enhanced nor altered the resistance response induced by salinity. Changes in nutritional quality and total phenolics did not reliably predict resistance to this generalist herbivore.

Future work can be designed to investigate which phenolic metabolites (particularly salicinoid phenolic glycosides, flavonoids, and condensed tannins) and nitrogenous metabolites (such as proteinase inhibitors and defensive alkaloids) are most responsive to salinity and herbivory as these metabolites are likely to regulate inducible resistance in poplar (Boeckler et al., 2011; Waśkiewicz et al., 2012). In addition, the salinity-associated changes we observed in hybrid poplar generalize across herbivore feeding modes. I propose a follow-up experiment in which plants are exposed to the same salinity treatments and then challenged (simultaneously or

sequentially) by representatives of additional guilds e.g., leaf miners, gall formers, and sap feeders while also quantifying physical defenses (cuticular wax amount, trichome density, and leaf toughness). Coupling these measures with the existing knowledge of nutritional changes would clarify which traits mediate resistance or tolerance under combined abiotic and biotic stress. Finally, integrating hormonal profiling of ABA-, JA-, and SA-mediated pathways (Kumar et al., 2019) could clarify the signaling crosstalk that underlies stress allocation in woody plants. In plants, ABA accumulation under salinity stress plays a central role in mediating stomatal closure, osmotic adjustment (LaRosa et al., 1987), while JA and SA are rapidly induced following insect feeding or wounding to activate defense-related genes (Ali et al., 2024). Future studies combining hormonal profiling and gene-expression analyses would clarify whether these pathways interact synergistically or competitively in hybrid poplar exposed to sequential stress factors.

CHAPTER 6 - LITERATURE CITED

Abdelhamid, M. T., Rady, M. M., Osman, A. S., & Abdalla, M. A. (2013). Exogenous application of proline alleviates salt-induced oxidative stress in *Phaseolus vulgaris* L. plants. *The Journal of Horticultural Science & Biotechnology*, *88*(4), 439–446. https://doi.org/10.1080/14620316.2013.11512989

Adu-Acheampong, S., & Samways, M. J. (2019). Traits and land transformation change the fortunes of grasshopper generalists vs. Specialists in a biodiversity hotspot. *Biosystems Diversity*, *27*(1), 26–32. https://doi.org/10.15421/011904

Agrawal, A. A. (2005). Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research,* 7, 651-667.

Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature Protocols*, *2*(4), 875–877. https://doi.org/10.1038/nprot.2007.102

Alamgir, A. N. M., & Ali, M. Y. (1999). Effect of salinity on leaf pigments, sugar and protein concentrations and chloroplast ATPase activity of rice (*Oryza sativa* L.). *Bangladesh Journal of Botany.* 28 (2), 145–149. https://doi.org/10.3923/ijb.2011.73.81

Ali, A., Maggio, A., Bressan, R. A., & Yun, D. J. (2019). Role and functional differences of HKT1-type transporters in plants under salt stress. *International Journal of Molecular Sciences 2019*, *20*(5), 1059. https://doi.org/10.3390/ijms20051059

Ali, J. G., & Agrawal, A. A. (2012). Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science*, *17*(5), 293–302. https://doi.org/10.1016/j.tplants.2012.02.006

Ali, J., Tonğa, A., Islam, T., Mir, S., Mukarram, M., Konôpková, A. S., & Chen, R. (2024). Defense strategies and associated phytohormonal regulation in Brassica plants in response to chewing and sap-sucking insects. *Frontiers in Plant Science*, *15*, 1376917. https://doi.org/10.3389/fpls.2024.1376917

Amtmann, A., Troufflard, S., & Armengaud, P. (2008). The effect of potassium nutrition on pest and disease resistance in plants. *Physiologia Plantarum*, *133*(4), 682–691. https://doi.org/10.1111/j.1399-3054.2008.01075.x

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, *55*(1), 373–399. https://doi.org/10.1146/annurev.arplant.55.031903.141701

Arif, Y., Singh, P., Siddiqui, H., Bajguz, A., & Hayat, S. (2020). Salinity induced physiological and biochemical changes in plants: an omic approach towards salt stress tolerance. *Plant Physiology and Biochemistry*, *156*, 64–77. https://doi.org/10.1016/j.plaphy.2020.08.042

Ashraf, M., & Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Science*, *166*(1), 3–16. https://doi.org/10.1016/j.plantsci.2003.10.024

Ashraf, M., & Waheed, A. (1993). Organic solute status and water relations of some salt-tolerant and salt-sensitive accessions of lentil (*Lens culinaris*). *Acta Botanica Neerlandica*, *42*(1), 63-72. https://doi-org.uml.idm.oclc.org/10.1111/j.1438-8677.1993.tb00678.x

Atkinson, N. J., & Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany*, *63*(10), 3523–3544. https://doi.org/10.1093/jxb/ers100

Aucejo-Romero, S., Gómez-Cadenas, A., & Jacas-Miret, J. A. (2004). Effects of NaCl-stressed citrus plants on life-history parameters of *Tetranychus urticae* (Acari: Tetranychidae). *Experimental and Applied Acarology*, *33*(1–2), 55–67. https://doi.org/10.1023/b:appa.0000030026.77800.0c.

Avila-Sakar, G., Markham, J., Renault, S., & Adorno, B. V. (2018). Nitrogen fixation does not alter the effects of salinity on soybean resistance to a generalist caterpillar. *International Journal of Plant Sciences*, *179*(7), 560–568. https://doi.org/10.1086/698711

Ayala-Astorga, G. I., & Alcaraz-Meléndez, L. (2010). Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold & Zuccarini) and *Paulownia fortunei* (Seemann & Hemsley) grown in vitro. *Electronic Journal of Biotechnology*, *13*(5). 13-14. https://doi.org/10.2225/vol13-issue5-fulltext-13

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Yang, S., Dong, R., Ali, B., & Rahimi, M. (2023). Salinity stress improves antioxidant potential by modulating physio-biochemical responses in *Moringa oleifera* Lam. *Scientific Reports 2023 13:1*, *13*(1), 1–17. https://doi.org/10.1038/s41598-023-29954-6

Balasubramaniam, T., Shen, G., Esmaeili, N., & Zhang, H. (2023). Plants' response mechanisms to salinity stress. *Plants (Basel)*, *12*(12), Article 2253. https://doi.org/10.3390/plants12122253

Barbehenn, R. V., & Martin, M. M. (1992). The protective role of the peritrophic membrane in the tannin-tolerant larvae of *Orgyia leucostigma* (Lepidoptera). *Journal of Insect Physiology*, *38*(12), 973–980. https://doi.org/10.1016/0022-1910(92)90006-Y

Barbehenn, R. V., & Peter Constabel, C. (2011). Tannins in plant–herbivore interactions. *Phytochemistry (Oxford)*, *72*(13), 1551–1565. https://doi.org/10.1016/j.phytochem.2011.01.040

Barbehenn, R., Weir, Q., & Salminen, J.-P. (2008). Oxidation of ingested phenolics in the tree-feeding caterpillar *Orgyia leucostigma* depends on foliar chemical composition. *Journal of Chemical Ecology*, *34*(6), 748–756. https://doi.org/10.1007/s10886-008-9478-3

Barchet, G. L. H., Dauwe, R., Guy, R. D., Schroeder, W. R., Soolanayakanahally, R. Y., Campbell, M. M., & Mansfield, S. D. (2014). Investigating the drought-stress response of hybrid poplar genotypes by metabolite profiling. *Tree Physiology*, *34*(11), 1203–1219. https://doi.org/10.1093/treephys/tpt080

Bernards, M. A., & Båstrup-Spohr, L. (2008). Phenylpropanoid metabolism induced by wounding and insect herbivory. In A. Schaller (Ed.), *Induced Plant Resistance to Herbivory* (pp. 189–211). Springer Netherlands. https://doi.org/10.1007/978-1-4020-8182-8_9

Bharath, P., Gahir, S., & Raghavendra, A. S. (2021). Abscisic acid-induced stomatal closure: an important component of plant defense against abiotic and biotic stress. *Frontiers in Plant Science*, *12*, Article 615114. https://doi.org/10.3389/fpls.2021.615114

Bhonwong, A., Stout, M. J., Attajarusit, J., & Tantasawat, P. (2009). Defensive role of tomato polyphenol oxidases against cotton bollworm (*Helicoverpa armigera*) and beet armyworm (*Spodoptera exigua*). *Journal of Chemical Ecology*, *35*(1), 28–38. https://doi.org/10.1007/s10886-008-9571-7

Bistgani, Z. E., Hashemi, M., DaCosta, M., Craker, L., Maggi, F., & Morshedloo, M. R. (2019). Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and *Thymus daenensis* Celak. *Industrial Crops and Products*, *135*, 311–320. https://doi.org/10.1016/j.indcrop.2019.04.055

Blumwald, E., Aharon, G. S., & Apse, M. P. (2000). [Rev. of *Sodium transport in plant cells*]. *BBA - Biomembranes*, *1465*(1), 140–151. https://doi.org/10.1016/S0005-2736(00)00135-8

Boeckler, G. A., Gershenzon, J., & Unsicker, S. B. (2011). Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. *Phytochemistry (Oxford)*, *72*(13), 1497–1509. https://doi.org/10.1016/j.phytochem.2011.01.038

Boeckler, G. A., Gershenzon, J., & Unsicker, S. B. (2013). Gypsy moth caterpillar feeding has only a marginal impact on phenolic compounds in old-growth black poplar. *Journal of Chemical Ecology*, *39*(10), 1301–1312. https://doi.org/10.1007/s10886-013-0350-8

Bolu, W. H., & Polle, A. N. D. R. E. A. (2004). Growth and stress reactions in roots and shoots of a salt-sensitive poplar species (*Populus* x *canescens*). *Tropical Ecology*, *45*(1), 161-172.

Bose, J., Rodrigo-Moreno, A., & Shabala, S. (2014). ROS homeostasis in halophytes in the context of salinity stress tolerance. *Journal of Experimental Botany*, *65*(5), 1241–1257. https://doi.org/10.1093/jxb/ert430

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Bradshaw, H. D., Ceulemans, R., Davis, J., & Stettler, R. (2000). Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. *Journal of Plant Growth Regulation*, *19*(3), 306–313. https://doi.org/10.1007/S003440000030/metrics

Brini, F., & Masmoudi, K. (2012). Ion transporters and abiotic stress tolerance in plants. International scholarly research notices, 2012(1), 927436. https://doi.org/10.5402/2012/927436

Broekgaarden, C., Poelman, E. H., Steenhuis, M. M., Voorrips, R. E., Dicke, M., & Vosman, B. (2007). Genotypic variation in genome-wide transcription profiles induced by insect feeding: *Brassica oleracea* - Pieris rapae interactions. *BMC Genomics*, *8*(1), Article 239. https://doi.org/10.1186/1471-2164-8-239

Brown, A. D., & Simpson, J. R. (1972). Water relations of sugar-tolerant yeasts: the role of intracellular polyols. *Journal of General Microbiology*, *72*(3), 589–591. https://doi.org/10.1099/00221287-72-3-589

Bryant, K., Fredericksen, B., Hudiburg, T., & Rosenthal, D. (2023). Physiological strategies for handling summer water stress differ among co-existing species and between juvenile and mature trees. *Frontiers in Forests and Global Change*, *5*, Article 1018789. https://doi.org/10.3389/ffgc.2022.1018789

Byrt, C. S., Munns, R., Burton, R. A., Gilliham, M., & Wege, S. (2018). Root cell wall solutions for crop plants in saline soils. *Plant Science (Limerick)*, *269*, 47–55. https://doi.org/10.1016/j.plantsci.2017.12.012

Cakmak, I., & Demiral, M. A. (2007). Response of *Tetranychus cinnabarinus* feeding on NaCl-stressed strawberry plants. *Phytoparasitica*, *35*(1), 37–49. https://doi.org/10.1007/BF02981060

Carvajal Acosta, A. N., Agrawal, A. A., & Mooney, K. (2023). Plant water-use strategies as mediators of herbivore drought response: Ecophysiology, host plant quality and functional traits. *The Journal of Ecology*, *111*(3), 687–700. https://doi.org/10.1111/1365-2745.14059

Chaves, M. M., Flexas, J., & Pinheiro, C. (2009). Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Annals of Botany*, *103*(4), 551–560. https://doi.org/10.1093/aob/mcn125

Cheeseman, J. M. (2015). Evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *The New Phytologist*, *206*(2), 557–570. https://doi.org/10.1111/nph.13217

Chele, K. H., Tinte, M. M., Piater, L. A., Dubery, I. A., & Tugizimana, F. (2021). Soil salinity, a serious environmental issue and plant responses: a metabolomics perspective. *Metabolites*, *11*(11), 724. https://doi.org/10.3390/metabo11110724

Chen, H., & Markham, J. (2021). Ancient CO_2 levels favor nitrogen fixing plants over a broader range of soil N compared to present. *Scientific Reports*, *11*(1), Article 3038. https://doi.org/10.1038/s41598-021-82701-7

Chen, S., & Polle, A. (2010). Salinity tolerance of *Populus*. *Plant Biology*, *12*(2), 317–333. https://doi.org/10.1111/J.1438-8677.2009.00301.X

Chen, S., Li, J., Fritz, E., Wang, S., & Hüttermann, A. (2002). Sodium and chloride distribution in roots and transport in three poplar genotypes under increasing NaCl stress. *Forest Ecology and Management*, *168*(1), 217–230. https://doi.org/10.1016/S0378-1127(01)00743-5

Chen, S., Li, J., Wang, S., Hüttermann, A., & Altman, A. (2001). Salt, nutrient uptake and transport, and ABA of *Populus euphratica*; a hybrid in response to increasing soil NaCl. *Trees (Berlin, West)*, *15*(3), 186–194. https://doi.org/10.1007/s004680100091

Chen, W., Zou, D., Guo, W., Xu, H., Shi, D., & Yang, C. (2009). Effects of salt stress on growth, photosynthesis and solute accumulation in three poplar cultivars. *Photosynthetica*, *47*(3), 415–421. https://doi.org/10.1007/s11099-009-0063-y

Chen, Y., Cao, C., Guo, Z., Zhang, Q., Li, S., Zhang, X., Gong, J., & Shen, Y. (2020). Herbivore exposure alters ion fluxes and improves salt tolerance in a desert shrub. *Plant, Cell and Environment*, *43*(2), 400–419. https://doi.org/10.1111/pce.13662

Cheong, M. S., & Yun, D. J. (2007). Salt-stress signaling. *Journal of Plant Biology*, *50*(2), 148-155. https://doi.org/10.1007/BF03030623

Chojak-Koźniewska, J., Linkiewicz, A., Sowa, S., Radzioch, M. A., & Kuźniak, E. (2017). Interactive effects of salt stress and *Pseudomonas syringae* pv. *lachrymans* infection in cucumber: Involvement of antioxidant enzymes, abscisic acid and salicylic acid. *Environmental and Experimental Botany*, *136*, 9–20. https://doi.org/10.1016/j.envexpbot.2017.01.004

Cipollini, D., Walters, D., & Voelckel, C. (2018). Costs of resistance in plants: from theory to evidence. *Annual plant reviews online*, 263-307. https://doi.org/10.1002/9781118829783.ch8

Coley, P. D. (1980). Effects of leaf age and plant life history patterns on herbivory. *Nature (London)*, *284*(5756), 545–546. https://doi.org/10.1038/284545a0

Conrath, U., Beckers, G. J. M., Langenbach, C. J. G., & Jaskiewicz, M. R. (2015). Priming for enhanced defense. *Annual Review of Phytopathology*, *53*(1), 97–119. https://doi.org/10.1146/annurev-phyto-080614-120132

Constabel, C. P., Yip, L., Joseph J. Patton, & Mary E. Christopher. (2000). Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. *Plant Physiology (Bethesda)*, *124*(1), 285–295. https://doi.org/10.1104/pp.124.1.285

Cortés, A. H. J. (2019). *Salinity Tolerance in Plants*. MDPI - Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/books978-3-03921-027-5

Cuevas, J., Daliakopoulos, I. N., del Moral, F., Hueso, J. J., & Tsanis, I. K. (2019). A review of soil-improving cropping systems for soil salinization. *Agronomy (Basel)*, *9*(6), Article 295. https://doi.org/10.3390/agronomy9060295

Cuin, T. A., & Shabala, S. (2008). Compatible solutes mitigate damaging effects of salt stress by reducing the impact of stress-induced reactive oxygen species. *Plant Signaling & Behavior*, *3*(3), 207–208. https://doi.org/10.4161/psb.3.3.4966

Cunningham, M. A., Snyder, E., Yonkin, D., Ross, M., & Elsen, T. (2008). Accumulation of deicing salts in soils in an urban environment. *Urban Ecosystems*, *11*(1), 17–31. https://doi.org/10.1007/s11252-007-0031-x

Dawood, M. F. A., Zaid, A., & Latef, A. A. H. A. (2022). Salicylic acid spraying-induced resilience strategies against the damaging impacts of drought and/or salinity stress in two varieties of *Vicia faba* L. seedlings. *Journal of Plant Growth Regulation*, *41*(5), 1919–1942. https://doi.org/10.1007/s00344-021-10381-8

De Tillesse, V., Nef, L., Charles, J., Hopkin, A., & Augustin, S. (2007). Damaging poplar insects. FAO: Rome, Italy.

del Río, L. A., Corpas, F. J., López-Huertas, E., & Palma, J. M. (2018). Plant superoxide dismutases: function under abiotic stress conditions. In J. M. Palma, F. J. Corpas, & D. K. Gupta (Eds.), *Antioxidants and Antioxidant Enzymes in Higher Plants* (pp. 1–26). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-75088-0_1

Denno, R. F., Larsson, S., & Olmstead, K. L. (1990). Role of enemy-free space and plant quality in host-plant selection by willow beetles. *Ecology (Durham)*, *71*(1), 124–137. https://doi.org/10.2307/1940253

Deutsch, C. A., Tewksbury, J. J., Tigchelaar, M., Battisti, D. S., Merrill, S. C., Huey, R. B., & Naylor, R. L. (2018). Increase in crop losses to insect pests in a warming climate. *Science (American Association for the Advancement of Science)*, *361*(6405), 916–919. https://doi.org/10.1126/science.aat3466

Dimarco, R. D., Nice, C. C., & Fordyce, J. A. (2012). Family matters: effect of host plant variation in chemical and mechanical defenses on a sequestering specialist herbivore. *Oecologia*, *170*(3), 687–693. https://doi.org/10.1007/s00442-012-2343-7

Divekar, P. A., Narayana, S., Divekar, B. A., Kumar, R., Gadratagi, B. G., Ray, A., Singh, A. K., Rani, V., Singh, V., Singh, A. K., Kumar, A., Singh, R. P., Meena, R. S., & Behera, T. K. (2022). Plant secondary metabolites as defense tools against herbivores for sustainable crop protection. *International Journal of Molecular Sciences*, *23*(5), A2690. https://doi.org/10.3390/ijms23052690

Dixit, G., Praveen, A., Tripathi, T., Yadav, V. K., & Verma, P. C. (2017). Herbivore-responsive cotton phenolics and their impact on insect performance and biochemistry. *Journal of Asia-Pacific Entomology*, *20*(2), 341–351. https://doi.org/10.1016/j.aspen.2017.02.002

Duffey, S. S., & Stout, M. J. (1996). Antinutritive and toxic components of plant defense against insects. *Archives of Insect Biochemistry and Physiology*, *32*(1), 3–37. https://doi.org/10.1002/(SICI)1520-6327(1996)32:1<3::AID-ARCH2>3.0.CO;2-1

Ehtaiwesh, A. F. (2022). The effect of salinity on nutrient availability and uptake in crop plants. *Scientific Journal of Applied Sciences of Sabratha University*, *9*(9), 55-73.

Eichele-Nelson, J. L., Wick, A. F., DeSutter, T. M., & Harmon, J. P. (2017). The effects of salinity on the herbivorous crop pest *Tetranychus urticae* (Trombidiformes: Tetranychidae) on soybean and corn. *Environmental Entomology*, *46*(4), 839–846. https://doi.org/10.1093/ee/nvx103

English-Loeb, G., Stout, M. J., & Duffey, S. S. (1997). Drought stress in tomatoes: changes in plant chemistry and potential nonlinear consequences for insect herbivores. *Oikos*, *79*(3), 456–468. https://doi.org/10.2307/3546888

Environment Canada. (2011). *Code of practice for the environmental management of road salts*. Government of Canada. https://www.canada.ca/en/environment-climate-change/services/pollutants/road-salts/code-practice-environmental-management.html

Equiza, M. A., Calvo-Polanco, M., Cirelli, D., Señorans, J., Wartenbe, M., Saunders, C., & Zwiazek, J. J. (2017). Long-term impact of road salt (NaCl) on soil and urban trees in Edmonton, Canada. *Urban Forestry & Urban Greening*, *21*, 16–28. https://doi.org/10.1016/j.ufug.2016.11.003

Eraslan, F., Inal, A., Savasturk, O., & Gunes, A. (2007). Changes in antioxidative system and membrane damage of lettuce in response to salinity and boron toxicity. *Scientia Horticulturae*, *114*(1), 5–10. https://doi.org/10.1016.2007.05.002

Erb, M., & Reymond, P. (2019). Molecular interactions between plants and insect herbivores. *Annual Review of Plant Biology*, *70*(Volume 70, 2019), 527–557. https://doi.org/10.1146-050718-095910/1

Fahad, S., Hussain, S., Matloob, A., Khan, F. A., Khaliq, A., Saud, S., Hassan, S., Shan, D., Khan, F., Ullah, N., Faiq, M., Khan, M. R., Tareen, A. K., Khan, A., Ullah, A., Ullah, N., & Huang, J. (2015). Phytohormones and plant responses to salinity stress: a review. *Plant Growth Regulation*, *75*(2), 391–404. https://doi.org/10.1007/s10725-014-0013-y

Faiola, C., & Taipale, D. (2020). Impact of insect herbivory on plant stress volatile emissions from trees: A synthesis of quantitative measurements and recommendations for future research. *Atmospheric Environment: X*, *5*, 100060. https://doi.org/10.1016/j.aeaoa.2019.100060

Fang, Y., & Xiong, L. (2015). General mechanisms of drought response and their application in drought resistance improvement in plants. *Cellular and Molecular Life Sciences*, *72*(4), 673–689. https://doi.org/10.1007/s00018-014-1767-0

Feeny, P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. *Ecology (Durham)*, *51*(4), 565–581. https://doi.org/10.2307/1934037

Flexas, J., Bota, J., Loreto, F., Cornic, G., & Sharkey, T. D. (2004). Diffusive and metabolic limitations to photosynthesis under drought and salinity in C_3 plants. *Plant biology*, *6*(03), 269-279. https://doi.org/10.1055/S-2004-820867

Flexas, J., Diaz-Espejo, A., Galmés, J., Kaldenhoff, R., Medrano, H., & Ribas-Carbo, M. (2007). Rapid variations of mesophyll conductance in response to changes in CO_2 concentration around leaves. *Plant, Cell and Environment*, *30*(10), 1284–1298. https://doi.org/10.1111/j.1365-3040.2007.01700.x

Flowers, T. J., & Colmer, T. D. (2015). Plant salt tolerance: adaptations in halophytes. *Annals of Botany*, *115*(3), 327–331. https://doi.org/10.1093/aob/mcu267

Flowers, T. J., Galal, H. K., & Bromham, L. (2010). Evolution of halophytes: multiple origins of salt tolerance in land plants. *Functional Plant Biology*, *37*(7), 604–612. https://doi.org/10.1071/fp09269

Franco-Navarro, J. D., Brumós, J., Rosales, M. A., Cubero-Font, P., Talón, M., & Colmenero-Flores, J. M. (2016). Chloride regulates leaf cell size and water relations in tobacco plants. *Journal of Experimental Botany*, *67*(3), 873–891. https://doi.org/10.1093/jxb/erv502

Frost, C. J., Mescher, M. C., Carlson, J. E., & de Moraes, C. M. (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology*, *146*(3), 818–824. https://doi.org/10.1104/PP.107.113027

Fujita, M., Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., & Shinozaki, K. (2006). Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology*, *9*(4), 436–442. https://doi.org/10.1016/J.PBI.2006.05.014

Fürstenberg-Hägg, J., Zagrobelny, M., & Bak, S. (2013). [Rev. of plant defense against insect herbivores]. *International Journal of Molecular Sciences*, *14*(5), 10242–10297. https://doi.org/10.3390/ijms140510242

Gadallah, M. A. A. (Assiut Univ. (Egypt). F. of S. (1999). Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. *Biologia Plantarum*, *42*(2), 249–257. https://doi.org/10.1023/a:1002164719609

Gandhi, K. J. K., & Herms, D. A. (2010). Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions*, *12*(2), 389–405. https://doi.org/10.1007/s10530-009-9627-9/figures/3

Gely, C., Laurance, S. G. W., & Stork, N. E. (2020). How do herbivorous insects respond to drought stress in trees? *Biological Reviews of the Cambridge Philosophical Society*, *95*(2), 434–448. https://doi.org/10.1111/brv.12571

Ghodoum Parizipour, M. H., Rajabpour, A., Jafari, S., & Tahmasebi, A. (2021). Host-targeted salt stress affects fitness and vector performance of bird cherry-oat aphid (*Rhopalosiphum padi* L.) on wheat. *Arthropod-Plant Interactions*, *15*(1), 47–58. https://doi.org/10.1007/s11829-020-09795-0

Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, *48*(12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016

Glauser, G., Grata, E., Dubugnon, L., Rudaz, S., Farmer, E. E., & Wolfender, J.-L. (2008). Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding. *The Journal of Biological Chemistry*, *283*(24), 16400–16407. https://doi.org/10.1074/jbc.m801760200

González-Klenner, F. J., Albornoz, M. V., Ávila-Sákar, G., & Verdugo, J. A. (2022). Tomato defense against whiteflies under drought stress: non-additive effects and cultivar-specific responses. *Plants (Basel)*, *11*(8), Article 1049. https://doi.org/10.3390/plants11081049

Gu, H., Wang, H., Liu, M., Shangguan, Z., Shi, H., Xu, W., Ren, F., Zhu, J., & He, J. S. (2022). Leaf N:P stoichiometry overrides the effect of individual nutrient content on insect herbivore population dynamics in a Tibetan alpine grassland. *Agriculture, Ecosystems & Environment*, *336*, 108032. https://doi.org/10.1016/J.AGEE.2022.108032

Gupta, B., & Huang, B. (2014). Mechanism of salinity tolerance in plants : physiological, biochemical, and molecular characterization. *International Journal of Genomics*, *2014*(2014), Article 701596. https://doi.org/10.1155/2014/701596

Gururani, M. A., Venkatesh, J., & Tran, L. S. P. (2015). Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant*, *8*(9), 1304–1320. https://doi.org/10.1016/j.molp.2015.05.005

Gutbrodt, B., Dorn, S., & Mody, K. (2012). Drought stress affects constitutive but not induced herbivore resistance in apple plants. *Arthropod-Plant Interactions*, *6*(2), 171–179. https://doi.org/10.1007/s11829-011-9173-0

Hale, B. K., Herms, D. A., Hansen, R. C., Clausen, T. P., & Arnold, D. (2005). Effects of drought stress and nutrient availability on dry matter allocation, phenolic glycosides, and rapid

induced resistance of poplar to two lymantriid defoliators. *Journal of Chemical Ecology*, *31*(11), 2601–2620. https://doi.org/10.1007/s10886-005-7616-8

Halliwell, B., & Gutteridge, J. M. C. (2015). Free radicals in biology and medicine (J. M. C. Gutteridge, Ed.; Fifth edition.). *Oxford University Press*. https://doi.org/10.1093/acprof:oso/9780198717478.001.0001

Han, P., Wang, Z., Lavoir, A.-V., Michel, T., Seassau, A., Zheng, W., Niu, C., & Desneux, N. (2016). Increased water salinity applied to tomato plants accelerates the development of the leaf miner Tuta absoluta through bottom-up effects. *Scientific Reports*, *6*(1), Article 32403. https://doi.org/10.1038/srep32403

Han, P., Wang, Z., Lavoir, A.-V., Michel, T., Seassau, A., Zheng, W., Niu, C., & Desneux, N. (2016). Increased water salinity applied to tomato plants accelerates the development of the leaf miner Tuta absoluta through bottom-up effects. *Scientific Reports*, *6*(1), Article 32403. https://doi.org/10.1038/srep32403

Hanley, M. E., Lamont, B. B., Fairbanks, M. M., & Rafferty, C. M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics*, *8*(4), 157–178. https://doi.org/10.1016/j.ppees.2007.01.001

Hasanuzzaman, M., Raihan, Md. R. H., Masud, A. A. C., Rahman, K., Nowroz, F., Rahman, M., Nahar, K., & Fujita, M. (2021). Regulation of reactive oxygen species and antioxidant defense in plants under salinity. *International Journal of Molecular Sciences*, *22*(17), 9326. https://doi.org/10.3390/ijms22179326

Hastwell, G. T., & Facelli, J. M. (2003). Differing effects of shade-induced facilitation on growth and survival during the establishment of a chenopod shrub. *The Journal of Ecology*, *91*(6), 941–950. https://doi.org/10.1046/j.1365-2745.2003.00832.x

He, J., Chen, F., Chen, S., Lv, G., Deng, Y., Fang, W., Liu, Z., Guan, Z., & He, C. (2011). Chrysanthemum leaf epidermal surface morphology and antioxidant and defense enzyme activity in response to aphid infestation. *Journal of Plant Physiology*, *168*(7), 687–693. https://doi.org/10.1016/j.jplph.2010.10.009

Hemminga, M. A., & van Soelen, J. (1988). Estuarine gradients and the growth and development of *Agapanthia villosoviridescens*, (Coleoptera), a stem-borer of the salt marsh halophyte *Aster tripolium*. *Oecologia*, *77*(3), 307–312. https://doi.org/10.1007/bf00378035/metrics

Hieke, S., Menzel, C. M., & Ludders, P. (2002). Shoot development, chlorophyll, gas exchange and carbohydrates in lychee seedlings (*Litchi chinensis*). *Tree Physiology*, *22*(13), 947–953. https://doi.org/10.1093/treephys/22.13.947

Horie, T., Karahara, I., & Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice*, *5*(1), 1–18. https://doi.org/10.1186/1939-8433-5-11/figures/6

Hossain, M. S., & Dietz, K.-J. (2016). Tuning of redox regulatory mechanisms, reactive oxygen species and redox homeostasis under salinity stress. *Frontiers in Plant Science*, *7*, 548. https://doi.org/10.3389/fpls.2016.00548

Howe, G. A., & Jander, G. (2008). Plant immunity to insect herbivores. *Annual Review of Plant Biology*, *59*(1), 41–66. https://doi.org/10.1146/annurev.arplant.59.032607.092825

Imada, S., Yamanaka, N., & Tamai, S. (2009). Effects of salinity on the growth, Na partitioning, and Na dynamics of a salt-tolerant tree, *Populus alba* L. *Journal of Arid Environments*, *73*(3), 245–251. https://doi.org/10.1016/j.jaridenv.2008.10.006

Inbar, M., Doostdar, H., & Mayer, R. T. (2001). Suitability of stressed and vigorous plants to various insect herbivores. *Oikos*, *94*(2), 228–235. https://doi.org/10.1034/j.1600-0706.2001.940203.x

Jamil, M., Ashraf, M., Rehman, S., Ahmad, M., & Rha, E. S. (2012). Salinity induced changes in cell membrane stability, protein and RNA contents. *African Journal of Biotechnology*, *11*(24), 6476–6483. https://doi.org/10.5897/ajb11.2590

Joshi, S., Nath, J., Singh, A. K., Pareek, A., & Joshi, R. (2022). Ion transporters and their regulatory signal transduction mechanisms for salinity tolerance in plants. *Physiologia Plantarum*, *174*(3), e13702. https://doi.org/10.1111/ppl.13702

Kalske, A., & Kessler, A. (2023). Herbivory selects for tolerance and constitutive defence across stages of community succession. *Proceedings of the Royal Society. B, Biological Sciences*, *290*(1993), 20222458. https://doi.org/10.1098/rspb.2022.2458

Karban, R. (2020). The ecology and evolution of induced responses to herbivory and how plants perceive risk. *Ecological Entomology*, *45*(1), 1–9. https://doi.org/10.1111/een.12771

Karban, R., & Baldwin, I. T. (1997). *Induced responses to herbivory* (1st ed.). University of Chicago Press.

Karban, R., & Myers, J. H. (1989). Induced Plant Responses to Herbivory. *Source: Annual Review of Ecology and Systematics*, *20*, 331–348. https://doi.org.uml.idm.oclc.org/10.1146/annurev.es.20.110189.001555

Karimi, S. M., Freund, M., Wager, B. M., Knoblauch, M., Fromm, J., Mueller, H. M., Ache, P., Krischke, M., Mueller, M. J., Müller, T., Dittrich, M., Geilfus, C.-M., Alfarhan, A. H., Hedrich, R., & Deeken, R. (2021). Under salt stress guard cells rewire ion transport and abscisic acid signaling. *The New Phytologist*, *231*(3), 1040–1055. https://doi.org/10.1111/nph.17376

Kautz, M., Meddens, A. J. H., Hall, R. J., & Arneth, A. (2017). Biotic disturbances in Northern Hemisphere forests - a synthesis of recent data, uncertainties and implications for forest

monitoring and modelling. *Global Ecology and Biogeography*, *26*(5/6), 533–552. https://doi.org/10.1111/geb.12558

Kempel, A., SchÃ¤dler, M., Chrobock, T., Fischer, M., & van Kleunen, M. (2011). Tradeoffs associated with constitutive and induced plant resistance against herbivory. *Proceedings of the National Academy of Sciences - PNAS*, *108*(14), 5685–5689. https://doi.org/10.1073/pnas.1016508108

Keunen, E., Peshev, D., Vangronsveld, J., Van Den Ende, W., & Cuypers, A. (2013). Plant sugars are crucial players in the oxidative challenge during abiotic stress: extending the traditional concept. *Plant, Cell and Environment*, *36*(7), 1242–1255. https://doi.org/10.1111/pce.12061

Kissoudis, C., van de Wiel, C., Visser, R. G. F., & van der Linden, G. (2014). Enhancing crop resilience to combined abiotic and biotic stress through the dissection of physiological and molecular crosstalk. *Frontiers in Plant Science*, *5*(MAY), 90871. https://doi.org/10.3389/fpls.2014.00207/xml/nlm

Kitajima, K., Llorens, A., Stefanescu, C., Timchenko, M. V., Lucas, P. W., & Wright, S. J. (2012). How cellulose-based leaf toughness and lamina density contribute to long leaf lifespans of shade-tolerant species. *The New Phytologist*, *195*(3), 640–652. https://doi.org/10.1111/j.1469-8137.2012.04203.x

Kitajima, M., & Butler, W. L. (1975). Quenching of chlorophyll fluorescence and primary photochemistry in chloroplasts by dibromothymoquinone. *Biochimica et Biophysica Acta. Bioenergetics*, *376*(1), 105–115. https://doi.org/10.1016/0005-2728(75)90209-1

Koch, K. G., Chapman, K., Louis, J., Heng-Moss, T., & Sarath, G. (2016). Plant Tolerance: A Unique Approach to Control Hemipteran Pests. *Frontiers in Plant Science*, *7*, 1363. https://doi.org/10.3389/fpls.2016.01363

Kopper, B. J., Jakobi, V. N., Osier, T. L., & Lindroth, R. L. (2002). Effects of paper birch condensed tannin on whitemarked tussock moth (Lepidoptera: Lymantriidae) performance. *Environmental Entomology*, *31*(1), 10–14. https://doi.org/10.1603/0046-225X-31.1.10

Krikorian, A. D. (1988). Mineral Nutrition of Higher Plants. Horst Marschner. *The Quarterly Review of Biology*, *63*(2), 226–227. https://doi.org/10.1086/415879

Kumar, K., Debnath, P., Singh, S., & Kumar, N. (2023). An overview of plant phenolics and their involvement in abiotic stress tolerance. *Stresses 2023*, *3*(3), 570–585. https://doi.org/10.3390/stresses3030040

Kumar, M., Kesawat, M. S., Ali, A., Lee, S. C., Gill, S. S., & Kim, H. U. (2019). Integration of Abscisic Acid Signaling with Other Signaling Pathways in Plant Stress Responses and Development. *Plants*, *8*(12), 592. https://doi.org/10.3390/plants8120592

Kumar, S., Kumar, S., & Mohapatra, T. (2021). Interaction between macro- and micro-nutrients in plants. *Frontiers in Plant Science*, *12*, 665583. https://doi.org/10.3389/fpls.2021.665583

Kuromori, T., Seo, M., & Shinozaki, K. (2018). ABA transport and plant water stress responses. *Trends in Plant Science*, *23*(6), 513–522. https://doi.org/10.1016/j.tplants.2018.04.001

LaRosa, P. C., Hasegawa, P. M., Rhodes, D., Clithero, J. M., Watad, A. E. A., & Bressan, R. A. (1987). Abscisic acid stimulated osmotic adjustment and its involvement in adaptation of tobacco cells to NaCl. *Plant Physiology (Bethesda)*, *85*(1), 174–181. https://doi.org/10.1104/pp.85.1.174

Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant, Cell and Environment*, *25*(2), 275–294. https://doi.org/10.1046/j.0016-8025.2001.00814.x

Li, C., Wang, P., Wei, Z., Liang, D., Liu, C., Yin, L., Jia, D., Fu, M., & Ma, F. (2012). The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *Journal of Pineal Research*, *53*(3), 298–306. https://doi.org/10.1111/j.1600-079X.2012.00999.x

Li, Y., Liang, W., Han, J., & Huang, Z. (2016). A novel TaSST gene from wheat contributes to enhanced resistance to salt stress in Arabidopsis thaliana and *Oryza sativa*. *Acta Physiologiae Plantarum*, *38*(5), 113. https://doi.org/10.1007/s11738-016-2130-x

Li, Z.-X., Tan, J.-F., Yao, N., & Xie, R.-H. (2024). From trade-off to synergy: how nutrient status modulates plant resistance to herbivorous insects? Advanced Biotechnology, 2(4), 37. https://doi.org/10.1007/s44307-024-00045-5

Liang, W., Ma, X., Wan, P., & Liu, L. (2018). Plant salt-tolerance mechanism: A review. *Biochemical and Biophysical Research Communications*, *495*(1), 286–291. https://doi.org/10.1016/j.bbrc.2017.11.043

Lin, T., Chen, J., Zhou, S., Yu, W., Chen, G., Chen, L., Wang, X., Shi, H., Han, S., & Zhang, F. (2020). Testing the elemental defense hypothesis with a woody plant species: Cadmium accumulation protects *Populus yunnanensis* from leaf herbivory and pathogen infection. *Chemosphere (Oxford)*, *247*, 125851. https://doi.org/10.1016/j.chemosphere.2020.125851

Liu, B., Zhao, F., Zhou, H., Xia, Y., & Wang, X. (2022). Photoprotection conferring plant tolerance to freezing stress through rescuing photosystem in evergreen Rhododendron. *Plant, Cell & Environment*, *45*(7), 2093–2108. https://doi.org/10.1111/pce.14322

Liu, Z. B., Zhang, W. J., Gong, X. D., Zhang, Q., & Zhou, L. R. (2015). A Cu/Zn superoxide dismutase from *Jatropha curcas* enhances salt tolerance of Arabidopsis thaliana. *Genetics and Molecular Research*, *14*(1), 2086–2098. https://doi.org/10.4238/2015.march.20.19

Long, J. D., & Porturas, L. D. (2014). Herbivore impacts on marsh production depend upon a compensatory continuum mediated by salinity stress. *PLOS ONE*, *9*(10), e110419. https://doi.org/10.1371/journal.pone.0110419

Luczynski, A. (University of B. C., Isman, M. B., & Raworth, D. A. (1990). Strawberry foliar phenolics and their relationship to development of the two spotted spider mite. *Journal of Economic Entomology*, *83*(2), 557–563. https://doi.org/10.1093/jee/83.2.557

MacGillivray, H. G. (1950). The white-marked tussock moth *(Hemerocampa leucostigma (Abbot & Smith))* (Doctoral dissertation).

Maffei, M. E., Mithöfer, A., & Boland, W. (2007). Insects feeding on plants: rapid signals and responses preceding the induction of phytochemical release. *Phytochemistry (Oxford)*, *68*(22), 2946–2959. https://doi.org/10.1016/j.phytochem.2007.07.016

Mahajan, S., Pandey, G. K., & Tuteja, N. (2008). Calcium- and salt-stress signaling in plants: Shedding light on SOS pathway. *Archives of Biochemistry and Biophysics*, *471*(2), 146–158. https://doi.org/10.1016/j.abb.2008.01.010

Mahmoudi, H., Huang, J., Gruber, M. Y., Kaddour, R., Lachaâl, M., Ouerghi, Z., & Hannoufa, A. (2010). The impact of genotype and salinity on physiological function, secondary metabolite accumulation, and antioxidative responses in lettuce. *Journal of Agricultural and Food Chemistry*, *58*(8), 5122–5130. https://doi.org/10.1021/jf904274v

Malook, S. ul, Qi, J., Hettenhausen, C., Xu, Y., Zhang, C., Zhang, J., Lu, C., Li, J., Wang, L., & Wu, J. (2019). The oriental armyworm (*Mythimna separata*) feeding induces systemic defence responses within and between maize leaves. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences*, *374*(1767), 20180307. https://doi.org/10.1098/rstb.2018.0307

Marin, A., Ferreres, F., Barberá, G. G., & Gil, M. I. (2015). Weather variability influences color and phenolic content of pigmented baby leaf lettuces throughout the season. *Journal of Agricultural and Food Chemistry*, *63*(6), 1673–1681. https://doi.org/10.1021/acs.jafc.5b00120

Marsack, J. M., & Connolly, B. M. (2022). Generalist herbivore response to volatile chemical induction varies along a gradient in soil salinization. *Scientific Reports*, *12*(1), Article 1689. https://doi.org/10.1038/s41598-022-05764-0

Marschner, P. (2011). *Marschner's Mineral Nutrition of Higher Plants: Third Edition*. https://doi.org/10.1016/C2009-0-63043-9

Martel, J. (Carleton U. (1998). Plant-mediated effects of soil salinity on a gall-inducing caterpillar (Lepidoptera: Tortricidae) and the influence of feeding guild. *European Journal of Entomology*, *95*(4), 545–557.

Mattson, W. J. (1980). Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*, *11*(1), 119–161. https://doi.org/10.1146/annurev.es.11.110180.001003

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, *51*(345), 659–668. https://doi.org/10.1093/jexbot/51.345.659

Mithöfer, A., & Boland, W. (2012). Plant defense against herbivores: chemical aspects. *Annual Review of Plant Biology*, *63*(1), 431–450. https://doi.org/10.1146/annurev-arplant-042110-103854

Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, *11*(1), 15–19. https://doi.org/10.1016/j.tplants.2005.11.002

Moran, C. (2023). *The interactions between insect herbivory resistance and salinity tolerance in hybrid poplar*.

Mousavi, S. A. R., Chauvin, A., Pascaud, F., Kellenberger, S., & Farmer, E. E. (2013). Glutamate receptor-like genes mediate leaf-to-leaf wound signalling. *Nature (London)*, *500*(7463), 422. https://doi.org/10.1038/nature12478

Movahedi, A., Almasi Zadeh Yaghuti, A., Wei, H., Rutland, P., Sun, W., Mousavi, M., Li, D., & Zhuge, Q. (2021). Plant Secondary Metabolites with an Overview of Populus. *International Journal of Molecular Sciences*, *22*(13), 6890. https://doi.org/10.3390/ijms22136890

Munck, I. A., Bennett, C. M., Camilli, K. S., & Nowak, R. S. (2010). Long-term impact of de-icing salts on tree health in the Lake Tahoe Basin: Environmental influences and interactions with insects and diseases. *Forest Ecology and Management*, *260*(7), 1218–1229. https://doi.org/10.1016/j.foreco.2010.07.015

Munemasa, S., Hauser, F., Park, J., Waadt, R., Brandt, B., & Schroeder, J. I. (2015). Mechanisms of abscisic acid-mediated control of stomatal aperture. *Current Opinion in Plant Biology*, *28*, 154–162. https://doi.org/10.1016/j.pbi.2015.10.010

Munns, R., & Munns, R. (2002). Comparative physiology of salt and water stress. *Plant, Cell & Environment*, *25*(2), 239–250. https://doi.org/10.1046/j.0016-8025.2001.00808.x

Munns, R., & Termaat, A. (1986). Whole-plant responses to salinity. *Functional Plant Biology*, *13*(1), 143-160. https://doi.org/10.1071/PP9860143

Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, *59*(1), 651–681. https://doi.org/10.1146/annurev.arplant.59.032607.092911

Musser, R. O., Hum-Musser, S. M., Eichenseer, H., Peiffer, M., Ervin, G., Murphy, J. B., & Felton, G. W. (2002). Caterpillar saliva beats plant defences. *Nature (London)*, *416*(6881), 599–600. https://doi.org/10.1038/416599a

Nabity, P. D., Heng-Moss, T. M., & Higley, L. G. (2006). Effects of insect herbivory on physiological and biochemical (oxidative enzyme) responses of the halophyte *Atriplex subspicata* (Chenopodiaceae). *Environmental Entomology*, *35*(6), 1677–1689. https://doi.org/10.1093/ee/35.6.1677

Najjar, J., Nelson, L. D., Chen, P., & Korth, K. L. (2018). Salt stress alters insect growth in chloride-includer varieties of soybean.

Navarro, J. M., Flores, P., Garrido, C., & Martinez, V. (2006). Changes in the contents of antioxidant compounds in pepper fruits at different ripening stages, as affected by salinity. *Food Chemistry*, *96*(1), 66–73. https://doi.org/10.1016/j.foodchem.2005.01.057

Nawaz, M., Sun, J., Shabbir, S., Khattak, W. A., Ren, G., Nie, X., Bo, Y., Javed, Q., Du, D., & Sonne, C. (2023). A review of plants strategies to resist biotic and abiotic environmental stressors. *The Science of the Total Environment*, *900*, 165832. https://doi.org/10.1016/j.scitotenv.2023.165832

Negrão, S., Schmöckel, S. M., & Tester, M. (2017). Evaluating physiological responses of plants to salinity stress. *Annals of Botany*, *119*(1), 1–12. https://doi.org/10.1093/aob/mcw191

Nguyen, D., Rieu, I., Mariani, C., & van Dam, N. M. (2016). How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Molecular Biology*, *91*(6), 727–740. https://doi.org/10.1007/s11103-016-0481-8

Niknam, S. R., & McComb, J. (2000). Salt tolerance screening of selected Australian woody species — a review. *Forest Ecology and Management*, *139*(1–3), 1–19. https://doi.org/10.1016/s0378-1127(99)00334-5

Noctor, G., Mhamdi, A., Chaouch, S., Han, Y. I., Neukermans, J., Marquez-garcia, B., Queval, G., & Foyer, C. H. (2012). Glutathione in plants: an integrated overview: Special Issue on Redox Signaling. *Plant, ell and Environment*, *35*(2), 454–484.

Novotny, V., Miller, S. E., Baje, L., Balagawi, S., Basset, Y., Cizek, L., Craft, K. J., Dem, F., Drew, R. A. I., Hulcr, J., Leps, J., Lewis, O. T., Pokon, R., Stewart, A. J. A., Allan Samuelson, G., & Weiblen, G. D. (2010). Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *The Journal of Animal Ecology*, *79*(6), 1193–1203. https://doi.org/10.1111/j.1365-2656.2010.01728.x

Núñez-Farfán, J., Fornoni, J., & Valverde, P. L. (2007). Evolution of resistance and tolerance to herbivores. *Annual Review of Ecology, Evolution, and Systematics*, *38*(1), 541–566. https://doi.org/10.1146/annurev.ecolsys.38.091206.095822

Ottow, E. A., Brinker, M., Teichmann, T., Fritz, E., Kaiser, W., Brosche, M., Kangasjarvi, J., Jiang, X., & Polle, A. (2005). *Populus euphratica* displays apoplastic sodium accumulation, osmotic adjustment by decreases in calcium and soluble carbohydrates, and develops leaf

succulence under salt stress. *Plant Physiology (Bethesda)*, *139*(4), 1762–1772. https://doi.org/10.1104/pp.105.069971

Ozturk, M., Turkyilmaz Unal, B., García-Caparrós, P., Khursheed, A., Gul, A., & Hasanuzzaman, M. (2021). Osmoregulation and its actions during the drought stress in plants. *Physiologia Plantarum*, *172*(2), 1321–1335. https://doi.org/10.1111/PPL.13297

Paganová, V., Hus, M., & Lichtnerová, H. (2022). Effect of Salt treatment on the growth, water status, and gas exchange of *Pyrus pyraster* L. (Burgsd.) and *Tilia cordata* Mill. seedlings. *Horticulturae*, *8*(6), Article 519. https://doi.org/10.3390/horticulturae8060519

Painter, R. H. (1951). Insect Resistance in Crop Plants. *Soil Science*, *72*(6), 481. https://doi.org/10.1097/00010694-195112000-00015

Pandolfi, C., Azzarello, E., Mancuso, S., & Shabala, S. (2016). Acclimation improves salt stress tolerance in *Zea mays* plants. *Journal of Plant Physiology*, 201, 1–8. https://doi.org/10.1016/j.jplph.2016.06.010

Pankoke, H., & Müller, C. (2013). Impact of defoliation on the regrowth capacity and the shoot metabolite profile of Plantago lanceolata L. *Plant Physiology and Biochemistry*, *71*, 325–333. https://doi.org/10.1016/j.plaphy.2013.07.016

Parida, A. K., & Das, A. B. (2005). Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety*, *60*(3), 324–349. https://doi.org/10.1016/j.ecoenv.2004.06.010

Pastierovič, F., Čepl, J., Kalyniukova, A., Mogilicherla, K., Hradecký, J., Bláha, J., & Tomášková, I. (2024). Time is of the essence: unveiling the rapid response of *Populus* to insect feeding. *Frontiers in Forests and Global Change*, *7*, 1376465. https://doi.org/10.3389/ffgc.2024.1376465

Paul, M. J., & Foyer, C. H. (2001). Perspectives in experimental botany. Sink regulation of photosynthesis. *Journal of Experimental Botany*, *52*(360), 1383. https://doi-org.uml.idm.oclc.org/10.1093/jexbot/52.360.1383

Pawar, S. v., Paranjape, S. M., Kalowsky, G. K., Peiffer, M., McCartney, N., Ali, J. G., & Felton, G. W. (2025). Tomato defenses under stress: the impact of salinity on direct defenses against insect herbivores. *Plant, Cell & Environment*, *48*(5), 3647–3659. https://doi.org/10.1111/pce.15353

Petridis, A., Therios, I., Samouris, G., & Tananaki, C. (2012). Salinity-induced changes in phenolic compounds in leaves and roots of four olive cultivars (*Olea europaea* L.) and their relationship to antioxidant activity. *Environmental and Experimental Botany*, *79*, 37–43. https://doi.org/10.1016/j.envexpbot.2012.01.007

Philippe, R. N., & Bohlmann, J. (2007). Poplar defense against insect herbivores. *Canadian Journal of Botany*, *85*(12), 1111–1126. https://doi.org/10.1139/B07-109

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, *28*(1), 489–521. https://doi.org/10.1146/annurev-cellbio-092910-154055

Plasencia, F. A., Estrada, Y., Flores, F. B., Ortíz-Atienza, A., Lozano, R., & Egea, I. (2021). The Ca^{2+} sensor calcineurin B–Like protein 10 in plants: emerging new crucial roles for plant abiotic stress tolerance. *Frontiers in Plant Science*, *11*, Article 599944. https://doi.org/10.3389/fpls.2020.599944

Polack, L. A., Pereyra, P. C., & Sarandón, S. J. (2011). Effects of plant stress and habitat manipulation on aphid control in greenhouse sweet peppers. *Journal of Sustainable Agriculture*, *35*(7), 699–725. https://doi.org/10.1080/10440046.2011.606489

Polle, A., & Chen, S. (2015). On the salty side of life: molecular, physiological and anatomical adaptation and acclimation of trees to extreme habitats. *Plant, Cell and Environment*, *38*(9), 1794–1816. https://doi.org/10.1111/pce.12440

Pottosin, I., & Shabala, S. (2014). Polyamines control of cation transport across plant membranes: implications for ion homeostasis and abiotic stress signaling. *Frontiers in plant science*, *5*, 154. https://doi.org/10.3389/fpls.2014.00154

Pujni, D., Chaudhary, A., & Rajam, M. V. (2007). Increased tolerance to salinity and drought in transgenic indica rice by mannitol accumulation. *Journal of Plant Biochemistry and Biotechnology*, *16*(1), 1–7. https://doi.org/10.1007/BF03321921

Qadir, M., Oster, J. D., Schubert, S., Noble, A. D., & Sahrawat, K. L. (2007). Phytoremediation of sodic and saline-sodic soils. *Advances in Agronomy*, *96*, 197–247. https://doi.org/10.1016/s0065-2113(07)96006-x

Qiu, Q.-S., Guo, Y., Dietrich, M. A., Schumaker, K. S., & Zhu, J.-K. (2002). Regulation of SOS1, A plasma membrane Na^+/H^+ Exchanger in *Arabidopsis thaliana*, by SOS2 and SOS3. *Proceedings of the National Academy of Sciences - PNAS*, *99*(12), 8436–8441. https://doi.org/10.1073/pnas.122224699

Quais, M. K., Ansari, N. A., Wang, G.-Y., Zhou, W.-W., & Zhu, Z.-R. (2019). Host plant salinity stress affects the development and population parameters of *Nilaparvata lugens* (Hemiptera: Delphacidae). *Environmental Entomology*, *48*(5), 1149–1161. https://doi.org/10.1093/ee/nvz084

Quijano-Medina, T., Turlings, T. C. J., Sosenski, P., Grandi, L., Cervera, J. C., Moreira, X., Abdala-Roberts, L., & Bardgett, R. (2021). Effects of soil salinity on the expression of direct and indirect defences in wild cotton *Gossypium hirsutum*. *The Journal of Ecology*, *109*(1), 354–368. https://doi.org/10.1111/1365-2745.13483

Quintero, F. J., Martinez-Atienza, J., Villalta, I., Jiang, X., Kim, W.-Y., Ali, Z., Fujii, H., Mendoza, I., Yun, D.-J., Zhu, J.-K., & Pardo, J. M. (2011). Activation of the plasma membrane Na/H antiporter Salt-Overly-Sensitive 1 (SOS1) by phosphorylation of an auto-inhibitory C-terminal domain. *Proceedings of the National Academy of Sciences - PNAS*, *108*(6), 2611–2616. https://doi.org/10.1073/pnas.1018921108

Quintero, F. J., Ohta, M., Shi, H., Zhu, J.-K., & Pardo, J. M. (2002). Reconstitution in yeast of the Arabidopsis SOS signaling pathway for Na^+ homeostasis. *Proceedings of the National Academy of Sciences - PNAS*, *99*(13), 9061–9066. https://doi.org/10.1073/pnas.132092099

Rahman, S., Rostás, M., & Vosteen, I. (2025). Drought aggravates plant stress by favouring aphids and weakening indirect defense in a sugar beet tritrophic system. *Journal of Pest Science*, *98*(1), 549–564. https://doi.org/10.1007/s10340-024-01799-6

Ramachandran, S., Renault, S., Markham, J., Verdugo, J., Albornoz, M., & Avila-Sakar, G. (2020). Lower nitrogen availability enhances resistance to whiteflies in tomato. *Plants (Basel)*, *9*(9), 1096. https://doi.org/10.3390/plants9091096

Ramagopal, S. (1987). Salinity stress induced tissue-specific proteins in barley seedlings. *Plant Physiology (Bethesda)*, *84*(2), 324–331. https://doi.org/10.1104/pp.84.2.324

Rapo, C. B., Schaffner, U., Eigenbrode, S. D., Hinz, H. L., Price, W. J., Morra, M., Gaskin, J., & Schwarzländer, M. (2019). Feeding intensity of insect herbivores is associated more closely with key metabolite profiles than phylogenetic relatedness of their potential hosts. *PeerJ (San Francisco, CA)*, *7*, Article e8203. https://doi.org/10.7717/peerj.8203

Rejeb, I., Pastor, V., & Mauch-Mani, B. (2014). [Rev. of *Plant Responses to Simultaneous Biotic and Abiotic Stress: Molecular Mechanisms*]. *Plants (Basel)*, *3*(4), 458–475. https://doi.org/10.3390/plants3040458

Renault, S. (2005). Response of red-osier dogwood (*Cornus stolonifera*) seedlings to sodium sulphate salinity: effects of supplemental calcium. *Physiologia Plantarum*, *123*(1), 75–81. https://doi.org/10.1111/j.1399-3054.2005.00444.x

Renault, S., Wolfe, S., Markham, J., & Avila-Sakar, G. (2016). Increased resistance to a generalist herbivore in a salinity-stressed non-halophytic plant. *AoB Plants*, *8*. https://doi.org/10.1093/aobpla/plw028

Rengasamy, P. (2010). Soil processes affecting crop production in salt-affected soils. *Functional Plant Biology*, *37*(7), 613–620. https://doi.org/10.1071/FP09249

Richardson, J., Isebrands, J. G., & Ball, J. B. (2014). Ecology and physiology of poplars and willows. *Poplars and willows: Trees for society and the environment*, 92-123.

Riyazuddin, R., Verma, R., Singh, K., Nisha, N., Keisham, M., Bhati, K. K., Kim, S. T., & Gupta, R. (2020). Ethylene: A master regulator of salinity stress tolerance in plants. *Biomolecules (Basel, Switzerland)*, *10*(6), 959. https://doi.org/10.3390/biom10060959

Rode, M., Lemoine, N. P., & Smith, M. D. (2017). Prospective evidence for independent nitrogen and phosphorus limitation of grasshopper (*Chorthippus curtipennis*) growth in a tallgrass prairie. *PloS One*, *12*(5), e0177754. https://doi.org/10.1371/journal.pone.0177754

Roe, A. D., Demidovich, M., & Dedes, J. (2018). Origins and history of laboratory insect stocks in a multispecies insect production facility, with the proposal of standardized nomenclature and designation of formal standard names. *Journal of Insect Science (Tucson, Ariz.)*, *18*(3). https://doi.org/10.1093/jisesa/iey037

Rusydi, A. F. (2018). Correlation between conductivity and total dissolved solid in various type of water: A review. *IOP Conference Series. Earth and Environmental Science*, *118*(1), 12019. https://doi.org/10.1088/1755-1315/118/1/012019

Sachdev, S., Ansari, S. A., Ansari, M. I., Fujita, M., & Hasanuzzaman, M. (2021). Abiotic stress and reactive oxygen species: generation, signaling, and defense mechanisms. *Antioxidants*, *10*(2), 277. https://doi.org/10.3390/antiox10020277

Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Shoukat, A., ... & Sarwar, M. I. (2019). A review: Impact of salinity on plant growth. *Natural Science*, *17*(1), 34-40. https://10.7537/marsnsj170119.06

Schile, L., & Mopper, S. (2006). deleterious effects of salinity stress on leafminers and their freshwater host. *Ecological Entomology*, *31*(4), 345–351. https://doi.org/10.1111/j.1365-2311.2006.00799.x

Schoonhoven, L. M., Loon, J. J. A. van, & Dicke, M. (2005). *Insect-plant biology / Louis M. Schoonhoven, Joop J.A. van Loon, Marcel Dicke* (2nd ed).

Schowalter, T. D. (2018). Biology and Management of the Whitemarked Tussock Moth (Lepidoptera: Erebidae). *Journal of Integrated Pest Management*, *9*(1). https://doi.org/10.1093/jipm/pmy016

Schultz, J. C., Appel, H. M., Ferrieri, A. P., & Arnold, T. M. (2013). Flexible resource allocation during plant defense responses. *Frontiers in Plant Science*, *4*, Article 324. https://doi.org/10.3389/fpls.2013.00324

Sewelam, N., Oshima, Y., Mitsuda, N., & Ohme-Takagi, M. (2014). A step towards understanding plant responses to multiple environmental stresses: a genome-wide study. *Plant, Cell and Environment*, *37*(9), 2024–2035. https://doi.org/10.1111/pce.12274

Shabala, S., & Mackay, A. (2011). Ion transport in halophytes. in Turkan (Ed.), *Advances in Botanical Research* (Vol. 57, pp. 151–199). Elsevier Science & Technology. https://doi.org/10.1016/B978-0-12-387692-8.00005-9

Shabala, S., Bose, J., & Hedrich, R. (2014). Salt bladders: do they matter? *Trends in Plant Science Xx*, 1–5. https://doi.org/10.1016/j.tplants.2014.09.001

Shahzad, M., Witzel, K., Zörb, C., & Mühling, K. H. (2012). Growth-related changes in subcellular ion patterns in maize leaves (*Zea mays* L.) under salt stress. *Journal of Agronomy and Crop Science (1986)*, *198*(1), 46–56. https://doi.org/10.1111/j.1439-037x.2011.00487.x

Shao, J., Markham, J., & Renault, S. (2020). Nitrogen fixation symbiosis and salt tolerance of the boreal woody species *Elaeagnus commutata*. *Acta Physiologiae Plantarum*, *42*(6), Article 100. https://doi.org/10.1007/s11738-020-03088-y

Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., & Zheng, B. (2019). Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules (Basel, Switzerland)*, *24*(13), 2452. https://doi.org/10.3390/molecules24132452

Shrivastava, P., & Kumar, R. (2015). Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi Journal of Biological Sciences*, *22*(2), 123–131. https://doi.org/10.1016/j.sjbs.2014.12.001

Silveira, J. A. G. da, Lima Junior, A. R. de, Queiroz, J. E., & Fausto, M. J. M. (2001). Effects of NaCl-salinity on growth and inorganic solute accumulation in young cashew plants. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *5*(2), 216–222. https://doi.org/10.1590/s1415-43662001000200007

Singh, M., Kumar, J., Singh, S., Singh, V. P., & Prasad, S. M. (2015). Roles of osmoprotectants in improving salinity and drought tolerance in plants: a review. *Reviews in Environmental Science and Biotechnology*, *14*(3), 407–426. https://doi.org/10.1007/s11157-015-9372-8

Singh, P., Choudhary, K. K., Chaudhary, N., Gupta, S., Sahu, M., Tejaswini, B., & Sarkar, S. (2022). Salt stress resilience in plants mediated through osmolyte accumulation and its crosstalk mechanism with phytohormones. *Frontiers in Plant Science*, *13*, 1006617. https://doi.org/10.3389/fpls.2022.1006617

Singh, S., & Kariyat, R. R. (2020). Exposure to polyphenol-rich purple corn pericarp extract restricts fall armyworm (*Spodoptera frugiperda*) growth. *Plant Signaling & Behavior*, *15*(9), 1784545. https://doi.org/10.1080/15592324.2020.1784545

Singh, S., Kaur, I., & Kariyat, R. (2021). The multifunctional roles of polyphenols in plant-herbivore interactions. *International Journal of Molecular Sciences 2021, 22*(3), 1442. https://doi.org/10.3390/ijms22031442

Sinha, R., Irulappan, V., Mohan-Raju, B., Suganthi, A., & Senthil-Kumar, M. (2019). Impact of drought stress on simultaneously occurring pathogen infection in field-grown chickpea. *Scientific Reports*, *9*(1), 5577. https://doi.org/10.1038/s41598-019-41463-z

Smirnoff, N., & Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Phytochemistry*, *28*(4), 1057–1060. https://doi.org/10.1016/0031-9422(89)80182-7

Sofy, M. R., Elhawat, N., & Tarek Alshaal. (2020). Glycine betaine counters salinity stress by maintaining high K^+/Na^+ ratio and antioxidant defense via limiting Na^+ uptake in common bean (*Phaseolus vulgaris* L.). *Ecotoxicology and Environmental Safety*, *200*, Article 110732. https://doi.org/10.1016/j.ecoenv.2020.110732

Stanton, B. J., Neale, D. B., & Li, S. (n.d.). *Populus Breeding: From the Classical to the Genomic Approach*. https://doi.org/10.1007/978-1-4419-1541-2_14

Stevens, M. T., & Lindroth, R. L. (2005). Induced resistance in the indeterminate growth of aspen (*Populus tremuloides*). *Oecologia*, *145*(2), 298–306. https://doi.org/10.1007/s00442-005-0128-y

Strauss, S. Y., & Agrawal, A. A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends in ecology & evolution*, *14*(5), 179–185. https://doi.org/10.1016/s0169-5347(98)01576-6

Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *The New Phytologist*, *203*(1), 32–43. https://doi.org/10.1111/nph.12797

Thaler, J. S., & Bostock, R. M. (2004). Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. *Ecology (Durham)*, *85*(1), 48–58. https://doi.org/10.1890/02-0710

Thomson, W. W. (1975). The structure and function of salt glands. *Plants in saline environments*, 118-146.

Tian, D., Peiffer, M., De Moraes, C. M., & Felton, G. W. (2014). Roles of ethylene and jasmonic acid in systemic induced defense in tomato (*Solanum lycopersicum*) against Helicoverpa zea. *Planta*, *239*(3), 577–589. https://doi.org/10.1007/s00425-013-1997-7

Tsai, C.-J., Kayal, W. el, & Harding, S. A. (2006). *Populus*, the new model system for investigating phenyl-propanoid complexity. *International Journal of Applied Science and Engineering*, *4*, 3.

Turlings, T. C. J., & Erb, M. (2018). Tritrophic interactions mediated by herbivore-induced plant volatiles: mechanisms, ecological relevance, and application potential. *Annual Review of Entomology*, *63*(1), 433–452. https://doi.org/10.1146/annurev-ento-020117-043507

Tuteja, N., & Mahajan, S. (2007). Calcium signaling network in plants: an overview. *Plant Signaling & Behavior*, *2*(2), 79. https://doi.org/10.4161/PSB.2.2.4176

Underwood, N., Rausher, M., & Cook, W. (2002). Bioassay versus chemical assay: measuring the impact of induced and constitutive resistance on herbivores in the field. *Oecologia*, *131*(2), 211–219. https://doi.org/10.1007/s00442-002-0867-y

Urbaneja-Bernat, P., Rodriguez-Saona, C., Valero, M. L., González-Cabrera, J., & Tena, A. (2024). Not just candy: A herbivore-induced defence-related plant protein in honeydew enhances natural enemy fitness. *Functional Ecology*, *38*(8), 1822–1834. https://doi.org/10.1111/1365-2435.14605

Valifard, M., Mohsenzadeh, S., Kholdebarin, B., & Rowshan, V. (2014). Effects of salt stress on volatile compounds, total phenolic content and antioxidant activities of *Salvia mirzayanii*. *South African Journal of Botany*, *93*, 92–97. https://doi.org/10.1016/j.sajb.2014.04.002

Visakorpi, K., Gripenberg, S., Malhi, Y., Bolas, C., Oliveras, I., Harris, N., Rifai, S., & Riutta, T. (2018). Small-scale indirect plant responses to insect herbivory could have major impacts on canopy photosynthesis and isoprene emission. *The New Phytologist*, *220*(3), 799–810. https://doi.org/10.1111/nph.15338

Volf, M., Hrcek, J., Julkunen-Tiitto, R., & Novotny, V. (2015). To each its own: differential response of specialist and generalist herbivores to plant defence in willows. *The Journal of Animal Ecology*, *84*(4), 1123–1132. https://doi.org/10.1111/1365-2656.12349

Walters, D., & Heil, M. (2007). Costs and trade-offs associated with induced resistance. *Physiological and Molecular Plant Pathology*, *71*(1–3), 3–17. https://doi.org/10.1016/J.PMPP.2007.09.008

Wang, F., Liu, J., Chen, M., Zhou, L., Li, Z., Zhao, Q., Pan, G., Zaidi, S.-H.-R., & Cheng, F. (2016). Involvement of abscisic acid in PSII photodamage and D1 protein turnover for light-induced premature senescence of rice flag leaves. *PloS One*,*11*(8), e0161203. https://doi.org/10.1371/journal.pone.0161203

Wang, R., Chen, S., Deng, L., Fritz, E., Hüttermann, A., & Polle, A. (2007). Leaf photosynthesis, fluorescence response to salinity and the relevance to chloroplast salt compartmentation and anti-oxidative stress in two poplars. *Trees (Berlin, West)*, *21*(5), 581–591. https://doi.org/10.1007/s00468-007-0154-y

Wang, R., Chen, S., Zhou, X., Shen, X., Deng, L., Zhu, H., Shao, J., Shi, Y., Dai, S., & Fritz, E. (2008). Ionic homeostasis and reactive oxygen species control in leaves and xylem sap of two poplars subjected to NaCl stress. *Tree Physiology*, *28*(6), 947–957. https://doi.org/10.1093/treephys/28.6.947

Wang, X., Chen, Z., & Sui, N. (2024). Sensitivity and responses of chloroplasts to salt stress in plants. *Frontiers in Plant Science*, *15*, Article 1374086. https://doi.org/10.3389/fpls.2024.1374086

Wang, Y., & Nii, N. (2000). Changes in chlorophyll, ribulose bisphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *The Journal of Horticultural Science & Biotechnology*, *75*(6), 623–627. https://doi.org/10.1080/14620316.2000.11511297

Wang, Y., Ying, Y., Chen, J., & Wang, X. (2004). Transgenic *Arabidopsis* overexpressing Mn-SOD enhanced salt-tolerance. *Plant Science (Limerick)*, *167*(4), 671–677. https://doi.org/10.1016/j.plantsci.2004.03.032

Waśkiewicz, A., Muzolf-Panek, M., & Goliński, P. (2012). Phenolic content changes in plants under salt stress. In P. Ahmad, M. M. Azooz, & M. N. V. Prasad (Eds.), *Ecophysiology and Responses of Plants under Salt Stress* (pp. 283–314). Springer New York. https://doi.org/10.1007/978-1-4614-4747-4_11

Wasternack, C., & Song, S. (2017). Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany*, *68*(6), 1303–1321. https://doi.org/10.1093/jxb/erw443

Watanabe, S., Kojima, K., Ide, Y., & Sasaki, S. (2000). Effects of saline and osmotic stress on proline and sugar accumulation in *Populus euphratica* in vitro. *Plant Cell, Tissue and Organ Culture*, *63*(3), 199–206. https://doi.org/10.1023/a:1010619503680

Wittyngham, S. S. (2021). Salinity and simulated herbivory influence spartina alterniflora traits and defense strategy. *Estuaries and Coasts*, *44*(4), 1183–1192. https://doi.org/10.1007/s12237-020-00841-x

Wolf, J., Straten, S., Pitann, B., & Mühling, K. H. (2019). Foliar Magnesium supply increases the abundance of RuBisCO of Mg-deficient maize plants. *Journal of Applied Botany and Food Quality*, *92*, 274–280. https://doi.org/10.5073/JABFQ.2019.092.038

Yang, Y., & Guo, Y. (2018). Elucidating the molecular mechanisms mediating plant salt-stress responses. *The New Phytologist*, *217*(2), 523–539. https://doi.org/10.1111/nph.14920

Young, B., Wagner, D., Doak, P., & Clausen, T. (2010). Induction of phenolic glycosides by quaking aspen (*Populus tremuloides*) leaves in relation to extrafloral nectaries and epidermal leaf mining. *Journal of chemical ecology*, *36*(4), 369–377. https://doi.org/10.1007/s10886-010-9763-9

Zeng, M. (2024). The mutual effect of nutrients on plant–herbivore interactions. *Plant Ecology*, *225*(10), 1035–1045. https://doi.org/10.1007/s11258-024-01452-3

Zhang, P., Liu, L., Wang, X., Wang, Z., Zhang, H., Chen, J., Liu, X., Wang, Y., & Li, C. (2021). Beneficial effects of exogenous melatonin on overcoming salt stress in sugar beets (*Beta vulgaris* L.). *Plants (Basel)*, *10*(5), 886. https://doi.org/10.3390/plants10050886

Zhang, Q., Wang, Q., Wyckhuys, K. A. G., Jin, S., & Lu, Y. (2024). Salinity stress alters plant-mediated interactions between above- and below-ground herbivores. *The Science of the Total Environment*, *940*, 173687. https://doi.org/10.1016/j.scitotenv.2024.173687

Zhong, J., Zhang, J., Zhang, Y., Ge, Y., He, W., Liang, C., Gao, Y., Zhu, Z., Machado, R. A. R., & Zhou, W. (2024). Heat stress reprograms herbivory-induced defense responses in potato plants. *BMC Plant Biology*, *24*(1), 677. https://doi.org/10.1186/s12870-024-05404-x

Zhou, S., Lou, Y. R., Tzin, V., & Jander, G. (2015). Alteration of plant primary metabolism in response to insect herbivory. *Plant physiology*, *169*(3), 1488–1498. https://doi.org/10.1104/pp.15.01405

Zhu, J.-K. (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiology (Bethesda)*, *124*(3), 941–948. https://doi.org/10.1104/pp.124.3.941

Zhu, J.-K. (2003). Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology*, *6*(5), 441–445. https://doi.org/10.1016/s1369-5266(03)00085-2

Zhu, M., Liu, Y., Cai, P., Duan, X., Sang, S., & Qiu, Z. (2022). Jasmonic acid pretreatment improves salt tolerance of wheat by regulating hormones biosynthesis and antioxidant capacity. *Frontiers in Plant Science*, *13*, 968477. https://doi.org/10.3389/fpls.2022.968477

Zörb, C., Geilfus, C. -M, Dietz, K. -J, & Weber, A. (2019). Salinity and crop yield. *Plant Biology (Stuttgart, Germany)*, *21*(S1), 31–38. https://doi.org/10.1111/plb.12884

Zörb, C., Schmitt, S., & Mühling, K. H. (2010). Proteomic changes in maize roots after short-term adjustment to saline growth conditions. *Proteomics (Weinheim)*, *10*(24), 4441–4449. https://doi.org/10.1002/pmic.201000231

CHAPTER 7 – APPENDIX

APPENDIX 1

Table A1. Modified Hoagland's nutrient solution (50%) used for fertilizing hybrid poplar cuttings in both experiment one and two, Concentrations are in mM for macronutrients and µM for micronutrients.

	Nutrient concentration
Macronutrients (mM)	
KH_2PO_4	0.5
KNO_3	2.5
$Ca(NO_3)_2 \cdot 4H_2O$	2.5
$MgSO_4$	1
$NH_4^+ \cdot NO_3^-$	
Micronutrients (µM)	
H_3BO_3	0.5
$MnCl_2 \cdot 4H_2O$	0.5
$ZnSO_4 \cdot 7H_2O$	0.5
$CuSO_4 \cdot 5H_2O$	0.5
MoO_3	0.5
Fe-EDTA	0.5

APPENDIX 2: ANOVA TABLES IN EXPERIMENT 1

Table A2.1. Results of one-way ANOVA for gas-exchange parameters in hybrid poplar exposed to salinity (0 vs 100 mM NaCl) after one, three, and five weeks of treatment.

Treatment duration (weeks)	Parameter	Df	F	Pr(>F)
1 week	Photosynthetic rate	1, 30	2.23	0.145
	Stomatal conductance	1, 30	2.56	0.120
	Transpiration	1, 30	3.47	0.072
	F_v/F_m (chlorophyll fluorescence)	1, 30	5.51	0.026
3 weeks	Photosynthetic rate	1, 22	1.40	0.250
	Stomatal conductance	1, 22	14.68	0.0009
	Transpiration	1, 22	15.84	0.0006
	F_v/F_m (chlorophyll fluorescence)	1, 22	0.84	0.370
5 weeks	Photosynthetic rate	1, 26	4.26	0.049
	Stomatal conductance	1, 26	34.38	3.5×10^{-6}
	Transpiration	1, 26	41.26	8.3×10^{-7}
	F_v/F_m (chlorophyll fluorescence)	1, 26	3.40	0.077

Table A2.2. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) and their interaction on total leaf phenolic content (mg g^{-1} DW) in hybrid poplar 48 h after exposure to herbivory following four weeks of salinity treatments (n = 15)

	Df	F	Pr(>F)
Salinity	1	3.374045	0.07154
Herbivory	1	0.780408	0.380793
Salinity: herbivory	1	0.358734	0.551625
Residuals	56	NA	NA

Table A2.3. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) and their interaction on total leaf phenolic content (mg g^{-1} DW) in hybrid poplar after six weeks of salinity treatments (n = 5).

	Df	F	Pr(>F)
Salinity	1	0.448	0.072
Herbivory	1	0.194	0.380
Salinity: herbivory	1	0.078	0.784
Residuals	16	NA	NA

Table A2.4. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) and their interaction on concentrations of elements (mg kg^{-1} DW) in hybrid poplar leaves exposed NaCl, 0 or 100 mM for four weeks and 48 h after insect herbivory treatments (n=5).

	S			I			S*I		
Elements	Df	F	Pr(>F)	Df	F	Pr(>F)	Df	F	Pr(>F)
Na	1, 16	14.314	0.00163	1, 16	0.651	0.43150	1, 16	0.556	0.467
Cl	1, 16	97.268	3.33e-08	1, 16	0.056	0.816	1, 16	0.069	0.796
N	1, 16	2.014	0.175	1, 16	0.006	0.938	1, 16	0.467	0.504
P	1, 16	15.262	0.00126	1, 16	0.280	0.60368	1, 16	0.552	0.468
K	1, 16	19.984	0.000387	1, 16	1.752	0.204241	1, 16	1.377	0.257
Ca	1, 16	0.018	0.896	1, 16	0.014	0.0907	1, 16	0.608	0.447
Mg	1, 16	40397	0.0523	1, 16	1.342	0.2637	1, 16	3.314	0.088
S	1, 16	45.590	4.65e-06	1, 16	0.642	0.435	1, 16	1.249	0.280
Fe	1, 16	15751	0.00110	1, 16	13.890	0.00183	1, 16	8.665	0.009
Mn	1, 16	119.432	7.88e-09	1, 16	0.226	0.641	1, 16	0.002	0.965
Cu	1, 16	2.009	0.1756	1, 16	10.637	0.0049	1, 16	0.482	0.498
Zn	1, 16	9.535	0.00706	1, 16	0.001	0.97153	1, 16	0.228	0.639
Mo	1, 16	63.361	5.92e-07	1, 16	0.760	0.3964	1, 16	3.244	0.090
B	1, 16	113.793	5.23e-06	1, 16	1.605	0.241	1, 16	0.203	0.665

Table A2. 5. Results of two-way ANOVA testing the effects of salinity (S; 0 or 100 mM NaCl) and herbivory (I; NH = no herbivory; H = herbivory) and their interaction (S*I) on concentrations of elements (mg kg^{-1} DW) in hybrid poplar leaves exposed NaCl 0 or 100 mM for six weeks and two weeks after first insect herbivory treatments(n=3).

	S			I			S*I		
Elements	Df	F	Pr(>F)	Df	F	Pr(>F)	Df	F	Pr(>F)
Na	1,8	47.390	0.000127	1,8	0.494	0.502	1,8	0.306	0.595
Cl	1,8	1033.898	9.53e-10	1,8	4.678	0.063	1,8	4.678	0.061
N	1,8	0.653	0.443	1,8	0.060	0.813	1,8	0.013	0.911
P	1,8	1.693	0.229	1,8	0.182	0.681	1,8	0.831	0.389
K	1,8	0.166	0.695	1,8	0.013	0.910	1,8	1.333	0.282
Ca	1,8	6.262	0.0368	1,8	0.568	0.473	1,8	0.295	0.602
Mg	1,8	1.256	0.295	1,8	0.848	0.384	1,8	0.463	0.516
S	1,8	0.389	0.550	1,8	0.014	0.908	1,8	1.661	0.233
Fe	1,8	3.140	0.114	1,8	1.906	0.205	1,8	0.894	0.372
Mn	1,8	118.287	4.52e-06	1,8	1.748	0.233	1,8	1.461	0.261
Cu	1,8	3.033	0.120	1,8	0.181	0.682	1,8	1.046	0.336
Zn	1,8	7.336	0.0267	1,8	0.011	0.9173	1,8	0.019	0.893
Mo	1,8	0.470	0.512	1,8	0.345	0.573	1,8	1.067	0.332
B	1,8	0.907	0.369	1,8	1.134	0.318	1,8	0.082	0.782

Table A2. 6. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) and their interaction on leaf ionic ratios (Na/, Na/Ca, and Na/Mg) in hybrid poplar after four weeks of salinity exposure and 48 h following insect herbivory and six weeks of salt treatments (n=3)

		Four weeks salinity treatments			Six weeks salinity treatments		
Ratio		Df	F	Pr(>F)	Df	F	Pr(>F)
Na/K	Salinity	1, 16	83.79	9.3×10^{-8}	1,8	41.26	0.0002
	Herbivory	1, 16	0.12	0.74	1,8	0.33	0.579
	Salinity × herbivory	1, 16	0.40	0.54	1,8	0.22	0.654
Na/Ca	Salinity	1, 16	66.77	4.2×10^{-7}	1,8	26.50	0.0009
	Herbivory	1, 16	0.44	0.52	1,8	0.36	0.564
	Salinity × herbivory	1, 16	0.66	0.43	1,8	0.12	0.737
Na/Mg	Salinity	1, 16	70.03	3.1×10^{-7}	1,8	31.88	0.0005
	Herbivory	1, 16	0.70	0.42	1,8	0.50	0.499
	Salinity × herbivory	1, 16	0.47	0.50	1,8	0.17	0.692

Table A2. 7. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) and their interaction on induced resistance to *O. leucostigma* in hybrid poplar (leaf-disc assay), after six weeks of salt treatment (0 or 100 mM NaCl). (n=5)

	Df	F value	Pr(>F)
Salinity	1	4.484	0.0484
Herbivory	1	0.752	0.3972
Salinity: herbivory	1	2.028	0.1716
Residuals	18	NA	NA

Table A2. 8. One-way ANOVA results for the effect of salinity (0 or 100 mM NaCl) on log-transformed relative growth rate (log(RGR)) in two lateral shoots (Lat1 and Lat2) of hybrid poplar.

	Df	F	Pr(>F)
log(RGR_Lat1)	1	87.35	4.22×10^{-13}
log(RGR_Lat2)	1	29.06	1.39×10^{-6}
Residuals	57	NA	NA

APPENDIX 3: ANOVA TABLES IN EXPERIMENT 2

Table A3.1. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory and their interaction on total phenolic content (mg g^{-1} DW) in hybrid poplar leaves after four weeks of salinity treatment (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory). n=10

	Df	F value	Pr(>F)
Salinity	1	0.651	0.4256
Herbivory	1	5.466	0.0256
Salinity: herbivory	1	0.249	0.6211
Residuals	33	NA	NA

Table A3.2. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory and their interaction on protein content (mg g^{-1} FW) in hybrid poplar leaves after four weeks of salinity treatment (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory). n=10

	Df	F value	Pr(>F)
Salinity	1	7.118	0.0115
Herbivory	1	0.026	0.8734
Salinity: herbivory	1	0.008	0.9279
Residuals	35	NA	NA

Table A3.3. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory (NH = no herbivory; H = herbivory) and their interaction on concentrations of elements (mg kg^{-1} DW) in hybrid poplar leaves after four weeks of salt treatments

	S			I			S*I		
Elements	Df	F	Pr(>F)	Df	F	Pr(>F)	Df	F	Pr(>F)
Na	1,8	170.085	0.0002	1,8	3.779	0.1238	1,8	3.764	0.1244
Cl	1,8	59.0494	5.67e-05	1,8	0.077	0.788	1,8	0.087	0.775
N	1,8	5.022	0.0263	1,8	0.467	0.0553	1,8	0.467	0.5135
P	1,8	6.691	0.0323	1,8	0.010	0.9242	1,8	2.100	0.1854
K	1,8	1.109	0.323	1,8	0.007	0.935	1,8	0.214	0.656
Ca	1,8	1.639	0.236	1,8	2.199	0.176	1,8	0.044	0.838
Mg	1,8	2.197	0.1766	1,8	5.782	0.0429	1,8	0.761	0.4083
S	1,8	9.473	0.0152	1,8	5.072	0.0544	1,8	1.447	0.2634
Fe	1,8	27.645	0.000766	1,8	5.937	0.040779	1,8	0.103	0.756955
Mn	1,8	4.522	0.0662	1,8	1.990	0.1960	1,8	0.965	0.3547
Cu	1,8	0.557	0.477	1,8	0.535	0.485	1,8	1.535	0.250
Zn	1,8	0.801	0.397	1,8	0.259	0.624	1,8	0.435	0.528
Mo	1,8	25.613	0.000976	1,8	1.550	0.248369	1,8	1.488	0.25733
B	1,8	113.793	5.23e-06	1,8	1.605	0.241	1,8	0.203	0.665

Table A3.4. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory and their interaction total phenolic content (mg g^{-1} DW) in hybrid poplar leaves after four weeks of salinity treatment (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory). n=10

Ratio		Df	F value	Pr(>F)
Na/K	Salinity	1,8	87.453	1.4e-05
	Herbivory	1,8	0.074	0.792
	Salinity × herbivory	1,8	0.000	0.996
Na/Ca	Salinity	1,8	65.426	4.03e-05
	Herbivory	1,8	0.000	1.000
	Salinity × herbivory	1,8	0.002	0.962
Na/Mg	Salinity	1,8	63.074	4.6e-05
	Herbivory	1,8	0.000	0.989
	Salinity × herbivory	1,8	0.029	0.869

Table A3.5. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory and their interaction on induced resistance measured through leaf disc assay after four weeks of salinity treatment (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory n=10

	Df	F value	Pr(>F)
Salinity	1	0.942	0.339
Herbivory	1	0.087	0.770
Salinity: herbivory	1	0.092	0.7631
Residuals	35	NA	NA

Table A3.6. Results of two-way ANOVA testing the effects of salinity (0 or 100 mM NaCl) and herbivory and their interaction on induced resistance measured through *in vivo* assay after four weeks of salinity treatment (0 or 100 mM NaCl) with and without herbivory (NH = no herbivory, H = herbivory). n=10

	Df	F value	Pr(>F)
Salinity	1	0.935	0.340
Herbivory	1	0.412	0.525
Salinity: herbivory	1	0.798	0.378
Residuals	35	NA	NA