Salinity Tolerance of Red-Osier Dogwood (*Cornus sericea*) from Southeastern Manitoba

by

Laura Davis

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Department of Biological Sciences
University of Manitoba
Winnipeg

ABSTRACT

To test the hypothesis that red-osier dogwood cuttings from a more stressful edaphic environment (dry site) would have a greater salinity tolerance compared with cuttings from a less stressful environment (moist site) a greenhouse experiment was designed. Cuttings collected from three sites in southeastern Manitoba varying in edaphic conditions (moisture) were exposed to 0, 25, 50, or 100 mM NaCl in hydroponics. After four weeks of treatment, physiological parameters were measured. No differences in salinity tolerance were observed between the sites; nevertheless, this study supported previous results suggesting that red-osier dogwood can limit the transport of Na^+ from the root to the shoot. To determine the presence of barriers to ion movement, the roots of red-osier dogwood exposed to NaCl were sectioned and observed using brightfield and fluorescence techniques. A modified outer cortical layer was observed suggesting the presence of an exodermis, which would be an additional barrier to Na^+ and Cl^- movement. Furthermore, the maturation of the endodermis and exodermis occurring closer to the root tip could also contribute to limiting the transport of Na^+ to the shoot.

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Chapter 1 INTRODUCTION

Salinity impacts the growth, development, and reproduction of a wide variety of plant species from different environments. However, the growing concern for future food supply and the spread of saline soils due to agricultural practices (Rengasamy, 2006) has resulted in the majority of research to be focused on agricultural species. Although understanding the effects of salinity on agricultural species is important, non-agricultural species should not be overlooked. In Canada, the increase production of mining wastes (Townley-Smith and Redmann, 1980; Renault *et al*., 2001*a ,b*; Madsen and Mulligan, 2006) and road de-icing practices in winter (Ramakrishna and Viraraghavan, 2005) have resulted in the accumulation of salt in the underlying and surrounding soil and water that are not naturally saline. The vegetation growing in these areas is not adapted to salinity and our knowledge of the impact of salinity on these species, especially woody species of northern plant communities, is limited.

The presence of NaCl in the soil decreases the water potential gradient between the soil and the roots (Munns and Termaat, 1986). Thus, the efficiency of the plant to uptake water is reduced creating an osmotic stress. With continued exposure of plant roots to NaCl, ionic stress could occur as the concentration of Na^+ and Cl^- in the plant tissues reach toxic levels (Flowers and Yeo, 1986; Castillo *et al*., 2007; Munns and Tester, 2008). On the other hand, Na^+ and Cl^- may compete with essential nutrients (K^+, Ca^{2+}, Mg^{2+}, NO_3^-, $H_2PO_4^-$) for uptake resulting in nutrient deficiencies (Greenway and Munns, 1980; Grattan and Grieve, 1999; Tester and Davenport, 2003). Furthermore, during salinity stress, the photosynthetic processes can be inhibited by the osmotic and/or

ionic stress, resulting in an oxidative stress (Hasegawa *et al*., 2000; Miller *et al*., 2010). This combination of stresses (osmotic, ionic, and oxidative) disrupts the performance of the plant by affecting the metabolic systems, which causes physiological, morphological, and anatomical changes within the plant that ultimately reduces plant growth (Zhu, 2001; Bernstein and Kafkafi, 2002; Pastori and Foyer, 2002).

Salinity tolerance varies widely between plant species (Greenway and Munns, 1980; Flowers *et al*., 1986; Glenn *et al*., 1999). While some species only reach their maximum growth potential under saline conditions, other species cannot survive even relatively low salt concentrations. Although plant species have an inherent degree of tolerance to salinity stress, their salt tolerance is influenced by the developmental stage, the concentration of the salt, the duration of exposure, and the environmental conditions to which the plant is exposed to (Storey and Walker, 1999; Pastori and Foyer, 2002; Bernstein and Kafkafi, 2002; Munns and Tester, 2008). Another factor that is not often considered, but could play a significant role in the stress tolerance, is the environmental conditions to which the parent plant was exposed to. The seedlings of red-osier dogwood (*Cornus sericea*), a fast growing woody shrub native to North America, have been shown to have moderate salinity tolerance compared to other boreal woody species (Renault *et al*, 1998, 2000). It has also been observed that salinity tolerance of red-osier dogwood seedlings differed between three provenances in Canada varying in temperature and precipitation (Renault, Personal Communication). Although this suggests that climatic differences may influence the tolerance of the offspring, it is not known if edaphic conditions would influence the NaCl tolerance of red-osier dogwood.

Shoot growth is usually more sensitive to salinity stress than root growth (Bernstein and Kafkafi, 2002; Munns and Tester, 2008). However, the roots are the first organ to encounter the salt in soil and control the uptake and transport of Na^+ and Cl^- to the shoot. Therefore, understanding the effect of salinity on roots is essential. It has been shown that red-osier dogwood seedlings have the ability to tolerate up to 50 mM NaCl by limiting the transport of Na^+ to the leaves (Renault *et al*., 2001*b*). However, the mechanisms utilized by the root to control the Na^+ transport to the shoot to limit the damage have not been determined in red-osier dogwood. A potential mechanism is the modification of cell walls to enhance the physical barriers within the roots to control the movement of ions (Hose *et al*., 2001; Enstone *et al*., 2003). These barriers include the exodermis and endodermis, which regulate the movement of solutes by developing Casparian bands and suberin lamellae (Enstone and Peterson, 1992*a, b*; Lux *et al*., 2004). The efficiency of the exodermis and endodermis in limiting transport of Na^+ and Cl^- could be increased by the maturation of these layers closer to the root tip and increased deposition of lignin and suberin (Hajibagheri *et al*., 1985; Hose *et al*., 2001; Karahara *et al*., 2004). To the best of my knowledge, the root anatomy of red-osier dogwood has not been investigated yet; thus, the presence of an exodermis in this species remains to be determined.

The first objective of my thesis was to determine if a more stressful edaphic environment (dry site) would result in an increase in the salinity tolerance of red-osier dogwood compared to a less stressful environment (moist site). The second objective was to determine changes in the root morphology and anatomy that could contribute to the reduced transport of Na^+ to the shoot of red-osier dogwood exposed to NaCl. To achieve

these objectives, three sites varying in soil conditions were selected from southeastern Manitoba; the first was a relatively moist site, the second a relatively dry site, and the third a bog site. Red-osier dogwood cuttings were collected from each site and two greenhouse hydroponic experiments were designed. The first experiment was designed to compare the salinity tolerance of the cuttings from each site by measuring the effects of NaCl on selected physiological parameters. During the second experiment, stained embedded sections were examined to observe the effect of NaCl on the root morphology and anatomy of red-osier dogwood cuttings. In addition, freehand root sections were observed with fluorescence and also stained with various dyes to detect the presence of an exodermis and other cell wall modifications that may contribute to the decrease in Na^+ transport to the shoot.

Chapter 2 LITERATURE REVIEW

2.1 INTRODUCTION

Human activities have been increasing the distribution and severity of saline soils. Understanding the impact of saline soils on plant growth and the salinity tolerance mechanisms utilized by plants will therefore be essential for maintaining food production and preserving native plant communities. However, the understanding of salinity stress has been limited by its complexity and the variability in the response of plant species. In this review the current knowledge of salinity stress, which includes osmotic, ionic, and oxidative stresses, will be discussed. In addition, the potential of increasing the ability of a plant to tolerate salinity stress through initial exposure to another stress, such as drought, cold, light, which is termed cross-tolerance, will be examined. This review will also present the current knowledge of the impact of these above aspects of salinity stress on red-osier dogwood, a non-agricultural woody species.

2.2 SALINE SOILS

Saline soils, occurring all over the world, create a challenging environment for plant growth. It has been estimated that more than 800 million ha of land in the world are affected by salinity (Rengasamy, 2006; Munns and Tester, 2008) with 16 million ha occurring in North America (Szabolcs, 1986; Rengasamy, 2006). Soils are considered to be saline when the electrical conductivity (EC) is greater than 4 dS m^{-1} (US Salinity Laboratory, 1954). Soils become naturally saline from the weathering of parental

materials or the deposition of salts carried in the rain and wind or in ground and surface waters (Burchill and Kenkel, 1991; Rengasamy, 2006; Munns and Tester, 2008). The most prevalent salt in saline soils is sodium chloride, although other salts such as calcium and magnesium chlorides or sulphates may dominate (Eilers *et al*., 1995; Munns and Tester, 2008) depending on the parental material and pH (US Salinity Laboratory, 1954). Saline soils are less common in humid climates because the increased precipitation leaches the salt from the root zone (US Salinity Laboratory, 1954; Rengasamy, 2006). However, salt may come to the surface in a humid environment if drainage is impeded and/or the water table is near the surface (Eilers *et al*., 1995). In North America, naturally saline soils occur in the relatively arid areas, which include the Great Plains (McKell *et al*., 1986; Florinsky *et al*., 2000). In Manitoba, the saline springs near Lake Winnipegosis produce the boreal salt pans (Burchill and Kenkel, 1991).

The effect of salinity on crop production is a major issue in agriculture as the concern for future food production grows (Flowers and Yeo, 1995; Rengasamy, 2006). Agricultural practices are increasing salt concentrations in both saline soils and soils that did not have a salinity problem. When perennial species are replaced with annual crop species, salt may be brought to the root zone by a rising water table caused by decrease in evapotranspiration from the shorter growing season and shallower root systems of the annual species (Eilers *et al*., 1995; Rengasamy, 2006). The water table may also rise with excess irrigation (Rengasamy, 2006). Additionally, irrigation may contribute to soil salinity if the water used for irrigation contains salts; with the decrease in freshwater supply around the world, the potential of this occurring has increased (Wallace, 2000; Qadir *et al*., 2007; Chen and Polle, 2010). In the Canadian Prairies, 3.5 million ha of land

has subsoil (60 - 120 cm) that is saline (EC greater than 8 dS m^{-1}) (Wiebe *et al*., 2007) with 240,000 to 275,000 ha of farmland affected by salinity in Manitoba (Eilers *et al*., 1990; Government of Alberta, 2010). Saline agricultural land in Manitoba is generally localized in depressions with poor drainage resulting in salt accumulation and a rising water table (Eilers *et al*., 1997; Florinsky *et al*., 2000).

Along with the salinization of agricultural soils, land may become saline from the accumulation of salts from mine wastes. Saline mine wastes include those from the oil sands (Renault *et al*., 1998, 2001a,b), potash mining (Townley-Smith and Redmann, 1980), coal mining (Zielinski *et al*., 2001; Madsen and Mulligan, 2006), and petroleum wells (Hamid *et al*., 2008; Dastgheib *et al*., 2011). Salt tolerant plants are valuable to the reclamation/revegetation of sites impacted by salinity. Furthermore, salinity stress not only affects the rural environment but the urban environment as well. For instance, the application of de-icing salts (e.g. NaCl, $CaCl_2$, $MgCl_2$, and KCl) to urban and rural roads may result in the accumulation of salts in the surrounding soil and water (Ramakrishna and Viraraghavan, 2005; Hanslin, 2011). In Canada, 5 million tonnes of de-icing salts are applied to roads each year (Environment Canada 2004) with 40,000 tonnes applied in Manitoba (Manitoba Conservation, 2009).

The vegetation on all these salinity impacted lands will be affected by the salts under a variety of climatic environments. Therefore, the understanding of how salinity stress affects the growth and development of plants from different environments will improve our ability to respond to the growing problem of saline soils.

2.3 Salinity Stress: Effects and Tolerance

2.3.1 Introduction

Plant species vary greatly in their ability to tolerate saline environments. Salinity tolerance ranges from species that require salt for maximal growth to species that cannot survive relatively low salt concentrations (Greenway and Munns, 1980; Flowers *et al*., 1986; Glenn *et al*., 1999). Based on their salinity tolerances plant species are divided into two main groups, the halophytes and the glycophytes. Halophytes have a superior ability to grow and survive in saline environments compared to glycophytes, but there is not a clear division between the two groups as salinity tolerance is a continuous scale. This continuity in salinity tolerance also results in relative extremes in salinity tolerance between species within the glycophytes and halophytes. For example, within the halophytes, exposure to 200 mM NaCl increased the shoot biomass of the herbaceous seepweed (*Suaeda maritima*) by approximately 65% while it decreased the shoot biomass of *Puccinellia peisonis* by approximately 50% (Flowers and Colmer, 2008). Within the glycophytes, cotton (*Gossypium hirsutum*) is a more salt tolerant species with a decrease of only 14% in biomass compared to bean (*Phaseolus vulgare*), with a 59% decrease in biomass (Gouia *et al*., 1994) when treated with 100 mM NaCl. Although extremes occur between species within the halophytes and glycophytes, determining where a species falls within these two groups can be difficult. This can be due to the fact that the degree of salinity tolerance of a species is not only influenced by their overall inherent tolerance to salinity stress, but also by the developmental stage of the plant, the severity and duration of the stress, and the environmental conditions in which the plant is growing (Storey and Walker, 1999; Bernstein and Kafkafi, 2002; Munns and Tester, 2008). To further

complicate the classification of a species salinity tolerance, variability in tolerance may occur within a genus or even within a species (Munns *et al*., 1995; Storey and Walker, 1999).

Salinity stress causes a decrease in the rate of cell production and cell size (Munns *et al*., 1995; Bernstein and Kafkafi, 2002; Munns and Tester, 2008). Therefore, plant biomass will be reduced by having fewer and smaller leaves produced and also by the reduction in lateral root and stem development. When the reduction in plant growth causes insufficient energy production to sustain the necessary metabolic functions, plant death could occur (Munns, 2002). Salinity tolerance is often determined by the ability of the plant to maintain a relatively high biomass. However, a slower growth rate may result in an increase in salt tolerance by reducing the demands for resources (i.e., water, nutrients, and photosynthates) and increase the resources available for protection and repair of tissues (Zhu, 2001; Achard *et al*., 2006; Flowers and Colmer, 2008). It is therefore difficult to separate the changes observed during the stress that will contribute to plant sensitivity from those related to the development of salinity tolerance mechanisms (Ashraf and Foolad, 2007).

Salinity stress in itself is complex as it is composed of osmotic, ionic, and oxidative stresses (Greenway and Munns, 1980; Munns and Tester; 2008) (Fig. 2.1). Determining how these three components contribute independently to the overall effect of salinity has been a challenging aspect of salinity stress research. In some species, such as moderately salt tolerant wheat (*Triticum* spp.), salinity stress can be separated into two distinct 'phases' with osmotic stress as the first phase and ionic stress as the second phase (Munns *et al*., 1995; Castillo *et al*., 2007; Munns and Tester, 2008). In other species like

lupin (*Lupinus albus*), distinction between osmotic and ionic stress is more difficult, because of the rapid accumulation of ions and low tolerance to ionic stress (Munns, 1988; Munns *et al*., 1995). Separating the effects of the osmotic, ionic, and oxidative components will assist in determining how salinity affects plant growth and development and the possible tolerance mechanisms.

2.3.2 OSMOTIC STRESS

Osmotic stress occurs with the addition of salt to the growth medium decreasing the water potential of the medium, which leads to a reduction in the driving force for water uptake (water potential gradient between the plant and the growth medium) (Fig. 2.2). As a result, the turgor pressure in the cells of the leaves and roots is reduced, causing a decrease in the expansion of leaves and elongation of roots (Yeo *et al*., 1991; Neumann, 1993; Bernstein and Kafkafi, 2002; Munns, 2002). In roots exposed to a severe osmotic stress, plasmolysis may occur and disrupt the plasmodesmata and plasma membranes (Munns, 2002); thus, affecting the uptake of water and ions and the duration required for recovery. The recovery from loss of turgor in the root and leaf cells occurs through osmotic adjustment (Cramer and Bowman, 1991; Rodriguez *et al*., 1997; Lu and Neumann, 1998). There are several potential osmolytes that a plant may use to decrease the osmotic potential within the plant to maintain the water balance (Glenn *et al*., 1999; Hasegawa *et al*., 2000; Flowers and Colmer, 2008). Halophytes tend to accumulate Na^+ and Cl^- from the soil, which are relatively energetically inexpensive osmolytes, to restore, and maintain the water potential gradient within the cells. This may also occur in glycophytes, but the benefit of accumulating the Na^+ and Cl^- for osmotic adjustment may

eventually be lost when the ions reach toxic levels in the cells due to the inefficient control of ion transport and compartmentalization (Hasegawa *et al*., 2000). Osmolytes may also include compatible solutes, such as sugars (sucrose, trehalose), polyols (sorbitol, mannitol, and inositol), amino acids (proline), quaternary ammonium compounds (glycine betaine), tertiary sulfonium compounds and K^+, which are osmolytes that do not interfere with the processes occurring within the cell (Bohnert *et al*., 1995; Glenn *et al*., 1999; Hasegawa *et al*., 2000; Ashraf and Foolad, 2007; Flowers and Colmer, 2008). Furthermore, compatible solutes are required in the cytoplasm of both halophytes and glycophytes to balance the accumulation of ions in the vacuole, thus increasing the energy cost. An additional role of many of the compatible solutes is as an osmoprotectant. Osmoprotectants can maintain the structure and activity of the enzymes and proteins, the structure and permeability of the cell walls and membranes, and scavenge reactive oxygen species that are produced as a result of the osmotic or ionic stress. Although the accumulation of osmolytes is often positively correlated with salinity tolerance (Greenway and Munns, 1980; Hasegawa *et al*., 2000), in some cases a greater increase in osmolytes occurs in the more sensitive species (Lloyd *et al*., 1990; Ashraf and Foolad, 2007; Chen *et al*., 2007; Widodo *et al*., 2009). A species with greater salinity tolerance may accumulate relatively low concentrations of compatible solutes by distributing osmolytes to specific locations and only at the level that is required for protection and osmotic balance (Hasegawa *et al*., 2000). The moderate osmolyte production will allow for more resources to be available for cellular growth, maintenance, repair, and other stress tolerance mechanisms (Zhu, 2001; Flowers and Colmer, 2008; Munns and Tester, 2008). Therefore, it can be difficult to interpret the significance of an

accumulation of osmolytes as the accumulation may reflect either a greater salinity tolerance or an indication of stress.

Recovery of the loss of turgor and cell elongation may occur within minutes to hours after salt treatment depending on the species and level of salt (Yeo *et al*., 1991; Cramer, 2003). In addition, the severity of the stress will determine if the rate of cell elongation returns to that observed before the stress or to a lower rate. In barley (*Hordeum vulgare*) and maize (*Zea mays*) the leaf elongation rates fully recovered within 5 h after treatment with 40 mM NaCl but a treatment of 120 mM NaCl resulted in only a partial recovery (Cramer, 2003). It is still not well understood what causes the reduction in the rate of root elongation during osmotic stress (Cramer and Bowman, 1991*a, b*; Neumann *et al*., 1994; Lu and Neumann, 1998; Cramer, 2003). Osmotic stress may reduce elongation rate of cells in the roots and leaves by affecting one or more of the mechanical and hydraulic components regulating cell expansion. These include the yield threshold (the minimum turgor pressure required for cell expansion to occur), the hydraulic conductivity, and the extensibility of the cell wall (Cramer, 2003). The predominant mechanism inhibiting cell elongation may also vary between species; for example, while in foxtail barley (*Hordeum jubatum*) it has been attributed to a decrease in hydraulic conductivity, in maize (*Zea mays*) an increase in the yield threshold was the cause of the decrease in leaf elongation (Cramer, 2003). Furthermore, changes in cell elongation could be the result of modifications in the composition and arrangement of cell wall material (polysaccharides, proteins, and pectins) (Zhong and Läuchli, 1993*b*), the inhibition of cell wall loosening through increased cross-linkages of cell wall polymers and lignification (Neumann *et al*., 1994; Neves *et al*., 2010), or even reduced

acidification of the apoplast (Taleisnik *et al*., 2009). Cell wall metabolism can also be altered by the modification of enzyme activities, gene expression and/or accumulation of hormones in response to salinity (Munns *et al*., 2000; Cramer and Quarrie, 2002; Taleisnik *et al*., 2009; Neves *et al*., 2010). For example, a reduction of expansins under salinity stress may contribute to the decrease in cell elongation by limiting cell wall loosening (Geilfus *et al*., 2010). Salinity stress also reduces root and shoot growth by decreasing the concentration of gibberellins (GA) and increasing the concentrations of abscisic acid (ABA) and ethylene, which enhances the accumulation and activity of the growth-repressing DELLA proteins (Achard *et al*., 2006). Further research is required to understand the complex changes involved in cell elongation in the leaves and roots under salinity stress and how it may vary among species.

A typical response to osmotic stress is the closure of the stomates caused by the rapid accumulation of ABA in the leaves resulting in a reduction in the transpiration rate (Robinson *et al*., 1997; Storey and Walker, 1999; Chaves *et al.*, 2009). This decrease in transpiration may contribute to an increase in water-use efficiency (ratio of carbon dioxide fixed by photosynthesis to transpiration rate) (Downton *et al*., 1985; Flowers *et al*., 1988); however, the decrease in stomatal conductance can also limit photosynthesis by reducing the CO_2 supply (Downton *et al*., 1985; Seemann and Critchley, 1985; Delfine *et al*., 1998; Chaves *et al*., 2009). It has also been shown that many other factors such as stress-induced changes in leaf morphology/anatomy (Downton *et al*., 1985; Delfine *et al*., 1998; Chaves *et al*., 2009), reduced enzyme content and/or activity (Seemann and Critchley, 1985; Delfine *et al*., 1998; Chaves *et al*., 2009), and reduced chlorophyll

content (Downton *et al*., 1985; Seemann and Critchley, 1985; Delfine *et al*., 1998) can contribute to the decrease in photosynthesis during salinity stress.

The duration of the dominance of the osmotic component over the ionic component of salinity stress will vary depending on the concentration of the salt and the ability of the plant to limit the accumulation of ions and the tolerance to the accumulated ions (Munns and Tester, 2008).

2.3.3 IONIC STRESS

The second 'phase' of salinity stress, ionic stress, is the result of the direct toxicity of Na^+ and Cl^-, and nutrient deficiencies in the plant (Fig. 2.1). In halophytes the accumulation of Na^+ and Cl^- may contribute to the maintenance of the water potential gradient through osmotic adjustment, as mentioned above, but in glycophytes the accumulation of these ions may rapidly result in toxicity (Flowers *et al*., 1986; Munns and Tester, 2008). The accumulation Na^+ and Cl^- in the cytoplasm and within organelles results in physical damage and directly interferes with biochemical and metabolic processes of the cell. The concentration at which Na^+ and Cl^- is toxic to the cells is not known, but enzymes from both halophytes and glycophytes are inhibited by a NaCl concentration of approximately 100 mM *in vitro* (Greenway and Osmond, 1972; Munns and Tester, 2008). Therefore, halophytes and glycophytes do not differ in their sensitivity to salt *in vitro* (Greenway and Osmond, 1972; Munns and Tester, 2008). The accumulated Na^+ may also directly affect the plasma membrane by replacing Ca^{2+} on the membrane, which alters the permeability of the membrane allowing the leakage of K^+

from the cell, as was observed in cotton (*Gossypium hirsutum*) (Cramer *et al*., 1985). In addition, Na^+ and Cl^- indirectly affects plant cells if accumulated in the apoplast at concentrations resulting in cellular dehydration (Flowers and Yeo, 1986; Flowers *et al*., 1991; Munns, 2002). It is still not clear if one of the two ions has a greater toxicity in plants (Munns and Tester, 2008), as it is difficult to change the concentration of one of the two ions in the nutrient solution without affecting the osmotic potential or the ratios of the other anions and cations present (Tavakkoli *et al*., 2011). It is often reported that NaCl induced injury is more strongly correlated with Na^+ than Cl^- in some plants (Tester and Davenport, 2003; Munns and Tester, 2008) like barley (*Hordeum vulgare*, Flowers and Hajibagheri, 2001) and wheat (*Triticum* spp., Schachtman and Munns, 1992). However, stronger correlation has also been found between injury and Cl^- in soybean (*Glycine max*, Valencia *et al*., 2008) and narrow-leaf trefoil (*Lotus tenuis*, Teakle *et al*., 2010). In woody species, such as Citrus (*Citrus* spp., Zerki and Parsons, 1992; Storey and Walker, 1999) and grapevine (*Vitis* spp., Tregeagle *et al*., 2010), it has been found that genotypes with greater control of the transport of Cl^- tend to have greater NaCl tolerance. In addition, it has also been suggested in barley (*Hordeum vulgare*) that Cl^- and Na^+ are both involved in determining NaCl toxicity in plants and that there is an additive or/interactive effect (Tavakkoli *et al*., 2011).

To prevent Na^+ and Cl^- from interfering with biochemical and metabolic processes, the Na^+ and Cl^- concentrations in the cytoplasm must remain below toxic levels (Flowers and Yeo, 1986; Munns and Tester, 2008). One mechanism used by plants is succulence, which may help the plant deal with increasing ion concentration by diluting the ions. However, as succulence has been observed in both salt tolerant and salt

sensitive species, it is not likely to play a major role in salinity tolerance. Another mechanism is the removal of excess Na^+ and Cl^- from the leaves through glands and bladders, but this mechanism is limited to a few halophytes (Flowers and Colmer, 2008). In addition, the quantity of Na^+ reabsorbed from the leaves by the phloem for export to the roots is insignificant compared to the transport to the leaves by the xylem (Flowers and Yeo, 1986; Munns, 2002). Therefore, without mechanisms to remove the Na^+ and Cl^- from the leaves, the plant must control the uptake, transport, and compartmentalization of Na^+ and Cl^-. To remove the potentially toxic Na^+ from the cytoplasm, Na^+ can be transported to the vacuole through the tonoplast Na^+/H^+antiporter (NHX) with the H^+ electrochemical potential gradient maintained by the vacuolar pyrophosphatase (AVP) and H^+-ATPase (Tester and Davenport, 2003; Munns and Tester, 2008; Kronzucker and Britto, 2011) (Fig. 2.3). The importance of compartmentalization of Na^+ in the vacuole has been shown by the increase in salinity tolerance with the over expression of NHX in *Arabidopsis thaliana* (Apse *et al*., 1999), rice (*Oryza sativa*, Fukuda *et al*., 2004), cotton (*Gossypium hirsutum*, Wu *et al*., 2004), and alfalfa (*Medicago sativa*, Li *et al*., 2011). Although compartmentalization of Cl^- has been found to correlate with an increase in salinity tolerance in some species such as grapevine (*Vitis* spp.) and citrus (*Citrus* spp.), it is still not clear how Cl^- is transported across the tonoplast (Munns and Tester, 2008; Teakle and Tyerman, 2010). The transport of Cl^- into the vacuole may involve both an active (Cl^-/nH^+ antiporter) and a passive transport component (anion channels), but additional research is needed to clarify the mechanisms of Cl^- transport in plants.

The amount of Na^+ and Cl^- accumulated in the shoot is determined by the transpiration rate and the concentration of the ions in the xylem sap (Flowers and Yeo,

1986). Therefore, the transport of Na^+ and Cl^- to the leaves may be limited by the closure of stomata that reduces the transpiration rate. Some halophytes without salt glands, such as sea aster (*Aster tripolium*), may further reduce stomatal conductance through a Na^+-sensing response pathway that deactivates the guard cells K^+ inward channels (Perera *et al*., 1994; Véry *et al*., 1998). Another potential mechanism to control ion accumulation is found in some glycophytes that preferentially transport Na^+ and Cl^- to the older leaves to reduce the accumulation of these ions in young growing tissue (Greenway and Munns, 1980; Flowers and Yeo, 1986, Lutts *et al*., 1996). However, if the uptake of ions into the roots and xylem is not controlled, the injury and senescence of mature leaves will limit resources for leaf production and ions will also accumulate in the growing leaves.

To control the accumulation of Na^+ and Cl^- in the plant, the plant must minimize the influx of these ions into the roots and maximize the efflux of the ions back into the external growth medium (Tester and Davenport, 2003; Munns and Tester, 2008). The influx of Na^+ across the plasma membrane occurs passively through voltage-independent non-selective cation channels and possibly via high-affinity K^+ transporters (HKT) (Tester and Davenport, 2003; Kronzucker and Britto, 2011) (Fig. 2.3). The removal of Na^+ from the cell occurs via Na^+/H^+ antiporters, and under salinity stress the efflux may be enhanced by the increased activation of SOS1, a Na^+/H^+ antiporter. Less is known about Cl^- transport across the plasma membrane under salinity stress. The uptake of Cl^- is believed to be predominately active through a $Cl^-/2H^+$ symporter (Flowers and Colmer, 2008; Teakle and Tyerman, 2010). However, passive uptake through anion channels may occur if the membrane is depolarized from the uptake of Na^+ and the Cl^- concentration in

the cytoplasm is low. Anion channels are also involved in the passive movement of Cl^- out of the cell.

In addition to ionic toxicity, nutrient imbalance and deficiency are likely to occur in plants with exposure to salinity (Greenway and Munns, 1980; Grattan and Grieve, 1999; Hu and Schmidhalter, 2005). This is due to the increased Na^+ to K^+, Ca^{2+}, and Mg^{2+} ratios and Cl^- to NO_3^-, and $H_2PO_4^-$ ratios in the rhizosphere, which is the result of the competition for uptake at the level of the roots. With exposure to NaCl, a high cytoplasmic Na^+/K^+ ratio has been shown to decrease protein synthesis and enzyme activity due to competition for binding sites (Grattan and Grieve, 1999; Tester and Davenport, 2003). The accumulation of Na^+ will also interfere with Ca^{2+} in maintaining membrane and cell wall structure that will affect ion transport and selectivity (Clarkson and Hanson, 1980; Grattan and Grieve, 1999). Furthermore, deficiencies in $H_2PO_4^-$, Mg^{2+}, and NO_3^- will affect biochemical and metabolic activities by limiting the formation and/or activity of molecules involved in energy production and assembly of cellular components such as ADP and ATP (adenosine di- and tri-phosphate), chlorophyll, and enzymes in the plant (Clarkson and Hanson, 1980; Hu and Schmidhalter, 2005; Rivelli *et al*., 2010). Therefore, reducing Na^+ and Cl^- accumulation by controlling the uptake and efflux of these ions will contribute to maintaining required levels of nutrients for cellular functions and plant growth. For example, sustaining levels of Ca^{2+} in cells may increase the salinity tolerance of plants by maintaining membrane stability and increasing the selectivity of ion channels and transporters to inhibit the uptake of Na^+ and Cl^- (Tester and Davenport, 2003; Teakle and Tyerman, 2010). Studies have shown that the addition of supplemental Ca^{2+} decreased Na^+ uptake and increased salinity tolerance in plum

(*Plum* spp., Bolat *et al*., 2006), tomato (*Lycopersicon esculentum*, Tuna *et al*., 2007), strawberry (*Fragaria* x *ananassa*, Kaya *et al*., 2002) and pistachio (*Pistacia vera*, Tavallali *et al*., 2008).

The presence of physical barriers in the roots may increase the selectivity of the ions being transported to the shoot by blocking the uncontrolled movement of ions through the apoplastic pathway (Enstone *et al*., 2003; Ma and Peterson, 2003). Blocking the apoplastic route forces the ions to move through the plasma membrane regulated symplastic pathway. These barriers include the Casparian bands and suberin lamellae of the endodermal and exodermal layers. The Casparian bands are formed by deposition of lignin and suberin in the anticlinal cell walls. The Casparian bands of the endodermis are present in almost all vascular plants and the distance of its formation from the root tip varies depending on the species and environmental conditions (Enstone *et al*., 2003; Ma and Peterson, 2003). The distance from the root tip at which the Casparian bands of the exodermis is formed varies between species and with environmental conditions but usually forms at a greater distance from the root tip than observed in the endodermis. Following the formation of the Casparian band, the hydrophobic suberin lamellae are deposited as secondary walls of the endodermal and exodermal cells. In the endodermis, suberin lamellae are first developed between the xylem poles and have been found not to sever the plasmodesmata connections. The suberized endodermis may have a role in protecting the root when the epidermis and cortex are lost, such as in some woody species or in some species under stress conditions (drought) (Enstone *et al*., 2003; Ma and Peterson, 2003). For example, the exodermis of drought stressed onion *(Alium cepa*) became the outer protective barrier after the death of the epidermal cells (Stasovski and

Peterson, 1993). Although the exodermis is not as commonly found in plants as the endodermis, it has been found in many roots of plant species examined (Perumalla *et al*., 1990). Peterson and Perumalla (1990) defined an exodermis as a hypodermis (the outer layer(s) of the cortex with modified cell shape) that develops Casparian bands. However, the Casparian bands in the exodermis are difficult to observe because the suberin lamellae usually forms immediately after the Casparian bands are formed in the thin exodermal walls (Hose *et al*., 2001; Enstone *et al*., 2003; Ma and Peterson, 2003). Methods of observing Casparian bands in the exodermis were not discovered until the 1980's, which now include staining with berberine (fluorescent dye), clearing of sections with NaOH, and the use of apoplastic dyes (Cellufluor, berberine hemisulphate) (Brundrett *et al*., 1988; Perumalla *et al*., 1990, Peterson and Perumalla, 1990; Meyer *et al*., 2009). Variations in the morphology of the exodermis include the uniform and dimorphic exodermis and the single (uniseriate) and multiple (multiseriate) layered exodermis (Hose *et al*., 2001; Enstone *et al*., 2003; Ma and Peterson, 2003). The uniform exodermis do not have Casparian bands if they do not have suberin lamellae, while all cells have Casparian bands in the dimorphic exodermis. The lack of Casparian bands will affect the role of the exodermis as an apoplastic barrier. In some species a multiseriate exodermis is formed, and the maturation of the exodermal layers may be accelerated and enhanced under variable environmental conditions. For example, the exposure of iris (*Iris germanica*) roots to air resulted in the Casparian bands and suberin lameallae of the second layer of the multiseriate exodermis to occur closer to the root tip (Meyer *et al*., 2009).

The presence and strengthening of these barriers may result in increased salinity tolerance. The Casparian bands in halophytes are thicker than in glycophytes (Tester and Davenport, 2003; Kronzucker and Britto, 2011). However, in glycophytes modifications to the endodermis and exodermis do occur. For example, exposure to 200 mM NaCl increased the thickness of endodermal Casparian bands in maize (*Zea mays*, Karahara *et al*., 2004) and caused formation of an exodermis in cotton (*Gossypium hirsutum*, Reinhardt and Rost, 1995*a*). The Casparian band and suberin lamellae formation in the endodermis and exodermis occurring closer to the root tip in Rangpur lime (*Citrus reticulate*, Walker *et al*., 1984), Etrog citron (*Citrus medica*, Walker *et al*., 1984), seepweed (*Suaeda maritima*, Hajibagheri *et al*., 1985), cotton (*Gossypium hirsutum*, Reinhardt and Rost, 1995*a, b*), maize (*Zea mays,* Karahara *et al*., 2004), and rice (*Oryza sativa*, Krishnamurthy *et al*., 2009). The accelerated maturation of these cells may contribute to a reduction of ion accumulation in the roots. However, it is difficult to determine if it is a salt tolerance mechanism or only the result of salinity stress decreasing the cell production and cell elongation (Karahara *et al*., 2004). In addition, the presence of the endodermis and exodermis does not necessarily mean that these layers are efficient barriers. The stage of development and the chemical composition of both the exodermis and endodermis, as well as the properties of the compound to be transported will determine the permeability of these layers (Hose *et al*., 2001; Enstone *et al*., 2003; Ma and Peterson, 2003). For example, in corn (*Zea mays*) and rice (*Oryza sativa*) roots, the Casparian bands did not completely block the movement of Cu^{2+} through the apoplast (Ranathunge *et al*., 2005). In addition, an increase in the suberization of root cells does not always result in the decrease in ion accumulation (Kronzucker and Britto, 2011).

Baxter *et al*. (2009) found that an Arabidopsis (*Arabidopsis thaliana*) mutant exhibited increased suberization of the root cells did not show a decrease in Na^+ accumulation but an increase in accumulation under non-saline conditions. The extent of the contribution of the exodermis and endodermis to salinity stress by decreasing the uptake of ions requires more research especially in woody roots.

2.3.4 OXIDATIVE STRESS

The osmotic and ionic components of salinity stress may cause an imbalance in the energy production pathways that results in oxidative stress (Fig. 2.1). Oxidative stress is the result of the production of reactive oxygen species (ROS) in the chloroplasts, peroxisomes, and mitochondria of the cell at a greater rate than the scavenging rate (Gill and Tuteja, 2010; Miller *et al*., 2010). This accumulation of ROS damages lipids, proteins, and DNA, which may eventually lead to cell death. During salinity stress, the efficiency of the photosynthetic pathway is reduced, which leads to ROS formation in the chloroplast with the transfer of electrons from reduced products of the photosynthetic pathway to oxygen producing O_2^- (superoxide) via the Mehler reaction (Asada, 2006; Gill and Tuteja, 2010; Miller *et al*., 2010). The O_2^- is then converted to H_2O_2 by superoxide dismutase (SOD). The production of ROS in the chloroplast may also occur with the transfer of energy from excited triplet-state chlorophyll to oxygen, which produces $^1O_2$ (singlet oxygen, Arora *et al*., 2002; Asada, 2006; Gill and Tuteja, 2010; Miller *et al*., 2010). To prevent cellular damage, $^1O_2$ must be quenched by antioxidants β - carotene and α - tocopherol and H_2O_2 converted to water via the water-water cycle (Halliwell-Asada pathway). This pathway involves the reduction of the antioxidants ascorbate and

glutathione, which is catalyzed by antioxidant enzymes (ascorbate peroxidase and glutathione reductase). Although uncontrolled accumulation of ROS results in cellular damage, ROS production has also been shown to be involved in stress signalling by initiating other stress response mechanisms (Gill and Tuteja, 2010; Miller *et al*., 2010). Therefore, the initial accumulation of ROS from the interference of metabolic activity under environmental and pathogen induced stress may result in tolerance to the stress, while insufficient scavenging of the ROS will result in oxidative stress. The overexpression of antioxidant enzymes have be found to increase the salinity tolerance in transgenic tobacco (*Nicotiana tabacum*, Badawi *et al*., 2004), poplar (*Populus davidiana* x *P. bolleana*, Wang *et al*., 2010), and *Arabidopsis* (Wang *et al*., 2004). However, Munns and Tester (2008) questioned the importance of reactive oxygen species in salinity stress as they suggested that plants would be exposed to increased levels of ROS in the variable environmental conditions (e.g. fluctuating light levels) and would therefore be prepared for the changes caused by salinity stress. This may be true if the moderate fluctuations in salinity levels do not occur with other stresses. However, if salinity stress is combined with light and temperature stress, which is likely to happen in arid environments where saline soils are abundant (Chaves *et al*., 2009), oxidative stress may be an important component of salinity stress.

2.4 Cross-Tolerance

Cross-tolerance occurs when a plant exposed to one type of stress initiates changes in the plant that will then contribute to the tolerance to a different stress (Cayuela *et al*., 2007; Tuteja, 2007). The initial exposure to a stress, signals to the plant to respond

by modifying the expression of genes (Wang *et al*., 2003; Tuteja, 2007; Roelofs *et al*., 2008), the regulation of transporters/pumps (Wang *et al*., 2003), and/or metabolic processes (Tuteja, 2007). The response of the plant to these signals from various abiotic stresses may result in the activation of similar signal transduction pathways. This may occur more when the stresses share similar stress components such as with salt, drought, and cold that share the osmotic stress, and oxidative component. The activation of signal transduction pathways may result in the up-regulation of transcription factors and gene expression responsible for the synthesis and accumulation of proteins, compatible solutes, and antioxidants that will help with subsequent stresses (Wang *et al*., 2003; Tuteja, 2007; Roelofs *et al*., 2008). These proteins and antioxidants may include proline, heat shock proteins (HSP), late embryogenesis abundant (LEA) proteins, and superoxide dismutase (SOD). The cross- tolerance therefore results in natural up-regulations of protective pathways that may be an alternative to genetic engineering.

Exposing plants to salt, drought, or heat stress have resulted in an increase tolerance to cold stress in *Solanum commersonii* (Ryu *et al*., 1995), chilling stress in *Zea mays* (Aroca *et al*., 2003), or salt stress in *Zea mays* (Gong *et al*., 2001) respectively. However, cross-tolerance does not always occur and the increase in tolerance may not be long-term (Cayuela *et al*., 2007). The increase in tolerance may also depend on the developmental stage of the plant when it is exposed to the initial stress. For example, the salinity tolerance of tomato (*Solanum lycopersicum*) was increased with a pretreatment of drought stress at the five-leaf stage but not at the two- and eight- leaf stage (Cayuela *et al*., 2007). The developmental stage may influence the ability of the plant to respond to the initial stress in terms of perceiving the stress and initiating the mechanisms for cell

defence and repair (Amzallag *et al*., 1993; Chinnusamy *et al*., 2004). Along with the developmental stage of the plant, the occurrence of cross-tolerance may also be affected by the inherent stress tolerance of the plant and the duration and severity of the first stress (pretreatment) and the following stress (treatment) (Amzallag *et al*., 1993; Balibrea, 1999; Cayuela *et al*., 2001).

In a natural environment, plants exposed to a higher level of stress may be better adapted to salinity stress than those from a less stressful environment (Nguyen *et al*., 2004; Marcar *et al*., 2002; Megdiche *et al*., 2007). This variation in salinity tolerance may be attributed to underlying genetic differences between the provenances (Nguyen *et al*., 2004) and/or from the adaptation to the stresses that the parent plant had been exposed to and passed on to its offspring (Amzallag, 2000; Marcar *et al*., 2002; Megdiche *et al*., 2007). For example, Megdiche *et al*. (2007) found an increase in leaf biomass and leaf number in sea rocket (*Cakile maritima*) from an arid habitat treated with 100 mM NaCl, while those from a humid environment showed a decrease in these parameters in response to the salinity stress. However, most of these studies (Nguyen *et al*., 2004; Megdiche *et al*., 2007) do not fully describe the provenances/ecotypes; therefore, the magnitude of the differences in climatic or soil characteristics in relation to potential stresses between the sites is difficult to establish. More research is also needed to understand how cross-tolerance may affect the salinity tolerance of non-agricultural woody species.

2.5 RED-OSIER DOGWOOD

Red-osier dogwood (*Cornus sericea* L. syn. *Cornus stolonifera* Michx) is a woody shrub with a distribution from the east coast to the west coast of Canada and the northern USA states, and from Alaska to northern Mexico (Smithberg and Weiser, 1968; Crane, 1989; Johnson *et al*., 1995). Red-osier dogwood can grow in riparian environments such as riverbanks and marshes as well as in dryer environments such as forest clearings and edges. This wide distribution exposes red-osier dogwood to a variety of climatic conditions results in variation in growth rates and timing of cold acclimation between different climatic areas (Smithberg and Weiser, 1968; Bray and Parsons, 1981). Research on red-osier dogwood has focused on its ability to tolerate freezing stress (Bray and Parsons, 1981; Karlson *et al*., 2003) as it can survive -269°C after acclimation to freezing (Guy *et al.*, 1986). Red-osier dogwood is also a potential species for land reclamation and soil stabilization because of the relatively easy propagation with cuttings, its rapid growth, and potential as wildlife shelter and food source (Crane, 1989). Furthermore, red-osier dogwood seedlings may be suitable for reclamation of oil sand tailings as they have been found to be relatively tolerant of the saline waters produced from oil sands operations compared with other boreal species such as raspberry (*Rubus idaeus*), trembling aspen (*Populus tremuloides*), Jack pine (*Pinus banksiana*), white spruce (*Picea glauca*), and tamarack *(Larix laricina*) (Renault *et al*., 1998). Red-osier dogwood is also a popular horticulture species because of the red stems in the winter providing colour to the landscape. The application of de-icing salts to roads each winter could expose red-osier dogwood to the salt spray from passing vehicles and the accumulation of salts within the surrounding soil (Ramakrishna and Viraraghavan, 2005;

Hanslin, 2011). Therefore, understanding the effect of salinity stress on red-osier dogwood is important.

Red-osier dogwood is able to limit the transport of Na^+ to the shoot when treated with up to 50 mM NaCl, although the Cl^- content in the shoot increased with increasing NaCl concentration (Renault *et al*., 2001). The reduced Na^+ accumulation in the leaves has also been found in other woody perennial species such as citrus (*Citrus* spp.) and grapevine (*Vitis* spp.) (Storey and Walker, 1999; Storey *et al*., 2003). Along with the accumulation of Na^+ and Cl^-, a reduction of photosynthesis and stomatal conductance, and limited osmotic adjustment may contribute to the reduction in growth of red-osier dogwood exposed to 100 mM NaCl for four weeks (Renault *et al*., 2001; Mustard and Renault, 2004). These previous studies on the effect of salinity stress were conducted on seedlings collected from the Canadian province Alberta. Variability in the response to salinity stress may occur between plants propagated by seeds and by cuttings. A lower shoot/root ratio found in seedlings compared to cuttings of glue gum (*Eucalyptus globules*) may have contributed to a higher survival rate to multiple drought periods (Sasse and Sands, 1996). As the ease of propagation of red-osier dogwood through cuttings makes it a preferred method of propagation, it is also important to determine if there is a difference in salinity response between seedlings and cuttings of red-osier dogwood. Furthermore, variability in the salinity tolerance of red-osier dogwood from different provenances has been found (Renault, Personal Communication). Three-month-old red-osier dogwood seedlings from Alberta (cold provenance) had a slower growth rate than seedlings from British Columbia (dry provenance), and New Brunswick (wet provenance) under control conditions. Alberta and British Columbia seedlings exposed to

50 mM NaCl had the least leaf injury after two weeks of treatment, and higher photosynthetic rate and photosynthetic water-use efficiency compared to seedlings from New Brunswick. This suggests that cross-tolerance may occur in red-osier dogwood, as exposure to cold and drought stress may increase the NaCl tolerance of red-osier dogwood. However, although the Alberta seedlings exposed to 100 mM NaCl for one week of treatment had the least leaf injury, after two weeks of exposure there were no differences between the seedlings from the three provenances. The benefit of cross-tolerance on a plant may therefore depend on the intensity and duration of the second stress, but more research is needed to determine the effects of cross-tolerance (i.e. the environmental conditions of the parent plant) on the NaCl tolerance in red-osier dogwood.

In red-osier dogwood seedlings, the decrease in shoot biomass is greater than the root biomass at higher NaCl concentrations (Renault *et al*., 2001). However, reduction in root growth does occur and the roots are the initial control point for ion uptake as they are in direct contact with NaCl. The effect of salinity stress on the development of the red-osier dogwood roots has not been determined. It is not known if an exodermis is present or if modifications to the endodermis occur with exposure to NaCl, a mechanism that could increase the control of ion uptake. It has also not been determined if there are changes occurring in root cell walls contributing to the reduction in root growth and uptake of ions. In red-osier dogwood shoots, an increase in the cell wall hemicellulose content occurs with salinity stress that may decrease growth by increasing cell wall cross-linkages (Mustard and Renault, 2004). Prior to determining the effects of salinity stress

on red-osier dogwood roots, the development and anatomy of red-osier dogwood roots remain to be studied.

2.6 Conclusion

Although salinity is not a major concern in North America, compared to Asia or Australia, the amount of land becoming saline through agricultural, mining, and road ice removal practices, has resulted in the exposure of plants not adapted to salinity stress to saline environments. The effect of salinity in these saline areas will depend on the plant species exposed, the type of salt, the developmental age of the plant, the degree and severity of the stress, and the environmental conditions. In addition, in the natural environment a plant is often exposed to many types of stress and the exposure to other stress factors (extreme temperatures, water availability) may affect the plant's response to salinity stress. The exposure to a different initial stress may benefit the plant through cross-tolerance mechanisms. In addition, although a large number of studies have been conducted, the complexity of salinity stress and the differences in the response of plants to salinity stress makes it difficult to determine the underlying mechanisms causing the biochemical, physiological, morphological, and genetic changes in the plant. Another gap in our knowledge of salinity stress is from the limited research of the effects of salinity stress on non-agricultural woody species especially in reference to the roots. The potential use of red-osier dogwood in the reclamation of saline mine tailings and also as a horticultural species in areas that use road de-icing salts makes it important to understand the effects of salinity stress on red-osier dogwood and to determine ways to increase its salinity tolerance.

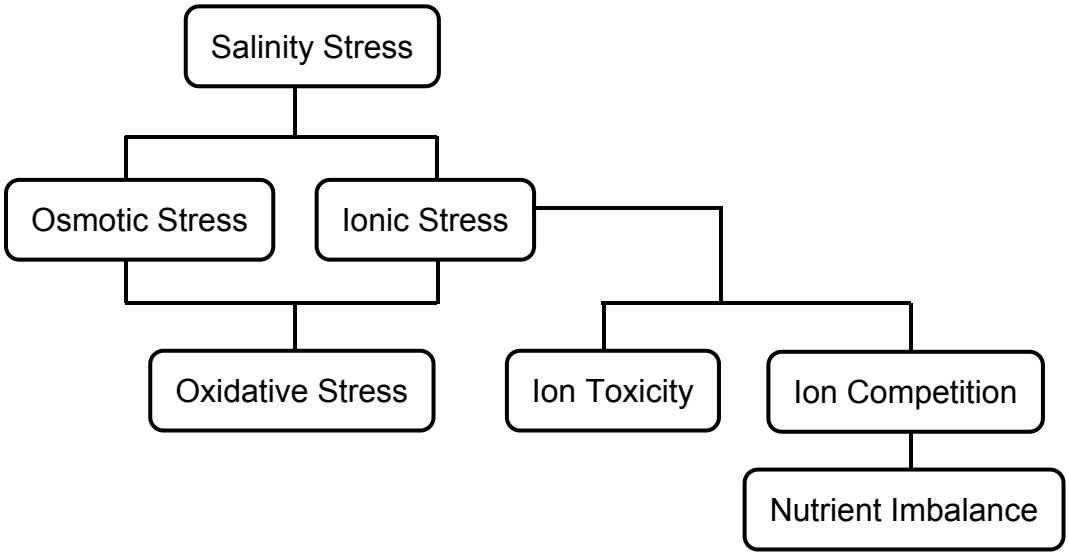


Figure 2.1. Diagram illustrating the relationships between the components of salinity stress. Salinity stress includes the effects of both osmotic and ionic stresses. The osmotic and ionic stresses cause disturbances in energy production pathways resulting in oxidative stress. The effects of ionic stress on the plant can be separated into the direct effects of Na^+ and Cl^- (ion toxicity) and the effects from the competition of these ions with the essential minerals resulting in nutrient imbalance.

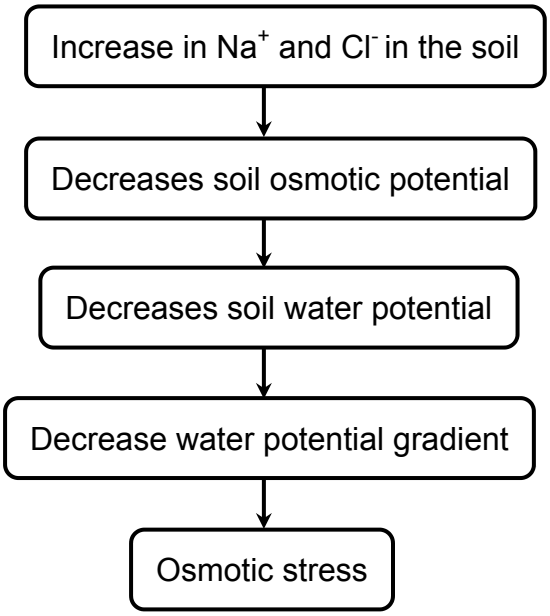


Figure 2.2. Flow diagram illustrating the effect of the addition of NaCl to the soil. The addition of NaCl to the soil increases the solute concentration of the soil, which decreases the osmotic potential of the soil. This results in the decrease in the water potential of the soil; therefore, the water potential gradient between the soil and the roots is reduced. The reduction in the driving force for water uptake causes the turgor pressure in the cells of the leaves and roots to be reduced and osmotic stress occurs.

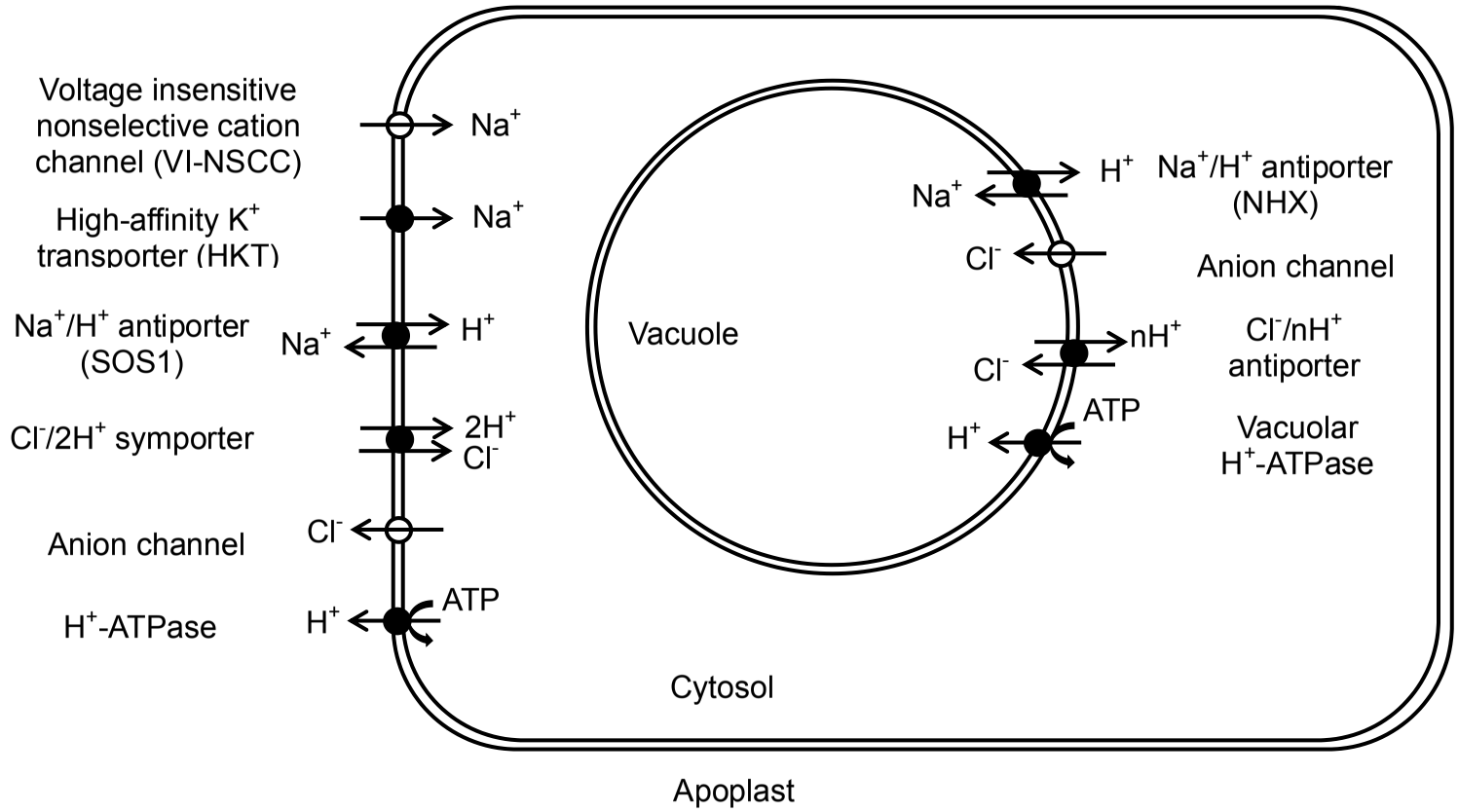


Figure 2.3. Potential transporters and channels involved in Na^+ and Cl^- transport through the plasma membrane and tonoplast.

Chapter 3 THE SALINITY TOLERANCE OF RED-OSIER DOGWOOD (*CORNUS SERICEA*) FROM THREE SITES WITH CONTRASTING EDAPHIC CONDITIONS IN SOUTHEASTERN MANITOBA.

3.1 INTRODUCTION

Red-osier dogwood is a cold tolerant woody shrub with a wide natural distribution in North America (Smithberg and Weiser, 1968; Crane, 1989; Johnson *et al*., 1995), which could expose red-osier dogwood to a wide variety of environmental conditions. It has been observed that red-osier dogwood from northern and southern provenances exhibited different growth rates that were maintained even when the plants were grown under the same conditions (Smithberg and Weiser, 1968). This suggests that red-osier dogwood is able to adapt to the environmental conditions (length of growing season and temperature) of the provenance. It has also been found that red-osier dogwood seedlings from relatively colder or drier provenances have a greater tolerance to moderate salinity stress than the seedlings from warmer and wetter provenance (Renault, Personal Communication), which suggests that cross-tolerance can occur in red-osier dogwood. Cross-tolerance occurs when an initial stress induces the modification of gene expression, transporters and/or metabolic processes through stress response pathways that will contribute to the tolerance of a different stress (Wang *et al*., 2003; Cayuela *et al*., 2007; Tuteja, 2007). This may take place more readily between salinity, drought, and cold stress since these three stresses share the osmotic stress and would therefore induce similar stress response pathways (Roelof *et al*., 2008).

The conditions required for cross-tolerance to occur are not well understood. Studies have reported the occurrence of cross-tolerance after exposure to a controlled and short pretreatment of heat shock or drought before exposure to saline conditions in maize (*Zea mays*, Gong *et al*., 2001) and tomato (*Solanum lycopersicum*, Cayuela *et al*., 2007), respectively. A few studies have also shown that a naturally stressful environment increases the salinity tolerance of a species compared with those from a relatively less stressful environment (Nguyen *et al*., 2004; Marcar *et al*., 2002; Megdiche *et al*., 2007). Cross-tolerance may be a factor to consider when selecting the provenance or the growth conditions of species used in land reclamation. Red-osier dogwood have been found growing in habitats ranging from bogs to relatively dry forest stands that would differ in the degree of stress. It is not known if the differences in the edaphic conditions would be sufficient to result in adaptation (genetic changes) that would increase the plants ability to tolerate salinity stress or if only an acclimation response was induced.

The objective of this experiment was to compare the salinity tolerance of red-osier dogwood cuttings collected from three selected sites varying in edaphic conditions by measuring gas exchange, injury, biomass, and ion accumulation after exposure to NaCl treatments. I hypothesized that the cuttings from the drier site would have greater salinity tolerance; as a result of their adaptation to a more stressful environment inducing the activation of a protective mechanism (cross-tolerance) thereby increasing their tolerance to salt stress.

3.2 Material and Methods

3.2.1 Plant material

Red-osier dogwood (*Cornus sericea* L. syn. *Cornus stolonifera* Michx) cuttings were collected from three sites in southeastern Manitoba differing in edaphic conditions. Site 1, the moist site, was approximately 119 km east of Winnipeg off of Hwy 1 (N 49° 38' 29.4", W 95° 29' 53.5"). The red-osier dogwood at this site were large compared to the other selected sites and had abundant growth (Fig. 3.1A). The red-osier dogwoods were growing along a gravel access road, at a lower elevation with a stream nearby (Fig. 3.2A). Site 2, the dry site, was approximately 112 km east of Winnipeg off of Hwy 1 (N 49° 38' 57.4", W 95° 35' 41.0"). The red-osier dogwoods appeared to be shorter and have less biomass than site 1 (Fig. 3.2B). These dogwoods were growing in a jack pine stand at a higher elevation than the access road (Fig. 3.1B). Site 3, the wet site, was 74.6 km south east of Winnipeg, in the Sandilands Provincial Forest (N 49° 27' 58.7", W 96° 14' 27.1"). The red-osier dogwoods appeared to be shorter than those growing in the site 1 and 2 but they had abundant lateral growth (Fig. 3.2C). Site 3 was a neutral pH bog dominated by short shrubs and grasses (Fig. 3.1C).

Vegetative characteristics of the red-osier dogwood from each of the three sites were measured. At site 1, five plants were selected along a 200 m long transect running parallel to the access road (3 m from the road). Plants were selected every 40 m. At site 2, two 100 m long transect lines were made parallel to the access road (7 m and 15 m from the road). The five selected red-osier dogwood plants were staggered along the two transect lines and were 20 m apart. At site 3, the five plants were 40 m apart and selected from the north, south, east, and west corners and also the middle of the site. The height of

the red-osier dogwood plants was measured from the soil to tip of the tallest branch. Five shoots were selected and the number of nodes from the tip to 25 cm was recorded. The average nodal length was determined by dividing the length of the branch (25 cm) by the number of nodes. The branch diameter below the first pair of mature leaves was also determined with digital calipers. The youngest pair of mature leaves was collected from each branch and measured with a leaf area meter (LI-COR, Nebraska, USA) to determine the average leaf area of the plant at each site. The leaves were then weighed, dried in a 65°C oven for 4 days and reweighed for dry weight determination. The specific leaf area (SLA, leaf area per unit of dry mass) and the leaf water content (Eq. 1) were determined.

$$\text{Water Content (\%)} = ((\text{Fresh Weight} - \text{Dry Weight}) / \text{Dry Weight}) * 100 \quad \text{(Eq. 1)}$$

3.2.2 SOIL SAMPLING

Soil samples were taken next to the red-osier dogwood plants selected for vegetative characteristics measurements at sites 1 and 2. At site 1, a 60 cm deep soil profile was dug at each sampling spot. A 250 mL Kubiena box was pushed into the soil profile as gently as possible to prevent compaction of the soil at 15 and 40 cm from the soil surface. The Kubiena box was carefully removed and trimmed to have sampled a 250 mL volume of soil (Fig. 3.3) for bulk density measurements. Two samples were taken from each depth at each of the five sampling spots for a total of 20 soil samples from site 1. At site 2, a 60 cm soil profile was dug, but a large coarse fraction component (>2 mm) did not make it possible to push the Kubiena box into the soil profile. For that reason, soil samples were dug out of the soil profile at the 15 and 40 cm depth. No soil samples were

taken at site 3, as it was a bog site. Instead, three water samples were collected from the surface water near each of the five red-osier dogwood plants measured for vegetative characteristics.

3.2.3 Soil Analysis

The protocols for gravimetric water content and bulk density were slightly different between site 1 and 2, due to the difference in sampling techniques. Therefore, the soil analysis protocols for each site will be described separately.

Soil samples from site 1, were weighed to determine the fresh weight of the 250 mL soil samples. To determine the bulk density of the of the fresh soil sample, approximately 10 g (fresh weight) was taken from each soil sample and placed in an oven at 105°C for 30 h and then re-weighed to obtain the dry weight (Blake and Hartge, 1986). The gravimetric water content was then calculated for the 10 g soil subsample using Eq. 2 (Gardner, 1986).

$$\text{Gravimetric Water Content} = (\text{Fresh Weight} - \text{Dry Weight}) / \text{Dry Weight} \quad \text{(Eq. 2)}$$

To avoid drying the total 250 mL soil sample, the gravimetric water content was then used to determine the dry weight from site 1, which was needed to determine bulk density (Eq. 3).

$$\text{Dry Weight} = \text{Fresh Weight} / (1 + \text{Gravimetric Water Content}) \quad \text{(Eq. 3)}$$

The bulk density was determined with the calculated oven-dry weight of the 250 mL soil sample (Eq. 4).

Bulk Density = Dry Weight / Volume of the Kubiena Box (Eq. 4)

The remaining soil samples were air-dried and sieved through a 2 mm sieve to be used for further analysis.

For site 2, the gravimetric water content of the fresh soil sample was determined from a 10 g subsample, as described for site 1 (Eq. 2). The remaining soil samples from sites 2 were air-dried and sieved through a 2 mm sieve to obtain the fine fraction (< 2 mm). The sieved samples were then used to fill the Kubiena box. The soil required to fill the Kubiena box was weighed for 'fresh' weight. A 10 g subsample of air-dried samples was used to determine the gravimetric water content of the air-dried sample (Eq. 2). The gravimetric water content was then used to determine the oven-dry weight of the 250 mL fine fraction soil sample (Eq. 3). This calculated oven-dry weight was later used to determine the bulk density of the fine fraction soil sample from site 2 (Eq. 4), which represented the maximum bulk density of the soil.

For pH and electrical conductivity measurements, a 5 g air-dried sample from each of the two samples from each depth and location was combined for sites 1 and 2. To each sample, 50 mL distilled water was added to a 1:5 (W:V) ratio and placed on a shaker for 1 h at room temperature (Kalra and Maynard, 1991). For site 3, the three water samples from the five sampling locations were analyzed. The samples from all three sites were then filtered through a Whatman #1 filter paper in a Buchner funnel under suction. The pH and electrical conductivity of the filtrate were immediately measured with a pH

meter (Orion 3-Star, Thermo Scientific, Beverly, Massachusetts USA) and with an electrical conductivity meter (Orion 3-Star, Thermo Scientific, Beverly, Massachusetts USA), respectively.

3.2.4 PLANT GROWTH

Red-osier dogwood cuttings were collected from the three selected sites in September 2007. The cuttings were taken from healthy branches with minimal lateral branch development and a length of at least 15 cm to obtain at least 3 nodes. If the collected cuttings had short internodes the cuttings were trimmed to a length of 13 cm. The leaves of the red-osier dogwood cuttings were removed and 2 cm of the stem, which included one node, was planted in a peat/sand (3:1, V:V) mixture in a 650 mL plug. The cuttings were grown in a greenhouse with a temperature of 22 ± 5°C and photoperiod of 16 hours that was supplemented with high-pressure sodium lamps (Philips Lighting Company, Somerset, NJ). After the cuttings had rooted, they were fertilized every four weeks with N/P/K (20-20-20) fertilizer (Plant-prod®, Sure-Gro IP Inc, Brantford, ON, Canada) at a rate of 1.2 mL L^{-1} water.

After growing in the peat/sand mixture for six months to allow sufficient root growth, the red-osier dogwood cuttings (6-9" tall) were randomly selected and transferred to containers containing 10 L of ½ strength modified Hoagland's solution: 0.5 mM KH_2PO_4; 2.5 mM KNO_3; 2.5 mM Ca $(NO_3)_2$*$4H_2O$; 1 mM $MgSO_4$; 0.023 mM H_3BO_3; 0.005 mM $MnCl_2$*$4H_2O$; 0.0004 mM $ZnSO_4$*$7H_2O$; 0.0002 mM $CuSO_4$*$5H_2O$; 0.00007 mM H_2MoO_4*H_2O; 0.007 mM Fe-EDTA. The solutions were aerated using irrigation

tubing and aquarium pumps, with one pump irrigating two 12 L containers (Fig. 3.4). Within each container (one replicate), six cuttings were transferred with two cutting from each site.

3.2.5 TREATMENTS

The red-osier dogwood cuttings were acclimated to hydroponics for one week. After the acclimation period, the red-osier dogwood cuttings were treated with 0 mM NaCl (121 mS m^{-1}), 25 mM NaCl (410 mS m^{-1}), 50 mM NaCl (700 mS m^{-1}), or 100 mM NaCl (1230 mS m^{-1}) for four weeks. The NaCl was added in 25 mM increments in the morning and evening until the final concentration was reached to reduce injury caused by rapid exposure to NaCl. Each treatment had six replicates with two plants from each site. The nutrient solution was initially replaced once a week for the first week of treatment, then twice a week for the remaining three weeks of treatment to maintain nutrient solution levels.

3.2.6 PHOTOSYNTHESIS, TRANSPIRATION, AND STOMATAL CONDUCTANCE

The photosynthetic rate, stomatal conductance, and transpiration rate of the youngest mature leaf were measured weekly between 10:00 and 14:00 following exposure to salinity with an Infrared Gas Analyzer (IRGA) (Model Li-1600, LI-COR, Nebraska, USA). The IRGA was programmed with a quantum flux of 500 µmoles m^{-2} s^{-1}, a flow rate of 400 µmoles s^{-1}, and a reference CO_2 of 400 µmoles s^{-1}, which were determined from running a light response curve. When the measured leaf did not

completely fill the IRGA leaf chamber, the leaf was traced and the area of the leaf was measured with a leaf area meter (LI-COR, Nebraska, USA). The photosynthetic rate, stomatal conductance, and transpiration rate were recalculated with the corrected leaf area. The water-use efficiency (%) was calculated by dividing the photosynthetic rate by the transpiration rate.

3.2.7 GROWTH AND INJURY

The lengths of all main and lateral stems were measured at the start of the NaCl treatment and again at harvest, after four weeks of treatment. Injury was also estimated with the following injury index:

0 = no symptoms of injury

1= < 25% injury

2 = 25-49% injury

3 = 50-75% injury

4 = > 75% injury

5 = death

The red-osier dogwood cuttings were then washed three times with distilled water and separated into roots, stems, and leaves. The plant tissues were weighed and then freeze-dried for three days to obtain the dry weight.

3.2.8 ELEMENTAL ANALYSIS

The freeze-dried plant tissues were ground for ion analysis. To measure the Cl^- concentration, subsamples of 0.025 g from each of the two plants from the same site in each replicate were combined. Ten mL of 0.5 M HNO_3 was added to the ground tissues and incubated for 30 min on a shaker (Rieger and Litvin, 1998; Renault and Affifi, 2009). After the 30 min, 200 μL of ISA (Ionic Standard Adjuster, 5 M $NaNO_3$) was added and the Cl^- content was measured after 1 min with a Cl^- selective electrode (Accumet, Fisher Scientific). For the Na^+, K^+, Mg^{2+}, and Ca^{2+} concentration, the two freeze-dried plant samples from each of the first four replicates were subsampled (0.25 g from each sample) and combined. The tissue samples were sent to Activation Laboratories Ltd. (Ancaster, Ontario, Canada) for cation analysis. The tissues were ashed and digested with HNO_3-H_2O_2 and then analyzed with ICP/MS (Inductively Coupled Plasma - Mass Spectrometry). Quality control included analysis of blanks, internal replicates, and standard reference materials.

3.2.9 DATA ANALYSIS

Data were analyzed using a two-way Analysis of Variance (ANOVA) (Appendix B). There was no significant interaction between the three selected sites and the NaCl treatments at $\alpha = 0.05$ (Tables A.1 – A.25). Therefore, sites within each treatment were compiled and analyzed using a one-way ANOVA. The means were compared using Tukey-Kramer HSD, and were considered to be significantly different at $\alpha = 0.05$. To determine if the vegetative characteristics and plant water content differed between the

cuttings of the three sites in absence of NaCl, the control cuttings were analyzed separately using a one-way ANOVA. The data analyses were performed using JMP 8.0.1 (SAS Institute Inc., Cary, NC).

Figure 3.1. Three selected sites in southeastern Manitoba where red-osier dogwood cuttings and soil samples were collected. (A) site 1; (B) site 2; (C) site 3.

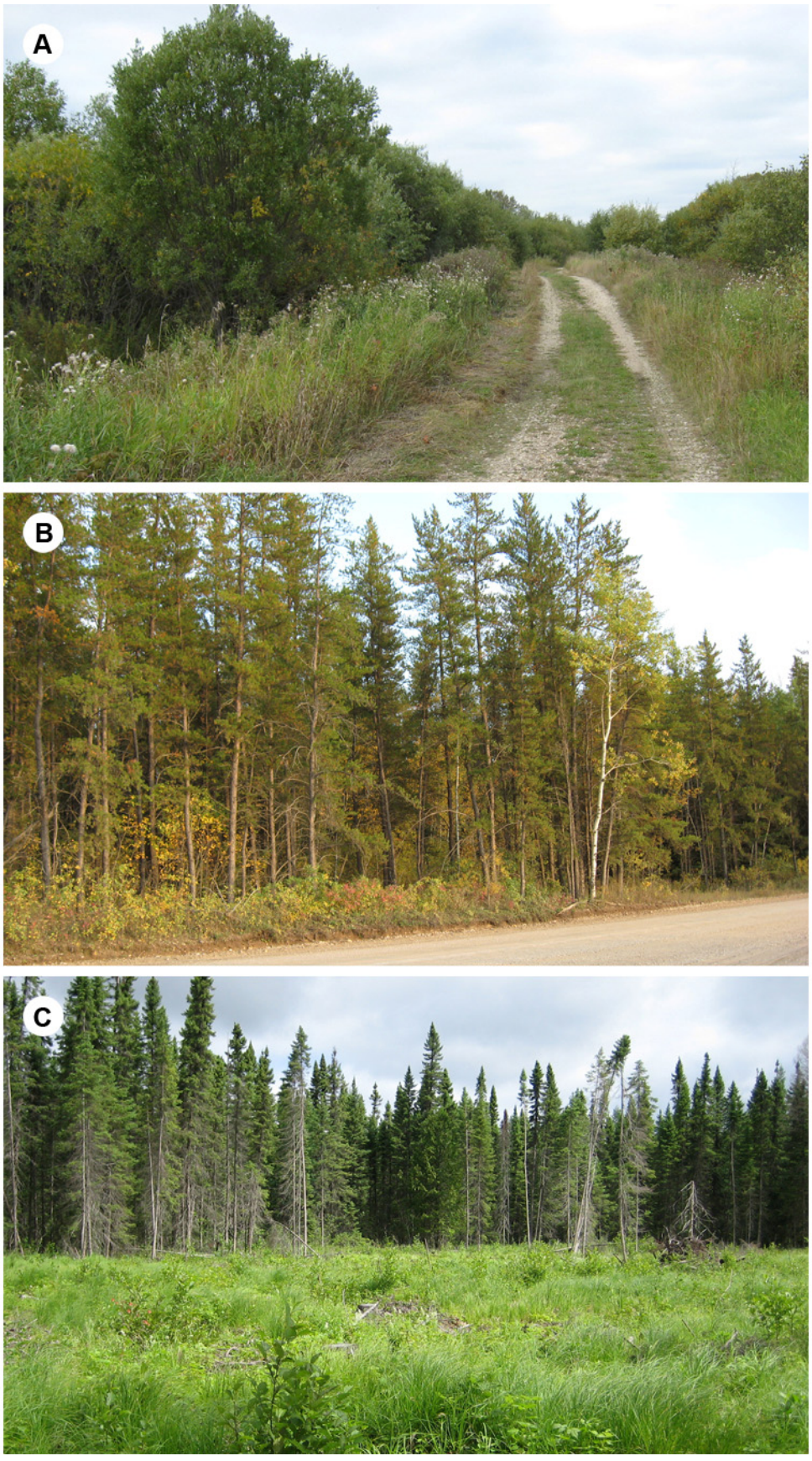

A
B
C

Figure 3.2. Red-osier dogwood plant from site 1 (A), site 2 (B), and site 3 (C) from which cuttings and measurements were made to determine differences in growth between sites. Scale bars = 15 cm.

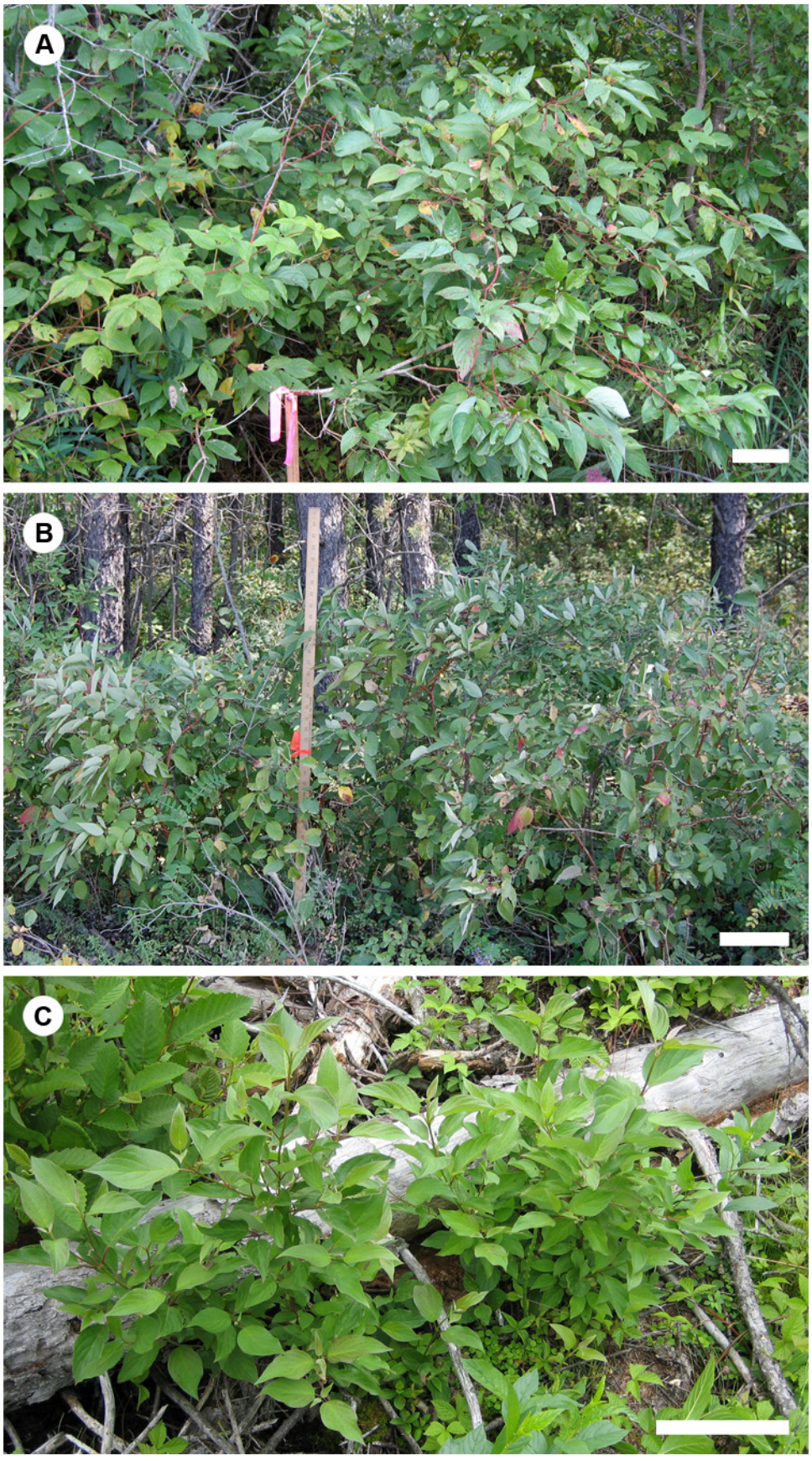

A
B
C

Figure 3.3. A 250 mL Kubiena box containing soil sampled from a site 1 soil profile at a depth of 15 cm. The Kubiena box would then be trimmed to remove excess soil and the soil was collected for bulk density analysis. Scale bar = 1 cm.

Figure 3.4. Greenhouse hydroponic setup to expose red-osier dogwood cuttings to 0, 25, 50, or 100 mM NaCl for four weeks.

3.3 Results

3.3.1 Field Measurements

3.3.1.1 Growth parameters

The red-osier dogwoods from site 1 were on average 0.9 m and 1.2 m taller than the red-osier dogwoods from sites 2 and 3 respectively (Table 3.1). The average internodal lengths within 25 cm from the branch tip at sites 1 and 3 were more than double the length found at site 2, but no significant differences were found in stem thickness measured below the first mature leaves between the three sites. The red-osier dogwood leaves from site 1 had greater leaf area than the ones from sites 2 (69.6%) and 3 (77.6%). The leaf dry weight at site 1 was also greater than at site 2, but not significantly different between sites 1 and 3. This resulted in a greater specific leaf area of red-osier dogwood at sites 1 and 2 than at site 3, suggesting that leaves from site 3 were thicker than the ones from sites 1 and 2. The water content was also significantly lower in the leaves at site 1 (18.4%) and site 2 (12.8%) than for site 3.

3.3.1.2 Soil Analysis

The three selected sites had very different soil profiles (Fig. 3.5). Site 1 had a thick organic layer with silt, sand, and clay content increasing with depth (Fig. 3.5A). Site 2 had relatively homogenous sand and gravel composition with a thin organic layer at the soil surface and pockets of organic material deposited through the soil profile (Fig. 3.5B). Site 3 was waterlogged and the soil was composed of peat (Fig. 3.5C).

Site 1 had a slightly lower pH than sites 2 and 3, although, all pH values were close to a neutral pH (6.2 – 8.0) (Table 3.2). The sites were not considered to be saline as the electrical conductivities were less than 4000 μS cm^{-1}. The electrical conductivity was the highest in the soil from site 1 at 15 cm (684.5 – 2105.0 μS cm^{-1}) and the lowest in the water samples from site 3 (262.2 – 435.0 μS cm^{-1}). The electrical conductivity decreased at 40 cm for site 1, while the opposite was observed at site 2. The gravimetric water content was the highest at 15 cm at site 1(0.57 – 3.21 W/W), but decreased considerably at 40 cm (0.19 – 0.77 W/W). At one soil sampling location in site 1, water began to enter the hole excavated to a depth of 80 cm indicating a shallow water table (Fig. 3.5A). The gravimetric water content of soil from site 2 was similar at both 15 and 40 cm depths (0.05 – 0.09 W/W). The bulk density was the lowest at site 1 at 15 cm (0.20 – 0.87 g cm^{-3}) but increased with depth (0.73 – 1.61 g cm^{-3}).

3.3.2 Greenhouse Experiment

3.3.2.1 Growth, Injury, and Water Content

Red-osier dogwood cuttings collected from the three sites and growing in hydroponics without NaCl treatment for four weeks did not significantly differ in their vegetative characteristics or water content (Appendix A). The one exception was observed with the root dry weight of cuttings from site 2 was less than for cuttings from site 3 (Table A.1). My results show that after four weeks of NaCl treatment there was no significant interaction between the three sites and the NaCl treatments (Appendix A); therefore, the data from the three sites were combined to determine the effect of NaCl on

red-osier dogwood cuttings from southeastern Manitoba. Within each site, the growth response of red-osier dogwood cuttings to the NaCl treatments was highly variable (Fig. 3.6 – 3.8). The control cuttings exhibited a similar biomass pattern as the 25 mM NaCl treated cuttings within each replicate (Fig. 3.6 – 3.8).

The NaCl induced injury (necrosis, chlorosis, and leaf margin curling, Fig. 3.9A, B) was first observed in the oldest mature leaves and later in the younger leaves (Fig. 3.10). The level of injury was significantly greater in the 25 and 50 mM NaCl treated cuttings than the controls, with no significant difference between the two treatments (Table 3.3). The greatest NaCl induced injury was observed in cuttings exposed to the 100 mM NaCl treatment. Necrosis of immature leaves and apical meristems, along with greater root death was observed in a few of the red-osier dogwood cuttings exposed to 100 mM NaCl (Fig. 3.6; 3.7E). Conversely, other 100 mM NaCl treated cuttings had relatively little leaf necrosis (Fig. 3.9C, E).

The root and shoot biomass of the 25 mM and 50 mM NaCl treated red-osier dogwood cuttings were not significantly affected after four weeks of treatment, but the root/shoot ratio was significantly increased (Table 3.3, Fig. 3.10). The stem dry weight was reduced in the 25 mM NaCl treated cuttings by 27.6% and a reduction of 57.8% and 64.4% in stem length was found with the 25 and 50 mM NaCl treatments, respectively. There was no significant effect of 25 and 50 mM NaCl on the water content of the red-osier dogwood cuttings. A significant reduction in the number of leaves was found with 25 mM NaCl (29.7%) and with 50 mM NaCl (30.2%), but there was no significant decrease in leaf dry weight. The 100 mM NaCl treatment severely affected the growth of red-osier dogwood by reducing the shoot and root biomass and decreasing the water

content of the plant. The shoots of the 100 mM NaCl treated cuttings were more affected than the roots as seen with the increase in the root/shoot ratio (30.1%). In addition, stem elongation after four weeks of 100 mM treatment was reduced by 93.4%.

Root growth correlated poorly with shoot growth (dry weight basis) after treatment with 50 and 100 mM NaCl (r^2 = 0.47 and 0.45, respectively). The cuttings exposed to 100 mM NaCl exhibiting lower level of stress (less injury and less shoot biomass reduction) showed a high variability in root growth. Some of the less stressed red-osier dogwood treated with 100 mM NaCl showed an increase in root diameter towards the tip (Fig. 3.9D, F). In contrast, the 0, 25, and 50 mM NaCl treated cuttings did not appear to have thickening of the distal portion of the roots relative to the older root tissue.

3.3.2.2 TRANSPIRATION, STOMATAL CONDUCTANCE, PHOTOSYNTHESIS, AND WUE

The stomatal conductance and transpiration rate of red-osier dogwood cuttings treated with 25 and 50 mM NaCl followed a similar trend (Fig. 3.11). Three weeks of treatment with 25 and 50 mM NaCl resulted in a significant reduction in the stomatal conductance and transpiration rate of red-osier dogwood. After the four weeks of 25 and 50 mM NaCl treatment, the transpiration rate was reduced by 22.2% and 42.6% respectively. The photosynthetic rate was less affected by NaCl than the transpiration rate with only the red-osier dogwood treated with 100 mM NaCl showing a significant reduction (Fig. 3.11B, 3.12A) The 100 mM NaCl treatment also resulted in a severe reduction in the stomatal conductance and transpiration rate after only one week and a reduction of 75.8% was observed after four weeks of treatment. The water-use efficiency

(WUE) was not significantly affected by the NaCl treatments with the exception of a 33.7% increase in WUE after four weeks of treatment with 50 mM NaCl (Fig. 3.12B).

3.3.2.3 TISSUE ION CONTENT

The Cl^- and Na^+ content of red-osier dogwood generally increased in root, stems, and leaves with increasing NaCl concentration (Fig. 3.13A, B). However, the Na^+ concentration was higher in the roots than the leaves up to 50 mM NaCl. With exposure to 100 mM NaCl, the Na^+ content was similar in the roots and the leaves. In contrast to the Na^+ accumulation, the Cl^- content was higher in the leaves than the roots. In addition, the increase in Cl^- content in the roots was similar with all NaCl treatments (11.2 – 15.9 mg g^{-1} DW).

The K^+ concentration appeared to be greater in the roots than the leaves of red-osier dogwood cuttings under control conditions. Conversely, the K^+ content decreased in the roots and stems and increased in the leaves with exposure to NaCl (Fig. 3.13C). The 50 and 100 mM NaCl treatments resulted in the greatest decrease in K^+ with a reduction of 56.6 and 75.5%, respectively, in the roots and the largest increase in the leaves (95.9 and 70.5%), respectively. However, the decrease in K^+ content in the stem with exposure to NaCl was not significantly different between treatments. The limited transport of Na^+ to the leaves and increased accumulation of K^+ in the leaves with exposure to 25 and 50 mM NaCl resulted in a higher Na^+/K^+ ratio in the leaves than the roots and stems (Fig. 3.14A).

In contrast to K^+, the greatest Ca^{2+} content was observed in the leaves (Fig. 3.14B). In addition, NaCl did not change the Ca^{2+} content in the roots, stems, and leaves to the same extent as found with K^+. The Ca^{2+} content was only significantly reduced in the roots by 17.6, 26.0, and 46.3% with 25, 50, and 100 mM NaCl respectively.

The Mg^+ content of red-osier dogwood tissues was not modified by treatment with 25 and 50 mM NaCl. Conversely, the Mg^+ content was significantly reduce by 38.4, 59.0, and 38.7% in the roots, stems, and leaves, respectively, after four weeks of 100 mM NaCl treatment (Fig. 3.14C).

Table 3.1. Vegetative characteristics of red-osier dogwood from the three selected sites. Different letters adjacent to the mean ± SE values indicate significant difference between treatments at $\alpha = 0.05$ with each growth parameter analyzed separately (n = 5).

Growth parameter	Site		
	1	2	3
Height (m)	2.21 ± 0.08^{a}	1.31 ± 0.13^{b}	0.98 ± 0.08^{b}
Number of nodes*	5.6 ± 1.1^{b}	11.2 ± 0.9^{a}	4.1 ± 0.5^{b}
Internodal length (cm)*	5.67 ± 1.07^{a}	2.75 ± 0.41^{b}	6.63 ± 0.91^{a}
Branch diameter (mm)**	2.21 ± 0.05^{a}	1.99 ± 0.06^{a}	2.10 ± 0.15^{a}
Leaf area (cm^2)	44.45 ± 3.02^{a}	26.21 ± 2.92^{b}	25.03 ± 4.60^{b}
Leaf dry weight (g)	0.24 ± 0.02^{a}	0.15 ± 0.02^{b}	0.21 ± 0.04^{ab}
Specific leaf area ($m^2\ kg^{-1}$ DW)	18.65 ± 0.72^{a}	17.55 ± 0.82^{a}	12.14 ± 0.55^{b}
Leaf water content ($g\ g^{-1}$ DW)	1.63 ± 0.07^{a}	1.53 ± 0.03^{a}	1.33 ± 0.02^{b}

* Measurements taken from within 25 cm from the first mature leaves.

** Measurements taken below the first mature leaves.

Figure 3.5. The 60 cm deep soil profiles excavated near a red-osier dogwood plant at site 1 (A) and site 2 (B) from which the soil samples were obtained. At site 3 (C), which was a bog, water samples were obtained from the surface water near a red-osier dogwood plant. Scale bar: C = 10 cm.

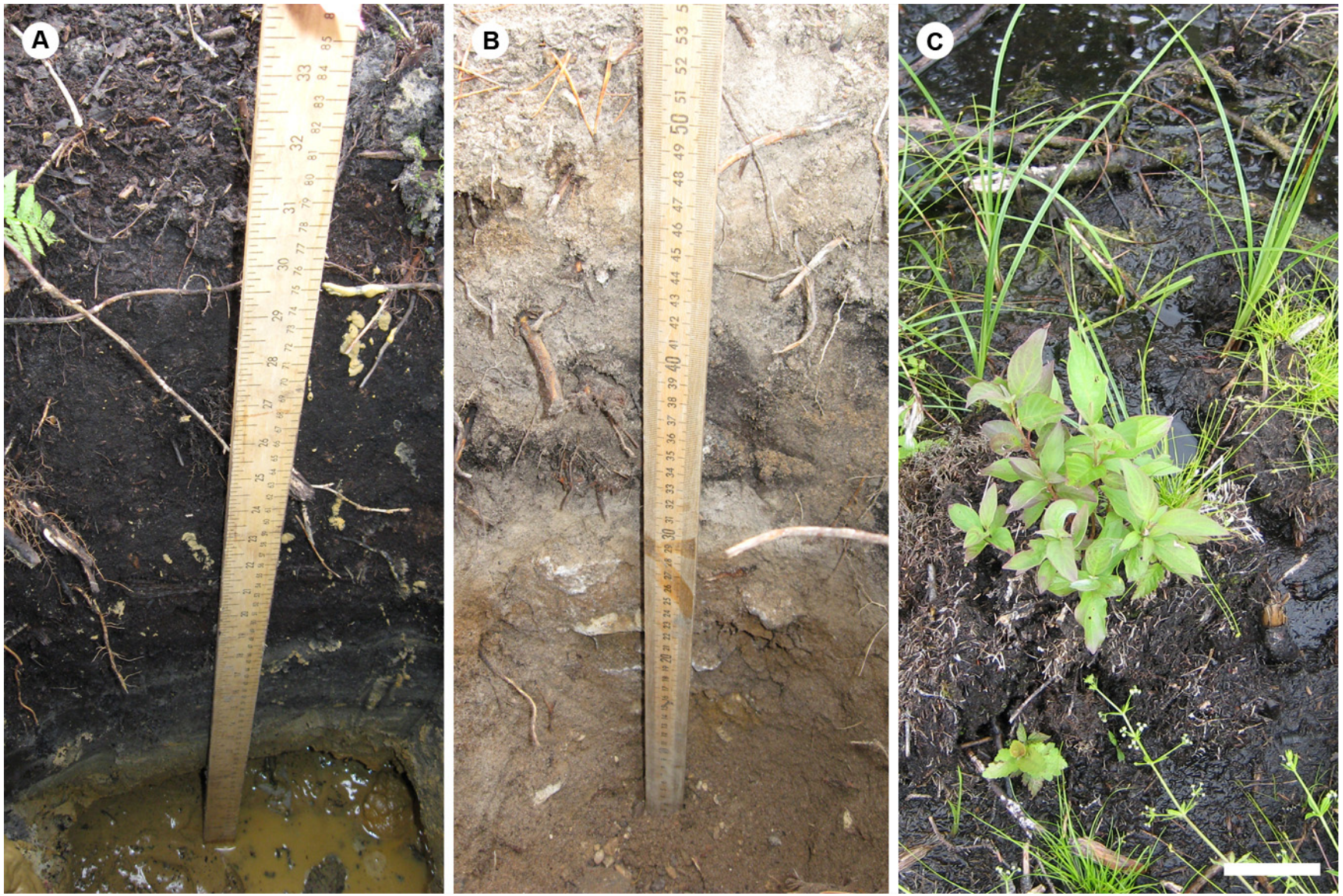

A
B
C

Table 3.2. Geochemical and physical properties of the soil collected from the three sites at 15 and 40 cm depths* (n = 5).

Depth (cm)	Site	pH	Electrical conductivity ($\mu S\ cm^{-1}$)	Gravimetric water content (W/W)	Bulk density ($g\ cm^{-3}$)**
15					
	1	6.2 – 7.0	684.5 – 2105.0	0.57 – 3.21	0.20 – 0.87
	2	7.1 – 7.9	346.5 – 618.0	0.05 – 0.09	1.05 – 1.23
	3	7.2 – 7.8	262.2 – 435.0	---	---
40					
	1	6.5 – 7.9	278.0 – 740.0	0.19 – 0.77	0.73 – 1.61
	2	7.5 – 8.0	549.5 – 646.5	0.05 – 0.07	1.23 – 1.43
	3	---	---	---	---

* Water samples were collected from site 3

** Bulk density was measured for only the fine fraction of the site 2 soil sample

--- denotes data not available

Figure 3.6. The extreme responses of red-osier dogwood cuttings from site 1 to four weeks of treatment with 25 (A, B), 50 (C, D), and 100 (E, F) mM NaCl. A, C, and E are the cuttings with the least growth and B, D, and F are the cuttings with the greatest growth. Scale bars = 5 cm.

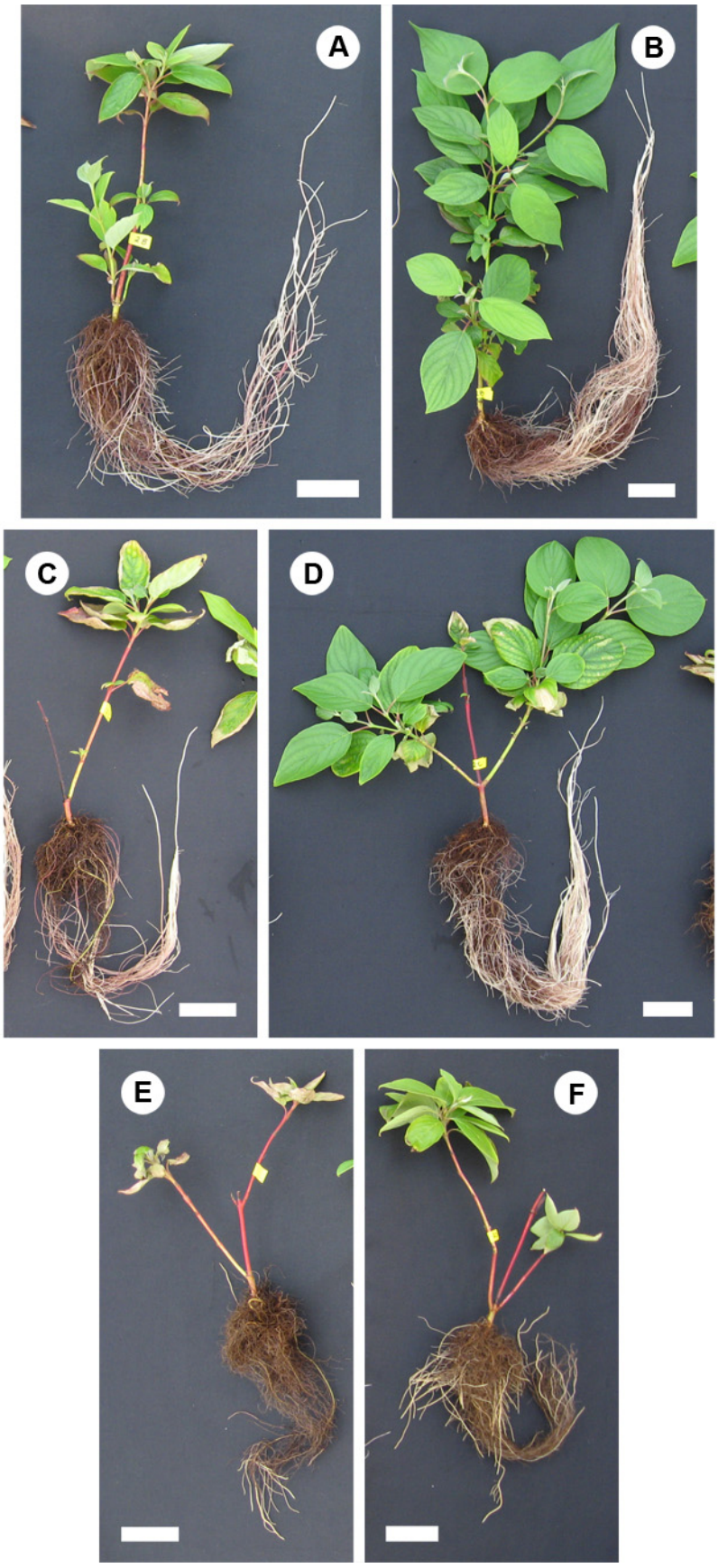

A
B
C
D
E
F

Figure 3.7. The extreme responses of red-osier dogwood cuttings from site 2 to four weeks of treatment with 25 (A, B), 50 (C, D), and 100 (E, F) mM NaCl. A, C, and E are the cuttings with the least growth and B, D, and F are the cuttings with the greatest growth. Scale bars = 5 cm.


A
B
C
D
E
F

Figure 3.8. The extreme responses of red-osier dogwood cuttings from site 3 to four weeks of treatment with 25 (A, B), 50 (C, D), and 100 (E, F) mM NaCl. A, C, and E are the cuttings with the least growth and B, D, and F are the cuttings with the greatest growth. Scale bars = 5 cm.

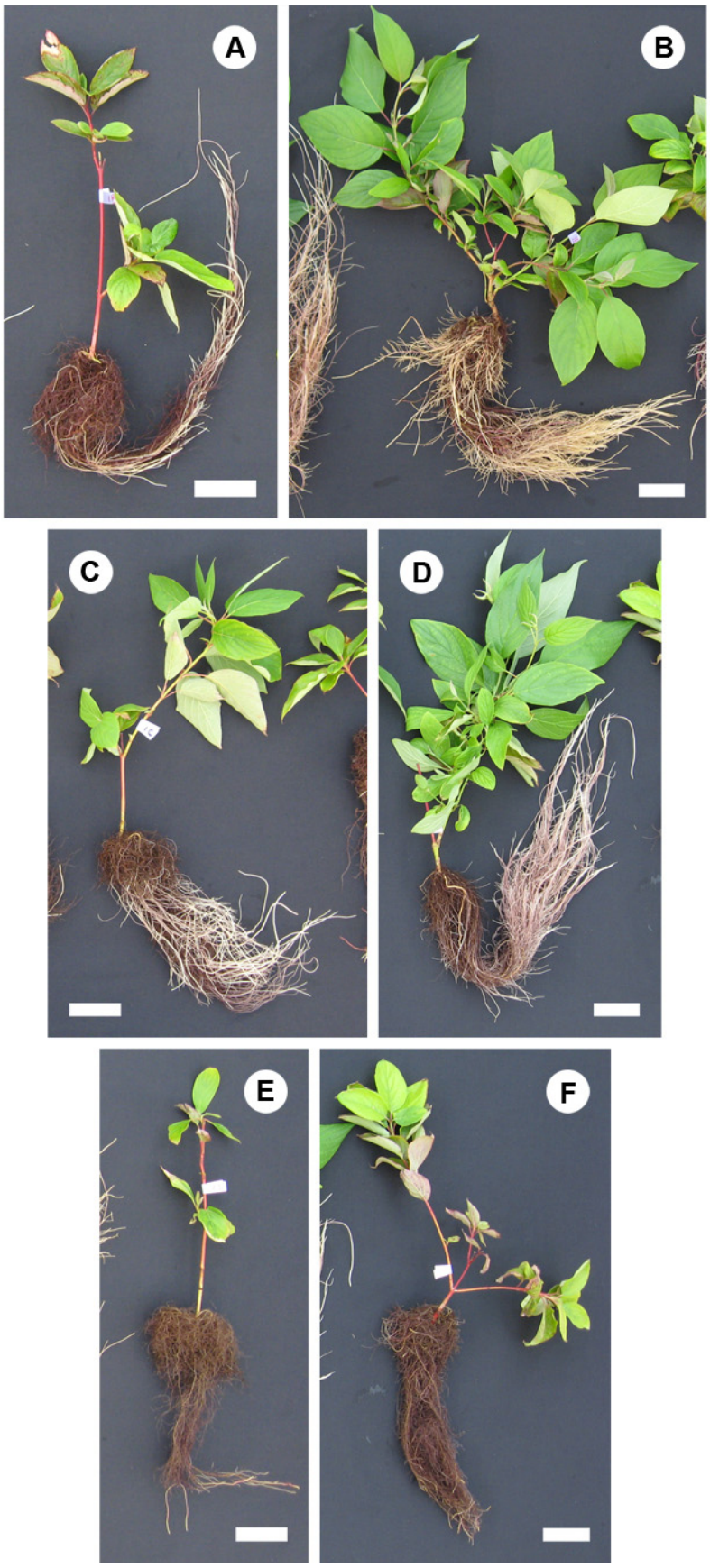

A
B
C
D
E
F

Figure 3.9. (A) Necrosis of red-osier dogwood leaf after four weeks of 50 mM NaCl treatment. (B) Leaf necrosis after four weeks of 100 mM NaCl treatment. (C) A 100 mM NaCl treated red-osier dogwood cutting with 3 – 5 cm of new root growth and exhibited a gradual increase in root diameter with proximity to the root tip. This cutting had moderate leaf injury and average shoot biomass. (D) Magnification of roots shown in C. (E) A red-osier dogwood exposed to 100 mM NaCl with approximately 1 cm of new root growth that increase in diameter with proximity to the root tip. This cutting had minimal leaf injury and above average shoot biomass. (F) Magnification of roots shown in E. Arrows indicate transition zone from the relatively thinner proximal portion of the root to the thicker distal portion of the root. Scale bars = (A, B) = 2 cm; (C, E) = 5 cm; (D) = 1 cm; (F) = 0.25 cm.

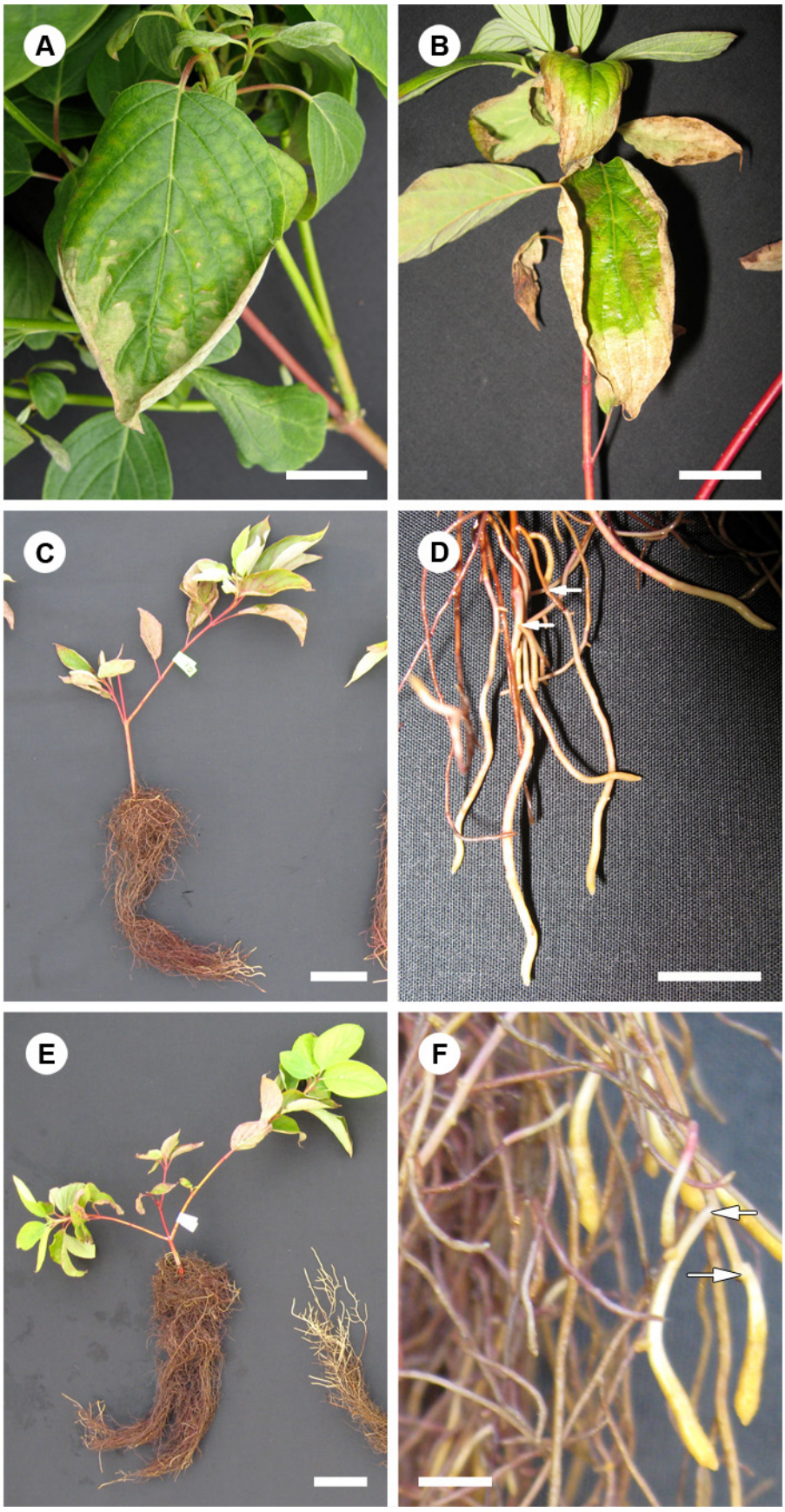

A
B
C
D
E
F

Figure 3.10. Red-osier dogwood cuttings from site 1 (1), site 2 (2), and site 3 (3) after four weeks of 0 (A), 25 (B), 50 (C), or 100 (D) mM NaCl treatment. Scale bars = 10 cm.


A
1
2
3
B
1
2
3
1
2
3
C
1
2
3
D

Table 3.3. Growth characteristics of red-osier dogwood after four weeks of treatment with 0, 25, 50, and 100 mM NaCl. Different letters adjacent to the mean ± SE values indicate significant difference between treatments at $\alpha = 0.05$ with each growth parameter analyzed separately (n = 6).

Growth parameter	NaCl treatment (mM)			
	0	25	50	100
Root dry weight (g)	2.99 ± 0.14^{a}	2.83 ± 0.27^{a}	2.96 ± 0.12^{a}	1.88 ± 0.11^{b}
Shoot dry weight (g)	5.25 ± 0.46^{a}	4.14 ± 0.50^{a}	4.19 ± 0.34^{a}	2.56 ± 0.13^{b}
Stem dry weight (g)	1.54 ± 0.15^{a}	1.12 ± 0.11^{b}	1.16 ± 0.11^{ab}	0.92 ± 0.05^{b}
Total leaf dry weight (g)	3.70 ± 0.32^{a}	3.03 ± 0.40^{a}	3.03 ± 0.26^{a}	1.64 ± 0.10^{b}
Root DW/shoot DW ratio	0.59 ± 0.03^{b}	0.69 ± 0.03^{a}	0.73 ± 0.03^{a}	0.76 ± 0.03^{a}
Increase in stem length (cm)	44.3 ± 8.8^{a}	18.7 ± 6.7^{b}	15.7 ± 3.9^{b}	2.9 ± 0.5^{b}
Number of leaves	71.4 ± 6.6^{a}	50.2 ± 7.9^{b}	49.8 ± 4.5^{b}	24.6 ± 1.7^{c}
Injury	0.0 ± 0.0^{c}	0.6 ± 0.1^{bc}	1.1 ± 0.2^{b}	3.0 ± 0.2^{a}

Table 3.4. Water content of red-osier dogwood tissues after four weeks of treatment with 0, 25, 50, and 100 mM NaCl. Different letters adjacent to the mean ± SE values indicate significant difference between treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 6).

Tissues	Water content (g g^{-1} DW)			
	NaCl treatment (mM)			
	0	25	50	100
Root	5.16 ± 0.16^{a}	4.84 ± 0.37^{a}	5.00 ± 0.22^{a}	3.11 ± 0.12^{b}
Shoot	1.89 ± 0.09^{a}	1.80 ± 0.15^{a}	2.11 ± 0.13^{a}	1.35 ± 0.06^{b}
Stem	1.91 ± 0.10^{a}	1.57 ± 0.14^{ab}	1.71 ± 0.29^{a}	1.04 ± 0.03^{b}
Leaf	1.89 ± 0.09^{ab}	1.89 ± 0.15^{ab}	2.32 ± 0.12^{a}	1.53 ± 0.12^{b}

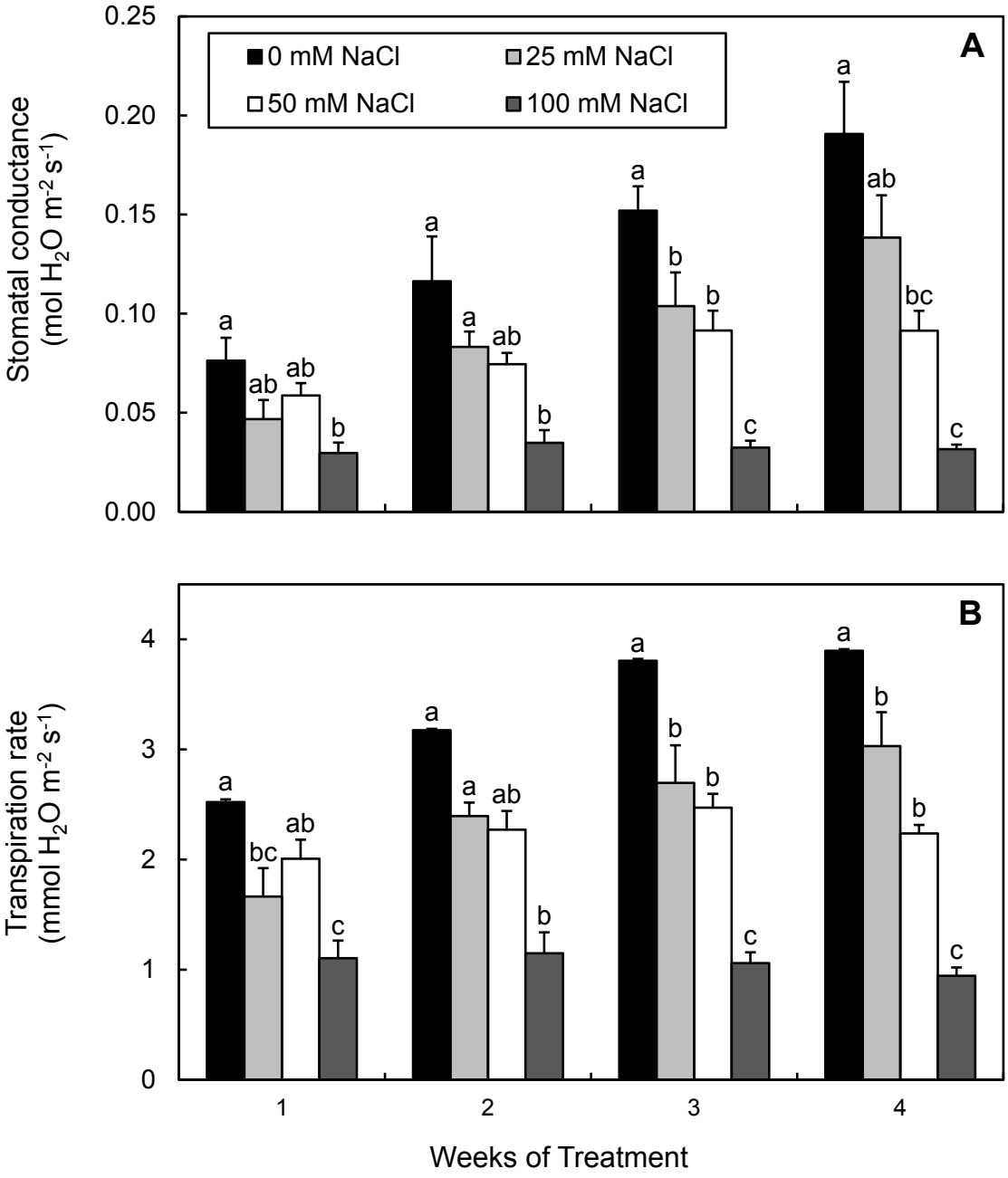


Figure 3.11. Stomatal conductance (A) and transpiration rate (B) of red-osier dogwood after each week of treatment with 0, 25, 50, or 100 mM NaCl. Different letters on top of error bars (SE) indicate significant difference between means at $\alpha = 0.05$ with each week analyzed separately (n = 6).

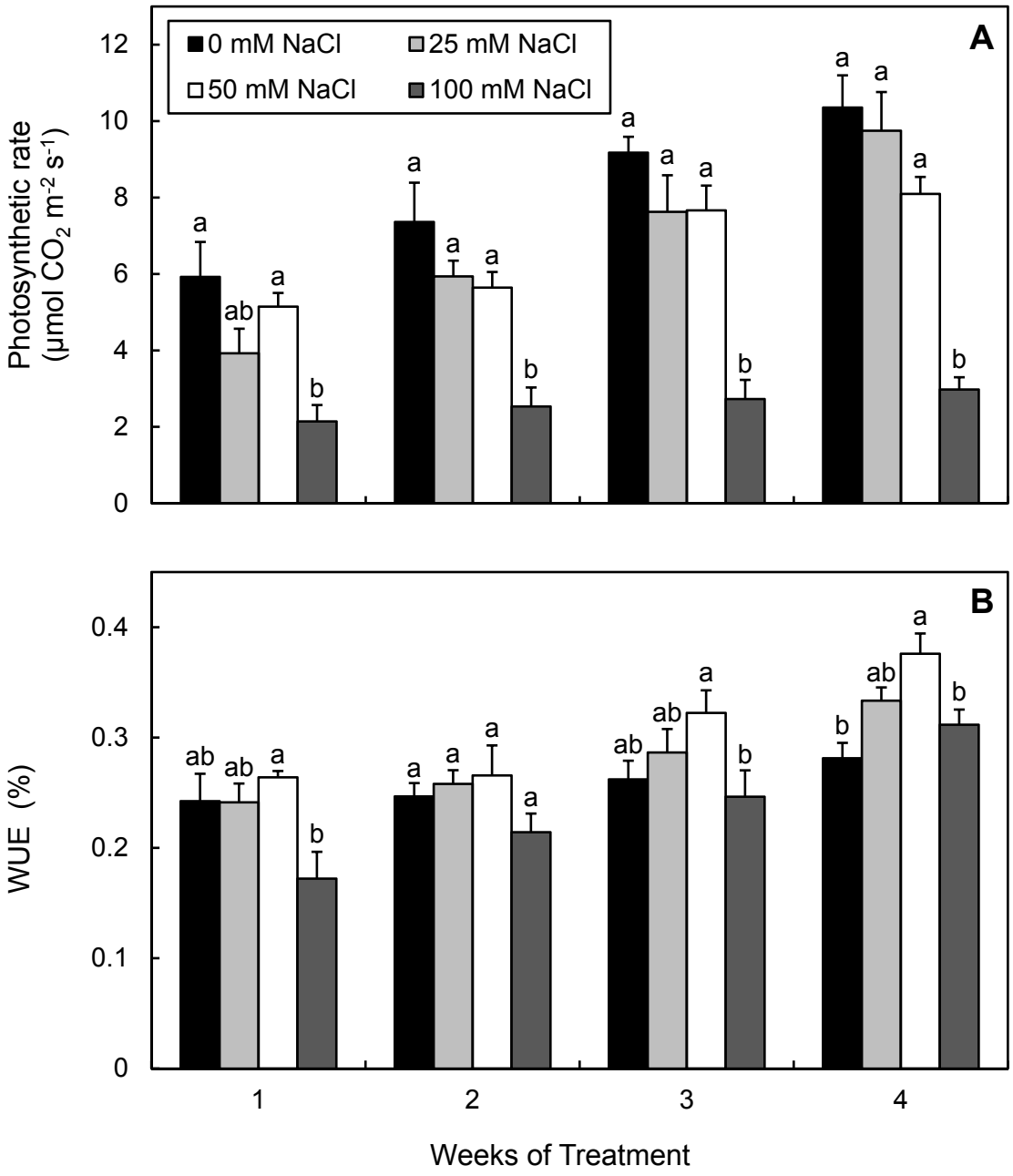


Figure 3.12. Photosynthetic rate (A) and water-use efficiency (WUE) (B) of red-osier dogwood after each week of treatment with 0, 25, 50, or 100 mM NaCl. Different letters on top of error bars (SE) indicate significant difference between means at $\alpha = 0.05$ with each week analyzed separately (n = 6).

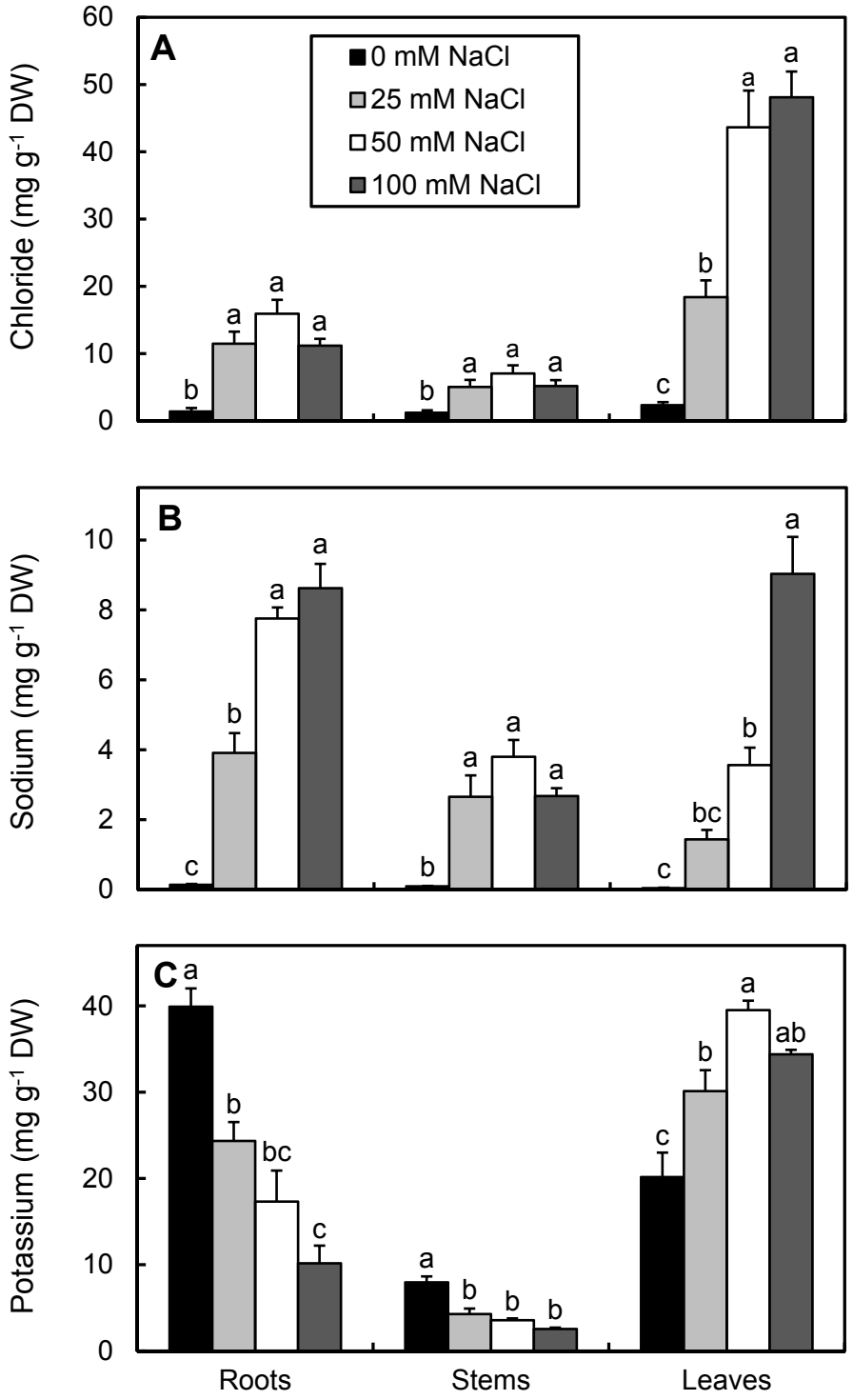


Figure 3.13. Chloride (A), sodium (B), and potassium (C) content in the roots, stems, and leaves of red-osier dogwood after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. Different letters on top of error bars (SE) indicate significant difference between means at $\alpha = 0.05$ with each tissue analyzed separately (A: n = 6; B – C: n = 4).

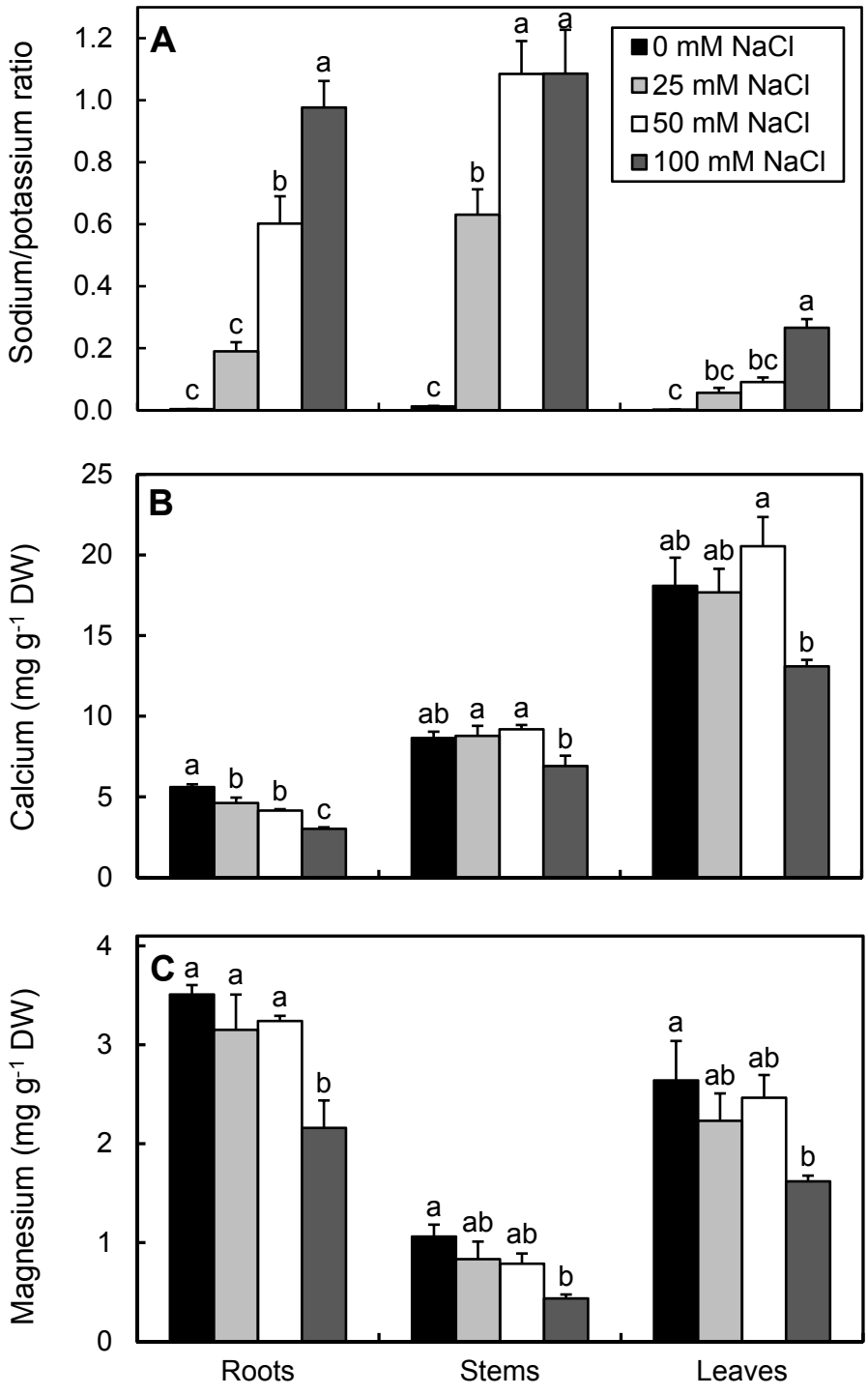


Figure 3.14. Sodium/potassium ratio (A) and the calcium (B) and magnesium (C) content in the roots, stems, and leaves of red-osier dogwood after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. Different letters on top of error bars (SE) indicate significant difference between means at $\alpha = 0.05$ with each tissue analyzed separately ($n = 4$).

3.4 DISCUSSION

3.4.1 SALINITY TOLERANCE OF RED-OSIER DOGWOOD CUTTINGS

The reduction in leaf and stem biomass that was observed with NaCl treatments was greater than the reduction in root biomass, which resulted in an increase in the root/shoot ratio. The greater reduction in shoot growth may help the plant survive the salt stress by reducing the demand for nutrient and water uptake (Chapin, 1980). However, red-osier dogwood cuttings exposed to the highest salt concentration, 100 mM NaCl had a large reduction in biomass after four weeks of treatment. Although death of cuttings was not observed in my experiment, plant death will eventually occur as the reduction in photosynthetic tissue becomes too severe (Munns and Termaat, 1986). Under moderate salinity stress, red-osier dogwood cuttings were able to maintain photosynthetic rates similar to the control plants, while the stomatal conductance and transpiration rate were decreased. A reduced transpiration may benefit the plant by increasing the water-use efficiency (Downton *et al*., 1985; Flowers *et al*., 1988) and decreasing Na^+ and Cl^- transport (Flowers and Yeo, 1986). Such a change in water-use efficiency was observed in red-osier dogwood, after four weeks of treatment with 50 mM NaCl; thus, helping the plant to tolerate the osmotic stress component of salinity stress by maintaining photosynthesis under reduced water uptake (Flowers *et al*., 1988).

A lower Na^+ content observed in the shoots compared with the roots after treatment with 25 and 50 mM NaCl, suggests that red-osier dogwood cuttings can limit the transport of Na^+ to the leaves. The limited accumulation of Na^+ in the leaves has been previously observed in red-osier dogwood seedlings (Renault *et al*., 2001*a, b*) as well as

in other woody species like citrus (*Citrus* spp., Storey and Walker, 1999) and grapevine (*Vitis* spp., Tregeagle *et al*., 2010) and also in soybean (*Glycine max*, Valencia *et al*., 2008). Conversely, the K^+ content decreased in the roots and increased in the leaves in red-osier dogwood cuttings. An increase in leaf K^+ contents also occurred in bean (*Phaseolus vulgaris*, Cachorro *et al*., 1993*a*) and potato (*Solanum tuberosum*, Potluri and Prasad, 1993) with increasing NaCl concentration in the external medium, due to the increased transport of K^+ from the roots to the leaves (Grattan and Grieve, 1999). The reallocation of K^+ from the roots and stems could contribute to the tolerance of red-osier dogwood to concentrations of NaCl (25 and 50 mM) by reducing the Na^+/K^+ ratio in the leaves; thus, maintaining enzyme activity and protein synthesis (Grattan and Grieve, 1999; Tester and Davenport, 2003). Greater salt tolerance was observed in barley (*Hordeum vulgare*, Flowers and Hajibagheri, 2001) genotypes with increased ability to maintain low Na^+/K^+ ratios.

Although red-osier dogwood cuttings limited the transport of Na^+ to the shoots with exposure to 25 and 50 mM NaCl, they were less efficient at limiting the transport of Cl^- to the shoots. This result supports earlier findings in red-osier dogwood seedlings (Renault *et al*., 2001*b*). The greater accumulation of Cl^- in the leaves suggested that the NaCl injury observed in the leaf at 50 mM NaCl was primarily caused by Cl^- and not Na^+; a similar result was observed in soybean (*Glycine max*, Valencia *et al*., 2008), narrow-leaf trefoil (*Lotus tenuis*, Teakle *et al*., 2010), and plum trees (*Prunus salicina*, Hoffman *et al*., 1989). However, at 100 mM NaCl both Na^+ and Cl^- accumulated to high concentrations within the red-osier dogwood leaves. The limited tolerance of red-osier

dogwood to high concentrations of NaCl could therefore be caused by the uptake and accumulation of both Na^+ and Cl^-.

The red-osier dogwood cuttings from southeastern Manitoba grown for six months before treatment had an overall similar response to 25 and 50 mM NaCl to the three-month-old seedlings from Alberta previously studied (Renault *et al*., 2001*b*). However, in this study all the cuttings survived the four weeks of exposure to 100 mM NaCl, while Renault *et al*. (2001*b*) found a survival rate of only 30% with the red-osier dogwood seedlings. This suggests that red-osier dogwood cuttings may have a greater salinity tolerance to higher NaCl concentrations than seedlings. A greater tolerance of cuttings was also found with Douglas-fir (*Pseudotsuga menziesii*) exposed to cold stress (Richie *et al*., 1992) but not with blue gum (*Eucalyptus globulus*) cuttings exposed to drought stress (Sasse and Sands, 1996). Sasse and Sands (1996) suggested that the blue glum cuttings were less tolerant due to a less developed root system than the seedlings (Sasse and Sands, 1996). Therefore, the longer period of growth prior to NaCl treatment of the red-osier dogwood cuttings (six months) compared with seedlings (three months) (Renault *et al*., 2001*b*) resulted in a greater root biomass that may have played a significant role in the greater survival rate of the cuttings exposed to 100 mM NaCl. Older plants have also been found to be more tolerant to drought (perennial ryegrass, *Lolium perenne*, Sugiyama and Nikara, 2004) and cold stress (rhododendron, *Rhododendron maximum*, Lim *et al*., 1999) compared to younger plants.

It is important to note that the red-osier dogwood cuttings may have higher salinity tolerance as a result of a greater control of Na^+ and Cl^- transport into the root than the seedlings. A previous experiment has shown that the Na^+ and Cl^- content of red-osier

dogwood tissues (roots, stems, and leaves) were similar or higher after only two weeks of treatment with 25, 50, and 100 mM NaCl in seedlings (Renault, Personal Communication) compared to cuttings after four weeks of treatment. For example, after four weeks of treatment with 50 mM NaCl, the Na^+ content of the cutting roots was less than half the content determined for seedling roots (7.7mg g^{-1} DW, cuttings; 16.5–18.7 mg g^{-1} DW, seedlings). In addition, although the Na^+ content of the leaves was similar between cuttings and seedlings with exposure to 50 mM NaCl, a greater K^+ content was maintained in the cutting leaves, resulting in a lower Na^+/K^+ ratio in the cuttings. The difference in ion uptake may be due to differences in root systems between cuttings and seedlings. Sasse and Sands (1997) suggested that the blue gum (*Eucalyptus globulus*) seedlings would have better water and ion uptake capabilities compare with cuttings because of a continuous production of new primary roots from the tap root that greatly increases the root area, while the number of primary roots remained constant in the cuttings after rooting. The overall root system morphology of red-osier dogwood appeared to be similar between cuttings and seedlings (Fig. A.1A, B) after growing in hydroponics, but the root area available for Na^+ and Cl^- uptake may still differ between cuttings and seedlings. To confirm that red-osier dogwood cuttings are more tolerant to salinity stress than seedlings, a study is required to directly compare the effect of NaCl on biomass, survival, and ion accumulation of cuttings and seedlings grown for a similar period prior to salt treatment.

An interesting observation from this study was the occurrence of two red-osier dogwood cuttings treated with 100 mM NaCl that showed a thicker root tip than the other plants in the same treatment. In addition, these plants had a relatively large shoot biomass

with reduced NaCl induced injury, which resulted in lower root/shoot ratio. Increased root diameter under NaCl stress compared to control roots has also been reported in other plants, such as maize (*Zea mays*, Evlagon *et al*., 1990), rice (*Oryza sativa*, Garcia *et al*., 1997), and citrus (*Citrus aurantium* and *C. reticulata*, Zekri, 1991), and white seablite (*Suaeda maritima*, Hajibagheri *et al*., 1985). The advantage of thicker roots under salinity stress is not understood, but it has been suggested it may reduce root surface area for Na^+ and Cl^- uptake while maintaining cell volume (Kurth *et al*., 1986) for compartmentalization. In a previous study comparing red-osier dogwood seedlings from different provenances, one seedling from British Columbia treated with 100 mM NaCl had very short tuberized lateral roots near the root tip (Renault, Personal Communication) (Fig. A.2B, C). This seedling from British Columbia also had drastically reduced injury and Na^+ uptake. It is not known if a reduction in Na^+ accumulation occurred with the cuttings in my study as the root tissues of two cuttings were combined in each replicate for ion tissue analysis. The limited number of red-osier dogwood plants with thickened roots under severe NaCl stress makes it difficult to determine the significance of this modification for salinity tolerance. Furthermore, the thickened root portion of the red-osier dogwood roots exposed to 100 mM NaCl did not always appear to be thicker than the controls. Therefore, root area measurements are required to confirm if the increased root diameter is greater than the control or just relative to other 100 mM NaCl treated roots.

3.4.2 Influence of Parent Environmental Conditions on Cuttings Growth

The sites selected for this study were assessed to determine the potential for exposure to drought and adaptation that will benefit the plant in future exposure to stress. The edaphic conditions at site 2 were determined to be the most conducive for drought stress because of the low gravimetric water content, gravel soil composition, and minimal organic content. In addition, the red-osier dogwood growing at site 2 had smaller leaves and shorter internodes than the moister soil conditions of sites 1 and 3, suggesting a more stressful growing condition and possibly an adaptive response to site conditions. A correlation of lower intrinsic growth rates with increased stress tolerance has been found with drought (Parsons, 1968a; Xiao *et al*., 2008, Yang and Miao, 2010), cold (Ashton, 1958; Parsons, 1968b), low nutrient availability (McGraw and Chapin, 1989; Arendt, 1997), herbivory (Price, 1991; Bergelson, 1994; Herms and Mattson, 1992), and low light intensities (Grime and Jeffrey, 1965). Medgiche *et al*. (2007, 2009) also found that the sea rocket (*Cakile maritima*) seedlings collected from an arid climate had a slower growth rate under control conditions compared with the seedlings from an arid environment that were growing faster (Megdiche *et al*., 2007, 2009). In my experiment, the difference in shoot growth observed in the field between the red-osier dogwood plants growing at the three sites was not observed in the greenhouse in the cuttings prior to salt treatments (Table A.1, A.3).

In my study, under control conditions there was a significantly lower root biomass in cuttings from site 2 compared to site 3 (Table. A.1, A.3), but this did not produce a significant difference in root/shoot ratio (Table A.1, A.3). In contrast, a greater root/shoot ratio was found in a previous study with seedlings from different provenances, in red-

osier dogwood from British Columbia compared to seedlings from Alberta and New Brunswick (Renault, Personal Communication). This difference was attributed to the potential adaptation of the British Columbia seedlings to a drier environment. An increase in the root/shoot ratio has been found in species under stressful environments where there is an increase in allocation of resources to the roots (Munns and Termaat, 1986; Kozlowski and Pallardy, 2002). This may aid the plant in reducing water loss through transpiration and maintaining water and nutrient acquisition (Kozlowski and Pallardy, 2002). It was, therefore, surprising that the cuttings from site 2 had a lower root biomass.

Overall, the lack of differences in growth between the red-osier dogwood cuttings from the three sites could be related to the fact that the cuttings were grown under the same optimal conditions for six months in the greenhouse. Furthermore, the similarity of growth between the red-osier dogwood cuttings from the three sites may indicate that the differences observed between sites in the field were not adaptive but an elastic change in response to the climatic and edaphic conditions.

3.4.3 Influence of Parent Environmental Conditions on Cuttings Salinity Tolerance

Exposure of the plants from site 2 to more stressful conditions did not result in a greater salinity tolerance compared with the cuttings from less stressful environments (sites 1 and 3). A greater salinity tolerance was observed in seedlings of the sea rocket (*Cakile maritima*) grown from seeds collected from a southern arid climatic area compared to those collected from a northern humid climatic area (Megdiche *et al*., 2007,

2009). Furthermore, Renault (Personal Communication) found greater salinity tolerance in red-osier dogwood seedlings collected from more stressful provenances in Alberta (colder) and British Columbia (drier) compared with seedlings from New Brunswick (milder climate). The similar climatic conditions (i.e. temperature and precipitation) of the three sites in my study may have contributed to the lack of differences in salinity tolerance between the three sites. Conversely, the stress conditions to which the parent plant was exposed may not always result in an increase in stress tolerance of its progeny. This was found with *Eucalyptus camaldulensis* seedlings where one of the most salinity tolerant seedlings was collected from a non-saline provenance (Marcar *et al*., 2002).

A limitation of the research conducted on the variability in salinity tolerance with respect to differences between provenances (Marcar *et al*., 2002; Nguyen *et al.,* 2004; Megdiche *et al*., 2007, 2009) is that both the climatic and edaphic factors of the provenance are usually not provided in the studies. If the most significant environmental and edaphic factor(s) causing the increased salinity tolerance could be determined, this would aid in future selection of provenances for plants that will have greater salt tolerance. It does appear, however, from this study and those by Megdiche *et al*. (2007, 2009) and Renault (Personal Communication) that cross-tolerance is more likely to occur when the environmental stress of the provenance is constant through the development of the plant, such as the cold or dry climate. A greater severity and duration of the stress produced by the climatic conditions may be required for differences in cross-tolerance to occur.

The lack of differences in growth of the red-osier dogwood cuttings of the three selected sites may also be due to the highly variable growth rates within each site after

four weeks of growth in hydroponics. Although, the cuttings had initially similar shoot size at the beginning of the treatment, after four weeks there were red-osier dogwood cuttings that had abundant growth while others had little growth with an even distribution between the extremes in all treatments. It is often thought that propagation from cuttings will result in homogenous growth (Blake and Filho, 1988), nevertheless cuttings growth is influenced by many factors, such as the growth rate and the age of the parent plant and also the morphology of the root system (Struve and McKeand, 1990, Baltunis *et al*., 2009, Ky-Dembele *et al*., 2010). The initial root development may determine the future growth and survival of the cuttings (Grossnickle and Russell, 1990; Sasse and Sands, 1996, 1997; Grossnickle, 2005) by defining soil area available for water and nutrient uptake by the root system (Struve and McKeand, 1990). However, this relationship does not always occur; it was not found with eastern white pine (*Pinus strobus*) cuttings after eight years of growth, where the growth and survival were not correlated to the initial root system morphology (Struve and McKeand, 1990).

The similarity in response of the three sites to salinity stress may not exclude the possibility that differences in salinity tolerance may occur if the cuttings were treated sooner after the cuttings had rooted or were exposed to a pretreatment (exposure to an initial stress) such as drought stress or salt stress before the NaCl treatment. Activation of the stress response pathways (Roelofs *et al*., 2008) by a pretreatment may be enhanced to a greater extent in red-osier dogwood cuttings from site 2 than from site 1 and 3. However, the enhancement of the stress response pathways may have been lost during the six months of growth under optimal conditions. Other studies apply the treatment stress either immediately after the pretreatment (Amzallag *et al*., 1990; Umezawa *et al*., 2000;

Cayuela *et al*., 2001; Djanaguiraman *et al*., 2006) or after a short recovery period (Cayuela *et al*., 2007). For example, Cayuela *et al*. (2007) exposed tomato (*Solanum lycopersicum*) seedlings to 100 mM NaCl after only two day of recovery from a 24 h pretreatment of drought stress. During acclimation to drought, stress response pathways may be activated to increase the quantities of osmolytes, stress-associated proteins (i.e., heat-shock proteins (Hsps) and late embryogenesis abundant (LEA)-type proteins), and antioxidants in plant tissues, which may contribute to a greater overall tolerance to stress (Wang *et al*., 2003). Initially, the red-osier dogwood cuttings from site 2 could have had a greater accumulation of these stress-associated molecules compared with cuttings from site 1 and 3. However, as the cuttings grew under optimal conditions, greater quantities of osmolytes, stress-associated proteins, and antioxidants were no longer needed for protection of cellular components and the concentrations could have been reduced. Therefore, a drought pretreatment may be required to re-acclimate red-osier dogwood cuttings to drought after growing under optimal conditions.

Chapter 4 ROOT MORPHOLOGY AND ANATOMY OF RED-OSIER DOGWOOD (*CORNUS SERICEA*) EXPOSED TO SALINITY STRESS

4.1 INTRODUCTION

The roots are essential for the uptake and transport of water and nutrients to the shoots, yet an equally important role of the roots is the prevention of toxic ions from entering and accumulating within the plant (Hose *et al*., 2001; Enstone *et al*., 2003; Storey *et al*., 2003; Baxter *et al*., 2009). With exposure to moderate salinity stress, red-osier dogwood limited the transport of Na^+ to the shoots (Renault *et al*., 2001*a, b*; Chapter 3). In addition, in the previous experiment (Chapter 3), the root growth of two red-osier dogwood cuttings treated with 100 mM NaCl had a greater diameter than the other cuttings in the same treatment. These plants also had a relatively large shoot biomass with little NaCl induced injury, which suggested a greater ability to limit transport of Na^+ and Cl^-. However, in red-osier dogwood the anatomical features that contributed to the inhibition of Na^+ transport is not known.

A potential mechanism for controlling ion uptake in red-osier dogwood roots is the development of barriers to limit the uncontrolled movement of ions through the apoplastic pathway (Hose *et al*., 2001; Enstone *et al*., 2003; Baxter *et al*., 2009). These barriers are the exodermis and endodermis, which are formed in the outer and inner layer of the cortex, respectively (Hose *et al*., 2001; Enstone *et al*., 2003; Ma and Peterson, 2003; Ranathunge and Schreiber, 2011). The endodermis is found in most vascular plants and the exodermis has been observed in around 84% of dicotyledonous species surveyed (Peterson, 1987). In the first stage of development of the endodermis and exodermis, the

Casparian bands are formed by the deposition of suberin and lignin in the anticlinal walls to inhibit the apoplastic movement of ions (Hose *et al*., 2001; Enstone *et al*., 2003; Ma and Peterson, 2003; Ranathunge and Schreiber, 2011). The deposition of the suberin lamellae occurs during the second stage of exodermis and endodermis development, which further limits ion transport by reducing the plasma membrane surface area available for ion uptake. With salinity stress, the exodermis and endodermis are found to mature closer to the root tip in citrus (*Citrus* spp., Walker *et al*., 1984), herbaceous seepweed (*Suaeda maritima*, Hajibagheri *et al*., 1985) and cotton (*Gossypium hirsutum*, Reinhardt and Rost, 1995*a, b*); thus, increasing the root length with barriers to ion uptake and transport (Hajibagheri *et al*., 1985; Karahara *et al*., 2004).

To the best of my knowledge the anatomy of red-osier dogwood has not been examined; therefore, the first objective of this study was to examine the development of the roots under control conditions. The second objective was to study the anatomy of red-osier dogwood to determine the changes that occur within the roots after exposure to various NaCl treatments. Special attention was given to the outer and inner cortical layers to determine if an exodermis is present and if modifications occurred in these layers to enhance the control of ion uptake in red-osier dogwood roots. This was done by two techniques: serial sectioning and freehand sectioning with brightfield and fluorescence microscopy. I hypothesized that with moderate salinity stress the maturation of the exodermis and endodermis would occur closer to the root tip to limit the transport of Na^+ and Cl^- to the shoot.

4.2 Material and Methods

4.2.1 Plant Growth

In September 2009 and August 2010, red-osier dogwood cuttings were collected from site 1 described in Chapter 3, Section 3.1.1. I selected cuttings from only one site as no significant differences between the three selected sites were found in the previous experiment (see Chapter 3, Section 3.1.9). Site 1 was chosen for further experimentation as red-osier dogwood from this site had the most vigorous growth. The cuttings were planted and grown under conditions previously described in Chapter 3, Section 3.1.4, but a few modifications were made to improve the success of rooting of the cuttings. The cuttings were dipped into 0.4% IBA (indole-3-butyric acid) rooting powder (Plant-Prod Stim-Root No. 2). In addition, to prevent the cuttings from drying, 50% of each leaf was cut to reduce transpiration and the planted cuttings were placed under a misting system until the cuttings rooted.

After six to eight months of growth in the peat/sand mixture, cuttings collected in September 2009 were transferred to hydroponics as previously described in Chapter 3, Section 3.1.4, except there was only one plant per replicate. An additional modification in the protocol was that the acclimation period was increased to two weeks to allow for a new steady root growth rate after transfer to hydroponics. The nutrient solution was replaced weekly to maintain nutrient solution level. After the two weeks of acclimation period, the 0 mM, 25 mM, 50 mM, and 100 mM NaCl treatments were started as previously described (Chapter 3, Section 3.1.5). To determine the increase in root length during the NaCl treatments, a thread was tied loosely around three adventitious roots of each plant between two laterals to prevent thread movement. The length from the thread

to the root tip was then measured at day 0, 1, 3, 5, and 7 for the first week of treatment and at day three and seven for the next three weeks. Selection of only adventitious roots was not always possible because many of the adventitious roots had ceased growing. In these cuttings, the selected laterals originated from the most proximal portion of the adventitious root and exhibited similar growth morphology to the adventitious roots.

The cuttings collected in August 2010 were rooted, grown, and treated with 50 and 100 mM NaCl as described for the September 2009 cuttings. These cuttings were grown for two to three months in the peat/sand and the cuttings.

4.2.2 Microscopy

4.2.2.1 Serial Sections

After four weeks of treatment, 1 cm segments were cut from five adventitious roots per plant and per treatment. The following segments were collected: root tip, and 1, 2, 3, 5, 7, 10, 15, and 25 cm from the root tip. The root segments were immediately placed in 1.6% paraformaldehyde and 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 6.9 (Yeung and Saxena, 2005). The sections were then placed under vacuum at 25 mm Hg for 20 - 40 min depending on the thickness of the tissue. The tissues were left in the fixative for 24 h at room temperature. The fixed tissue segments were then dehydrated in methyl cellosolve (2-methoxyethanol) for 24 h at room temperature, followed by two changes of absolute ethanol at room temperature for 24 h each. The dehydrated tissues were left in absolute ethanol at 4°C until the tissues could be further processed.

The dehydrated tissues were transferred to a 1:1 (V:V) mixture of absolute ethanol and Leica Historesin solution which consisted of 5 g of activator (dibenzolperoxide) dissolved in 500 mL (2-hydroxyethyl)-methacrylate (Leica Canada, Toronto) for 24 h (Yeung and Saxena, 2005). This was followed by two 24 h washes of 100 % Historesin solution. The root tissues were placed under vacuum for 20 min after each 100 % Historesin solution change. In the next step, the root segments were embedded in a mixture of 7.5 mL 100% Historesin solution, 0.15 mL polyethylene glycol (400 M.W.), and 0.5 mL hardener for 24 h.

To attain transverse root sections, 0.125 mm^3 infiltrated carrot pieces were fixed and dehydrated as described for the root segments. Before the infiltration step, two holes were made through the carrot pieces with a dissecting needle. After the carrots were infiltrated, the infiltrated root segments were trimmed to a length 0.5 cm and placed within the holes in the carrot pieces to hold the root segments upright during the embedding process.

Longitudinal and transverse serial sections (3 μm) were made with a Ralf glass knife on a Leica RM2145 autocut rotary microtome. Before the sections were collected for the transverse root sections, the blocks were first cut in 1 mm to remove damaged tissue from initial root segment collection. A thin layer of polyethylene glycol was applied to the edges of the blocks to improve the ribbon formation of the sections by increasing the "stickiness" of the sections (Yeung and Saxena, 2005). The ribbons were then placed in distilled water on a glass and dried on a 35°C slide warmer overnight. To improve contrast for measurements and for general histochemistry, serial sections were stained with 0.1 % toluidine blue in 0.1 M benzoate buffer, pH 4.4 for 2 min. Toluidine

blue is a metachromatic dye that stains primary walls pink (pectins) and lignified secondary walls blue-green (O'Brien and McCully, 1981; Peterson *et al*., 2008).

Sections were examined under a Nikon Optiphot microscope (Nikon, Tokyo) with white light. Photographs of the sections were taken with a Q-color 3 camera (Olympus America Inc.). Measurements of the root sections were made with ImageJ software (NIH, USA). The total root area, stele area, the cortex thickness, which includes the hypodermis and endodermis, were measured and the number of endodermal cells and cortex layers were counted. To distinguish between different xylem developmental stages, the xylem cells were initially counted and then classified into a designated count from 50 to 150 depending on the xylem developmental stage (see Fig. 4.1). The different xylem stages were defined as the following with the designated xylem cell count in brackets:

Stage 1 (individual lignified xylem cells counted): protoxylem cells and the first metaxylem cells were in distinct poles (Fig. 4.1A).

Stage 2 (50): metaxylem had started to develop between the xylem poles (Fig. 4.2B).

Stage 3 (75): metaxylem had completely filled between the xylem poles and secondary xylem development had just started between the protoxylem poles (Fig. 4.2C).

Stage 4 (100): secondary xylem had progressed around the protoxylem poles, but had not formed a ring around the primary vascular tissue (Fig. 4.2D).

Stage 5 (125): secondary xylem was developed in a thick ring around the primary xylem (Fig. 4.2E).

Stage 6 (150): abundant secondary xylem development and the primary vascular tissue comprising only a small portion of the root area (Fig. 4.2F).

After observing all the sections at all distances from the first replicate, the 1, 3, 5, 10, 15, and 25 cm distances were chosen for transverse sections and the root tip for longitudinal sections. Three of the five root segments collected at each distance from each plant in five replicates were analyzed. The 25 mM NaCl treated cuttings were not further studied as there were no significant differences in increase root length (Fig. 4.1) or root anatomy (data not shown) compared with the controls.

4.2.2.2 FREEHAND SECTIONS

Freehand transverse sections of the roots from the August 2010 red-osier dogwood cuttings were made at 1, 3, 5, 10, 15, and 25 cm from the root tip. Fresh or 50% ethanol-preserved sections were stained with various stains. To stain multiple sections at once, sections were placed in section holders constructed from BEEM®embedding capsules (BEEM Inc., West Chester, PA, USA) (Peterson *et al*., 2008) (Fig. 4.2). To construct the section holders the tapered bottom of the capsule was removed. Then the top portion of the capsule's lid was removed to leave a ring to hold a piece of nylon mesh with a 48 μm opening (Small Parts Inc., Seattle, WA) in place.

To detect Casparian bands, sections were stained with 0.1% (W/V) berberine hemisulphate (C.I. 75160) for 15 min and counterstained with 0.5% (W/V) aniline blue (C.I. 42755) for 30 min (Brundrett *et al*., 1988). The sections were then mounted in 0.1% (w/v) ferric chloride ($FeCl_3$) in 50% glycerine to reduce the destaining of sections that

could occur when placed in glycerine alone. The sections were observed with UV light (excitation filter 330–380 nm, dichroic mirror 400 nm, and a barrier filter 420 nm). Berberine hemisulphate is a fluorochrome that stains cell walls containing phenolics (Brundrett *et al*., 1988). The aniline blue counterstain has been found to reduce the unspecific staining of berberine hemisulphate and also the fluorescence of the suberin lamellae to observe the Casparian bands in the exodermis. To observe the autofluorescence of tissues, sections were mounted in 0.1% $FeCl_3$ and observed with UV light. Sections were also stained with 0.01% Calcofluor White M2R for 1 min and mounted in deionized water to observe β-1,4glucans (cellulose and hemicellulose) with UV light (Peterson *et al*., 2008).

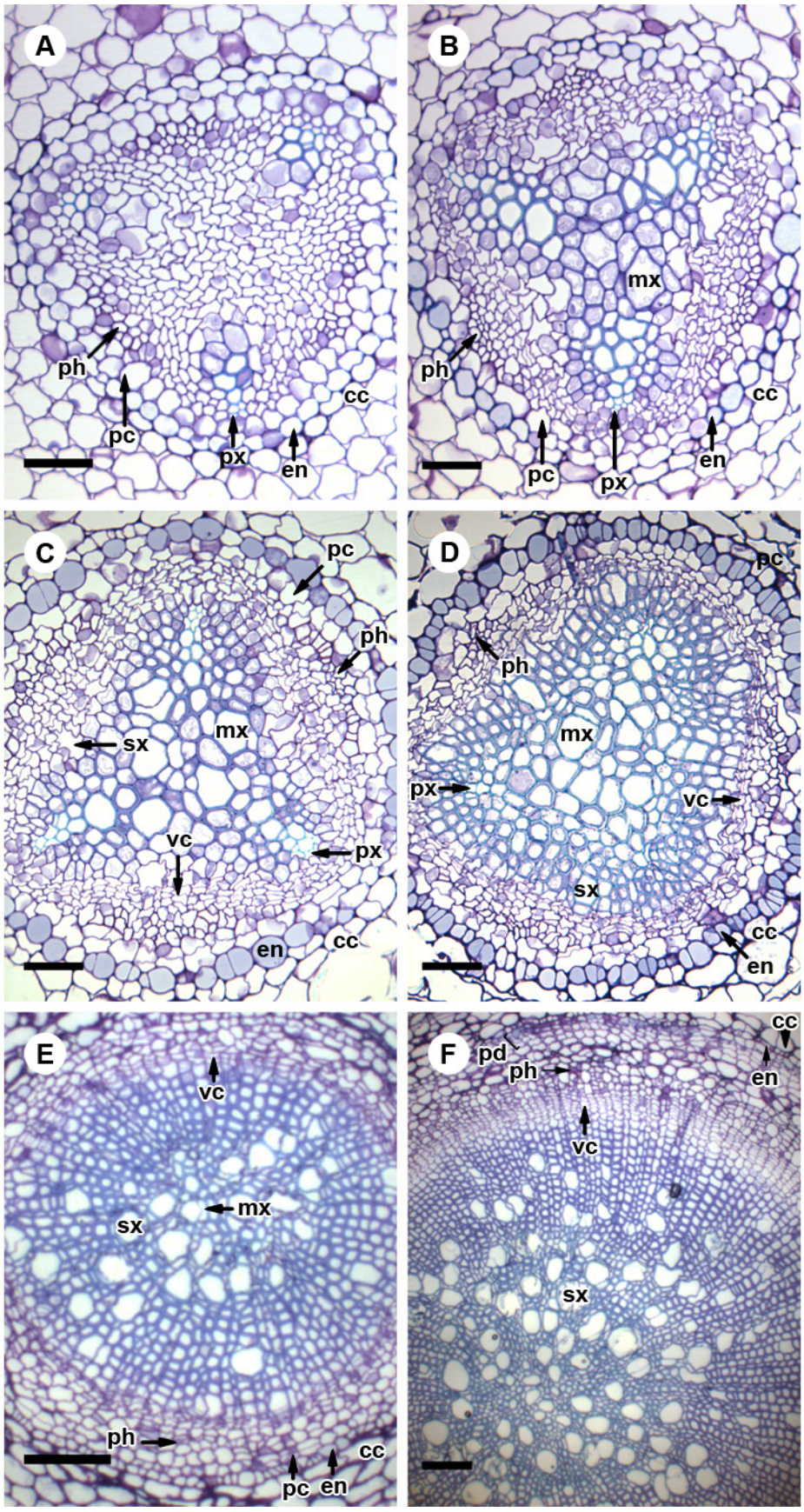
To differentiate between suberin and lignin, sections were stained with Sudan red 7B or phloroglucinol-HCl. To detect suberin, the lipid stain Sudan red 7B was prepared by dissolving 0.1% (w/v) Sudan red 7B (C.I. 26050) in a solution of equal parts polyethylene glycol (M.W. 400) and 90% (V/V) glycerol (Brundrett *et al*., 1991). The sections were stained for 1 h and washed thoroughly with deionized water. The stained sections were then mounted in 75% (V/V) glycerol and viewed with white light. Lignified tissues were identified by staining sections in a saturated solution of phloroglucinol in 20% HCl (7.3 g L^{-1}) (Jensen, 1962). The sections were directly mounted in the phloroglucinol-HCl solution and observed immediately with white light. The lignified tissues stained red.

The root sections were examined using a Nikon Optiphot microscope (Nikon, Tokyo) with white light. Photographs of the sections were taken with a Q-color 3 camera (Olympus America Inc.).

4.2.3 DATA ANALYSIS

Data were analyzed using a one-way Analysis of Variance (ANOVA) The means were compared using Tukey-Kramer HSD, and were considered to be significantly different at $\alpha = 0.05$. The data analyses were performed using JMP 8.0.1 (SAS Institute Inc., Cary, NC).

Figure 4.1.The different xylem developmental stages determined from transverse serial sectioned red-osier dogwood root cell counts. (A) Stage 1, individual xylem cells counted. (B) Stage 2, 50 xylem cells. (C) Stage 3, 75 xylem cells. (D) Stage 4, 100 xylem cells. (E) Stage 5, 125 xylem cells. (F) Stage 6, 150 xylem cells. Abbreviations: cc, central cortex; en, endodermis; pc, pericycle; ph, phloem tissue; vc, vascular cambial zone; sx, secondary xylem; px, protoxylem; mx, metaxylem. Staining procedure = toluidine blue. Scale bars: (A – D) = 50 μm; (E – F) = 100 μm.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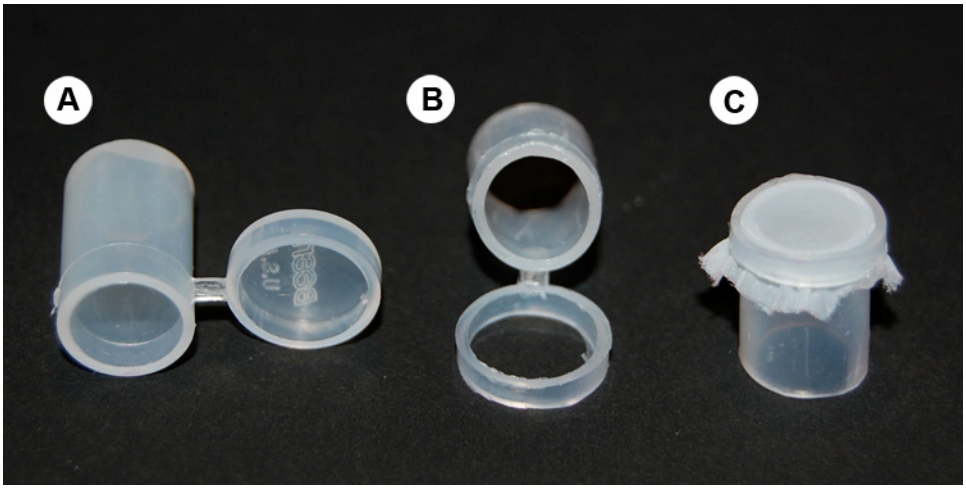


Figure 4.2. Section holders used for staining multiple sections at once. (A) BEEM® capsule. (B) BEEM® capsule with tapered bottom and top portion of the lid removed. (C) Section holder with a piece of 48µm size opening nylon mesh in place.

4.3 RESULTS

4.3.1 ROOT GROWTH

Root length of red-osier dogwood cuttings decreased with increasing NaCl concentration (Fig. 4.3). There was no significant difference in root length between treatments until after one week for the 100 mM NaCl treated cuttings and after four weeks for the 50 mM NaCl cuttings. After the four weeks of treatment, the root length of the 50 and 100 mM NaCl treated cuttings was reduced by 47.9 and 80.5%, respectively, compared with the controls (0 mM NaCl). In contrast, the 25 mM NaCl treatment did not significantly change the root length of red-osier dogwood cuttings. The control cuttings had abundant adventitious root growth that had a white colouration starting at the root tips and darkened to a golden brown with proximity to the base of the root (Fig. 4.4). The cuttings exposed to NaCl had a greater proportion of root tissue with a dark brown colouration (Fig. 4.5, 4.6). The proportion of darker roots increased with increasing NaCl concentration.

The red-osier dogwood cuttings used for the freehand sections were grown for only three months in the peat/sand mixtures; nonetheless these cuttings had a greater increase in root length after four weeks of treatment compared to their respective serial sectioned cuttings grown for six months before treatment (Fig. 4.3). The increase in root length was 37.4 ± 1.9 cm, 18.4 ± 2.8 cm, and 7.2 ± 0.8 cm for the controls, 50 mM NaCl, and 100 mM NaCl treated cuttings, respectively.

The diameter of the root generally appeared to gradually increase as the root matured with the exception of two of the six cuttings exposed to 50 mM NaCl (Fig. 4.5C,

D) and three out of the six cuttings exposed to 100 mM NaCl (Fig. 4.6C, D). In these cuttings, the distal portion of the root from these cuttings appeared thickened compared to the other cuttings of the same treatment. In addition, the transition zone from the thinner older root tissue to the younger thicker tissue was very short and distinct.

4.3.2 Root Anatomy of Red-Osier Dogwood

The increase in root length of control red-osier dogwood cuttings was 25.3 ± 3.0 cm; therefore, the portion of the root sectioned was produced during the treatment period (Fig. 4.3). The general observation that root diameter appeared to increase with distance from the root tip was confirmed with the measurement of the cross-sectional root area (Table 4.1, 4.3). The increase in the stele/root area ratio between 10 and 25 cm corresponded to an increase in secondary development of the xylem and phloem tissue (Table 4.1; Fig. 4.7E). The secondary growth continued to result in an increase in root area at 25 cm, but the growth also resulted in the compression of the cortical cells and a decrease in the number of cortical cell layers (Table 4.1; Figure 4.7F).

The xylem remained in stage 1 of development (primary root tissue) until after 10 cm from the root tip (Table 4.1; Fig. 4.7A – D, 4.8A – D). The difference in elongation rates between the cuttings used for serial sectioning and freehand sectioning affected the distances at which the Casparian bands and suberin lamellae development occurred (Table 4.1, 4.2). Therefore, the developmental stage of the xylem tissue was used as the reference point to determine where modifications of the endodermal and exodermal cell walls occurred in the serial sectioned cuttings after being determined in the freehand

sectioned cuttings (Table 4.1, 4.2). Casparian bands were present in all the radial walls of the endodermis in sections with the xylem development at early to mid-stage 1, which was approximately 3 cm from the root tip (Fig. 4.8B, 4.9B). With UV light, the Casparian bands in the radial walls fluoresced blue-white and appeared to be localized to the centre region of the radial wall in transverse sections. Band plasmolysis, which occurs when the plasma membrane is connected to a portion of the radial wall, was also detected in endodermal cells with only Casparian bands after staining with Sudan red 7B (Fig. 4.10C, D) or phloroglucinol-HCl (Fig. 4.11C). At 15 cm, or late stage 2 of xylem development, fluorescence of the transverse and radial walls was first detected in the endodermal cells located between the xylem poles (protoxylem groups) (Fig. 4.8E, 4.9E). In red-osier dogwood roots, triarch (Fig. 4.7) and tetrarch were the common protoxylem distribution patterns found. The fluorescent layer in the endodermal cell walls stained positive for suberin with Sudan red 7B (Fig. 4.10E, F), but not for lignin with phloroglucinol-HCl (Fig. 4.11E, F). Between 15 and 25 cm from the root tip, the xylem matured from late stage 2 to stage 4 in the freehand sections (Fig.4.8F) or stage 5 in the serial sections (Fig. 4.7F). At stage 4 of xylem development, approximately 70% of the endodermal cells lacked suberin lamellae. The endodermal cells did not develop tertiary walls after suberin lamellae development (Fig.4.7F, 4.10F, 4.11F).

In parallel with the observed increase in stele area (Table 4.1), anticlinal cell divisions of suberized endodermal cells were observed (Fig. 4.10F). The number of endodermal cells increased by a factor of two at 15 cm and a factor of three at 25 cm (Table 4.1). With increasing secondary growth, multiple divisions occurred within a single endodermal cell (Fig. 4.12). Casparian bands were not detected in the radial walls

produced after the division of endodermal cells (Fig 4.9F, 4.12B). In some of the new endodermal cells, suberin lamellae were detected under UV light (Fig. 4.12C) and in sections stained with Sudan red 7B (Fig. 4.12F). However, this did not occur in most of newly divided endodermal cells (Fig 4.12A, B). The new endodermal walls did not appear well defined in serial sections (Fig. 4.12D) and appeared thin and wavy in freehand sections after staining with calcofluor (Fig. 4.12E). In addition, the endodermal cells appeared stretched and the walls fluoresced faintly in sections 50 to 60 cm from the root tip where substantial secondary development had occurred, (Fig. 4.12G, Fig. 4.13D). At stage 6 of xylem development in the control cuttings, periderm formation internal to the endodermal layer was observed (Fig. 4.13A). The periclinal division of the pericycle was observed (Fig. 4.12B) and the outer layer of the periderm eventually developed suberin lamellae (Fig. 13D, E). At this stage of root development, the epidermis, hypodermis, and outer cortex layers separated from the internal root tissue during freehand sectioning.

The outer layer of the cortex of red-osier dogwood roots appeared modified in cross-section in relation to the shape and wall composition compared with the central cortical cells (Fig. 4.14 – 4.16). The cells in this outer layer generally appeared hexagonal in shape and lacked intercellular spaces between the radial walls and between the epidermis and adjacent cortex layer. However, with fluorescence and berberine-aniline blue staining procedure, the presence of Casparian bands in the hypodermis could not be clearly detected (Fig. 4.17C, D). Therefore, this outer layer was identified as a hypodermis. In addition, the fluorescence of unstained sections (autofluorescence) was clearer and there was less fluorescence of walls lacking suberin and lignin compared with

sections stained with berberine-aniline blue (Fig. 4.17). Therefore, sections observed under UV light were not stained with berberine-aniline blue.

At 1 cm from the root tip, only the epidermal cells fluoresced in the outer layers of the red-osier dogwood root (Fig. 4.14A). However, at 3 cm, 40% of the hypodermal cells had faintly fluorescing walls, with the fluorescence slightly brighter at the outer tangential and radial walls (Table. 4.2; Fig. 4.14B). The root sections at 3 cm were positive for suberin with Sudan red 7B (Fig. 4.15B), but not for lignin after staining with phloroglucinol-HCl (Fig. 4.16B) indicating the fluorescing walls were primarily due to the presence of suberin lamellae. Although, in some sections a few hypodermal walls were found to faintly stain red (Fig. 4.16C, D), which may indicate a slight lignification of the cell walls. However, the presence of lignin in the hypodermal layer was not conclusive, because unspecific faint red staining was seen in other cells with phloroglucinol-HCl staining that were not fluorescent under UV light (Fig. 4.16F). The number of hypodermal cells containing suberin lamellae and the strength of the fluorescence increased with increasing distance from the root tip (Table 4.1; Fig. 4.14, 4.15). In some sections, fluorescence of the cell walls of the central cortical cells adjacent the hypodermis were observed and the walls stained red with Sudan red 7B (Fig 4.15D). Infrequently, the hypodermis did not contain suberin lamellae but the adjacent cortical cell walls did (Fig 4.15D). It was also observed that the epidermal cells fluoresced (Fig. 4.14) and stained reddish-orange with Sudan red 7B (Fig. 4.15) and yellow with phloroglucinol-HCl (Fig. 4.16).

4.3.3 Effect of NaCl on Red-Osier Dogwood

After the four weeks of treatment, it was observed that the red-osier dogwood cuttings from replicates 2 and 3 of the 50 mM NaCl treatment (Table 4.4) and replicates 2, 4, and 6 from the 100 mM NaCl treatment had relatively larger transverse root area near the root tip compared to the other cuttings of the same treatment (Table 4.5). In addition, these cuttings appeared to have less injury (necrotic tissue) compared with other cuttings of the same treatment (Fig. 4.5 – 4.6). These roots appeared to be thicker near the root tip and then gradually or abruptly became thinner before showing an increase in thickness again towards the base of the root (Table 4.4, 4.5; Fig. 4.5C, D, 4.6C, D). The thinner transition region of the root appeared to coincide with the initial application of the NaCl treatment, which was determined from the data on the increase in root length (Table 4.4, 4.5). The 50 mM NaCl cuttings with thicker roots had a smaller increase in root length compared to the other 50 mM NaCl cuttings (Table 4.7), but the root cap cells appeared larger in longitudinal sections and more similar to the control root cap (Fig. 4.18, 4.19). In addition, the thickened portion of the root from 50 mM NaCl treated cuttings had a larger transverse root area than the controls (Table 4.3, 4.4). On the other hand, the 100 mM NaCl exposed cuttings with thicker roots generally had a greater increase in root length compared with the cuttings without thickened roots (Table 4.8). The root cap cells of the thickened root tips observed in longitudinal microscopy sections were similar to the control root tips, but the root cap cells were smaller and less organized than the controls (Fig. 4.18, 4.20C, D). Whereas, the root tips of the 100 mM NaCl treated red-osier dogwood cuttings without thickening had collapsed cells in the root cap and less meristematic tissue compared to the control root tips (Fig. 4.20A, B). The

collapsed cells did not appear to be an artifact of the tissue preparation as the constriction of the root tips were observed before the sections from the roots were collected. In addition to root cap modifications, vacuolation of the cortical cells with NaCl treatment appeared closer to the root tip than in the controls (Fig. 4.18 – 4.20).

The average increase in root length of cuttings after treatment with 50 mM and 100 mM NaCl was 13.2 ± 1.7 cm and 4.9 ± 1.1 cm, respectively. Consequently, the root sections collected after 5 cm from 50 mM NaCl exposed cuttings and 1 cm from 100 mM NaCl exposed cuttings must have been produced prior to the four weeks of NaCl treatment. The differences in root growth rates between treatments did not appear to modify the overall sequence of development of root tissues was similar (Table 4.1, Fig. 4.7, 4.21 – 4.23), but the distance from the root tip at which developmental changes occurred was closer with increasing NaCl concentration. To adjust for different root lengths, the xylem stage was determined for the root tissue of a similar age (determine from the data on the increase in root length). The developmental stage of xylem at the same tissue age was slightly less mature with NaCl treatment (Tables 4.6 – 4.8, Fig. 4.21). For example, after four weeks of treatment, the xylem developmental stage of the control roots at 25 cm was at late stage 4 of development (Fig. 4.7, 4.20). The xylem developmental stage of 50 mM NaCl treated roots, sectioned from root tissue of a similar age (15 cm from the root) was slightly less mature at only early stage 4 (Fig. 4.21, 4.22). The rate of xylem maturation of roots exposed to 100 mM NaCl appeared to be further reduced with xylem at stage 2 of development in sections at 5 cm from the root tip (Fig. 4.21, 4.23).

Endodermal development in contrast appeared to develop more rapidly in cuttings exposed to NaCl if xylem developmental stage was used as an indication of time (Table 4.2, Fig. 4.21). At stage 4 of xylem development in root sections from control cuttings, 28% of the endodermal cells contained suberin lamellae (Table 4.2, Fig. 4.8F, 4.21). Conversely in cuttings treated with 50 mM NaCl, 75% of the endodermal cells contained suberin lamellae when xylem was at late stage 3 (Table 4.2, Fig. 4.21, 4.24D, E,). In addition, when the xylem was at stage 2 of development in sections collected from 100 mM NaCl treated roots, suberin lamellae was detected in 54% of the endodermal cells (Table 4.2, Fig. 4.21, 4.25C). Even though the maturation of the endodermal cells occurred closer to the root tip with NaCl treatment, the overall sequence of endodermal development was similar in all treatments (Fig. 4.8, 4.24 – 4.27). At 1 cm from the root tip (early stage 1 of xylem development), Casparian bands were detected in the radial walls of all the endodermal cells in root sections from both the 50 (Fig. 4.24A, 4.26A) and 100 (Fig. 4.25A, 4.27A) mM NaCl treatments. Fluorescence of the tangential and radial walls occurred between 5 and 10 cm (stage 2 of xylem development) for the 50 mM NaCl roots (Fig. 4.24C, D) and between 3 and 5 cm (late stage 1 of xylem development) for the 100 mM NaCl roots (Fig. 4.25B, D). The proportion of fluorescent endodermal cells increased with increasing distance from the root tip. The fluorescence of the walls of the endodermal cells appeared to be primarily due to the presence of suberin lamellae because suberin was detected (Fig. 4.28F, 4.29C, E) and lignin was not (Fig. 4.28G, 4.29D, F, G) in root sections. Although, there was one exception found in the transverse sections at 25 cm from the root tip in a red-osier dogwood cutting exposed

to 50 mM NaCl (Fig. 4.28H). In these sections, phloroglucinol-HCl stained the tangential and radial walls of the endodermis red.

The effect of NaCl on the development of the hypodermis was similar to the effect NaCl had on endodermal development, with the hypodermis appearing to mature closer to the root tip and at a faster rate (Table 4.2, Fig. 4.21). In the root sections from control cuttings, the fluorescence of hypodermal walls was not detected until 3 cm from the root tip (Table 4.2; Fig. 4.14), whereas in the 50 mM NaCl treated cuttings the outer tangential walls of the hypodermis exhibited fluorescence at 1 cm from the root tip (Table 4.2; Fig. 4.30). The hypodermal cells were even more mature at 1 cm in the 100 mM treated roots with 87% of the hypodermal cells fluoresced and the walls showed bright fluorescence although the walls appeared slightly wavy (Table 4.2; Fig. 4.30A). In the 50 and 100 mM NaCl treated roots, greater than 95% of the endodermal cells had fluorescent cell walls at 5 and 10 cm from the root tip, respectively (Table 4.2). When the percentage of hypodermal cells with fluorescent walls in root sections at late stage 1 of xylem development were compared between treatments, the root sections from controls had a lower proportion of fluorescent cells than the NaCl treated cuttings (Table 4.2). At late stage 1 of xylem development, only 90% of the hypodermal cells fluoresced in root sections from the controls compared to 97% and 99% of hypodermal cells from 50 and 100 mM treated roots, respectively.

The fluorescent cell walls of the hypodermis stained positive for suberin and rarely for lignin in all three treatments (Fig. 4.32, 4.33), which was also observed in the controls. There was a noteworthy exception; lignin was detected in roots from a 50 mM NaCl cutting that exhibited epidermal cell loss and injury along the entire length of the

root. This cutting did not exhibit any obvious differences in shoot and root morphology compared to the other 50 mM NaCl treated cuttings (Fig. 4.34). The hypodermal cell walls below the injured or lost epidermal cells had increased fluorescence, especially from the outer tangential walls (Fig.4.35). The outer tangential walls and outer radial walls in addition stained dark red with phloroglucinol-HCl and Sudan red 7B (Fig. 4.36). Given that lignin was detected in hypodermal cell walls along with suberin, the fluorescence cell walls observed in photomicrographs of root sections under UV light were only referred to as fluorescent walls.

Generally, only cells in the hypodermal layers were fluorescent, but the cortical layers (usually between one and two layers) below the hypodermis were also found to fluoresce with UV light. This was observed in all treatments but appeared to occur more often with NaCl treatment (Fig. 4.30E, 4.32F, 4.33C), especially in the roots from the cutting with abundant epidermal cell loss (Fig. 4.35D). The outermost central cortical layer with fluorescent walls did not form a continuous layer around the root circumference, but only in few isolated groups of cortical cells. The number of the fluorescent cortical cells also appeared to vary between sections taken at the same distance. Suberin lamellae (Fig. 4.32F, 4.33C, 4.36 D, F) and to a lesser extent lignin (Fig. 4.32E, 4.36G, H) were detected in these fluorescent cortical cells.

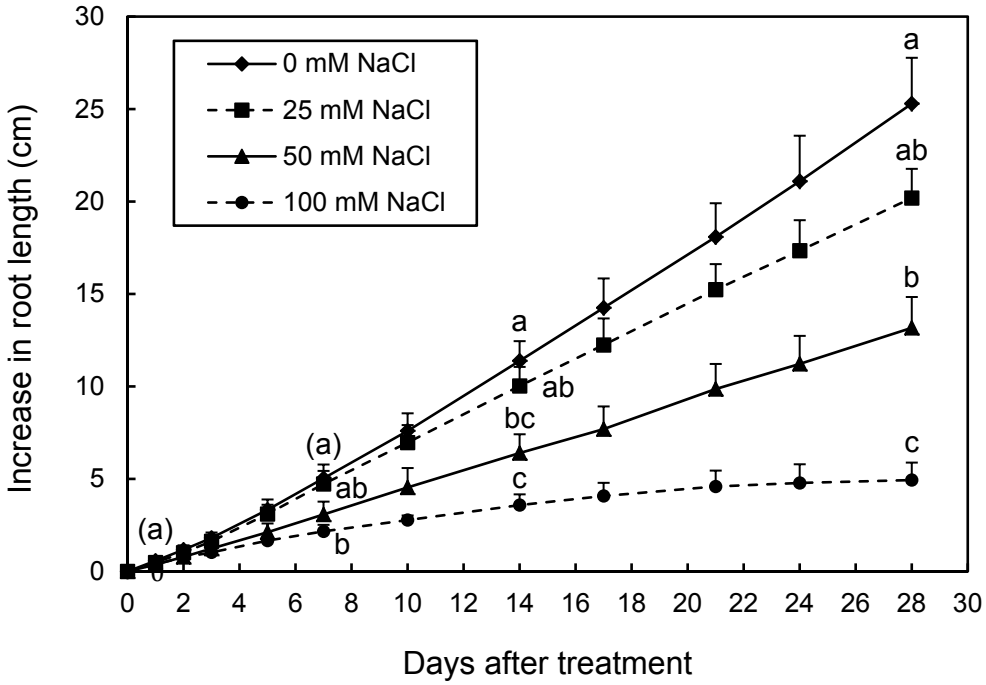


Figure 4.3. Increase in root length of red-osier dogwood during four weeks of treatment with 0, 25, 50, and 100 mM NaCl. Different letters next to error bars (SE) indicate significant difference between means at $\alpha = 0.05$ with each day analyzed separately ($n = 7$). Letters in brackets indicate same letter for all treatments at 0 – 5 days and for 0 and 25 mM NaCl treated plants at day 7. If significant levels did not change, the letters were not repeated for the following values.

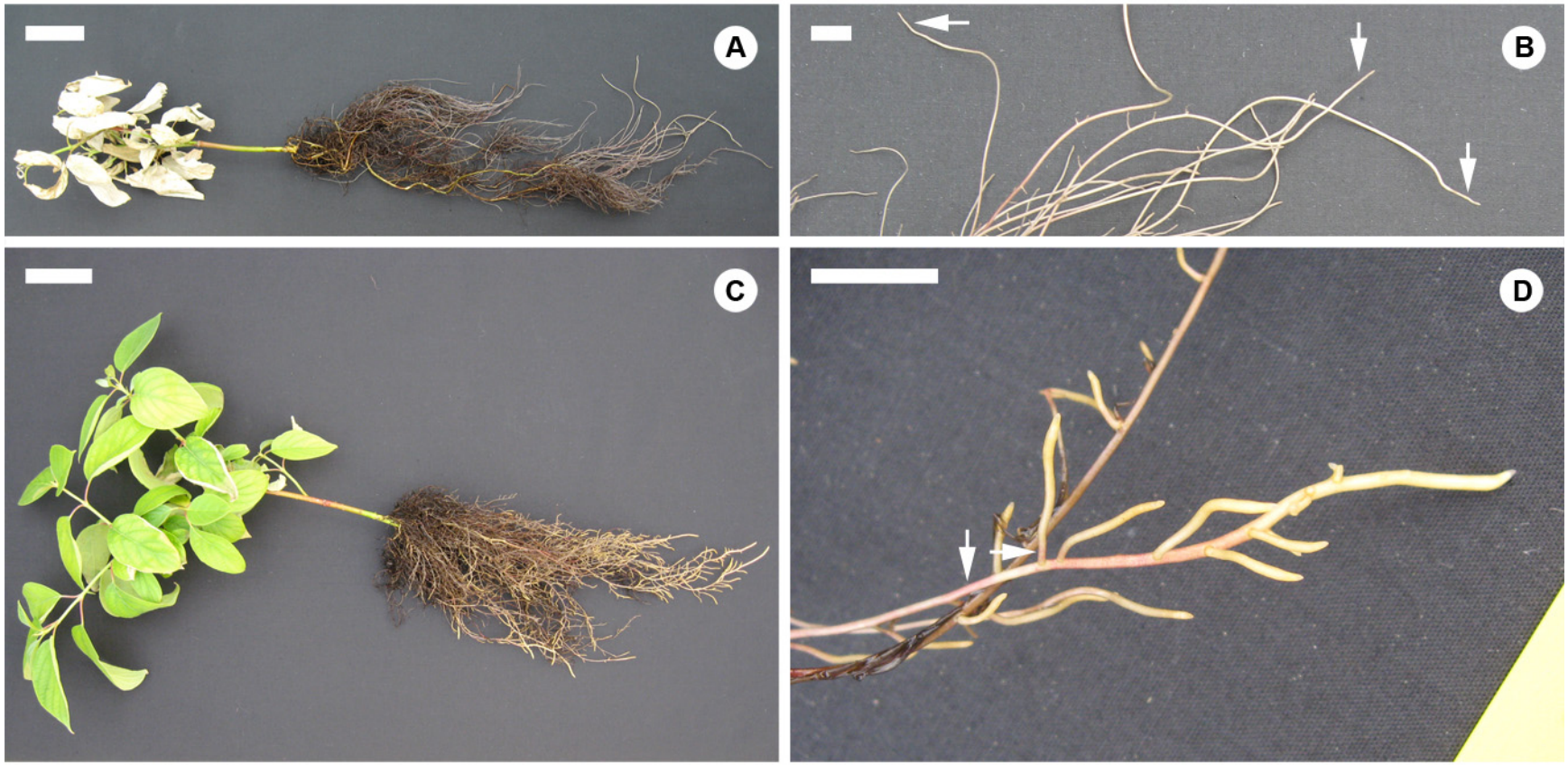
Figure 4.4. (A) Red-osier dogwood after four weeks of treatment: control plant. (B) Enlargement of the root system showing the colouration of the roots and the absence of root tip thickening. The roots were white at the root tips and gradually became golden brown near the stem. Scale bars: (A) = 10 cm; (B) = 5cm.


A
B

Figure 4.5. (A) A 50 mM NaCl treated red-osier dogwood without thickened roots after four weeks of treatment. NaCl induced leaf necrosis can be observed and the older root tissue had a very dark brown colouration near the stem. (B) Enlargement of the root system of the 50 mM NaCl treated cutting without thickened roots. (C) A 50 mM NaCl treated cuttings with the distal portion of the roots thickened compared to the proximal portion of the root. Very little leaf necrosis was observed and the older root growth had more of a golden brown colouration similar to the controls. (D) Enlargement of the distal portion of the roots showing the transition from the thinner older root growth to the thicker new root growth (Arrows pointing to the transition zones). Scale bars: (A – C) = 5 cm; (D) = 1 cm.


A
B
C
D

Figure 4.6. (A) 100 mM NaCl treated red-osier dogwood cutting without thickened roots after four weeks of treatment. The leaves were all necrotic and there was little root biomass. (B) Enlargement of the root system of the 50 mM NaCl treated cutting without thickened roots and root tips were very thin. (C) 100 mM NaCl treated cutting with the distal portion of the roots thickened compared to the proximal portion of the root. The cutting had relatively little leaf necrosis and an overall healthier appearance of the root system compared to other 100 mM NaCl treated cuttings. (D) Enlargement of the distal portion of the roots showing the transition from the thinner older root growth to the thicker new root growth (Arrows at the transition zones). Scale bars (A, C) = 5 cm; (B, D) = 1 cm.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C
D

Table 4.1. Root anatomy measurements of red-osier dogwood at various distances from the tip (serial sections) after exposure for four weeks to 0, 50, or 100 mM NaCl. Mean ± S.E. followed by different letters indicate significant difference between treatments mean values at $\alpha = 0.05$ with each column and root distance analyzed separately (n = 6).

NaCl (mM)	Distance (cm)	Root area (mm^2)	Stele/root area (%)	Cortex thickness (μm)	No. of cortex layers	No. of endodermal cells	Count of xylem cells
0	1	0.65 ± 0.07 ^{a}	9.3 ± 0.5 ^{a}	286 ± 18 ^{a}	12.7 ± 0.5 ^{a}	63.8 ± 3.4 ^{a}	3.3 ± 0.4 ^{b}
0	3	0.69 ± 0.07 ^{a}	8.6 ± 0.3 ^{a}	298 ± 18 ^{a}	12.9 ± 0.5 ^{a}	62.1 ± 3.5 ^{a}	7.4 ± 0.6 ^{a}
0	5	0.72 ± 0.09 ^{a}	9.1 ± 0.4 ^{a}	301 ± 20 ^{a}	13.1 ± 0.4 ^{a}	62.9 ± 3.7 ^{a}	9.7 ± 0.7 ^{a}
0	10	0.75 ± 0.11 ^{a}	15.5 ± 1.7 ^{a}	270 ± 25 ^{a}	13.2 ± 0.4 ^{a}	67.3 ± 4.7 ^{a}	35.0 ± 9.7 ^{b}
0	15	0.81 ± 0.06 ^{a}	22.8 ± 2.9 ^{a}	239 ± 18 ^{a}	12.8 ± 0.5 ^{a}	96.1 ± 14.1 ^{a}	76.4 ± 7.6 ^{a}
0	25	0.95 ± 0.05 ^{a}	42.4 ± 2.9 ^{a}	175 ± 14 ^{a}	10.0 ± 0.5 ^{a}	197.3 ± 10.2 ^{a}	113.9 ± 3.0 ^{a}
50	1	0.77 ± 0.13 ^{a}	7.2 ± 0.5 ^{b}	325 ± 37 ^{a}	13.6 ± 0.6 ^{a}	62.7 ± 2.1 ^{a}	6.9 ± 1.2 ^{ab}
50	3	0.80 ± 0.11 ^{a}	7.5 ± 0.3 ^{a}	331 ± 27 ^{a}	13.8 ± 0.6 ^{a}	60.2 ± 2.2 ^{a}	11.5 ± 1.5 ^{a}
50	5	0.76 ± 0.09 ^{a}	9.1 ± 0.6 ^{a}	307 ± 23 ^{a}	13.3 ± 0.4 ^{a}	59.0 ± 3.0 ^{a}	27.2 ± 10.7 ^{ab}
50	10	0.71 ± 0.11 ^{a}	19.9 ± 5.5 ^{a}	250 ± 38 ^{a}	11.7 ± 1.3 ^{ab}	81.1 ± 14.8 ^{a}	65.6 ± 17.4 ^{ab}
50	15	0.84 ± 0.12 ^{a}	29.7 ± 7.7 ^{a}	213 ± 37 ^{a}	10.6 ± 1.3 ^{a}	119.5 ± 30.7 ^{a}	95.3 ± 11.9 ^{a}
50	25	1.44 ± 0.32 ^{a}	53.4 ± 8.0 ^{a}	153 ± 26 ^{a}	7.9 ± 1.0 ^{a}	226.0 ± 36.2 ^{a}	126.1 ± 8.4 ^{a}
100	1	0.67 ± 0.07 ^{a}	7.0 ± 0.5 ^{b}	303 ± 22 ^{a}	12.4 ± 0.3 ^{a}	54.6 ± 1.4 ^{b}	9.8 ± 1.5 ^{a}
100	3	0.70 ± 0.09 ^{a}	8.4 ± 0.6 ^{a}	303 ± 21 ^{a}	12.2 ± 0.4 ^{a}	55.8 ± 2.0 ^{a}	24.7 ± 9.7 ^{a}
100	5	0.66 ± 0.07 ^{a}	12.1 ± 2.1 ^{a}	275 ± 20 ^{a}	11.5 ± 0.4 ^{b}	58.0 ± 1.6 ^{a}	49.6 ± 13.0 ^{a}
100	10	0.59 ± 0.09 ^{a}	22.2 ± 2.7 ^{a}	207 ± 16 ^{a}	10.2 ± 0.4 ^{b}	81.4 ± 9.6 ^{a}	95.0 ± 6.3 ^{a}
100	15	0.65 ± 0.11 ^{a}	27.6 ± 4.8 ^{a}	196 ± 16 ^{a}	9.9 ± 0.5 ^{a}	100.5 ± 16.5 ^{a}	95.8 ± 15.0 ^{a}

Table 4.2. Root anatomy measurements (mean ± S.E.) of red-osier dogwood at various distances from the tip (freehand sections) after exposure to four weeks of 0, 50, or 100 mM NaCl. Increase in root length was 37.4 ± 1.9 cm for the control cuttings, 18.4 ± 2.8 cm from the 50 mM treated cuttings and 7.2 ± 0.8 cm for the 100 mM treated cuttings. * next to the percentage of endodermal cell with suberin lamellae indicates the first distance at which Casparian bands were detected.

NaCl (mM)	Distance	Percentage of exodermal cells with fluorescent walls	No. of cortex layers	Percentage of endodermal cells with suberin lamellae	Count of xylem cells
0	1	0.0 ± 0.0	13.7 ± 1.5	0.0 ± 0.0	5.4 ± 1.6
0	3	42.5 ± 7.3	13.4 ± 1.0	0.0 ± 0.0*	9.6 ± 1.8
0	5	52.9 ± 15.3	13.4 ± 0.6	0.0 ± 0.0	9.8 ± 1.1
0	10	80.6 ± 5.1	13.3 ± 0.7	0.0 ± 0.0	15.4 ± 1.0
0	15	90.1 ± 4.7	12.8 ± 0.4	3.4 ± 3.7	37.4 ± 6.2
0	25	95.6 ± 2.4	12.4 ±0.6	28.0 ± 11.9	100.0 ± 0.0
50	1	0.0 ± 0.0	13.2 ± 0.2	0.0 ± 0.0*	3.8 ± 0.5
50	3	88.8 ± 6.4	13.2 ± 0.2	0.0 ± 0.0	6.3 ± 0.6
50	5	93.5 ± 2.5	12.1 ± 0.5	0.1 ± 0.1	10.4 ± 1.2
50	10	97.2 ± 1.8	12.3 ± 0.8	29.3 ± 28.3	31.0 ± 11.7
50	15	99.8 ± 0.2	11.6 ± 0.5	75.0 ±15.5	81.3 ± 9.3
50	25	96.7 ± 4.1	10.0 ± 0.7	93.3 ± 4.1	125.0 ± 8.8
100	1	87.5 ± 1.4	11.5 ± 0.5	9.4 ± 10.8*	8.1 ± 1.0
100	3	92.1 ± 0.6	12.3 ± 1.1	11.4 ± 15.7	15.5 ± 7.8
100	5	98.5 ± 0.5	11.1 ± 0.6	54.5 ± 10.0	36.0 ± 5.9
100	10	100.0 ± 0.0	10.6 ± 0.1	96.3 ± 5.2	95.8 ± 5.9
100	15	99.9 ± 0.1	9.4 ± 0.2	98.8 ± 1.1	100.0 ± 7.7
100	25	100.0 ± 0.0	7.7 ± 0.6	100.0 ± 0.0	134.4 ± 6.9

Figure 4.7. Transverse sections from control red-osier dogwood roots used to determine the developmental stage of the roots at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 0 mM NaCl treatment. (A) At 1 cm, early stage 1 of development. (B) At 3 cm, early to mid-stage 1 of development. (C) At 5 cm, mid-stage 1 of development. (D) At 10 cm, late stage 1 of development. (E) At 15 cm, late stage 2 of development, with a vascular cambium formed as well as division of many of the endodermal cells. (F) At 25 cm, stage 5 of development. Reduced number of cortex layers because of the squishing of the inner cortex cells with the secondary development of the vascular tissue. Abundant division of endodermal cells had occurred to maintain the integrity of the layer. Some of the epidermal cells had been sloughed off. Sections stained with toluidine blue. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex; en, endodermis; pc, pericycle; ph, phloem tissue; vc, vascular cambial zone; sx, secondary xylem; px, protoxylem; mx, metaxylem. Staining procedure = toluidine blue. Scale bars = 100 μm.

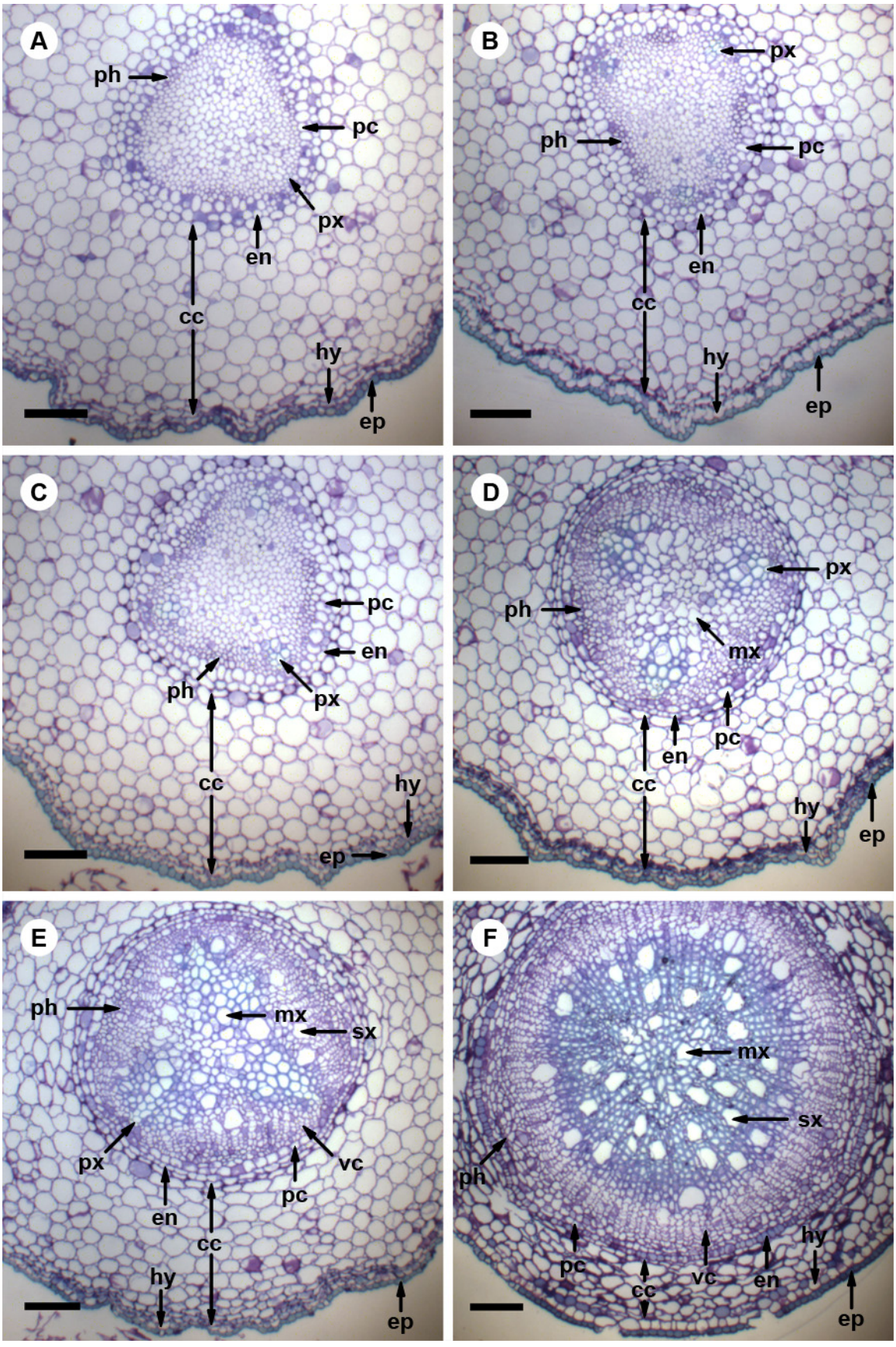
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Figure 4.8. Freehand transverse sections from red-osier dogwood roots used to illustrate the development of the xylem tissue and endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 0 mM NaCl treatment. (A) Xylem development: early stage 1. No Casparian bands detected in the radial walls of the endodermal cells. (B) Xylem development: early to mid-stage 1. Casparian band fluorescence (white arrowheads) was observed in the radial walls of the endodermal cells. (C) Xylem development: mid-stage 1. Only Casparian bands in the radial endodermal cell walls (D) Xylem development: late stage 1. Only Casparian bands in the radial endodermal cell walls. (E) Xylem development: stage 2. Approximately 10% of the endodermal cells had autofluorescence of the cell walls indicating suberin lamellae (red arrows). Endodermal cells containing suberin lamellae occurring between xylem poles. (F) Xylem development: stage 4. Suberin lamellae in approximately 30% of the endodermal cells. Endodermal cells with suberin lamellae appeared to be more elongated in cross-section than cells with only Casparian bands. Sections were viewed under UV light to observe autofluorescence. Abbreviations: en, endodermis; px, protoxylem; mx, metaxylem; sx, secondary xylem. Scale bars = 50 μm.

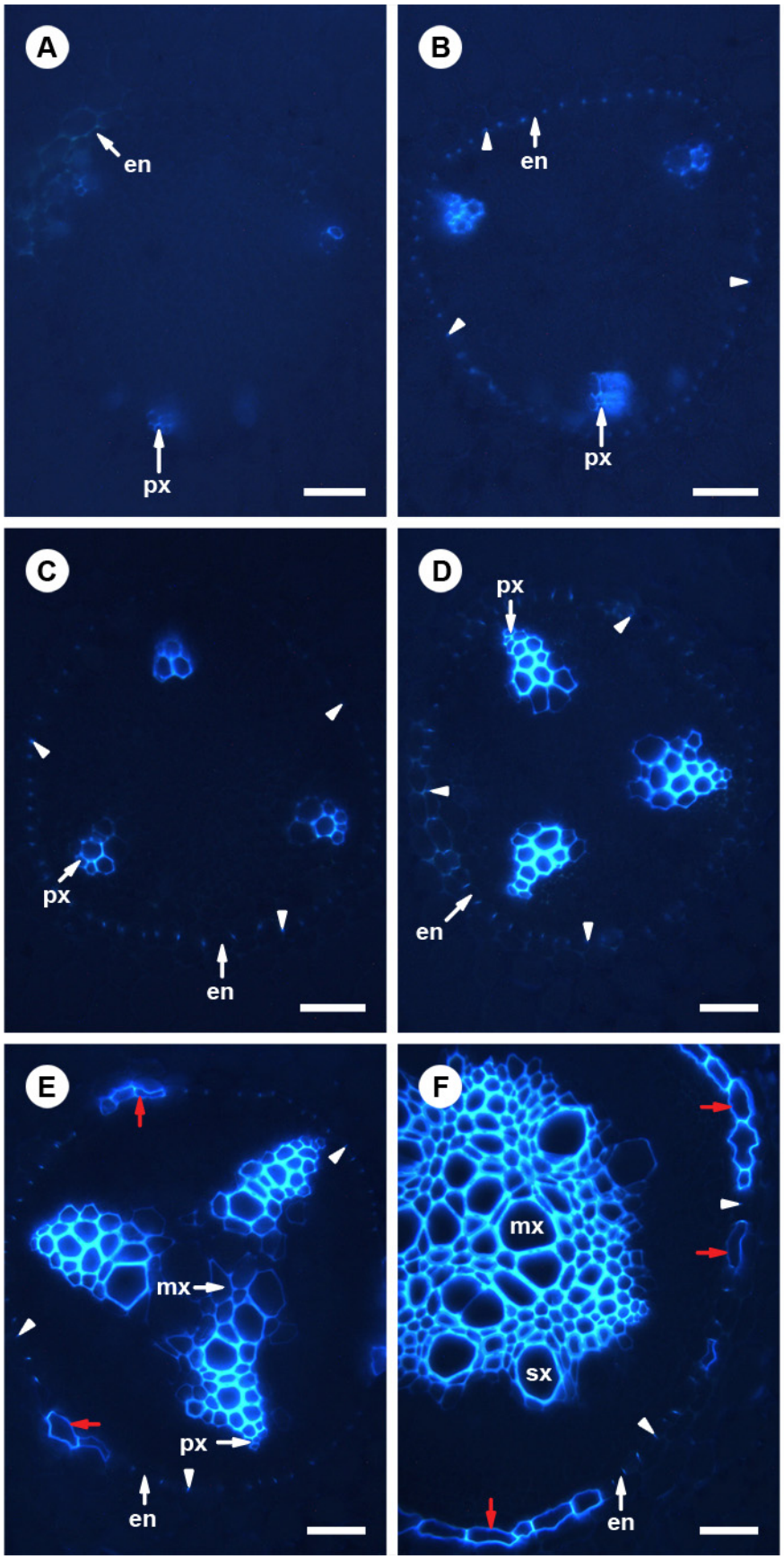
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Figure 4.9. Freehand transverse sections from red-osier dogwood roots used to illustrate the development of endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 0 mM NaCl treatment. (A) No Casparian bands detected in the radial walls of the endodermal cells. (B) Casparian band fluorescence (white arrowheads) was observed in the radial walls of the endodermal cells. (C – D) Only Casparian bands in the radial endodermal cell walls fluoresced. (E) Autofluorescence (red arrows) of endodermal cell walls containing suberin lamellae. (F) Passage cells (asterisk) were still present in the endodermis. Division of endodermal cells (double white arrowhead) was observed. Sections were viewed under UV light to observe autofluorescence. Abbreviations: cc, central cortex; endodermis; px, protoxylem. Scale bars = 10 μm.

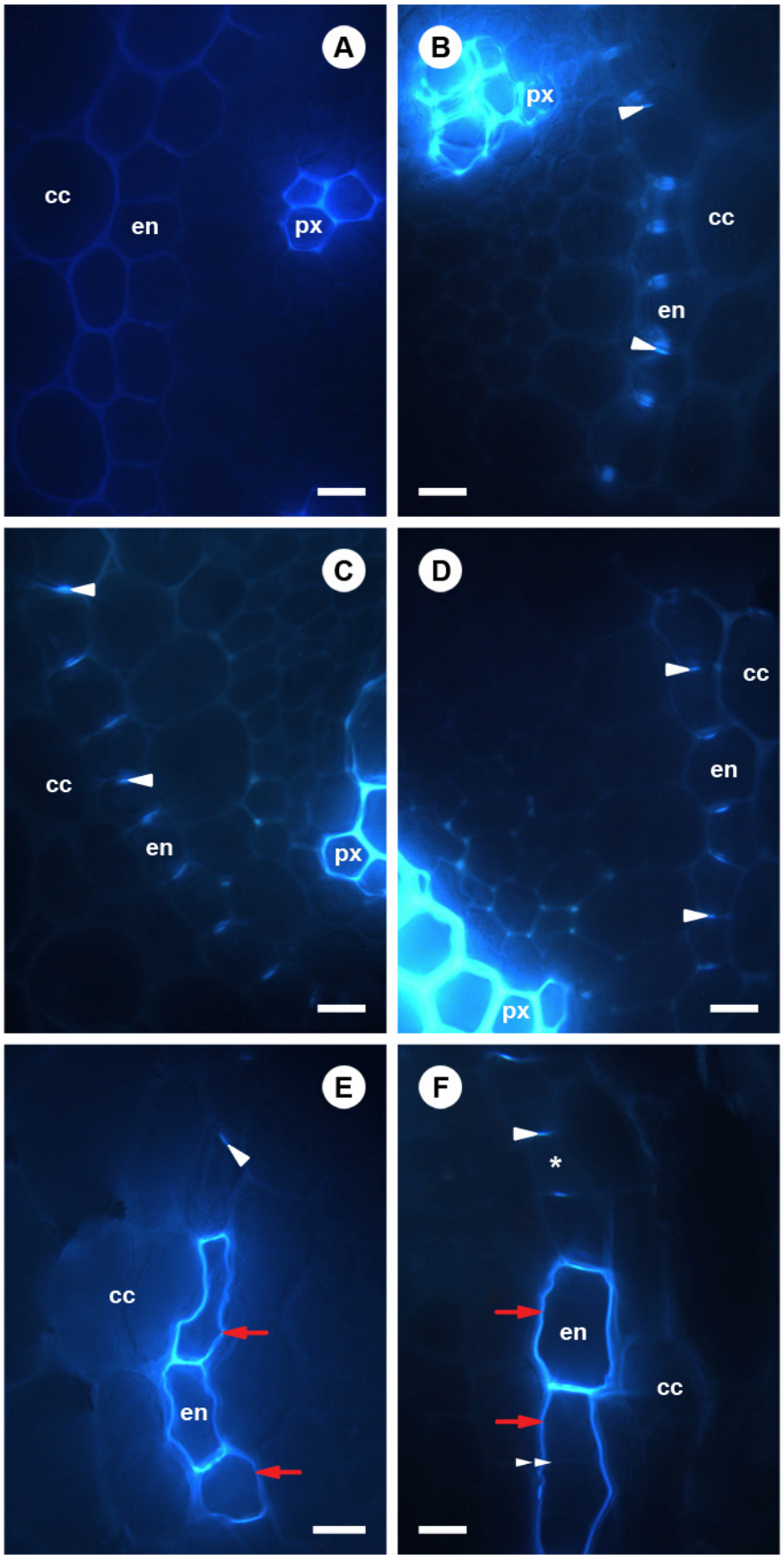
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Figure 4.10. Freehand transverse sections from control red-osier dogwood roots stained with Sudan red 7B to detect the presence of suberin lamellae in the endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks treatment. (A – B) No suberin lamellae detected. (C – D) Suberin lamellae continued not to be detected. Band plasmolysis was observed in endodermal cells (between black arrows). (E) The walls of some of the endodermal cells stained red indicating suberin lamellae (black arrows). (F) The new walls of the divided endodermal cell did not contain suberin lamellae (double black arrowheads). Abbreviations: cc, central cortex; en, endodermis; pc, pericycle; px, protoxylem. Scale bars = 10 μm.

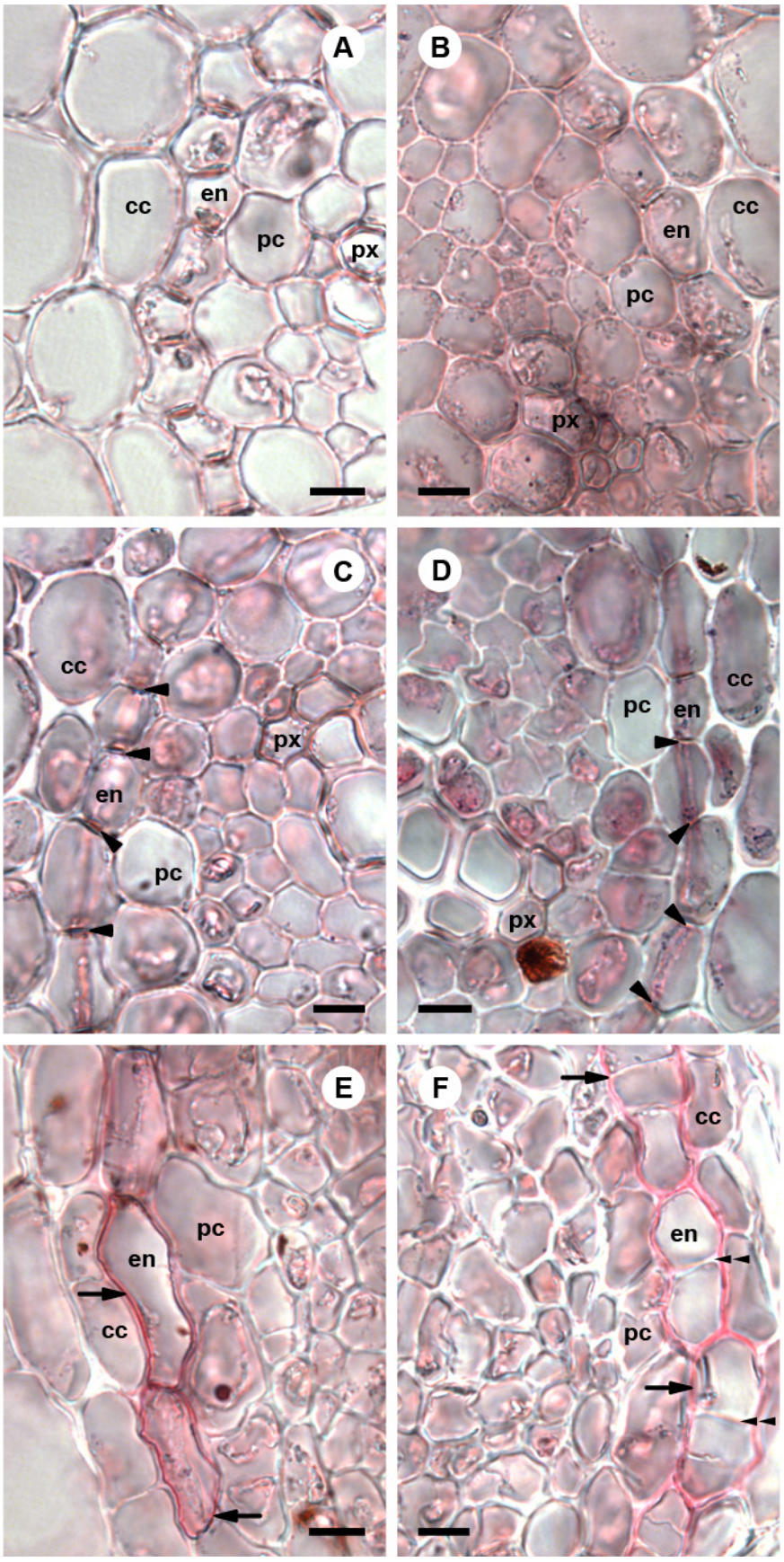
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Figure 4.11. Freehand transverse sections from red-osier dogwood roots stained with phloroglucinol-HCl to detect the presence of lignin in the endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 0 mM NaCl treatment. Endodermal cells did not contain significant red staining that would have indicated the presence of lignin. Abbreviations: cc, central cortex; en, endodermis; pc, pericylcle; px, protoxylem. Scale bars = 10 μm.

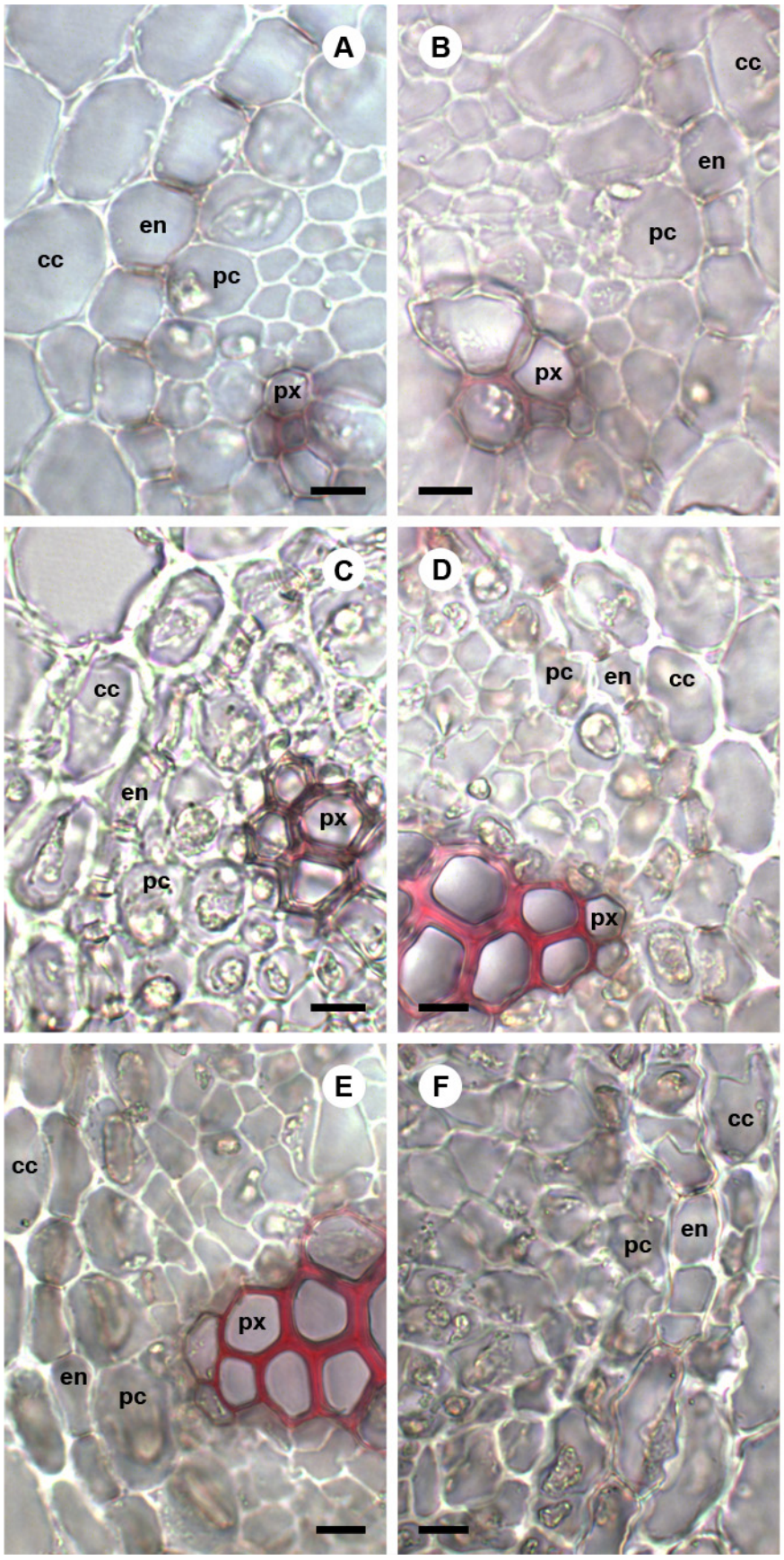
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Figure 4.12. Transverse sections from red-osier dogwood roots after four weeks of NaCl treatment used to illustrate endodermal cell division. (A) 50 mM NaCl treatment. Freehand section, 25 cm from the root tip. Observed under UV and white light. Anticlinal divisions (black double arrowheads) of the endodermal cells formed multiple new cells. Faint fluorescence of the original endodermal cells suberin lamellae (black arrows). Original endodermal radial walls indicated by black arrowheads. (B) Same section as in A, but under UV light only. The new walls of the divided endodermal walls (white double arrowheads) did not fluoresce and the new cells were enclosed by the original cells suberin lamella (red arrows). Original endodermal radial walls indicated by white arrowheads. (C) 100 mM NaCl treatment. Freehand section, 15 cm from the root tip. Observed under UV light. Double red arrowheads indicate where suberization of the new walls of a divided endodermal cells. (D) 0 mM treatment. Serial section, 25 cm from the root tip. Stained with toluidine blue. The original cell (defined between the two black arrowheads) appears to have divided once to form two cells and the two cells divided again to form four new cells. The radial cell wall where the first division occurs appears to be well-defined (double black arrowhead), while the area between the most recently divided cells lack a distinct cell wall (double white arrowheads). (E) 100 mM treatment. Freehand section, 25 cm from the root tip. Stained with calcofluor and observed under UV light. Walls of the endodermal cells vary from thick (original cell walls) to thin (newly formed radial walls indicated by double white arrowheads). Original endodermal radial walls indicated by white arrowheads. (F) 50 mM treatment. Freehand section, 25 cm from the root tip. Stained with Sudan red 7B. Radial walls of newly divided cells lacked suberin lamellae (double white arrowheads), but suberin lamellae appeared to have formed in older divided endodermal cells (double black arrowheads). Suberin lamellae of tangential walls indicated by red arrows. (G) 100 mM NaCl. Freehand sections, 25 cm from the root tip. Observed under UV light. With periderm formation, suberin lamellae (red arrows) appeared thinner and were difficult to distinguish under UV light. Abbreviations: hy, hypodermis; cc, central cortex; en, endodermis; pc, pericycle; pd, periderm; sx, secondary xylem. Scale bars: (A, B, D, E, F) = 10 μm; (C, G) = 50 μm.

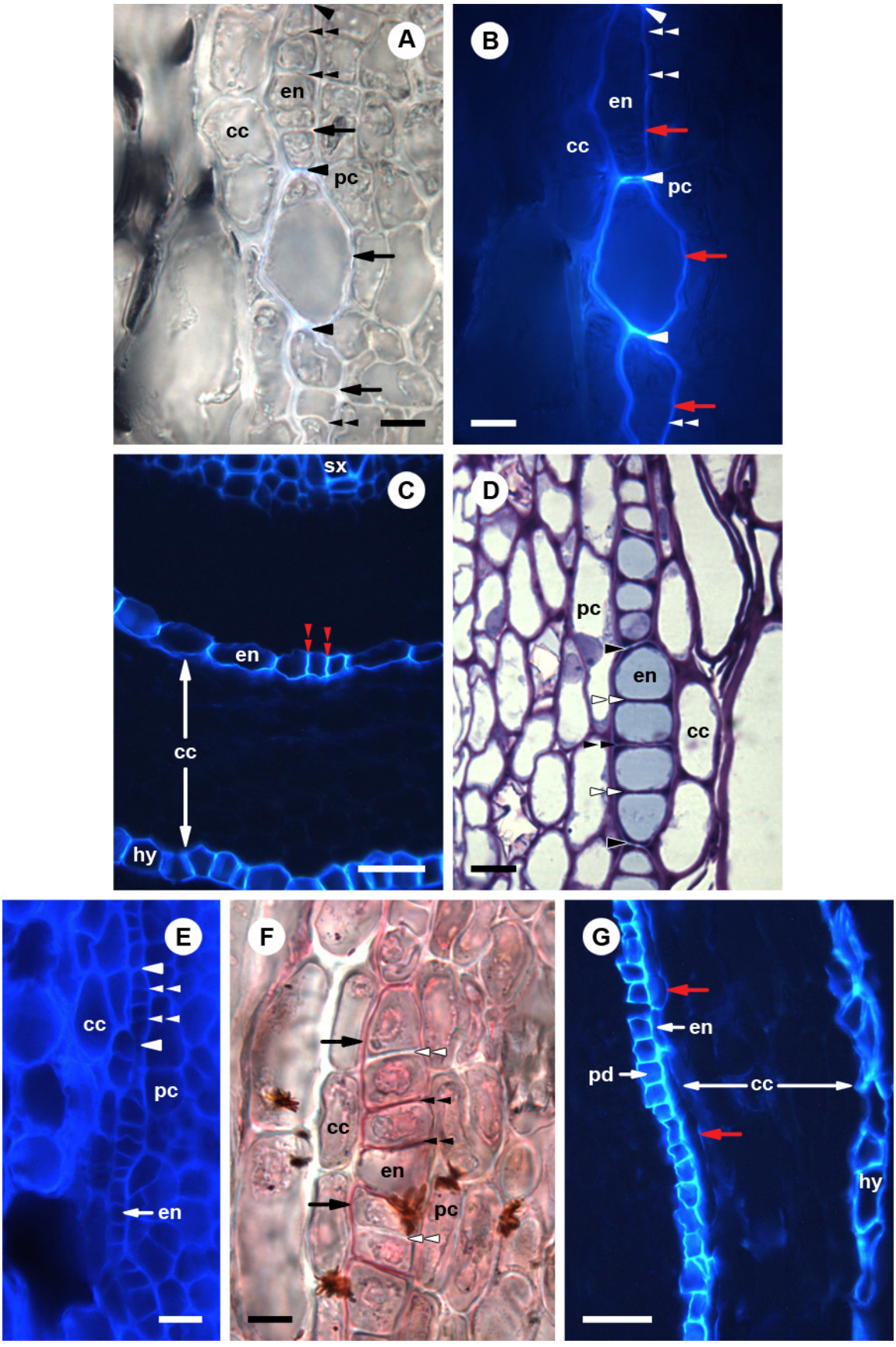
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Figure 4.13. Periderm formation in red-osier dogwood roots after four weeks of NaCl treatment (transverse sections). Periderm formation was similar in all treatments. (A) 0 mM NaCl treatment. Serial section, 25 cm from the root tip and stained with toluidine blue. Extensive secondary xylem growth. Central cortex layer were being squished and lost. In parallel, the epidermal cells were also being lost. Periderm appeared to be four to five layers deep. (B) 100 mM NaCl. Freehand section, 25 cm from the root tip. Sections stained with calcofluor and observed under UV light. White arrows indicate the newly form thin walls of the periderm. (C) 0 mM NaCl. Freehand section, 65 cm from the root tip. The epidermis and the cortex were lost except for the inner most central cortex layer and endodermis during the sectioning of the root. The periderm is approximately 5 layers deep. (D) Magnification of the area in the rectangle in C. Sections observed under UV light. Suberin lamellae of endodermal cells appear thin and faint (red arrows) compared to the outer layer of the periderm (red arrowheads). (E) 0 mM NaCl treatment. Freehand section, 65 cm from the root tip. Stained with Sudan red 7B. The endodermal layer (black arrows) and parts of the outer peridermal layer (white arrowheads) stained positively for suberin lamellae. (F) 0 mM NaCl treatment. Freehand section, 65 cm from the root tip. Stained with phloroglucinol-HCl. Lignin was not detected in the periderm. Abbreviations: ep, epidermis; cc, central cortex; en, endodermis; pd, periderm; ph, phloem tissue; vc, vascular cambial zone; sx, secondary xylem. Scale bars: (A, C) = 50 µm; (B, D, E, F) = 10 µm.

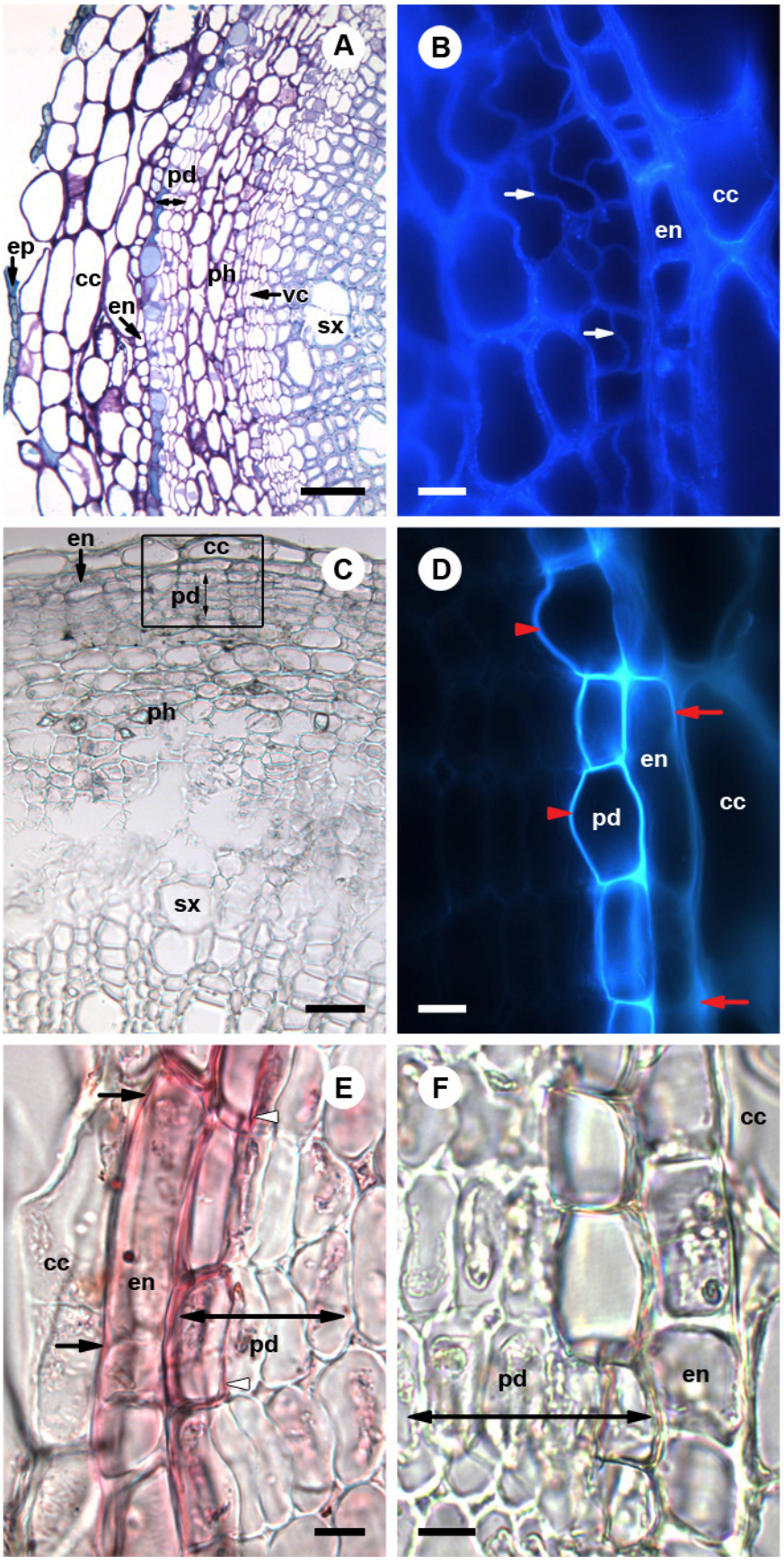
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Figure 4.14. Freehand transverse sections from control red-osier dogwood roots used to illustrate the development of hypodermal cell wall modifications at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of treatment. (A) Epidermal cell walls fluoresced blue (yellow arrowheads), but no fluorescence of the hypodermal cell walls was detected. (B) Hypodermal cell walls with blue fluorescence (red arrowheads) appeared faint and thin. Epidermal cell walls fluoresced blue and the internal contents fluoresced yellow (double yellow arrowheads). (C) The intensity of the fluorescence of hypodermal cell walls had increased from the previous observed distance, but the intensity of the cell wall fluorescence varied around the circumference of the cell. (D) The fluorescence of the hypodermal cells clearly outlined the circumference of some of the endodermal cells. (E) The number of hypodermal cells with thick and intense fluorescent walls increased. (F) Most of the hypodermal cells at 25 cm from the root tip showed fluorescence. The intensity and thickness of the wall fluorescence was relatively intense in all cell walls compared to closer the root tip. Sections were viewed under UV light to observe autofluorescence. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

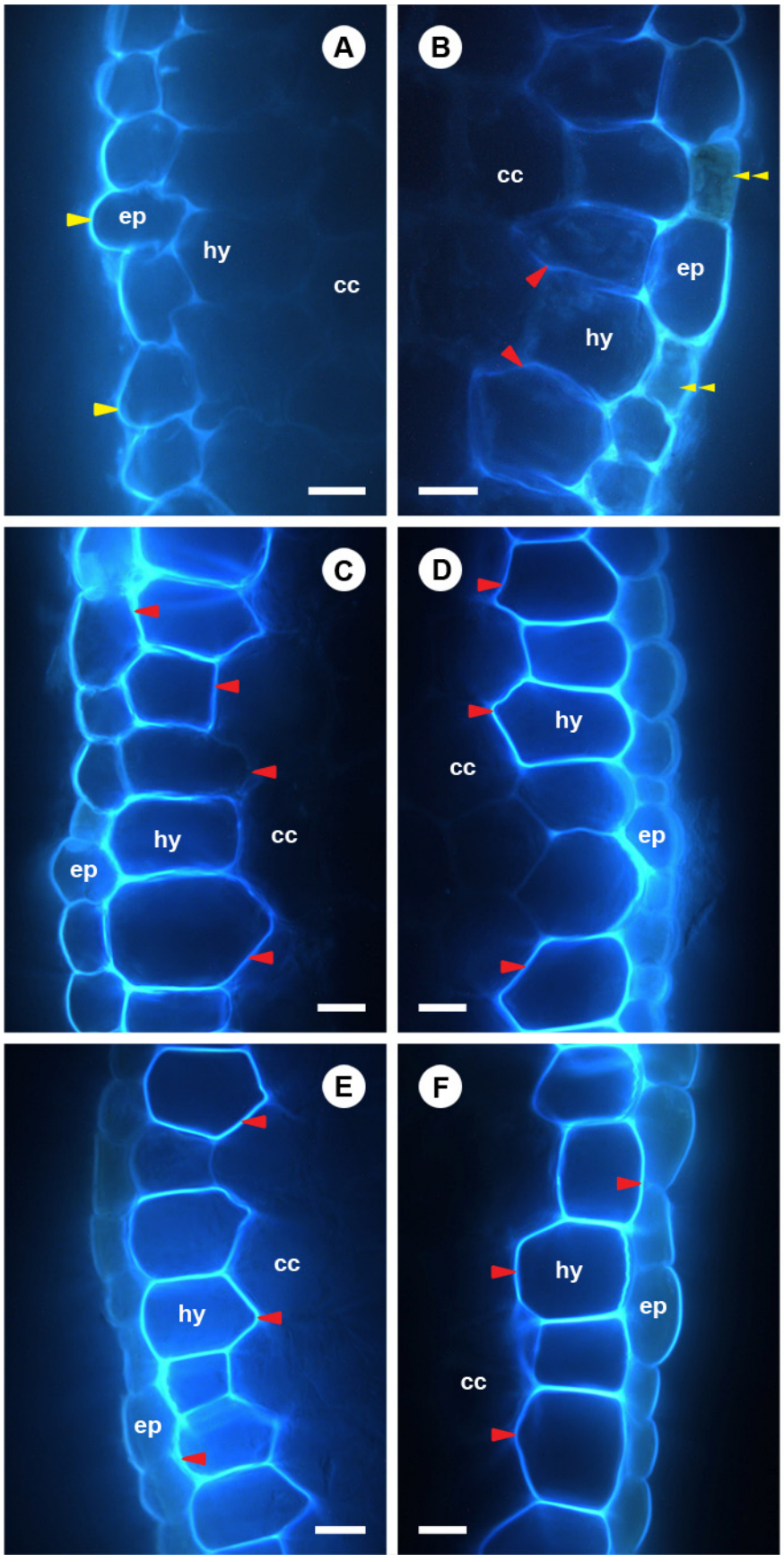
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Figure 4.15. Freehand transverse sections from control red-osier dogwood roots stained with Sudan red 7B to detect the presence of suberin lamellae in the hypodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of treatment. (A) No indication of suberin lamellae in hypodermal cell walls. (B) Outer tangential walls and outer radial walls of some of the hypodermal cells were positive for suberin (black arrows). (C) Suberin lamellae extended to all the walls of the hypodermal cells. Reddish-orange staining of epidermal cells (white arrowheads). (D) Suberin lamellae occasionally contained in the cortex cells below a hypodermal layer without suberin lamellae (white concave-based arrowhead). (E – F) Suberin lamellae in the hypodermal cell walls. Epidermal cells stained orange-red. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

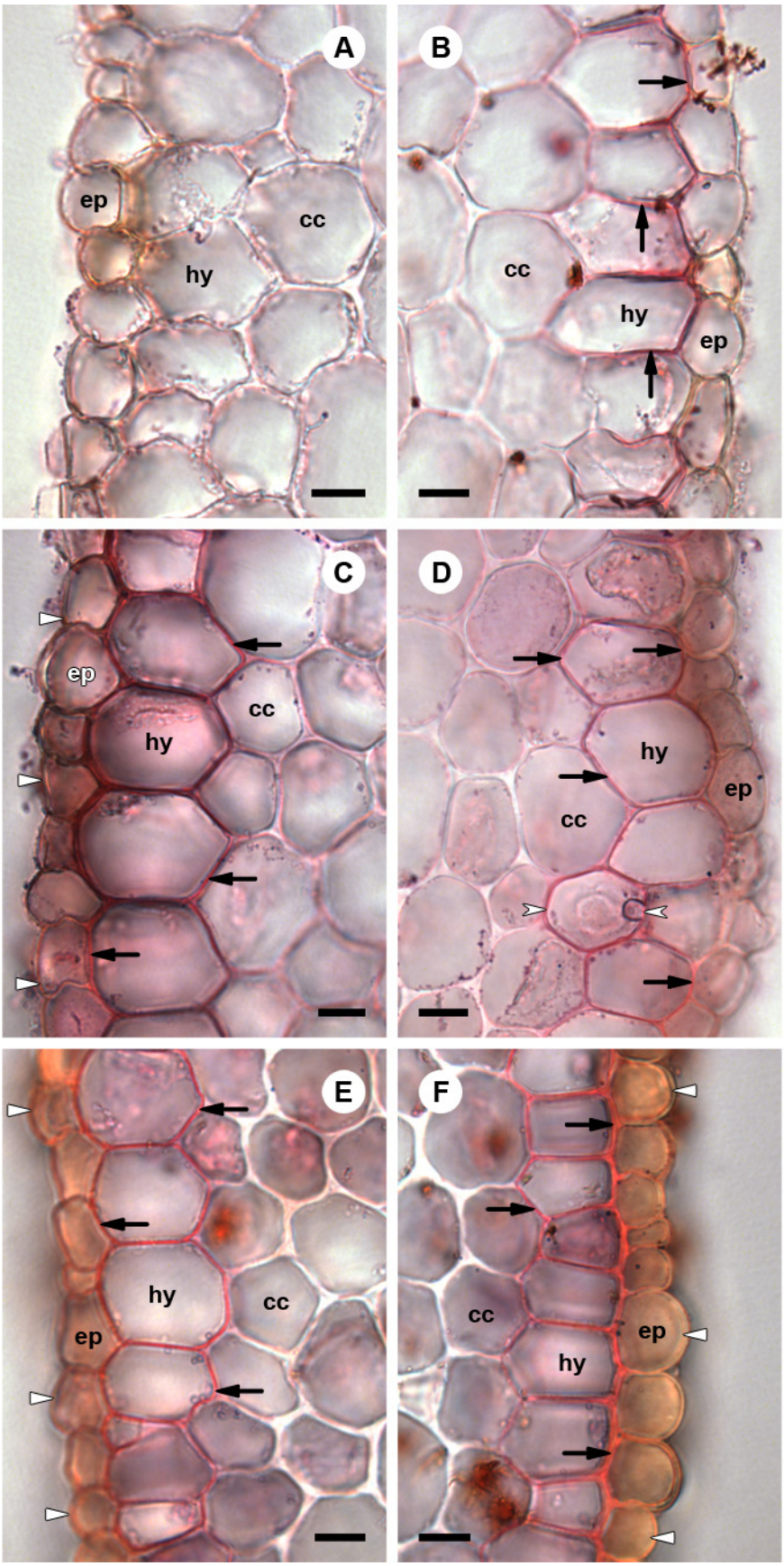
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Figure 4.16. Freehand transverse sections from control red-osier dogwood roots stained with phloroglucinol-HCl to detect the presence of lignin in the hypodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks treatment. (A – B) Lignin was not observed in the hypodermal cells. Epidermal cells stained yellow (white arrowheads). (C – D) Faint staining of hypodermal cell walls (black concave-based arrows. (E) Lignin not detected in hypodermal cell walls. (F) Unspecific staining observed in cortical cells (black arrowheads). Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

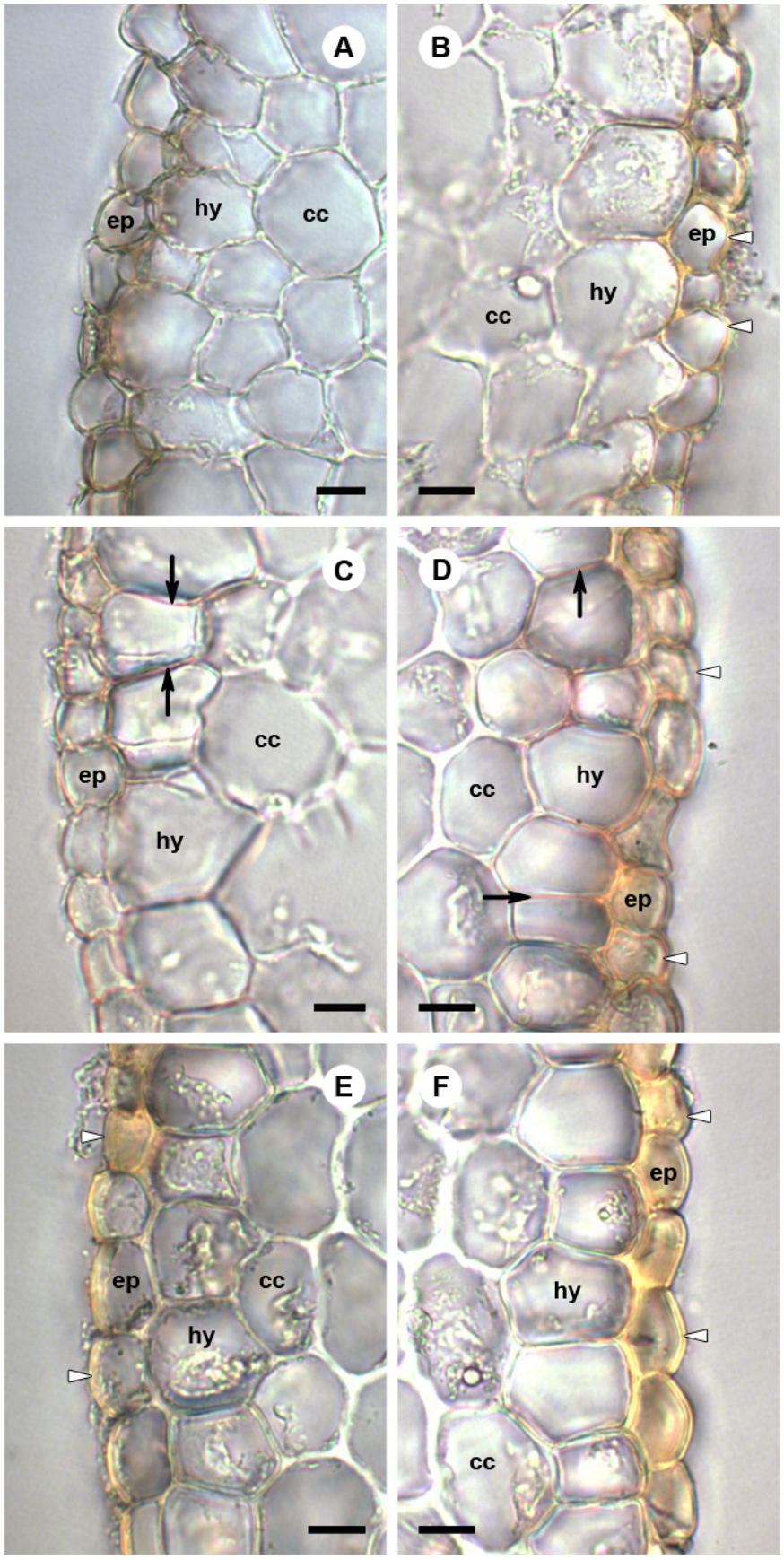
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Figure 4.17. Comparison of freehand transverse sections from red-osier dogwood roots stained with berberine-hemisulphate and counterstained with aniline blue (A, C, E) to sections not stained (B, D, F) after four weeks of 100 mM NaCl treatment. All sections were observed under UV light. (A) 15 cm from the root tip. Xylem tissue intensely fluoresced yellow. The walls between two fluorescing cells in the hypodermis or the walls between the fluorescing hypodermal cell and the adjacent fluorescing cortical cell fluoresced yellow (double red arrowheads). The other fluorescent cell walls appeared blue (red arrowheads) and was less intense than the yellow fluorescent cell walls. (B) 15 cm from the root tip. The fluorescent cell walls appeared blue (red arrowheads), except between two adjacent fluorescent cells, which appeared more white-blue (double red arrowheads). Fluorescence of the xylem tissue was less intense than seen with berberine hemisulphate and aniline blue staining procedure (A). (C) 5 cm from the root tip. Lack of fluorescence of epidermal cell walls (yellow arrowheads). Hypodermal cell walls fluoresced (red arrowheads) difficult to observe because of the fluorescence of the adjacent cortical cells (white concave-based arrowheads). (D) 5 cm from the root tip. Epidermal cells fluoresced yellow (yellow arrowheads) and the hypodermal cells fluoresced blue (red arrowheads). (E) 15 cm from the root tip. At higher magnification, the fluorescence of the suberin lamellae of endodermal cells (red arrows) was masked by the fluorescence of cell walls without suberin lamellae in the endodermis, central cortex and vascular tissue (white concave-based arrowheads). (F) 15 cm from the root tip. Autofluorescence of the endodermal cells containing suberin lamellae (red arrows) was easily detected because of the lack of fluorescence of surrounding tissue. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex; en, endodermis; pc, pericycle; mx, metaxylem; sx, secondary xylem. Scale bars: (A – B) = 50 μm; (C – F) = 10 μm.

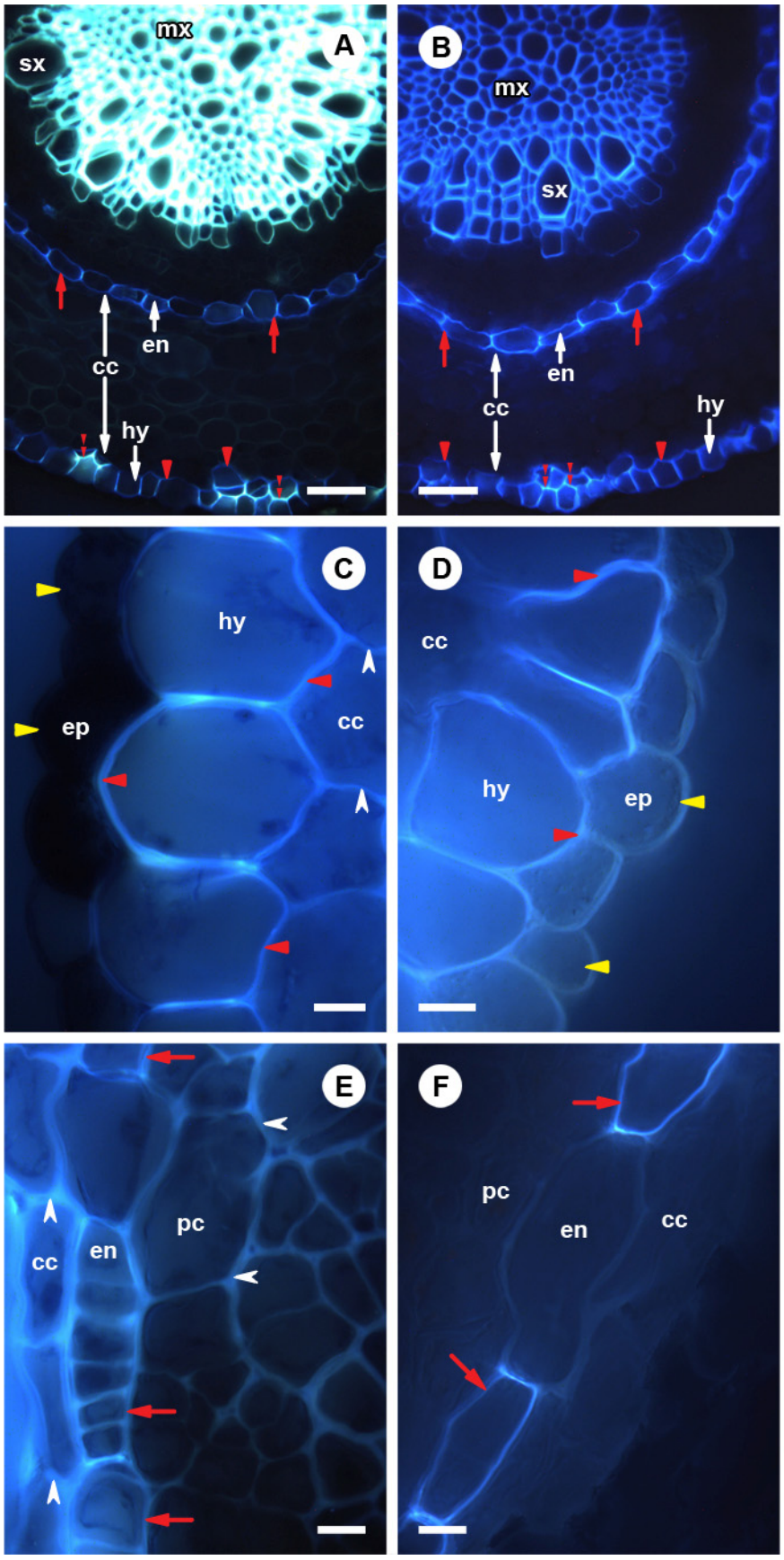
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Table 4.3. Root anatomy measurements of control red-osier dogwood roots at various distances from the root tip after four weeks of treatment to illustrate differences between replicates. Values are mean ± S.E. for each replicate (Rep 1: n = 5, Rep 2 – 6: n = 3). The treatments mean values are shown in Table 4.1.

Rep	Distance (cm)	Total Root Area (mm^2)	Stele/root area (%)	Cortex thickness (µm)	No. of cortex layers	Cortical cell diameter (µm)
1	1	0.47 ± 0.03	10.0 ± 0.3	241± 8	12.8 ± 0.4	18.8 ± 0.6
	3	0.52 ± 0.05	8.9 ± 0.4	264 ± 14	12.6 ± 0.6	21.0 ± 0.8
	5	0.46 ± 0.07	10.6 ± 0.9	234 ± 21	12.5 ± 0.3	18.6 ± 1.3
	10	0.47 ± 0.03	23.0 ± 1.5	193 ± 14	12.8 ± 1.0	15.3 ± 1.2
	15	0.69 ± 0.11	35.3 ± 5.9	177 ± 36	11.0 ± 1.3	15.6 ± 1.5
	25	0.79 ± 0.11	54.6 ± 2.7	116 ± 8	8.4 ± 1.1	14.2 ± 1.1
2	1	0.78 ± 0.10	10.1 ± 0.7	310 ± 16	13.7 ± 0.4	22.7 ± 0.6
	3	0.79 ± 0.04	8.2 ± 0.1	324 ± 13	13.7 ± 0.4	23.7 ± 0.7
	5	0.89 ± 0.10	9.0 ± 0.8	342 ± 23	13.3 ± 0.4	25.7 ± 1.7
	10	0.96 ± 0.16	12.3 ± 1.6	330 ± 35	14.0 ± 0.7	23.5 ± 1.6
	15	0.88 ± 0.06	19.7 ± 1.7	270 ± 9	14.0 ± 0.7	19.3 ± 0.4
	25	1.02 ± 0.13	43.8 ± 3.9	173 ± 24	9.3 ± 0.8	18.4 ± 1.1
3	1	0.75 ± 0.08	9.9 ± 0.5	302 ± 21	12.7 ± 0.4	23.9 ± 2.3
	3	0.81 ± 0.02	9.3 ± 0.2	311 ± 12	13.0 ± 0.7	24.0 ± 0.9
	5	0.84 ± 0.08	9.3 ± 0.2	318 ± 30	13.7 ± 0.4	23.3 ± 2.0
	10	0.97 ± 0.11	14.5 ± 1.0	315 ± 25	13.7 ± 0.4	23.0 ± 1.2
	15	0.92 ± 0.02	17.8 ± 0.7	269 ± 8	13.3 ± 0.4	20.2 ± 1.2
	25	0.93 ± 0.11	40.4 ± 3.9	176 ± 19	10.7 ± 1.1	16.5 ± 1.2
4	1	0.46 ± 0.04	7.4 ± 0.3	236 ± 16	10.7 ± 0.4	22.1 ± 1.1
	3	0.47 ± 0.03	7.6 ± 0.1	235 ± 6	10.7 ± 0.4	22.1 ± 0.3
	5	0.55 ± 0.03	7.7 ± 0.2	258 ± 19	11.7 ± 0.4	22.1 ± 1.0
	10	0.49 ± 0.06	15.8 ± 0.9	213 ± 19	11.7 ± 0.4	18.2 ± 1.4
	15	0.63 ± 0.11	22.5 ± 2.9	204 ± 29	11.7 ± 0.8	17.4 ± 1.4
	25	0.86 ± 0.01	37.2 ± 4.7	184 ± 22	10.0 ± 0.7	18.4 ± 0.9
5	1	0.83 ± 0.07	8.1 ± 0.3	339 ± 17	13.3 ± 1.1	25.5 ± 0.8
	3	0.88 ± 0.07	8.4 ± 0.1	341 ± 14	13.7 ± 0.4	24.9 ± 0.3
	5	0.93 ± 0.10	8.7 ± 0.2	345 ± 7	14.0 ± 0.7	24.7 ± 1.1
	10	0.95 ± 0.12	14.1 ± 0.8	306 ± 26	13.3 ± 0.4	22.9 ± 1.2
	15	0.94 0.13	18.9 ± 3.2	269 ± 33	13.3 ± 1.1	20.1 ± 1.1
	25	1.02 ± 0.12	40.2 ± 1.2	188 ± 20	10.3 ± 0.8	18.1 ± 0.6
6	1	0.64 ± 0.06	10.0 ± 0.6	289 ± 19	13.3 ± 0.4	21.7 ± 1.4
	3	0.69 ± 0.07	9.4 ± 0.7	314 ± 17	13.7 ± 0.4	23.0 ± 1.5
	5	0.67 ± 0.06	9.2 ± 0.1	309 ± 26	13.7 ± 0.4	22.5 ± 1.3
	10	0.66 ± 0.06	13.5 ± 0.7	263 ± 20	14.0 ± 0.0	18.8 ± 1.4
	15	0.79 ± 0.10	22.7 ± 4.5	246 ± 22	13.3 ± 0.8	18.4 ± 1.1
	25	1.06 ± 0.03	38.1 ± 10.1	210 ± 43	11.3 ± 1.8	18.4 ± 1.3

Table 4.4. Root anatomy measurements of red-osier dogwood roots at various distances from the root tip after exposure to four weeks of 50 mM NaCl to illustrate differences between replicates. Values are mean ± S.E. for each replicate (Rep 1: n = 5, Rep 2 – 6: n = 3). The treatments mean values are shown in Table 4.1. * indicates the replicates exhibiting thickening of the distal portion of the root. << indicates the distance(s) from the root tip that corresponds to the increase in root length during the treatment.

Rep	Distance (cm)	Total root area (mm^2)	Stele/root area (%)	Cortex thickness (µm)	No. of cortex layers	Cortical cell diameter (µm)
1	1	0.42 ± 0.03	8.6 ± 0.2	238 ± 12	12.4 ± 0.4	19.2 ± 0.6
	3	0.46 ± 0.06	7.4 ± 0.2	258 ± 24	12.0 ± 0.7	21.5 ± 1.4
	5	0.43 ± 0.05	8.6 ± 0.4	242 ± 17	12.2 ± 0.4	19.8 ± 1.0
	10	0.47 ± 0.03	12.7 ± 0.63	235 ± 12	11.2 ± 0.4	20.9 ± 0.5
	15<<	0.53 ± 0.09	21.2 ± 1.8	188 ± 11	10.4 ± 0.9	18.3 ± 1.2
	25<<	0.67 ± 0.14	38.2 ± 8.5	165 ± 20	8.4 ± 0.6	19.4 ± 1.1
2*	1	1.06 ± 0.14	6.3 ± 0.4	404 ± 40	14.7 ± 0.8	27.5 ± 1.1
	3	1.03 ± 0.17	7.3 ± 0.3	384 ± 41	14.3 ± 0.4	26.7 ± 2.1
	5<<	0.88 ± 0.11	10.7 ± 0.4	321 ± 20	13.7 ± 0.8	23.5 ± 1.0
	10<<	0.49 ± 0.06	31.2 ± 3.8	160 ± 24	9.7 ± 1.1	16.5 ± 1.1
	15	0.74 ± 0.22	45.4 ± 0.4	136 ± 17	8.0 ± 1.2	17.1 ± 0.5
	25	1.95 ± 0.45	68.0 ± 2.6	112 ± 11	5.7 ± 0.4	19.9 ± 2.3
3*	1	1.19 ± 0.00	5.6 ± 0.3	445 ± 9	14.7 ± 0.4	30.3 ± 0.6
	3	1.10 ± 0.06	6.6 ± 0.3	412 ± 19	14.3 ± 0.4	28.7 ± 0.5
	5<<	0.91 ± 0.06	10.7 ± 1.6	336 ± 25	12.7 ± 0.4	26.4 ± 1.2
	10<<	0.70 ± 0.10	39.6 ± 1.6	158 ± 16	7.7 ± 0.4	20.5 ± 1.4
	15	1.21 ± 0.21	57.2 ± 4.5	125 ± 10	6.7 ± 0.4	18.9 ± 2.3
	25	2.56 ± 0.26	74.2 ± 1.2	116 ± 11	6.0 ± 0.0	19.3 ± 1.8
4	1	0.56 ± 0.05	7.7 ± 0.3	263 ± 17	12.0 ± 0.0	21.9 ± 1.4
	3	0.62 ± 0.05	7.6 ± 0.6	281 ± 14	12.7 ± 0.4	22.2 ± 0.4
	5	0.59 ± 0.02	8.8 ± 0.8	259 ± 15	13.0 ± 0.7	19.9 ± 0.5
	10<<	0.62 ± 0.03	13.9 ± 0.7	251 ± 10	12.3 ± 0.4	20.4 ± 0.5
	15<<	0.64 ± 0.04	18.1 ± 1.8	229 ± 15	12.0 ± 0.7	19.1 ± 0.2
	25	0.87 ± 0.33	49.6 ± 8.0	125 ± 15	7.3 ± 0.8	17.0 ± 0.5
5	1	0.70 ± 0.04	7.8 ± 0.2	277 ± 17	13.0 ± 0.7	21.3 ± 0.6
	3	0.74 ± 0.02	7.7 ± 0.7	295 ± 11	14.0 ± 0.7	21.1 ± 0.5
	5	0.82 ± 0.02	8.0 ± 0.4	307 ± 17	13.7 ± 0.4	22.5 ± 0.6
	10	0.91 ± 0.02	11.3 ± 2.0	327 ± 23	13.7 ± 1.1	24.0 ± 1.6
	15<<	0.76 ± 0.06	20.7 ± 1.6	253 ± 8	12.0 ± 0.0	21.1 ± 0.7
	25<<	1.47 ± 0.22	62.6 ± 0.9	135 ± 1	8.0 ± 0.7	17.0 ± 1.4
6	1	0.71 ± 0.13	7.3 ± 0.4	324 ± 34	15.0 ± 0.7	21.6 ± 1.6
	3	0.87 ± 0.14	8.3 ± 0.5	355 ± 34	15.7 ± 0.8	22.6 ± 1.0
	5	0.96 ± 0.08	7.9 ± 0.7	379 ± 19	14.7 ± 0.4	25.8 ± 0.6
	10	1.08 ± 0.11	10.9 ± 0.5	369 ± 19	15.7 ± 0.4	23.5 ± 0.9
	15<<	1.15 ± 0.07	15.4 ± 0.5	349 ± 21	14.7 ± 0.8	23.9 ± 1.9
	25<<	1.09 ± 0.02	28.1 ± 2.1	266 ± 5	12.0 ± 0.7	22.2 ± 0.9

Table 4.5. Root anatomy measurements of red-osier dogwood roots at various distances from the root tip after exposure to four weeks of 100 mM NaCl to illustrate differences between replicates. Values are mean ± S.E. for each replicate (Rep 1: n = 5, Rep 2 – 6: n = 3). The treatments mean values are shown in Table 4.1. * indicates the replicates exhibiting thickening of the distal portion of the root. << indicates the distance(s) from the root tip that corresponds to the increase in root length during the treatment.

Rep	Distance (cm)	Total root area (mm^2)	Stele/root area (%)	Cortex thickness (μm)	No. of cortex layers	Cortical cell diameter (μm)
1	1<<	0.50 ± 0.04	9.0 ± 0.6	262 ± 17	11.8 ± 0.5	22.2 ± 1.1
	3<<	0.44 ± 0.04	9.4 ± 0.4	242 ± 14	11.8 ± 0.4	20.7 ± 1.5
	5	0.46 ± 0.02	9.7 ± 0.3	242 ± 4	12.0 ± 0.5	20.2 ± 0.5
	10	0.48 ± 0.08	19.4 ± 4.5	209 ± 37	11.0 ± 0.8	18.9 ± 2.6
2*	1	0.63 ± 0.02	6.5 ± 0.3	295 ± 8	12.0 ± 0.0	24.6 ± 0.7
	3	0.72 ± 0.03	8.0 ± 0.2	319 ± 10	12.7 ± 0.4	25.2 ± 0.7
	5<<	0.66 ± 0.04	14.9 ± 1.8	260 ± 1	11.7 ± 0.4	22.3 ± 0.8
	10<<	0.38 ± 0.03	30.0 ± 3.4	143 ± 4	8.7 ± 0.4	16.6 ± 0.8
3	1	0.53 ± 0.03	6.4 ± 0.4	262 ± 8	12.3 ± 0.4	21.3 ± 0.8
	3	0.58 ± 0.06	6.6 ± 0.4	276 ± 25	11.3 ± 0.4	24.3 ± 1.4
	5<<	0.77 ± 0.08	7.5 ± 0.2	325 ± 26	12.0 ± 0.7	27.1 ± 0.7
	10<<	0.54 ± 0.08	20.1 ± 1.3	203 ± 5	10.3 ± 0.4	19.7 ± 0.3
	15	0.52 ± 0.07	15.7 ± 2.3	223 ± 26	11.0 ± 0.7	20.2 ± 1.9
4*	1	0.77 ± 0.05	7.9 ± 0.2	320 ± 14	12.7 ± 0.4	25.2 ± 0.4
	3	0.87 ± 0.05	8.3 ± 0.4	348 ± 12	13.7 ± 0.4	25.5 ± 0.9
	5<<	0.88 ± 0.06	10.5 ± 0.9	334 ± 20	12.3 ± 0.4	27.1 ± 1.4
	10<<	0.72 ± 0.08	23.2 ± 0.8	229 ± 12	10.5 ± 0.7	21.8 ± 0.3
	15	0.68 ± 0.04	34.4 ± 5.4	174 ± 26	9.7 ± 1.1	17.9 ± 1.3
5	1	0.64 ± 0.01	6.5 ± 0.4	288 ± 13	11.7 ± 0.8	24.8 ± 1.5
	3<<	0.57 ± 0.02	8.0 ± 0.3	273 ±13	11.7 ± 0.4	23.5 ± 1.6
	5<<	0.57 ± 0.06	9.6 ± 0.3	254 ± 21	11.0 ± 0.7	23.0 ± 1.1
	10	0.47 ± 0.05	13.2 ± 0.6	214 ± 9	10.0 ± 0.7	21.6 ± 2.2
	15	0.50 ± 0.04	28.8 ± 6.7	170 ± 34	9.0 ± 0.7	18.8 ± 2.5
6*	1	0.95 ± 0.18	5.8 ± 0.4	391 ± 37	13.7 ± 0.4	28.5 ± 1.9
	3<<	1.00 ± 0.18	10.3 ± 1.4	363 ± 48	12.3 ± 0.8	29.2 ± 2.1
	5<<	0.63 ± 0.05	20.1 ± 4.0	233 ± 28	10.0 ± 1.2	23.4 ± 1.3
	10	0.92 ± 0.05	27.4 ± 2.6	246 ± 17	10.7 ± 0.4	23.1 ± 1.3
	15	0.91 ± 0.05	31.6 ± 6.8	217 ± 37	10.0 ± 0.7	21.5 ± 2.2

Figure 4.18. Longitudinal section of a root tip from a control red-osier dogwood cutting. (A) A healthy root tip with healthy and numerous cells in the root cap and apical meristem area. (B) Enlargement of the root cap. Root cap cells are large with a homogenous shape and regular alignment. (C) Enlargement of the apical meristem area with abundant cell division. Sections stained with toluidine blue. Abbreviations: rc, root cap; pm, promeristem. Staining procedure = toluidine blue. Scale bars: (A) = 100 μm; (B – C) = 50 μm.

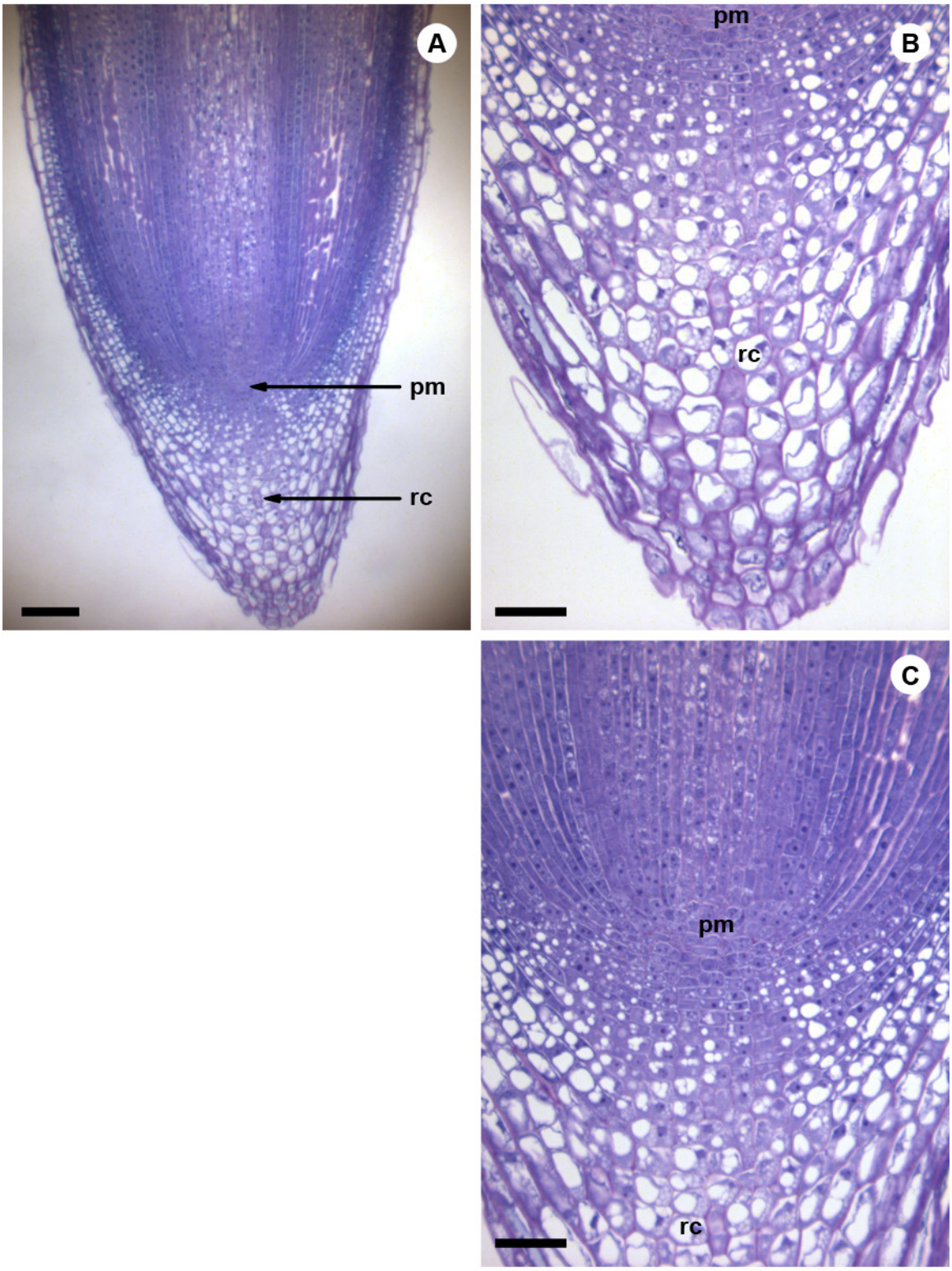
A
pm
rc
B
pm
rc
C
pm
rc

Figure 4.19. Longitudinal sections of root tips from 50 mM NaCl red-osier dogwood cuttings. (A) Root tip from a root not exhibiting thickening of the distal portion of the root (see Fig. 4.5A, B). (B) Enlargement of the root cap. Root cap cells appeared relatively smaller than the controls (see Fig. 4.18B). (C) Root tip from a root with the distal portion thickened relative to the proximal portion of the root (see Fig. 4.5C, D). (D) Enlargement of the root cap. Root cap cell appear relatively large compared to the thinner roots exposed to 50 mM NaCl and appear similar to the controls (see Fig. 4.18B). Staining procedure = toluidine blue. Scale bars: (A, C) = 100 μm; (B, D) = 50 μm.

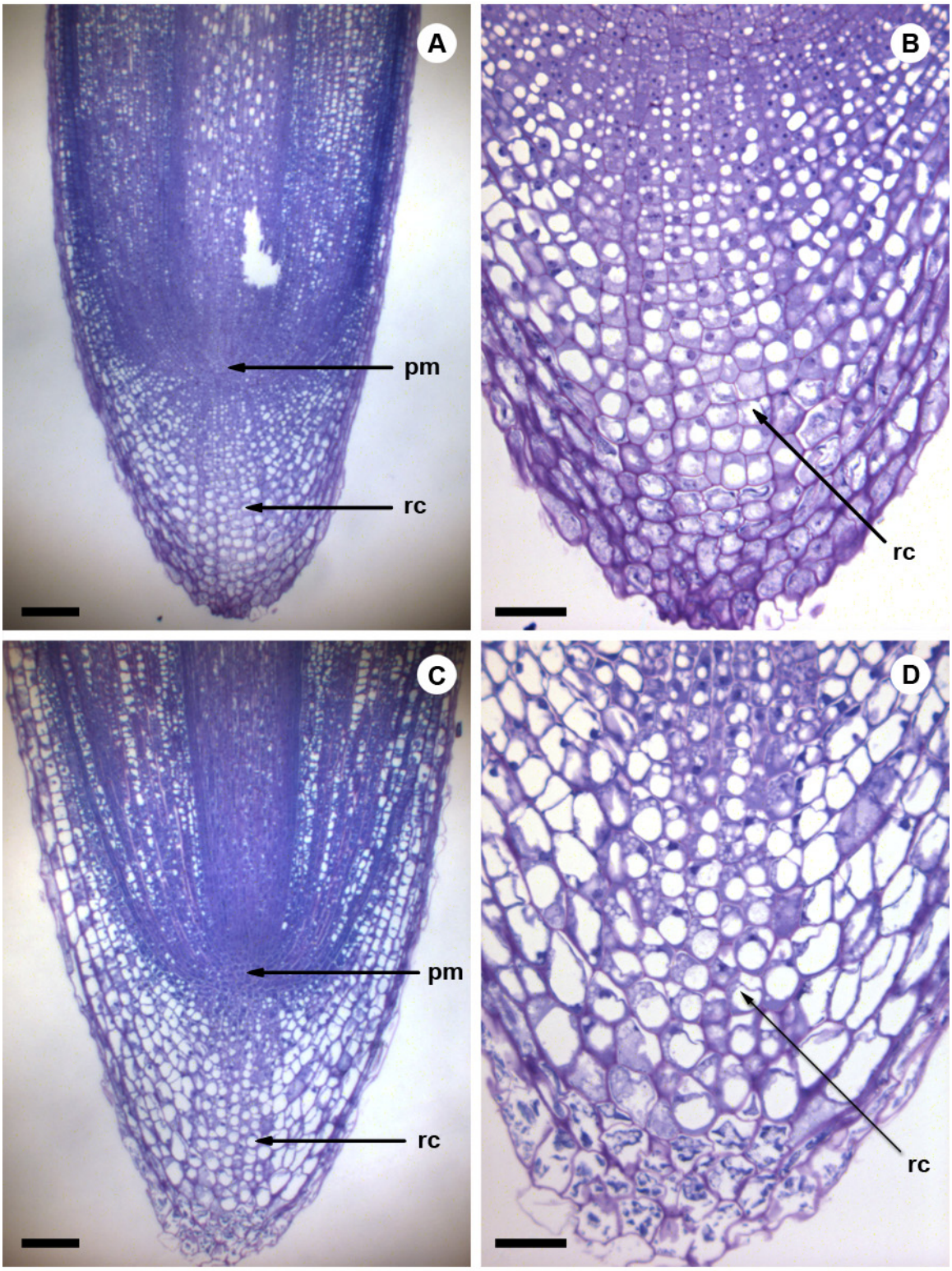
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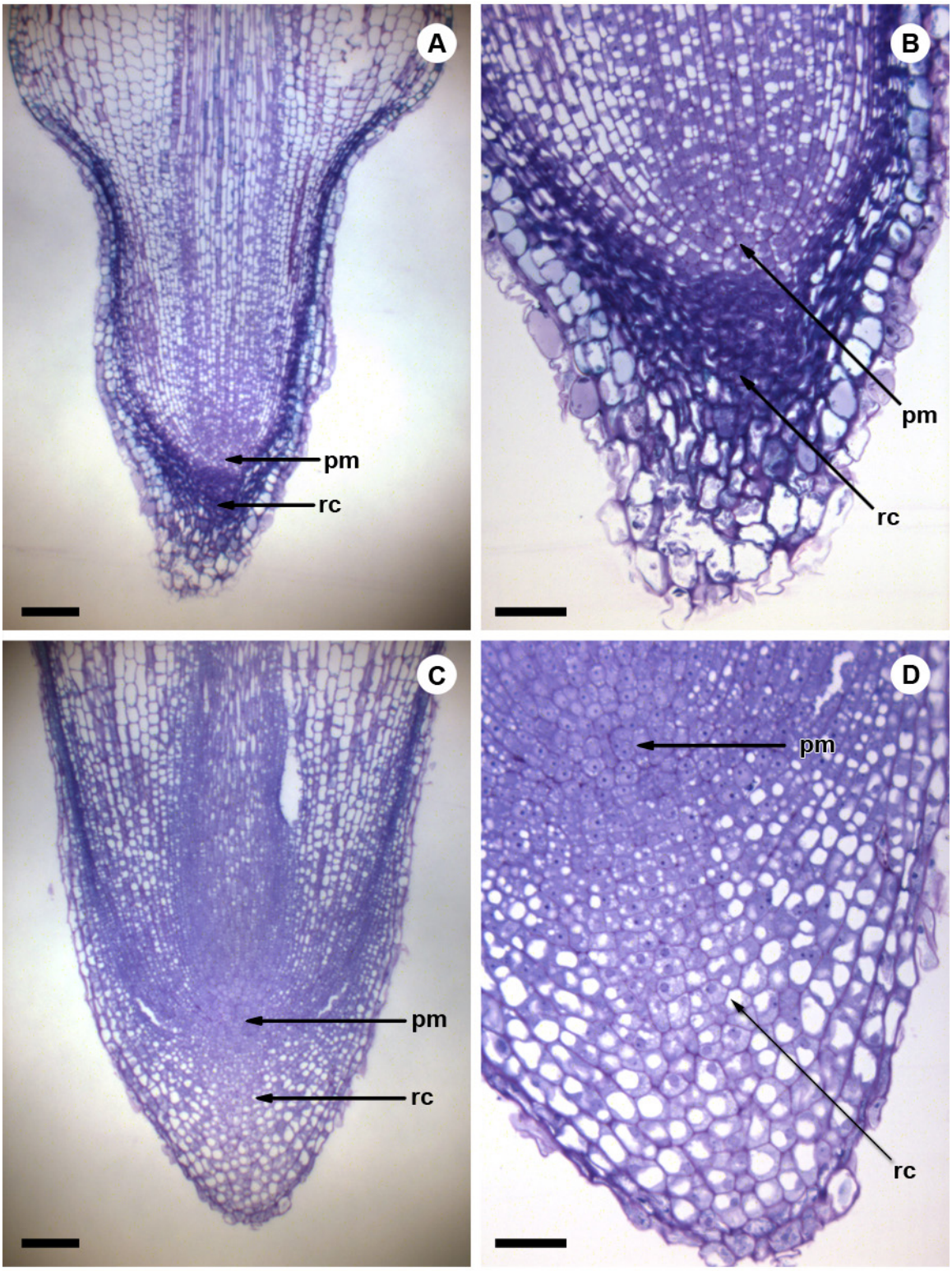
Figure 4.20. Longitudinal sections of adventitious root tips from 100 mM NaCl red-osier dogwood cuttings. (A) The root tip of a cutting that had stopped growing and appeared to have damaged root tips (see Figure 4.6A, B). (B) Enlargement of the root tip. Root cap cells have collapsed and reduced activity of the apical meristem. (C) A root tip of a cutting that had not stopped growing after four weeks of 100 mM NaCl treatment and were thick in diameter (see Figure 4.6C, D). (D) Enlargement of the root tip. Root cap cells have not collapsed and there is greater meristematic activity than in cuttings that had stopped growing (see Fig. 4.20A). Sections stained with toluidine blue. Abbreviations: rc, root cap; pm, promeristem. Staining procedure = toluidine blue. Scale bars: (A, C) = 100 μm; (B, D) = 50 μm.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rc
B
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C
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Table 4.6. The count of xylem cells (mean ± S.E., n = 6) at 1, 3, 5, 10, 15, and 25 cm from the root tip and the increase in root length of control red-osier dogwood cuttings after four weeks of treatment. The number of roots measured to determine the increase in root length in each replicate is given in the brackets next to the mean ± S.E. The increase in root length under salinity treatment was used to estimate the xylem cell count of four- week-old root tissue (indicated by *). This method was used to standardize the xylem count measurements between treatments as they varied in their rate of root elongation.

Replicate	Increase in root length (cm)	Count of xylem cells					
		Root distances (cm)					
		1	3	5	10	15	25
1	17.8 ± 0.8 (3)	4.4 ± 0.6	8.2 ± 0.8	10.0 ± 1.9	68.8 ± 7.2	100.0 ± 8.8 *	125.0 ± 0.0
2	25.7 ± 1.7 (2)	3.1 ± 0.6	9.8 ± 1.2	12.1 ± 2.1	23.9 ± 7.6	66.7 ± 10.2	116.7 ± 10.2 *
3	33.5 ± 2.8 (2)	3.1 ± 0.6	7.4 ± 1.0	9.6 ± 1.2	17.5 ± 0.6	50.0 ± 0.0	108.3 ± 10.2 *
4	30.3 ± 6.8 (2)	3.3 ± 0.2	5.9 ± 0.3	7.2 ± 0.4	56.1 ± 23.1	83.3 ± 10.2	116.7 ± 10.2 *
5	33.4 ± 0.6 (2)	1.9 ± 0.4	6.5 ± 0.7	10.6 ± 0.3	21.1 ± 2.3	75.0 ± 0.0	108.3 ± 10.2 *
6	26.27 ± 2.8 (3)	3.9 ± 0.4	6.8 ± 0.5	8.9 ± 0.4	22.7 ± 2.9	83.3 ± 10.2	108.3 ± 10.2 *

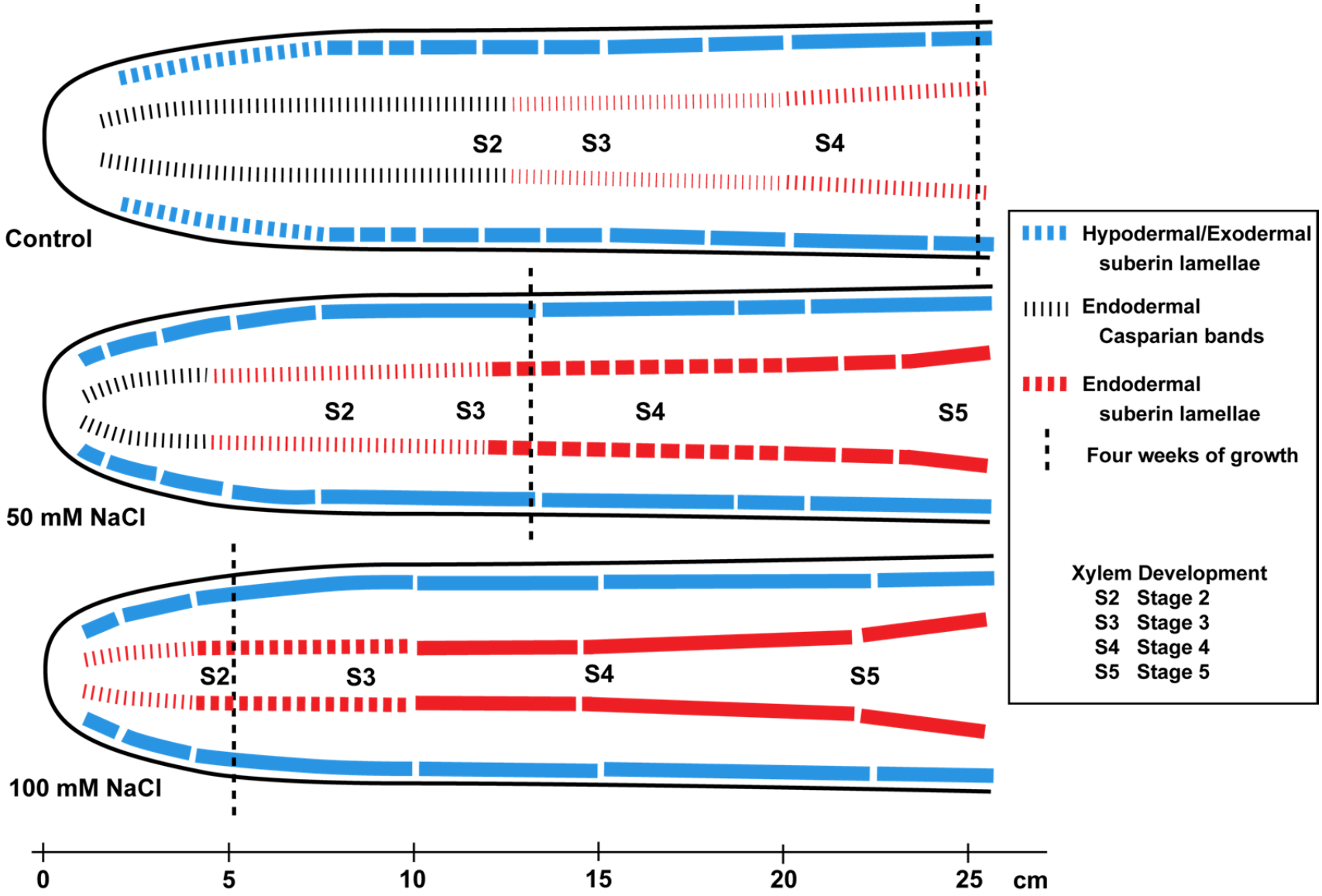
Table 4.7. The count of xylem cells (mean ± S.E., n = 6) at 1, 3, 5, 10, 15, and 25 cm from the root tip and the increase in root length of red-osier dogwood cuttings after exposure to four weeks of 50 mM NaCl. The number of roots measured to determine the increase in root length in each replicate is given in the brackets next to the mean ± S.E. The increase in root length under salinity treatment was used to estimate the xylem cell count of four- week-old root tissue (indicated by *). This method was used to standardize the xylem count measurements between treatments as they varied in their rate of root elongation.

Replicate	Increase in root length (cm)	Count of xylem cells					
		Root distances (cm)					
		1	3	5	10	15	25
1	18.1 ± 6.36 (2)	4.4 ± 1.0	8.8 ± 0.8	10.3 ± 0.6	25.7 ± 7.5	80.0 ± 10.5 *	115.0 ± 11.2
2	9.2 ± 4.0 (2)	8.2 ± 1.0	13.7 ± 0.5	47.0 ± 3.7	100.0 ± 0.0 *	125.0 ± 0.0	141.7 ± 10.2
3	6.3 ± 0.6 (3)	10.7 ± 1.2	16.9 ± 3.0	66.7 ± 10.2 *	125.0 ± 0.0	133.3 ± 10.2	150.0 ± 0.0
4	14.7 ± 3.0 (3)	3.7 ± 0.2	7.7 ± 0.0	11.4 ± 0.5	50.0 ± 0.0	75.0 ± 0.0 *	116.7 ± 10.2
5	15.4 (1)	8.3 ± 1.0	11.8 ± 0.6	17.3 ± 2.5	34.3 ± 10.3	83.3 ± 10.2 *	133.3 ± 10.2
6	21.2 ± 1.7 (3)	6.4 ± 0.5	10.2 ± 1.4	10.6 ± 1.8	58.3 ± 10.2	75.0 ± 0.0	100.0 ± 0.0 *

Table 4.8. The count of xylem cells (mean ± S.E., n = 6) at 1, 3, 5, 10, and 15 from the root tip and the increase in root length of red-osier dogwood cuttings after exposure to four weeks of 100 mM NaCl. The number of roots measured to determine the increase in root length in each replicate is given in the brackets next to the mean ± S.E The increase in root length under salinity treatment was used to estimate the xylem cell count of four- week-old root tissue (indicated by *). This method was used to standardize the xylem count measurements between treatments as they varied in their rate of root elongation. The roots of 100 mM NaCl were too short to section at 25 cm from the root tip and 15 cm (---).

Replicate	Increase in root length (cm)	Count of xylem cells				
		Root distances (cm)				
		1	3	5	10	15
1	1.9 (1)	5.5 ± 0.4 *	7.4 ± 0.3 *	11.3 ± 0.9	80.0 ± 10.5	---
2	5.8 (1)	8.0 ± 0.4	22.8 ± 5.5	75.0 ± 0.0 *	100.0 ± 0.0	---
3	7.8 ± 0.5 (3)	7.7 ± 1.5	11.0 ± 0.2	19.8 ± 4.0 *	91.7 ± 10.2 *	58.3 ± 10.2
4	7.3 ± 0.6 (2)	10.3 ± 0.9	24.6 ± 5.7	50.1 ± 17.6 *	100.0 ± 0.0 *	108.3 ± 10.2
5	2.9 ± 1.1 (2)	12.3 ± 1.0	15.8 ± 1.9 *	58.3 ± 10.2	75.0 ± 0.0	100.0 ± 17.7
6	4.2 ± 1.3 (3)	14.8 ± 2.8	66.7 ± 10.2 *	83.3 ± 20.4 *	108.3 ± 10.2	116.7 ± 10.2

Figure 4.21. Schematic diagram of red-osier dogwood roots after four weeks of treatment with 0 (A), 50 (B), and 100 (C) mM NaCl. Transverse root sections were collected at 1, 3, 5, 10, 15, and 25 cm from the root tip. Vertical dashed lines indicate the length of root grown during the four weeks of NaCl treatment. S2 (stage 2 of xylem development) was defined where metaxylem was starting to develop but not completely filling between the protoxylem poles. Prior to stage 2 (stage 1 of xylem development), the protoxylem cells and the first metaxylem cells were in distinct poles (groups). S3 (stage 3 of xylem development) was defined where the metaxylem had completely filled between the xylem poles and secondary xylem development had just started between the protoxylem poles. S4 (stage 4 of xylem development) was defined where secondary xylem had progressed around the protoxylem poles. S5 (stage 5 of xylem development) defined where the secondary xylem was developed in a thick ring around the primary xylem. Small-dashed black lines indicate the presence of Casparian bands in the endodermal cell radial walls (no suberin lamellae). Casparian bands were present simultaneously in the endodermal radial walls. Red dashed lines indicate the suberin lameallae in the endodermal cells; the length of the dash of the line represents the proportion of endodermal cells containing suberin lamellae (i.e., small dashes represent distances where only a few endodermal cells with suberin lamellae). As Casparian bands were not detected in the hypodermis, the blue lines represent the proportion of the hypodermal cells with suberin lamellae.


Control
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S3
S4
50 mM NaCl
S2
S3
S4
S5
100 mM NaCl
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S3
S4
S5
0
5
10
15
20
25
cm
Hypodermal/Exodermal suberin lamellae
Endodermal Casparian bands
Endodermal suberin lamellae
Four weeks of growth
Xylem Development
S2 Stage 2
S3 Stage 3
S4 Stage 4
S5 Stage 5

Figure 4.22. Transverse sections from red-osier dogwood roots were used to determine the xylem developmental stage at various distances from the tip after four weeks of 50 mM NaCl treatment. (A) At 1 cm, early to mid-stage 1 of development. (B) At 3 cm, mid-stage 1 of development. (C) At 5 cm, late stage 1 of development. (D) At 10 cm, stage 3 of development. (E) At 15 cm, late stage 4 of development. The endodermal cells were dividing as the secondary xylem and phloem are produced. The inner central cortical cells were starting to be squished. (F) At 25 cm, stage 6 of development. Reduced number of cortex layers because of the squishing of the inner cortex cells with the abundant secondary development of the vascular tissue. Abundant division of endodermal cells had occurred but the central cortex was squished and some of the epidermal cells had been sloughed off. Sections stained with toluidine blue. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex; en, endodermis; pc, pericycle; ph, phloem tissue; vc, vascular cambial zone; sx, secondary xylem; px, protoxylem; mx, metaxylem. Staining procedure = toluidine blue. Scale bars = 100 μm.

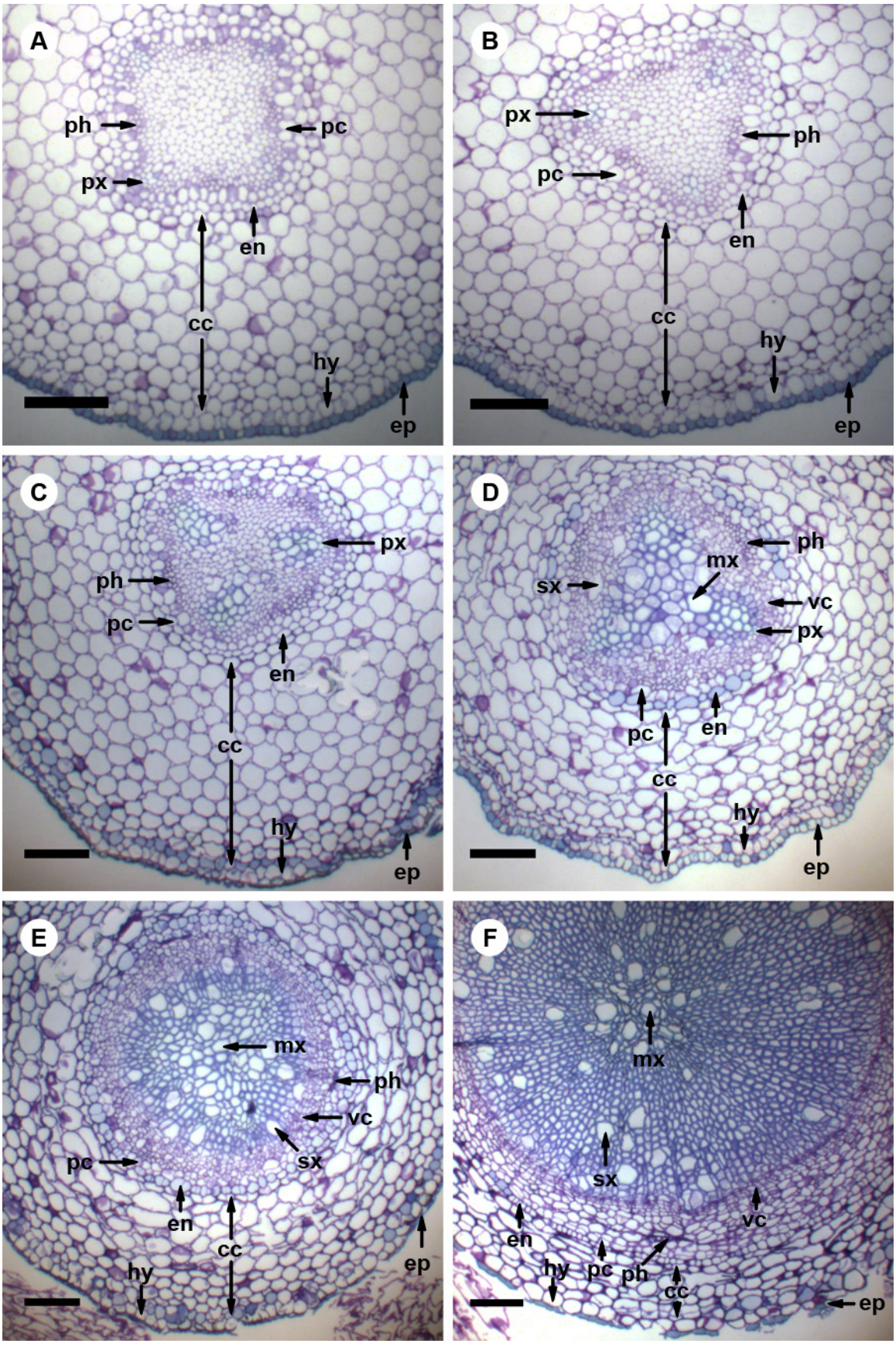
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Figure 4.23. Transverse sections from red-osier dogwood roots were used to determine the xylem developmental stage of the roots at various distances from the root tip after four weeks of 100 mM NaCl treatment. The roots of 100 mM NaCl were too short to section at 25 cm from the root tip. (A) At 1 cm, mid-stage 1 of development. (B) At 3 cm, late stage 1 of development. (C) At 5 cm, stage 3 of development. (D) At 10 cm, late stage 4 of development. A few of the endodermal cells were dividing. (E) At 15 cm, stage 5 of development. Reduced number of cortex layers because of the squishing of the inner cortex cells with the secondary development of the vascular tissue. Division of endodermal cells had occurred. The central cortex was squished and some of the epidermal cells had been sloughed off. Sections stained with toluidine blue. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex; en, endodermis; pc, pericycle; ph, phloem tissue; vc, vascular cambial zone; sx, secondary xylem; px, protoxylem; mx, metaxylem. Staining procedure = toluidine blue. Scale bars = 100 μm.

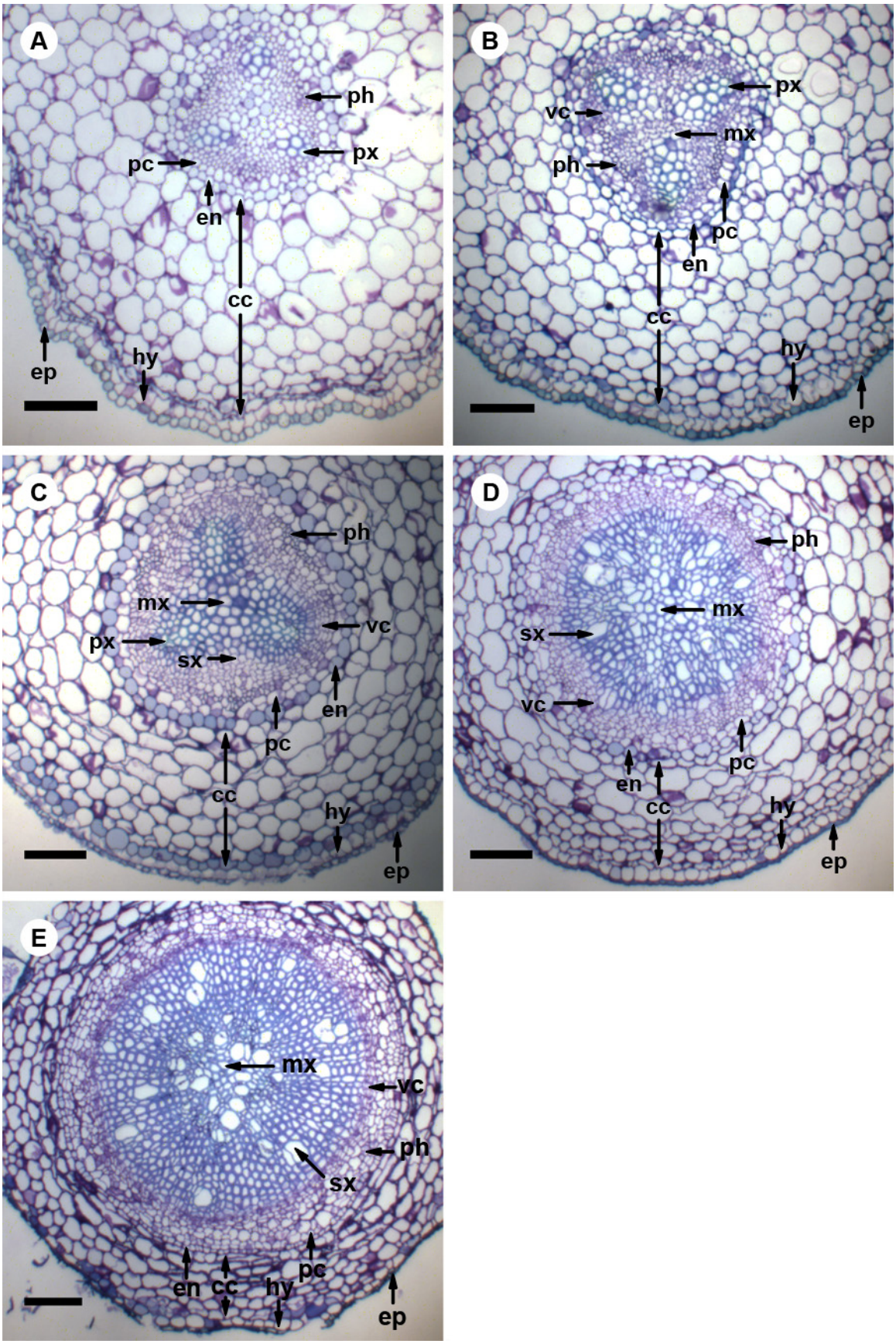
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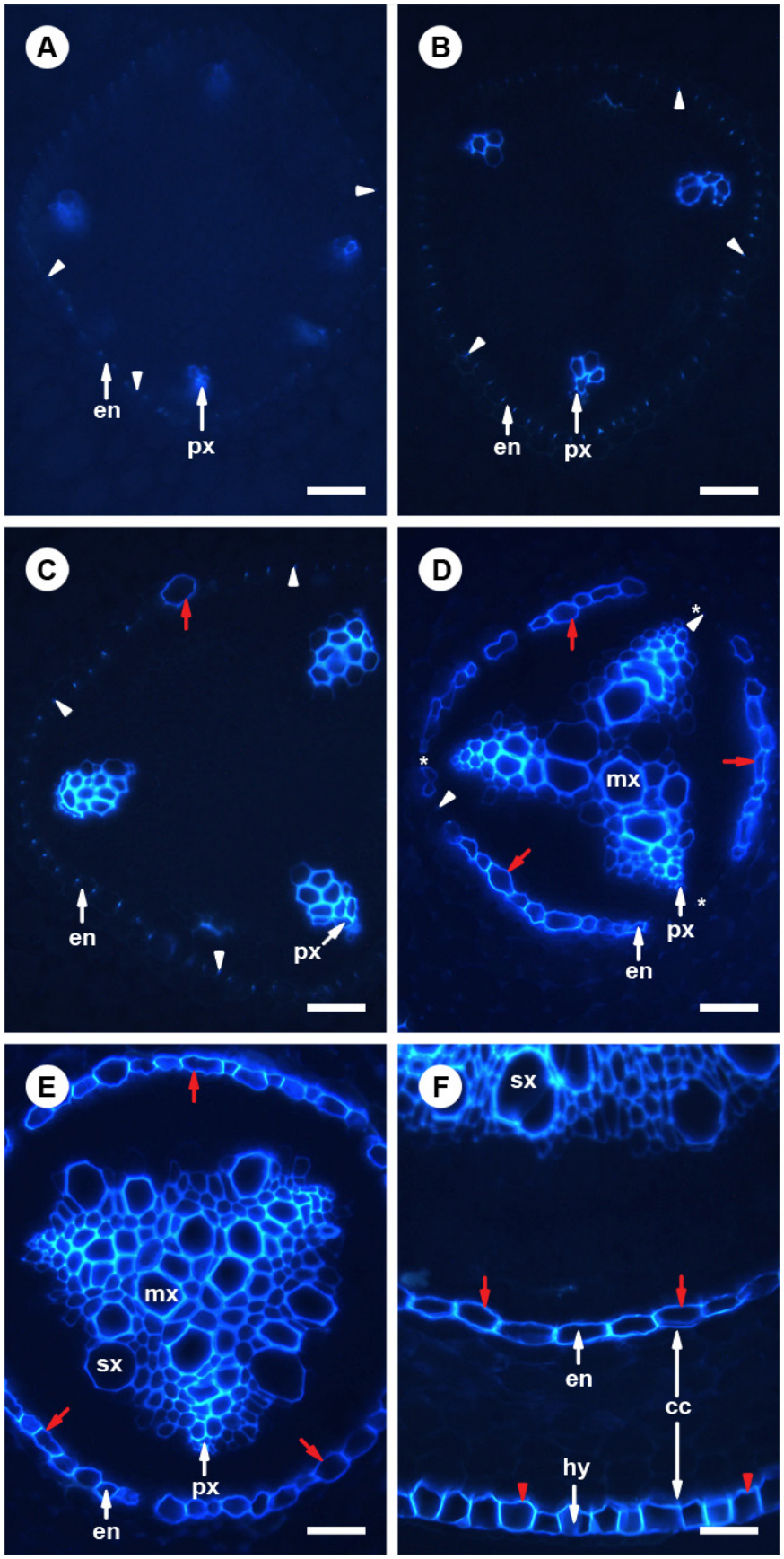
Figure 4.24. Freehand transverse sections from red-osier dogwood roots used to illustrate the development of the xylem tissue and endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 50 mM NaCl treatment. (A) Xylem development: early stage 1. Casparian bands fluoresced (white arrowheads) in the radial walls of the endodermal cells (B) Xylem development: early to mid-stage 1. Only Casparian bands detected in the endodermal cell walls. (C) Xylem development: mid- to late stage 1. Suberin lamellae autofluorescence (red arrows) was observed in one endodermal cell. (D) Xylem development: late stage 2. 70% of the endodermal cells contained suberin lamellae. Passage cells located opposite the xylem poles (asterisks). (E) Xylem development: early stage 4. 90% of the endodermal cells with suberin lamellae. (F) Xylem development: stage 5 of development. Suberin lamellae in 93% of the endodermal cells. Endodermal cells appeared elongated in cross-section. Sections were viewed under UV light to observe autofluorescence. Abbreviations: hy, hypodermis; cc, central cortex; en, endodermis; px, protoxylem; mx, metaxylem; sx, secondary xylem. Red arrowheads indicate autofluorescence of hypodermal cell walls. Scale bars = 50 μm.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en
cc
hy

Figure 4.25. Freehand transverse sections from red-osier dogwood roots to illustrate the development of the xylem tissue and endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 100 mM NaCl treatment. (A) Xylem development: early to mid-stage 1. Casparian bands fluoresced (white arrowheads) in radial endodermal cell walls. (B) Xylem development: mid-stage 1. Only Casparian bands observed in the endodermis. (C) Xylem development: late stage 1. Half of the endodermal cells contained suberin lamellae (red arrows). Passage cells occurred more abundantly at the xylem poles (asterisks). (D) Xylem development: early stage 4. Suberin lamellae present in 96% of the endodermal cells. (E) Xylem development: early stage 5. Suberin lamellae present in all of the endodermal cells. (F) Xylem development: mid- to late stage 5. All the endodermal cells contained suberin lamellae. Sections were viewed under UV light to observe autofluorescence. Abbreviations: hy, hypodermis; cc, central cortex; en, endodermis; px, protoxylem; mx, metaxylem; sx, secondary xylem. Red arrowheads indicate autofluorescence of hypodermal cell walls. Scale bars = 50 μm.

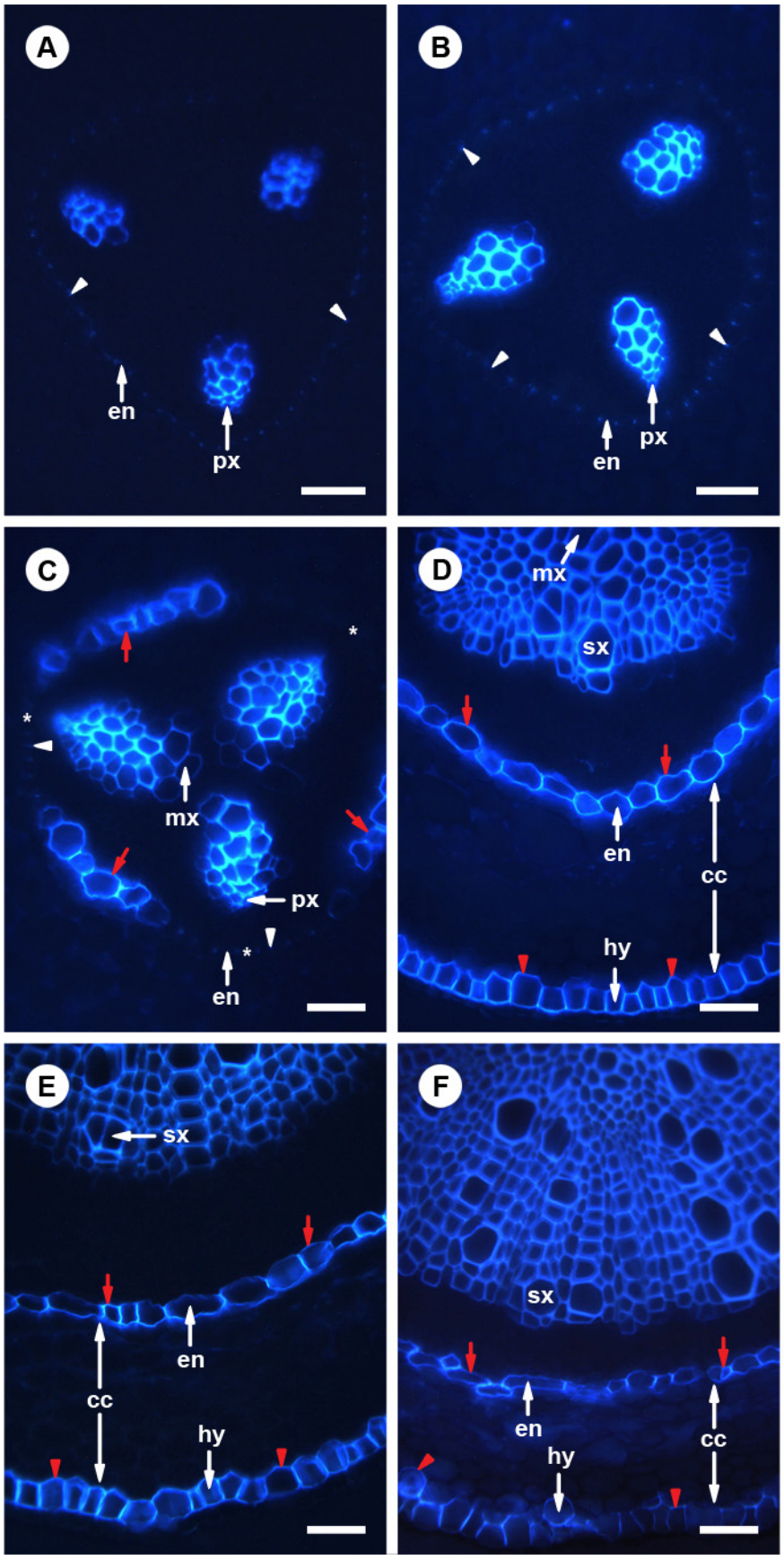
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Figure 4.26. Freehand transverse sections from red-osier dogwood roots used to illustrate the development of endodermal cells at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 50 mM NaCl treatment. (A) Casparian bands (white arrowheads) fluoresced in the radial walls of the endodermal cells. (B, C) Casparian bands fluoresced in endodermal cell walls. (D) Some of the endodermal cells had developed suberin lamellae (red arrows). (E) Majority of the endodermal cells with suberin lamellae, but passage cells were still present within the endodermis (asterisk). (F) New cells formed within a single suberized endodermal cell (double white arrowheads). Sections were viewed under UV light to observe autofluorescence. Abbreviations: cc, central cortex; en, endodermis; px, protoxylem. Scale bars = 10 μm.

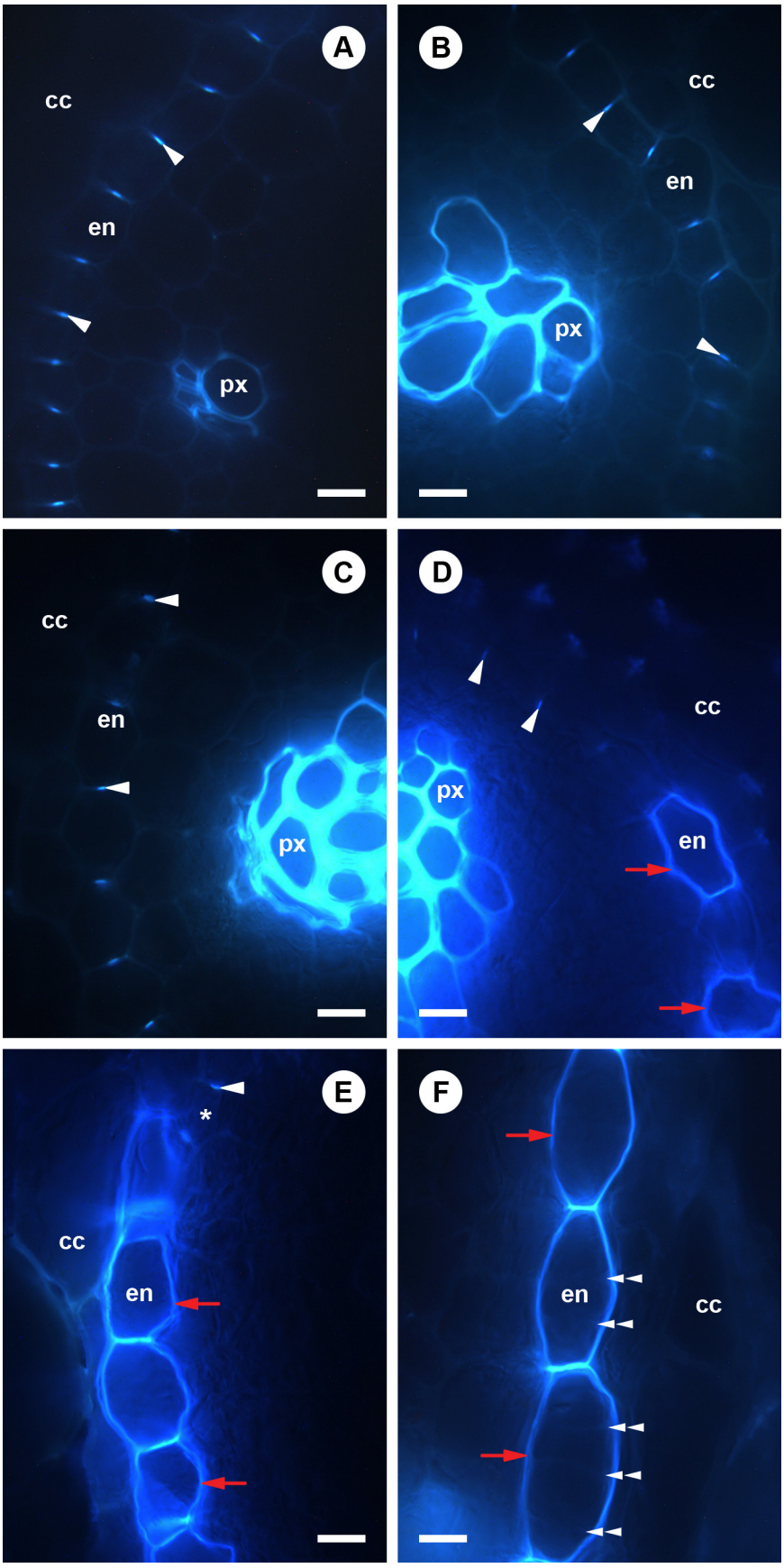
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Figure 4.27. Freehand transverse sections of red-osier dogwood roots used to illustrate the development of the endodermal cells at 1 (A), 5 (B), and 15 (C), and 25 (D) cm from the root tip after four weeks of 100 mM NaCl treatment. (A) Casparian bands (white arrowheads) fluoresced in radial endodermal cell walls. (B) Suberin lamellae fluorescence (red arrow) observed in half of the endodermal cells. (C) All the endodermal cells contained suberin lamellae. (D) Endodermal cells that had undergone multiple cell divisions (double white arrowheads) appeared elongated. The suberized cell wall of the original cell remained and there was a lack of suberization of the new cell walls. Sections were viewed under UV light to observe autofluorescence. Abbreviations: cc, central cortex; en, endodermis; px, protoxylem. White arrowheads indicate Casparian bands; Scale bars = 10 μm.

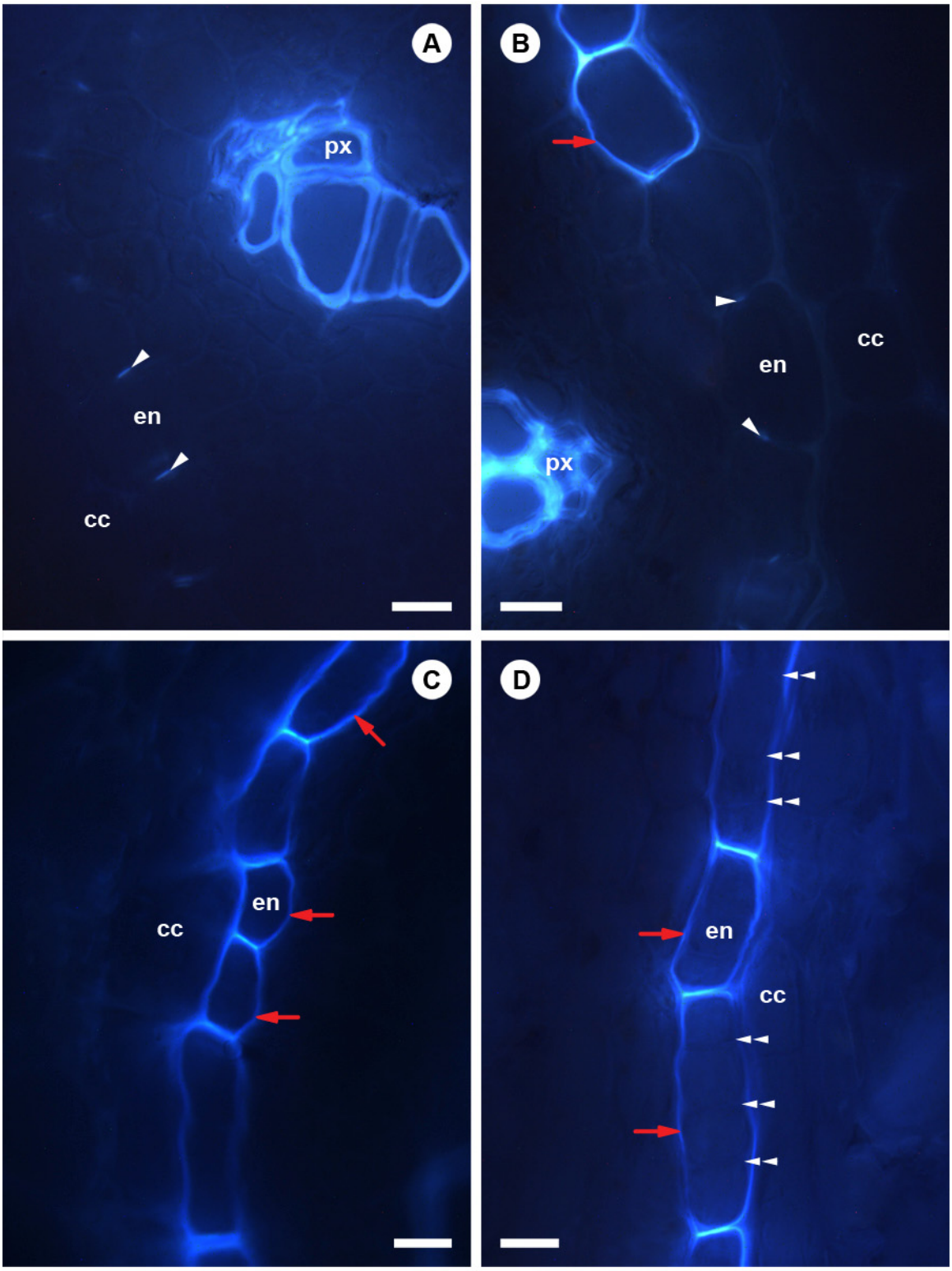
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Figure 4.28. Freehand transverse sections from red-osier dogwood roots stained with Sudan red 7B (B, D, F) or phloroglucinol-HCl (A, C, E, G, H) to detect the presence of suberin and lignin, respectively, in the endodermis after four weeks of 50 mM NaCl treatment. Sections were collected at 1 (A), 3 (B, C), 5 (D, E), 15 (F, G), and 25 (H) cm from the root tip. (A) Lignin was not detected in the endodermal cells. Band plasmolysis was observed in endodermal cells (between arrowheads). (B) Suberin lamellae were not detected. Casparian bands were observed as dark areas within the endodermal cell walls (black arrows). (C) Lignin not observed in the endodermis. Band plasmolysis occurred in endodermal cells (between arrowheads). (D) Lack of suberin lamellae in endodermal walls. Casparian bands were observed as dark areas within the cell walls (black arrows). (E) Lignin was not detected in the endodermal cells. (F) Endodermal cells contained suberin lamellae, but suberin was absent from the new walls of the divided endodermal cells (double black arrowheads). (G) Lignin was not observed in the endodermal cells. Casparian bands were observed in the radial walls as dark areas. (H) Faint red staining of endodermal cell walls indicates the presence of lignin in the endodermal cell walls (black arrows with concave-based arrowheads). Abbreviations: cc, central cortex; en, endodermis; pc, pericycle; px, protoxylem. Scale bars = 10 μm.

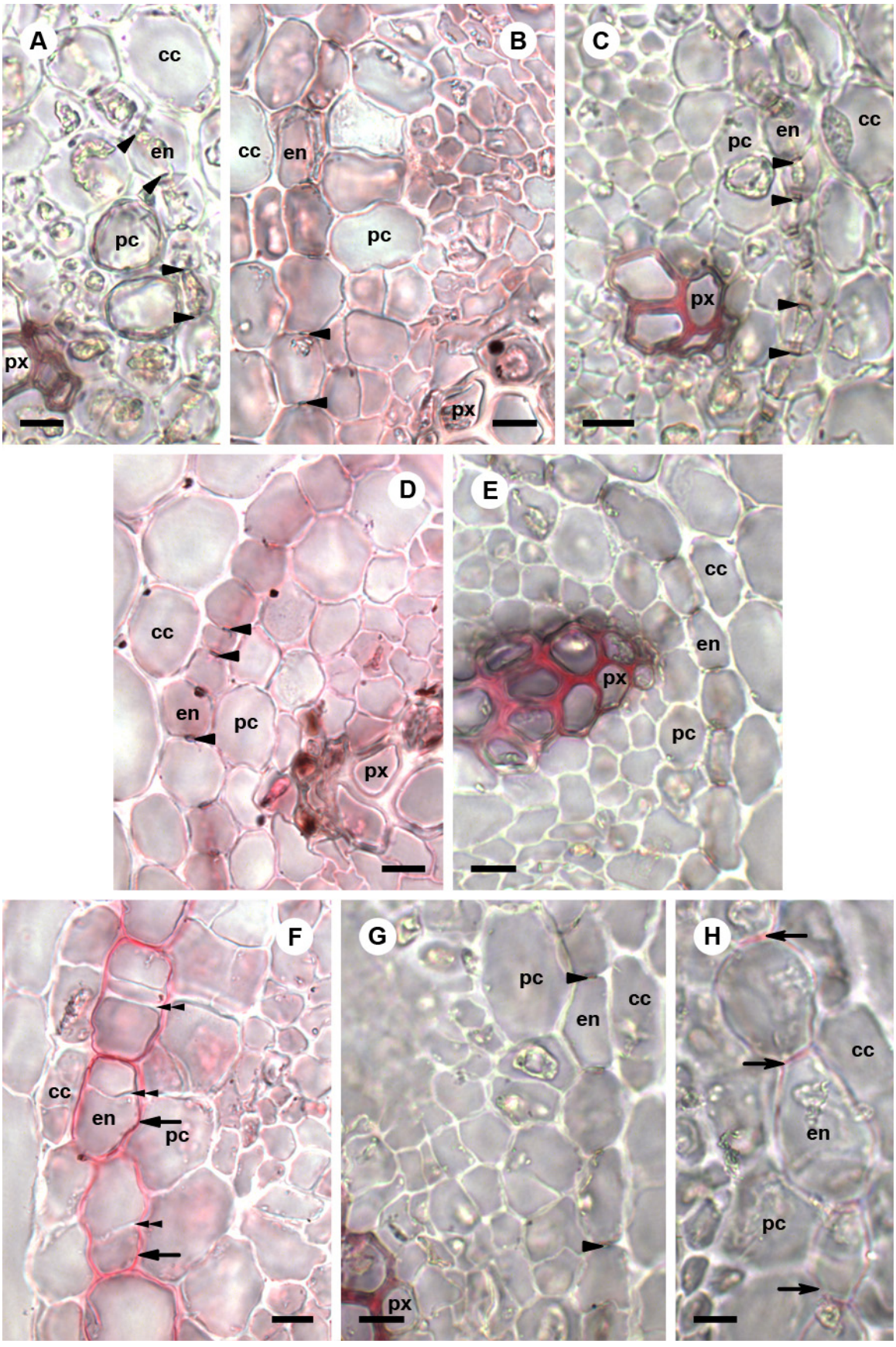
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Figure 4.29. Freehand transverse sections from red-osier dogwood roots stained with Sudan red 7B (A, C, E) or phloroglucinol-HCl (B, D, F, G) to detect the presence of suberin lamellae or lignin, respectively, in the endodermis after four weeks of 100 mM NaCl treatment. Sections were collected at 1 (A, B), 5 (C, D), 15 (E, F), and 25 (G) cm from the root tip. (A) Suberin lamellae were not present in the endodermis. Casparian bands appeared as dark areas at the centre of the radial endodermal walls (black arrowheads). (B) Lignin not observed in the endodermal cells. (C) Suberin lamellae (black arrows) stained red in the endodermal cells. (D) Lignin was not detected in the endodermal cells. (E) Endodermal cells contained suberin lamellae, but suberin was absent from the new walls of the divided endodermal cells (double black arrowheads). (F – G) Lignin not detected in the endodermal walls. Abbreviations: cc, central cortex; en, endodermis; pc, pericycle; px, protoxylem. Scale bars = 10 μm.

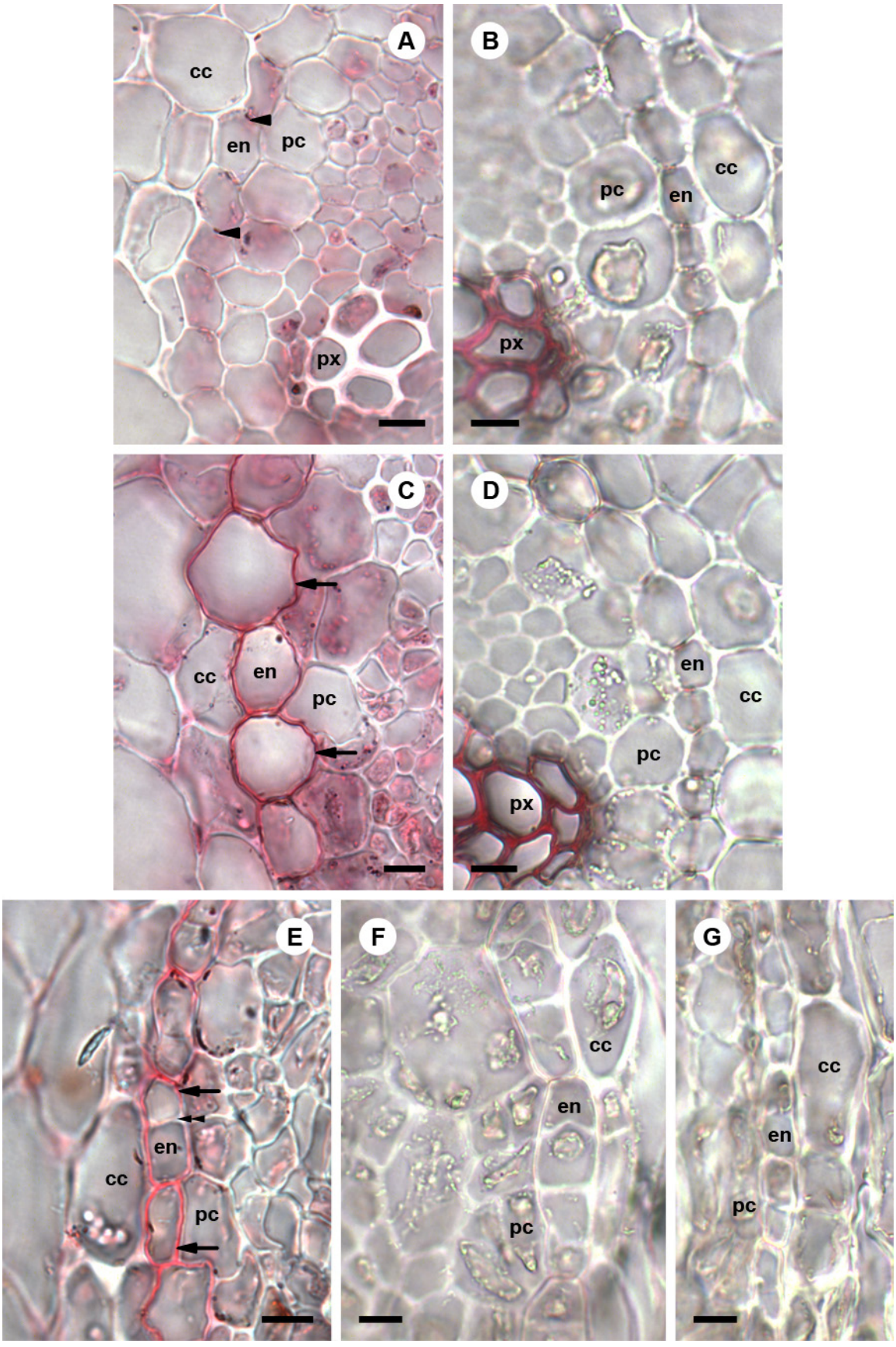
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Figure 4.30. Freehand transverse sections from red-osier dogwood roots used to illustrate the development of hypodermal cell wall modifications at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root tip after four weeks of 50 mM NaCl treatment. (A) Fluorescence of the outer tangential walls and the outer radial walls (red arrowheads). (B) Fluorescence of all hypodermal cell walls. (C) The width and the strength of the hypodermal cell wall fluorescence increased resulting in a fluorescent layer around the cell perimeter. The strength of the fluorescence varied between cells of the hypodermis. (D) The intensity of hypodermal cell wall fluorescence continued to increase. (E) A few cortical cells were observed to have fluorescence cell walls (white concave-based arrowheads). (F). Fluorescence of the hypodermal cell walls appeared to be similar to the hypodermal cells at 15 cm from the root tip. Sections were viewed under UV light to observe autofluorescence. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

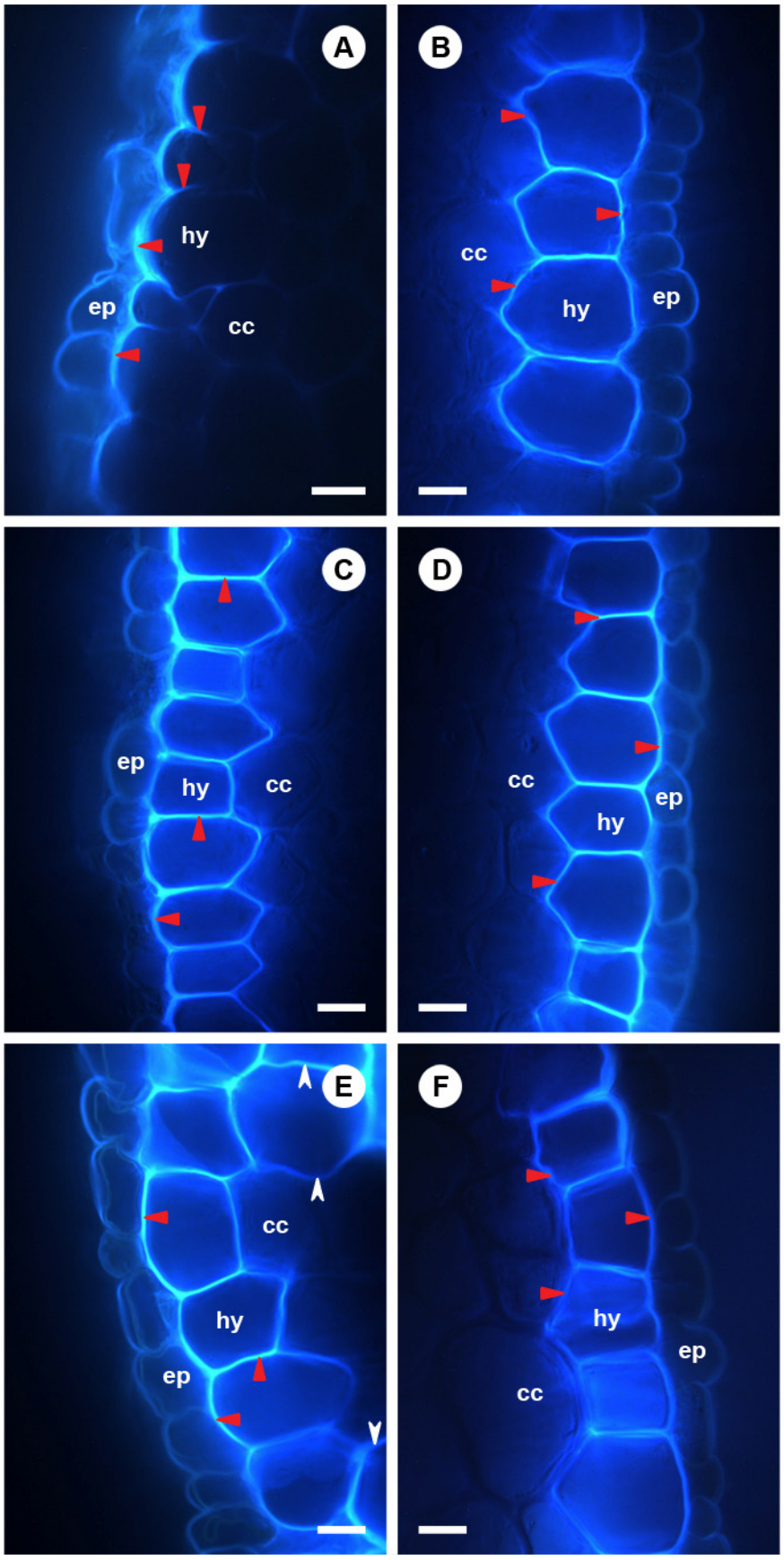
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Figure 4.31. Freehand transverse sections from red-osier dogwood roots used to illustrate the development of hypodermal cell wall modifications at 1 (A), 5 (B), 15 (C), and 25 (D) cm from the root tip after four weeks of 100 mM NaCl treatment. (A) Intense blue fluorescence was detected in many of the hypodermal cell walls (red arrows). (B) The intensity and the sharpness of the fluorescence hypodermal cell walls were increased. The yellow fluorescence of the epidermal cells was distinct in some sections (yellow arrowheads). (C) The hypodermal cell shape was clearly outlined by the fluorescence of the hypodermal cell walls. (D) The intensity of hypodermal cell wall fluorescence appeared to be reduced compared to the fluorescence at 15 cm from the root tip. Sections were viewed under UV light to observe autofluorescence. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

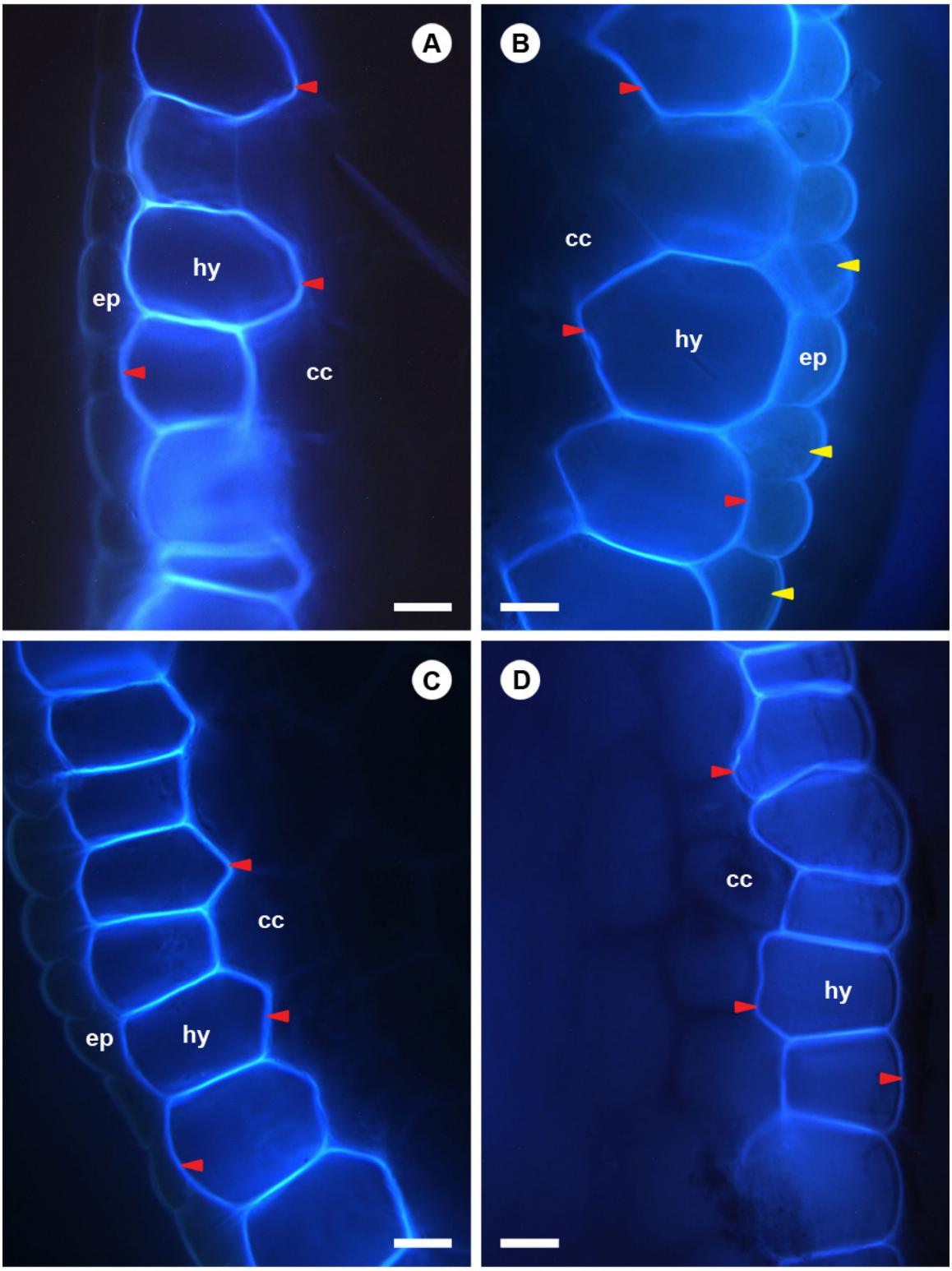
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Figure 4.32. Freehand transverse sections from red-osier dogwood roots stained with Sudan red 7B (A, C, E, F) or phloroglucinol-HCl (B, D, G) to detect the presence of suberin or lignin, respectively, in the hypodermal cells after four weeks of 50 mM NaCl treatment. Sections were collected at 3 (A, B), 5 (C, D), and 15 (E – G) cm from the root tip. (A) Hypodermal cells contained thin suberin lamellae (black arrows). Epidermis stained a reddish-orange with Sudan red 7B (white arrowheads). (B) Lignin was not detected in the hypodermal cells. (C) Suberin lamellae detected in endodermal cells as thin red layer around the hypodermal cells. Epidermal cells stained a dark red-orange. (D) Lignin was not detected in the hypodermal cells. (E) Suberin lamellae observed in the hypodermal cells as a thin layer around the circumference of the cell. Suberin lamellae were occasionally detected in the cortical cells (white concave-based arrows). (F) In other roots, suberin lamellae occurred in multiple layers of cortical cells adjacent to the hypodermis. (G) No lignin was detected in the hypodermis. Epidermal cells were a brownish-yellow (white arrowheads). Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

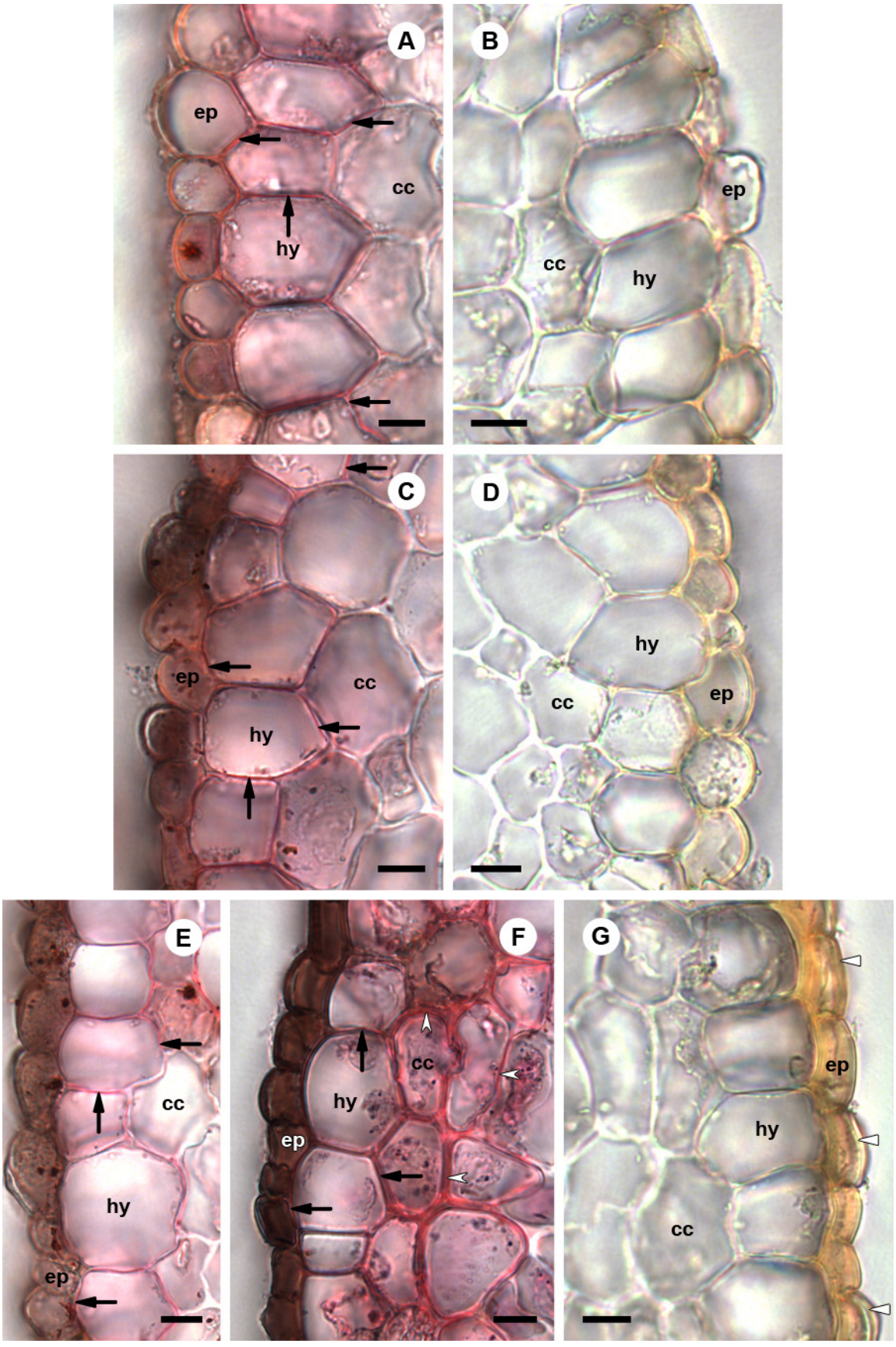
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Figure 4.33. Freehand transverse sections from red-osier dogwood roots stained with Sudan red 7B (A, C, F) or phloroglucinol-HCl (B, D, E, G, H) to detect the presence of suberin or lignin, respectively, in the hypodermal cells after four weeks of 100 mM NaCl treatment. Sections were collected at 1 (A, B), 5 (C - E), 15 (F, G), and 25 (H) cm from the root tip. (A) Hypodermal cells contained suberin lamellae (black arrows). (B) Lignin not observed in hypodermal cell walls (black concave-based arrows). (C) Suberin lamellae were observed in portions of the underlying central cortical cells (white concave-based arrowheads). Epidermal cells stained a reddish-brown (white arrowheads). (D) Lignin was not detected in the hypodermis (concave-based arrows). Epidermal cells appeared yellow to dark brown. (E) Lignin detected in the hypodermal and underlying cortical cells (black concave-based arrowheads) of root portions with missing epidermal cells (white asterisks). (F) Epidermal cells appeared dark reddish brown, but only the hypodermis contained suberin lamellae. (G) No lignin was detected. Epidermal cells stained yellow with phloroglucinol-HCl. (H) Weak red appearance of portions of hypodermal cell walls. Epidermal cells appeared brownish-orange and there was limited epidermal cell loss. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

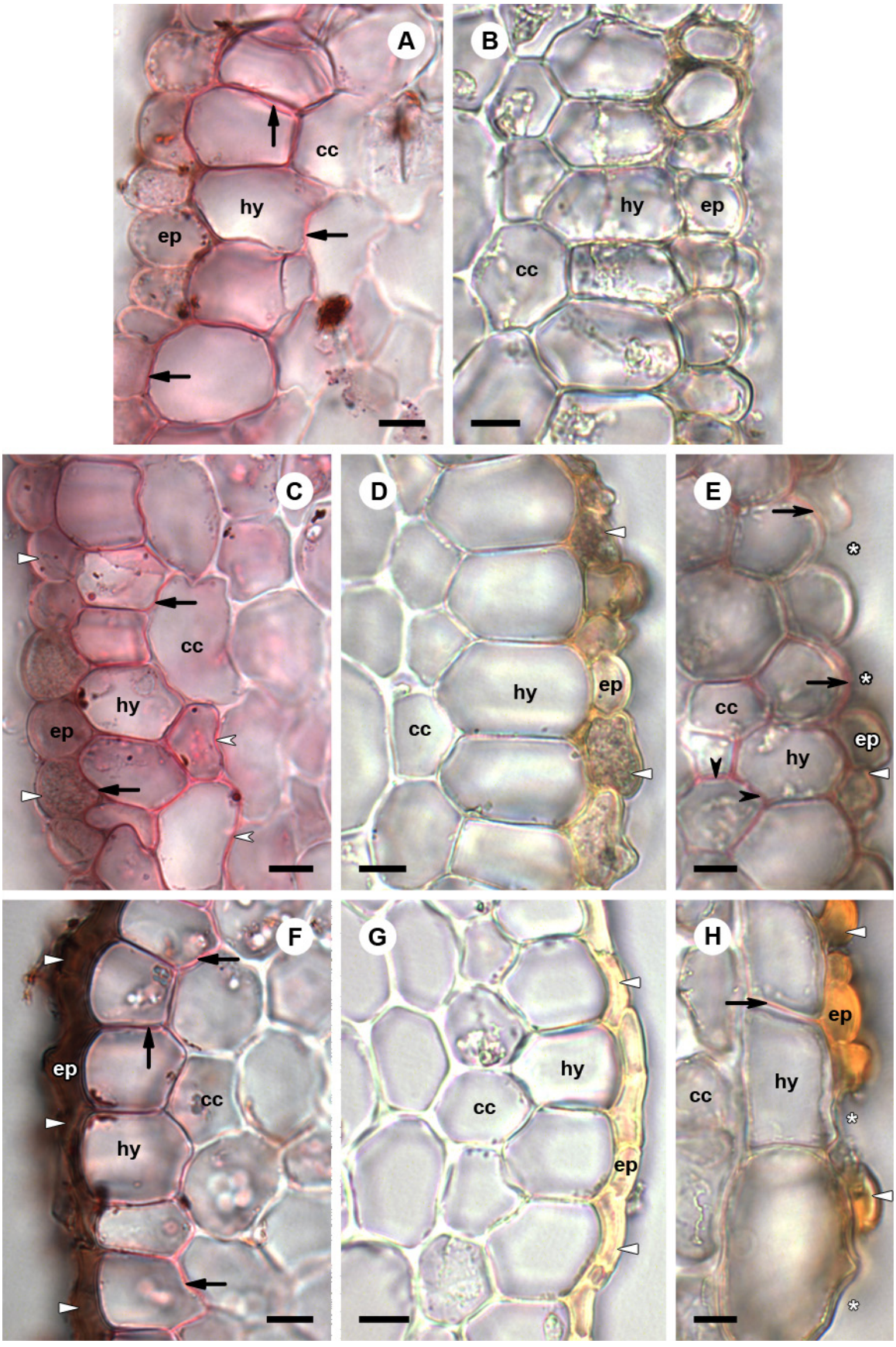
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Figure 4.34.Visual comparison of two red-osier dogwood cuttings with similar NaCl injury and increase in root length after four weeks of 50 mM NaCl treatment. The root sections collected from the cutting in (A – B) exhibited limited epidermal cell loss and injury (see Fig. 4.30, 4.32). While, the root sections collected from (C – D) had abundant epidermal cell loss and lignin was detected in the underlying hypodermal cell walls (see Fig. 4.35 – 4.36). Scale bars: (A, C) = 10 cm; (B, D) = 5 cm.


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Figure 4.35. Freehand transverse sections from roots from a red-osier dogwood cutting that exhibited significant epidermal cell loss after four weeks of treatment with 50 mM NaCl. Significant epidermal lost was not observed in the other cuttings treated with 50 mM NaCl (see Fig. 4.30). Root sections were taken at 1 (A), 3 (B), 5 (C), 10 (D), 15 (E), and 25 (F) cm from the root. (A) Outer tangential walls of the hypodermis fluoresced intensely (double red arrowheads). Faint fluorescence was observed in some of the radial walls and inner tangential walls of the hypodermis (red arrowheads). Some of the adjacent cortical cells also exhibited faint fluorescence (white concave-based arrowheads). Epidermal cells were present, but were smaller and the cell walls appeared to be collapsing (yellow arrowheads). (B) Almost all the hypodermal cell walls exhibited autofluorescence, but the hypodermal cells underlying the lost epidermal cells (yellow asterisks) exhibited increased intensity and thickness of the cell wall fluorescence (double red arrowheads). (C) Contrasting fluorescence of the outer tangential hypodermal walls (red arrowheads) underlying epidermal cells that did not appear damaged (yellow arrowheads) to the hypodermal cells without overlying epidermal cells (yellow asterisks). The intensity of the outer tangential hypodermal cell walls fluorescence was greater in the cells underlying lost epidermal cells (double red arrowheads). (D) All the epidermal cells were lost. Increased intensity of fluorescence of all the hypodermal cell walls was also observed. In parallel, fluorescence of adjacent cortical cell walls was also found (white concave-based arrowheads). (E – F) Epidermal cell injury or loss was continued to be observed at greater distances from the root tip. The corresponding increase in the thickness and intensity of the cell wall fluorescence and as well as the fluorescence of cortical cell walls were also observed. Sections were viewed under UV light to observe autofluorescence. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.

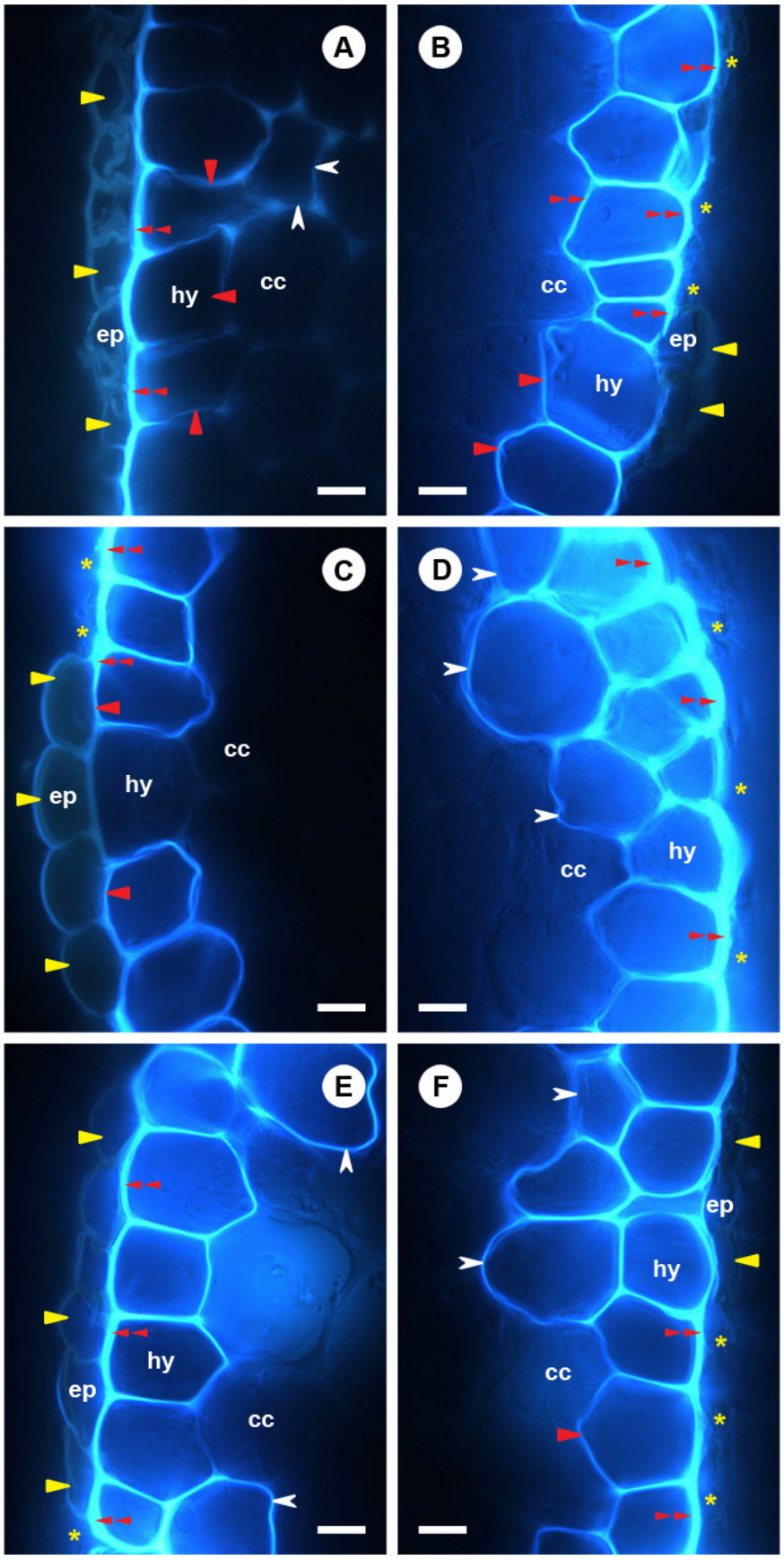
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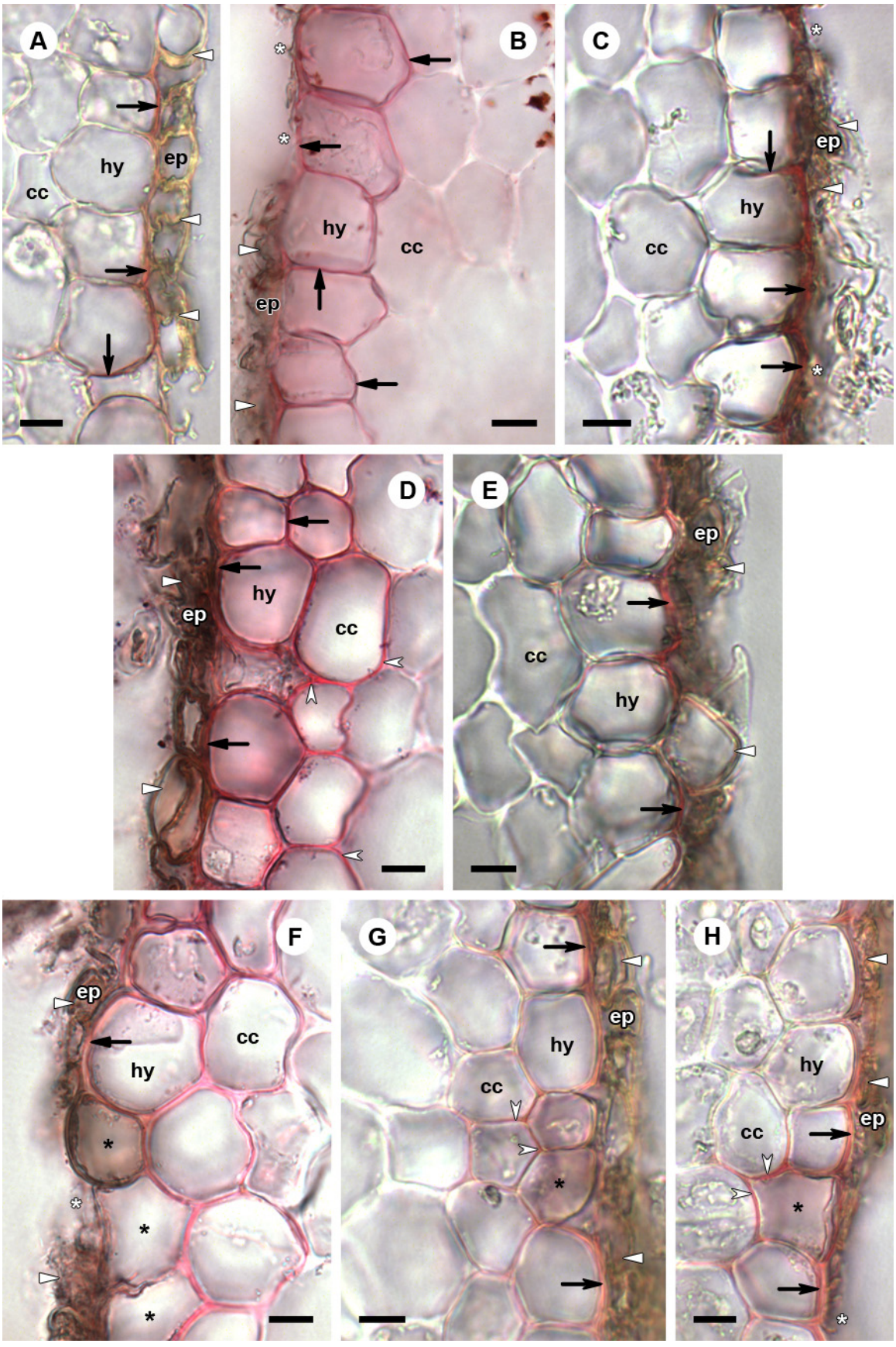
Figure 4.36. Freehand transverse sections from roots from a red-osier dogwood cutting that exhibited significant epidermal cell loss after four weeks of treatment with 50 mM NaCl that was not found in the other cuttings under the same treatment. Root sections were taken at 1 (A), 3 (B, C), 5 (D, E), 15 (F, G), and 25 (H) cm from the root and stained with Sudan red 7B (B, D, F) or phloroglucinol-HCl (A, C, E, G, H) to detect the presence of suberin or lignin, respectively, in the hypodermal cells. (A) Lignin present in the outer tangential walls and some of the outer radial walls of the hypodermis (black concave-based arrows). Epidermal cells appeared small and the radial walls collapsing (white arrowheads). (B) Hypodermal cells contained thin suberin lamellae (black arrows). Epidermal cells appeared injured (white arrowheads) or lost (white asterisk). (C) Thickened lignified outer tangential wall. Epidermal cell walls sloughed off. (D) Thickened suberin lamellae of hypodermal and adjacent central cortical cells (white concave-based arrowheads). Epidermal cells dark reddish-brown and were starting to be sloughed off. (E) Epidermal cell loss was continued to be observed at greater distance from the root tip. Lignification of the hypodermal cell walls appeared to be still confined to the outer tangential walls and the outer portion of the radial walls. (F) Central cortical cells underlying hypodermal cell with (black arrows) and without (black asterisks) suberin lamellae contained suberin lamellae (white concave-based arrowheads). (G – H) In areas where the hypodermal cells appeared injured, lignin was detected in the walls of underlying cortical cells. Epidermal cells were still present. Abbreviations: ep, epidermis; hy, hypodermis; cc, central cortex. Scale bars = 10 μm.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4.4 DISCUSSION

4.4.1 ROOT ELONGATION

The inhibition of root elongation in red-osier dogwood exposed to 50 and 100 mM NaCl may reduce the uptake of water and nutrients from the soil (Neumann *et al*., 1994, Bernstein and Kafkafi, 2002). The effect of salinity on root length differs from its effect on root biomass (Chapter 3) as 50 mM NaCl treatment did not result in a significant decrease in root biomass after four weeks of treatment compared with the controls. This conflicting result may be explained by the relatively large biomass of the root system at the beginning of the treatment period concealing the inhibition of the increase in root length with exposure to 50 mM NaCl. In addition, a significant reduction in the increase in root length was not observed until after two weeks of treatment. Therefore, a longer period of treatment may have been required to result in a significant decrease in root biomass. For example, at the highest concentration of salt, 100 mM NaCl, the increase in root length was already inhibited by 57% after one week while a significant decrease in root biomass was observed only after four weeks of treatment.

The smaller meristematic zone observed in the longitudinal sections from cutting root tips exposed to 100 mM NaCl compared to the controls suggests that the higher concentration of salt could reduce cell production. This reduction in the size of the meristem may be the result of a decrease in cell division and increase in cell death (Bernstein and Kafkafi, 2002; Ogawa *et al*., 2006). Ogawa *et al*. (2006) found that after exposing rye (*Secale cereale*) to 250 mM NaCl for 7 h, the number of cells with newly replicated DNA was drastically reduced indicating a decrease in cell division in the root

tips. In addition, the number of cells with DNA fragmentation, an initial indicator of eventual cell death, was greatly increased. The effect of NaCl on cell division in the root meristem through nuclear and DNA degradation, mitotic abnormalities, and a reduction in meristem size, was also reported in soybean (150 – 350 mM NaCl) (*Glycine max*, Liu *et al*., 2000), potato (500 mM NaCl) (*Solanum tuberosum*, Richardson *et al*., 2001), Arabidopsis (0.5 – 1.5% NaCl) (*Arabidopsis thaliana*, Burssens *et al*., 2000; West *et al*., 2004), and onion (40 – 160 mM NaCl) (*Allium cepa*, Teerarak *et al*., 2009). Changes in the meristematic activity may also have reduced the elongation of roots exposed to 50 mM NaCl.

Observations of longitudinal microscopy sections suggest that red-osier dogwood roots treated with 100 mM NaCl produce shorter cortical cells. A reduction in mature epidermal and cortical cell length is a commonly reported character of roots exposed to NaCl (Kurth *et al*., 1986; Zidan *et al*., 1990; Gersani *et al*., 1993; Karahara *et al*., 2004; West *et al*., 2004). However, the underlying mechanism(s) involved in the inhibition of cell elongation are not well understood (Bernstein and Kafkafi, 2002). A decrease in cell turgor immediately after exposure to NaCl may be the primary cause of the reduced cell elongation in the short term (Bernstein and Kafkafi, 2002; Munns and Tester, 2008). However in the long term, osmotic adjustment is likely to allow for maintenance of turgor as observed in maize (*Zea mays*, Neumann *et al*., 1994; Neumann, 1995). This suggests that NaCl is likely to reduce cell elongation by cell wall hardening (Neumann *et al*., 1994; Neumann, 1995) and/or reduced hydraulic conductance (Azaizeh and Steudle, 1991). Salinity stress may affect the process of cell wall loosening by inhibiting the breakage of cell wall cross-linkages and the degradation of cell wall polymers during cell

elongation (Zhong and Läuchli, 1993*b*). Neumann *et al*. (1994) suggested that the decrease in root elongation was due to the hardening of cell walls in maize (*Zea mays*) as they found that the turgor pressure required for cell elongation (yield threshold pressure) was increased and the cell extensibility was reduced with exposure to NaCl. An increase in the lignification of the cell wall may also contribute to cell wall hardening in bean (*Phaseolus vulagris*, Cachorro *et al*., 1993*b*) and soybean (*Glycine max*, Neves *et al*., 2010). However, interpretation of studies that only measured lignin content and not the increase in root length and/or the pattern of vascular tissue development are problematic (Cachorro *et al*., 1993*b*; Schreiber *et al*., 1999; Sánchez-Aguayo *et al*., 2004; Neves *et al*., 2010), since NaCl has been found to reduce the length of the zone of division and elongation of the roots (Zidan *et al*., 1990; Zhong and Läuchli, 1993*a*). This reduction may result from the maturation of the root tissues occurring closer to the root tip (Azaizeh and Steudle, 1991; Reinhardt and Rost, 1995*b*), which in turn increases the proportion of lignified tissue in the root. In my study for example, the root length was reduced and the xylem development was more advanced closer to the root tip in NaCl treated cuttings than in the controls. Consequently, the increase in lignin content reported for bean (*Phaseolus vulgaris*, Cachorro *et al*., 1993*b*), maize (*Zea mays*, Schreiber *et al*., 1999), and soybean (*Glycine max*, Neves *et al*., 2010) roots could have been the result of a decrease in root length and not the cause of the inhibition of the cell elongation. For instance, Lin and Kao (2001) did not find an increase in lignification in rice (*Oryza sativa*) roots in parallel with the decrease in root growth. The authors suggested that an increase in peroxidase activity contributed to cell wall hardening by catalyzing cross-linkages in the cell wall. Another factor that may contribute to a reduction in root growth

is the effect of salt stress on hormone levels and the distribution of the hormones within the root that may initiate the inhibition of root elongation (Zhong and Läuchli, 1993*a*; Munns *et al*., 2000; Achard *et al*., 2006). For example, a decrease in root growth after 22 days of exposure to 100 mM NaCl corresponded with an increase in the content of abscisic acid (ABA) and the ethylene precursor (ACC, 1-aminocyclopropane-1-carboxylic acid) in tomato roots (*Solanum lycopersicum*) (Albacete *et al*., 2008). An increase in ABA and ethylene content could inhibit root growth under salinity stress by promoting the growth-repressing DELLA proteins (Achard *et al*., 2006; Wolters and Jürgens, 2009). However, salinity affects the levels of a variety of other hormones (e.g. gibberellin (GA), auxin (IAA), cytokinins) and the ratios of the hormones in the root (Wolters and Jürgens, 2009; Javid *et* al., 2011) that may also contribute to the decrease in root elongation. For example, a decrease in gibberellin was observed in Arabidosposis (*Arabidopsis* sp.) in parallel with a reduction in root growth after exposure to 100 mM NaCl (Achard *et al*., 2006). In addition, the auxin level in tomato (*Solanum lycopersicum*) roots exposed to salinity stress has been reported to either decrease (Dunlap and Binzel, 2006) or increase (Albacete *et al*., 2008).

Which of the above factors is the dominant cause of the reduction in cell elongation with exposure to NaCl may depend on the species, the environmental conditions, and the duration and severity of NaCl treatment.

4.4.2 ROOT CAP

Along with a reduction in root growth, a disruption of the arrangement of the root cap cells was observed with 50 and 100 mM NaCl treatment. The root cap cells of red-osier dogwood cuttings exposed to 100 mM NaCl appeared less organized (i.e., cell files are difficult to identify) than those in the control root caps or had collapsed. A disruption in the arrangement of root cap cells with NaCl treatment has also been observed in rice (*Oryza sativa*, Rahman *et al*., 2001) and in pea (*Pisum sativum*, Hodson and Mayer, 1987). Root caps have been shown to play an important role in the direction of root growth by perceiving environmental signals (Sievers *et al*., 2002; Eapen *et al*., 2005). These signals include gravity (Morita, 2010; Wolverton *et al*., 2011), water gradients (Eapen *et al*., 2005; Taniguchi *et al*., 2010), mechanical impedance (Goss and Russell, 1980; Semchenko *et al*., 2008), and toxic elements (Bennet and Breen, 1992). An immediate reduction in root elongation was observed in maize (*Zea mays*) in response to mild mechanical impedance, but not in roots with the root caps removed (Goss and Russell, 1980). This suggests that a signal was perceived by the root cap and relayed to the meristematic and the elongating cells possibly via a Ca^{2+} transduction pathway (Sievers *et al*., 2002). It has been suggested that the cells in the columella closest to the meristem have the most control over the gravitropic response of the root (Blancaflor *et al*., 1998). Therefore, the disruption of the root cap by salinity stress may inhibit the gravitropic response of red-osier dogwood roots. Furthermore, NaCl may affect the direction of root growth by causing the degradation and displacement of amyloplasts within the root cap cells, which reduces the ability of the root to perceive and respond to gravity (Takahashi *et al*., 2003; Sun *et al*., 2008). The effect of 50 and 100 mM NaCl on

the amyloplast distribution and maintenance in red-osier dogwood root cap cells remains to be investigated. However, it was observed that the root tips of red-osier dogwood roots treated with 100 mM NaCl were more frequently and severely curved than the control root tips even when grown in hydroponics. In saline soils, the loss of the response of roots to gravity may benefit the plant by bending of the roots away from the deeper soils with high NaCl concentrations (Li and Zhang 2008; Sun *et al*., 2008). It still remains to be determined if the bending of the root occurs in response to the change Na^+ and K^+ concentrations in the root cap (Sun *et al*., 2008) or as a result of a loss of gravity perception. Root bending in response to environmental conditions has also been indicated in the response of roots to water gradients (hydrotropism, Takahashi *et al*., 2003; Eapen *et al*., 2005) and the presence of aluminate (Eleftheriou e*t al*., 1993).

The root cap could also protect the meristematic and elongating cells by accumulating Na^+ and thus excluding it from the root (Ferdose *et al*., 2009; Oh *et al*., 2009). For example, ion accumulation was suggested in rice (*Oryza sativa*), as the cells of the root cap were larger and contained more vacuoles in the salt treated plants than in the controls (Rahman *et al*., 2001). Furthermore, the length of the root cap was greater in more tolerant rice seedlings than the less tolerant seedlings (Ferdose *et al*., 2009). In red-osier dogwood exposed to 50 and 100 mM NaCl, the larger cells of the root cap containing large vacuoles in the more salt tolerant cuttings with the thicker roots suggest that accumulation of ions in the root cap could have occurred. Other studies suggest that plasma membrane proteins, such as PMP3 (plasma membrane protein), of the root cap are involved in controlling Na^+ uptake (Inada *et al*., 2005; Ferdose *et al*., 2009).

Additional studies need to be conducted to fully understand the role of the role cap during salinity stress as it is often ignored in studies on root development.

4.4.3 ROOT THICKENING

The thickening of the root may be the result of an increase in radial elongation of cortical cells as observed in the longitudinal sections of Arabidopsis (*Arabidopsis thaliana*, Burssens *et al*., 2000) and pea (*Pisum sativum*, Chiatante *et al.*, 1999) roots after exposure to 2 weeks of 1% NaCl treatment and 24 h of PEG (-1.7 M Pa), respectively. In my experiment, increased radial diameter of the cortical cells was also observed in transverse sections of the thickened distal portion of the red-osier dogwood cuttings roots after exposure to 50 and 100 mM NaCl compared with other roots of the same treatment that did not show the increase in diameter. The roots exposed to 50 mM NaCl also had an increased number of cortical cell layers. This combination of the increase in cortical cell diameter and number of cortical cell layers contributed to the greater root diameter. Conversely, exposure to 340 mM NaCl only increased the cortex cell diameter in seepweed (*Suaeda maritima*), which resulted in the increase in root diameter compared with the controls (Hajibagheri *et al*., 1985). In red-osier dogwood exposed to 100 mM NaCl, although some cuttings exhibited root thickening, this was only relative to other cuttings of the same treatment. Furthermore, root thickening did not occur in all cuttings that showed limited shoot growth inhibition and reduced leaf injury, making it difficult to draw conclusions. Nevertheless, thickened roots have been observed previously in one three-month-old red-osier dogwood seedling exposed to 100 mM NaCl for two weeks. In that experiment, the thickenings were localized in the lateral roots

(Renault, Personal Communication) (Fig. A.1). It was also observed that this seedling did not exhibit NaCl induced injury and the Na^+ concentration in the shoot was drastically lower than in other seedlings exposed to 100 mM NaCl. Does this mean that the thickened roots were more efficient at controlling Na^+ uptake? To answer this question the experiment needs to be repeated to obtain more replicates. Furthermore, the root anatomy would need to be investigated in parallel to the ion uptake. On the other hand, a greater cortex cell size in red-osier dogwood may limit NaCl induced injury by increasing the vacuole volume for Na^+ and Cl^- compartmentalization. Furthermore, short tuberized lateral roots of rapeseed (*Brassica napus*, Vartanian *et al*., 1992) and white mustard (*Sinapis alba*, Vartanian, 1981) produced in response to drought were found to accumulate proline and starch, respectively. This accumulation of proline or starch in the cortical root cells under water stress may contribute to osmoregulation and may also be a source of metabolites utilized during rehydration. However, not all species accumulate starch in the radially expanded cortical root cells in response to drought stress (*Arabidopsis thaliana*, Couot-Gastellier and Vartanian, 1995) and it is not known if osmolytes accumulate in thickened roots in response to NaCl stress.

4.4.4 Root Hairs

In the soil, root hairs are important in nutrient uptake and have been shown to increase in density and length to facilitate nutrient uptake in response to low mineral concentration, especially phosphorous and nitrate, (Jungk, 2001; Ridge and Katsumi, 2002). Conversely, a reduction in root hair density and length suggested in Arabidopsis (*Arabidopsis thaliana*) exposed to NaCl treatment may be beneficial by reducing root

area for the uptake of Na^+ and Cl^- (Wang *et al*., 2008). However, root hairs were not observed on the red-osier dogwood roots after six weeks in hydroponics. The absence of root hairs is common in aquatic plants, such as red mangrove (*Rhizosphora mangle*, Gill and Tomlinson, 1977) and white cattail (*Typha glauca*, Seago and Marsh, 1989). Species that normally form root hairs in soil are also found to lack root hairs after developing in hydroponic solutions (Fahn, 1982). In addition, Angeles *et al*. (1986) reported that flood induced adventitious roots of American elm (*Ulmus americana*) submersed in water did not form root hairs, but the roots in the soil did. Since environmental conditions have such as strong influence on the presence of root hairs, roots of red-osier dogwood should be collected from the field and seedlings/cuttings propagated in the greenhouse to determine if red-osier dogwoods lack root hairs or the absence of root hairs was due to the propagation method and/or growth conditions

4.4.5 HYPODERMIS/EXODERMIS AND ENDODERMIS

The microscopy results clearly show the presence of a hypodermal layer in red-osier dogwood roots. The hypodermal cells had a distinct hexagonal shape relative to other cortical cells and were tightly packed together with no intercellular spaces in the hypodermis as well as between the adjacent layers (epidermis and cortex layer). This anatomical feature is commonly found in other species such as Rangpur lime (*Citrus* x *limonia*, Storey and Walker, 1999), onion (*Allium cepa*, Ma and Peterson, 2001), gentian (*Gentiana asclepiadea,* Šottníková and Lux, 2003), maize (*Zea mays*, Karahara *et al*., 2004), seagrass (*Posidonia oceanica*, Belzunce *et al*., 2008). The hypodermal cells formed suberin lamellae as the layer matured, but Casparian bands were not detected in

the radial walls. Consequently, the outer layer of the cortex was initially defined as a hypodermis and not an exodermis, which is a hypodermis with Casparian bands (Peterson and Perumalla, 1990). However, a survey of 181 angiosperm species roots showed that all species with suberin lamellae in hypodermal cells also contained Casparian bands (Perumalla *et al*., 1990). The authors suggested that Casparian bands would always be present in the anticlinal walls if suberin lamellae were detected in the hypodermis. Therefore, it is likely that Casparian bands were present in the red-osier dogwood, but not detected by the method we used.

In contrast to the exodermis, the endodermal Casparian bands clearly fluoresced and were observed in all radial walls of the red-osier dogwood roots. In addition, the suberin lamellae were deposited in a more organized pattern than in the hypodermis. The endodermal cells between the xylem poles developed suberin lamellae first and then gradually towards the xylem poles. The endodermal cells did not undergo any additional cell wall modification, i.e. tertiary walls. Therefore, the root development of red-osier dogwood followed the general development pattern common to many woody species (Lemon and Considine, 1993; Ma and Peterson, 2003). An interesting feature of red-osier dogwood roots was that anticlinal divisions were observed in the endodermal cells after the suberin lamellae had formed and corresponded to an increase in the stele area. With increasing vascular tissue development, the number of divisions within a single endodermal cell increased and the radial diameter of the original cell increased. Therefore, the division of endodermal cells was able to maintain the integrity of the endodermal layer during secondary growth. However, in red-osier dogwood Casparian bands were not detected in the new radial walls and suberin lamellae only formed in a

few cells. Similarly, the newly formed cell walls (up to 19) within a single endodermal cell did not contain Casparian bands or suberin lamellae in gentian (*Gentiana asclepiadea*), (Šottníková and Lux, 2003). In contrast, Casparian bands and suberin lamella developed in all new endodermal cell walls of primrose (*Primula acaulis*, Lux and Luxová, 2003/4), broad bean (*Vicia faba*), garden balsam (*Impatiens balsamina*), and sunflower (*Helianthus annus*) (Weerdenburg and Peterson, 1984).

Although Casparian band and suberin lamellae are not formed in the newly divided endodermal cells in red-osier dogwood roots, the suberin lamellae of the original endodermal cell appear to remain around the newly formed endodermal cells, which may maintain the barrier function of the layer. In addition, the modifications of the new walls may not be not be required as it is common in woody species for the endodermis to be shed and the periderm to become the outermost layer of the root (Ma and Peterson, 2003). In red-osier dogwood cuttings, anatomical features of the oldest root tissue suggested that the endodermis would eventually be lost. These anatomical features included the periderm originating from the pericycle and the outer cells of the periderm layer forming suberin lamellae. In addition, after periderm formation the endodermal cells of red-osier dogwood roots appeared stretched and thin. Furthermore, during the maturation of the periderm the cortex separated easily from the rest of the root during freehand sectioning.

Our results showed that the maturation of the hypodermis and endodermis occurred closer to the root tip in red-osier dogwood cuttings after NaCl treatment. This result supports earlier findings in maize (*Zea mays*, Karahara *et al*., 2004), cotton (*Gossypium hirsutum*, Reinhardt and Rost, 1995*a, b*), and seepweed (*Suaeda maritima*,

Hajibagheri *et al*., 1985). The closer development of the exodermis and endodermis to the root tip has also been observed in response to other stresses such as drought (North and Nobel, 1991), cold (Clarkson *et al*., 1987), and heavy metal stress (Lux *et al*., 2004). The increase in root surface with controlled apoplastic movement may decrease the accumulation of toxic solutes, as it has been shown to prevent the transport of apoplastic dyes (Enstone and Peterson, 1992*a, b*) and sulphate ions (Peterson, 1987). For example, lower cadmium accumulation was observed in willow clones (*Salix* spp.) with the endodermal Casparian strips occurring closer to the root tip than clones with Casparian bands developing further away from the root tip (Lux *et al*., 2004). In addition, an increase in the radial width of the endodermal Casparian bands may contribute to the effectiveness of the barrier and was observed in the halophyte seepweed (*Suaeda maritima*, Hajibagheri *et al*., 1985) and the glycophyte maize (*Zea mays*, Karahara *et al*., 2004). However, not only the thickness of the Casparian bands (i.e., the amount of suberin and lignin) but the arrangement of the suberin and lignin in the cell wall will determine the efficiency of the Casparian bands to block ion movement through the root (Schreiber *et al*., 1999; Ranathunge and Schreiber, 2011). Furthermore, the continuity of the exodermis and endodermis may be disrupted with the formation of lateral roots, which originate from the pericycle. The disrupted endodermis and exodermis provide an uncontrolled entry point for Na^+ in rice (*Oryza sativa*) and corn (*Zea mays*) roots (Ranathunge *et al*., 2005; Zhou *et al*., 2011). The contribution of lateral root produced disruption of the apoplastic barriers to the overall ion uptake in red-osier dogwood roots exposed to NaCl still needs to be determined.

The younger roots (the roots of cuttings grown for only three months in soil) used for fluorescence microscopy had a faster growth rate in all treatments compared to the older roots (cuttings grown for six months in soil) used for serial sectioning. The slower growth rate of the six-month-old cuttings may have been the result of the older roots having an inherent slower root growth compared with younger roots of the three-month-old cuttings. This may also relate to the length of the root, since it was observed in pea (*Pisum sativum*) seedlings that roots between 40 – 90 mm long grew faster than roots 90 – 130 mm long even though the seedlings were the same age (Rost and Baum, 1988). In addition, although the plants were growing under relatively controlled conditions in the greenhouse, the red-osier dogwood cuttings treated in October - November (three-month-old cuttings) may have been exposed to higher temperatures and light intensities than the cuttings treated in February – March (six-month-old cuttings) that would have resulted in higher growth rates. The effect of the season on plant growth in a greenhouse was also observed in tomato (*Lycopersicon esculentum*) seedlings, with tomato grown in the fall having a larger biomass than seedlings grown in the spring (Vavrina *et al*., 1998). The growth rate of the roots will affect the distance from the root tip at which the Casparian bands and suberin lamellae of the endodermis and exodermis develop (Wilcox, 1962; Perumalla and Peterson, 1986). In my study, the maturation of the endodermis and exodermis occurred closer to the root tip in the six-month-old cuttings with the slower growth rate compared with the three-month-old cuttings with the faster growth rate. This highlights the difficultly to compare roots with different growth rates in response to diverse treatments. Since Casparian bands develop closer to the root tip salinity stress may accelerate the development of the apoplastic barriers (Hajibagheri *et al*., 1985;

Reinhardt and Rost, 1995*a, b*); but caution should be taken when interpreting these results, as the effect of the stress on the root growth rate should be considered (Karahara *et al*., 2004). For example, in maize (*Zea mays*) the time required to form the Casparian bands was not reduced with 200 mM NaCl treatment (Karahara *et al*., 2004) compared with the control roots. Therefore, the Casparian bands occurred closer to the root tip in maize not from an acceleration of Casparian band development but from a decrease in the apparent rate of cell production, the number of cells between the meristem and Casparian bands, and a reduction in cell length. In red-osier dogwood the NaCl treatment significantly reduced the elongation of the root. Therefore, the xylem development stage was used in combination with the length of the new root growth to determine the effect of NaCl treatment on the development of the endodermis and exodermis. The xylem development appeared to be slightly delayed with increasing NaCl concentration, but the proportion of cell with suberin lamellae at Stage 1 of xylem development was greater with NaCl treatment. This suggests that NaCl may have accelerated the development of the hypodermis and exodermis, but further research is required to confirm this.

4.4.6 EPIDERMIS

In my study, the epidermis from red-osier dogwood appeared injured in localized areas of the roots and the frequency of injured epidermal cell increased with NaCl treatment. In addition, the epidermis was almost completely lost and the hypodermis and the underlying cortex layers had increased lignification and suberization in one cutting exposed to 50 mM NaCl. However, the loss of the epidermal layer may benefit the plant by reducing the uptake of Na^+ and Cl^- through the loss of epidermal surface area for ion

transport (Liu *et al*., 2007) and the increased suberization and lignification of the outer cortical cell layers observed in red-osier dogwood roots. The epidermis does not appear to play a significant role in controlling ion uptake into roots as suberized epidermal cells has been shown not to inhibit the movement of the apoplastic dye Cellufluor and the arrangement of suberin in diffuse bands in onion (*Allium cepa*) did not suggest a barrier to symplastic transport (Peterson *et al*., 1978; Perumalla *et al*., 1990). The limited control of ion uptake may also occur in red-osier dogwood roots, since the epidermal cell walls with or without NaCl treatment fluoresced relatively faintly under UV light and stained weakly positive with Sudan red 7B compared with the hypodermal and endodermal cell walls. This suggests the presence of suberin in the cell walls, but the weakness of the positive tests may also indicate that the suberin may not act as a barrier.

The cause of the epidermal cell injury/death observed in red-osier dogwood root could not be conclusively determined. The epidermal cell injury of red-osier dogwood roots may have occurred during transplanting of the cuttings (Enstone and Peterson, 1998), but the proportion of injured epidermal cells increased with NaCl concentration. Under natural conditions the epidermis can be lost as a result of microbial activity (Enstone *et al*., 2003) or drought (Stasovski and Peterson, 1991; Kamula *et al*., 1994). However, the absence of these factors in hydroponics results in the maintenance of the epidermis (Barrowcough and Peterson, 1994). Epidermal cells also die in roots with dimorphic exodermis because the suberin lamellae deposited in the long cells sever the plasmodesmata connection with the adjacent epidermal cell, which prevents the photosynthates from the leaf to be transported to the epidermal cell (Barrowclough and Peterson, 1994). However, it is not known if Na^+ and Cl^- ions affect the plasmodesmata

connections between the epidermis and exodermis, which may alter ion uptake and epidermal cell vitality.

Overall, the results from my study suggest that modifications of root morphology and anatomy contribute to increase the salinity tolerance of plant. Therefore, understanding salinity tolerance in red-osier dogwood requires further investigation of the impact of NaCl on root growth.

Chapter 5 CONCLUSION AND FUTURE DIRECTIONS

In the current study the red-osier dogwood cuttings collected from a stressful environment (dry edaphic conditions) did not respond differently than cuttings from a less stressful environment (moist edaphic conditions). There are several factors that may explain the lack of difference in salinity tolerance between the three sites. The first factor is that although the three sites had different edaphic conditions, all sites had similar climatic conditions (i.e., temperature and precipitation). This suggests that the differences in edaphic conditions between the sites may not have been sufficiently severe or constant to induce adaptive change to the drier conditions at site 2 in the red-osier dogwood plants. The similarity of growth of cuttings grown under the optimal greenhouse conditions also suggests that the less vigorous growth of the red-osier dogwood parent plants at site 2 was likely due to the limited resources at the site. This leads to the second possible factor, which is that the potential increase in salinity tolerance was lost as the cuttings grew under optimum conditions for six months (i.e., deacclimation could have occurred). The third factor is that the highly variable growth of cuttings within each site after four weeks of growth in hydroponics could have prevented us to detect a difference in salinity tolerance. To determine which factor caused the lack of difference in salinity tolerance between red-osier dogwood cuttings, a future project should initiate the salinity treatment two to three months after planting. This would be the shortest period of growth that would allow for sufficient root growth. At this stage of development, it would also be easier to select a more homogenous set of cuttings. In parallel, red-osier dogwood cuttings should be exposed to a drought pretreatment to determine if the pretreatment

reacclimates red-osier dogwood to drought stress and increases the salinity tolerance of red-osier dogwood. This knowledge would aid in determining the best propagation methods for red-osier dogwoods to enhance their tolerance to stress after transplanting.

My findings supports previous studies (Renault *et al*., 2001*b*) suggesting that red-osier dogwood tolerates 25 and 50 mM NaCl by limiting the transport of Na^+ to the shoots. In order to elucidate potential mechanisms utilized by red-osier dogwood to limit the transport of Na^+, the roots of red-osier dogwood cuttings were examined to determine if anatomical changes occurred with NaCl treatment. Although this study was not able to confirm the presence of Casparian bands within the radial walls of the hypodermis, which could increase the efficiency of the layer to block ion movement, previous studies have found that all species surveyed with suberin lamellae also possess Casparian bands (Perumalla *et al*., 1990). Therefore, it is highly probable that Casparian bands are also formed in the hypodermis of red-osier dogwood but berberine-aniline blue staining method (Brundrett *et al*., 1988) used in this study was not able to differentiate the fluorescence of the Casparian bands from the suberin lamella. The berberine-aniline blue method may be improved by the use of clearing solutions, such as lactic acid saturated with chloral hydrate, to further reduce the interference of autofluorescent cellular material and enhance the staining of the Casparian bands (Lux *et al*., 2005).

The distance between the root cap and the deposition of the Casparian bands and suberin lamellae of the exodermis and endodermis decreased in red osier dogwood with increasing NaCl concentration. Furthermore, the results of this study suggested that NaCl treatments accelerated the development of the hypodermis and endodermis relative to the maturation of the xylem tissue. However, to confirm this acceleration of the Casparian

band and suberin lamella development with exposure to NaCl, the number and length of endodermal cells between the meristem and the deposition of the Casparian bands and suberin lamellae as well as the apparent rate of cell production are required (Karahara *et al*., 2004). However, measuring these parameters in red-osier dogwood roots would be difficult and time consuming. It is thus of greater importance to determine the efficiency of the hypodermis/exodermis and endodermis to block solute transport in red-osier dogwood roots. If these layers do not inhibit solute transport, accelerated development of the endodermis and exodermis would not limit the transport Na^+ to the shoot. Exposing roots to berberine hemisulphate would indicate the apoplastic permeability of the exodermis and endodermis (Enstone and Peterson, 1992*a, b*; Meyer et al., 2009). In addition, it would be important to determine if the emergence of lateral roots disrupt the apoplastic barriers in red-osier dogwood producing a significant entry point for Na^+ and Cl^- in red-osier dogwood roots. Determining the effectiveness of the hypodermis/exodermis as a barrier to apoplastic transport in red-osier dogwood would contribute to expanding our knowledge of solute transport in woody species.

Red-osier dogwood may also limit the transport of Na^+ to the shoot by sequestering ions in the vacuoles of the root. After exposure to 50 and 100 mM NaCl the vacuolation of the cortical cells appeared to occur closer to the root tip. The importance of a total vacuole volume the roots in increasing salinity tolerance was indicated by the limited NaCl induced injury in red-osier dogwood cuttings with larger root diameters. The challenge in determining the significance of root thickening is to obtain enough cuttings exhibiting root thickening. In this study all cuttings were harvested but in the

future if this trait is observed, the plant should be kept and cuttings made to determine if root thickening would be observed in the clones with exposure to NaCl.

Understanding the effect of salinity stress on red-osier dogwood is valuable in assessing its potential for reclamation/revegetation of sites impacted by salinity. To understand the full potential of red-osier dogwood, further research is required to determine the environmental conditions before and during the stress that could contribute to an increase in salinity tolerance. This information will not only be beneficial for the survival and growth of red-osier dogwood but could also be applied to other northern woody species. In addition, the ability of a plant to modify its root growth in response to stress was shown to be important in my study in the overall tolerance of red-osier dogwood cuttings. However, our knowledge of root growth under saline conditions is limited and further investigations are required to fully assess the roots role in the overall tolerance of a plant species.

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APPENDIX A

Table A.1. Growth characteristics of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately ($n = 6$). The control root dry weight (g) was significantly different between sites when analyzed separately (see Table A.3). Different letters adjacent to the mean ± SE values indicate significant difference between sites

Site	NaCl (mM)			
	0	25	50	100
		Root dry weight (g)		
1	3.03 ± 0.30^{ab}	2.65 ± 0.27	3.05 ± 0.29	2.11 ± 0.20
2	2.26 ± 0.22^{b}	2.39 ± 0.30	2.84 ± 0.26	1.51 ± 0.07
3	3.67 ± 0.36^{a}	3.44 ± 0.47	2.99 ± 0.16	2.02 ± 0.15
		Shoot dry weight (g)		
1	5.79 ± 0.91	3.93 ± 0.62	4.11 ± 0.46	2.43 ± 0.19
2	3.95 ± 0.41	3.53 ± 0.51	4.17 ± 0.53	2.40 ± 0.15
3	6.01 ± 0.55	4.97 ± 0.54	4.29 ± 0.18	2.86 ± 0.36
		Stem dry weight (g)		
1	1.87 ± 0.32	1.19 ± 0.12	1.38 ± 0.12	1.13 ± 0.15
2	1.14 ± 0.09	0.97 ± 0.11	1.08 ± 0.17	0.76 ± 0.05
3	1.62 ± 0.17	1.19 ± 0.15	1.01 ± 0.12	0.87 ± 0.08
		Leaf dry weight (g)		
1	3.92 ± 0.61	2.74 ± 0.52	2.74 ± 0.35	1.31 ± 0.06
2	2.81 ± 0.33	2.56 ± 0.40	3.09 ± 0.39	1.63 ± 0.13
3	4.38 ± 0.39	3.78 ± 0.39	3.27 ± 0.15	1.99 ± 0.30
		Root DW/shoot DW ratio		
1	0.55 ± 0.05	0.70 ± 0.03	0.75 ± 0.04	0.88 ± 0.06
2	0.59 ± 0.03	0.69 ± 0.04	0.73 ± 0.02	0.66 ± 0.05
3	0.61 ± 0.03	0.70 ± 0.06	0.70 ± 0.04	0.75 ± 0.08

Table A.2. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the growth characteristics of red-osier dogwood ($\alpha = 0.05$).

Source	DF	Sum of Squares	F Ratio	Prob > F
		Root dry weight (g)		
Site	2	7.332	9.840	<0.001
Treat	3	15.002	13.423	<0.001
Site*Treat	6	3.591	1.607	0.161
		Shoot dry weight (g)		
Site	2	12.490	5.082	0.009
Treat	3	66.091	17.928	<0.001
Site*Treat	6	10.309	1.398	0.230
		Stem dry weight (g)		
Site	2	1.965	8.329	0.001
Treat	3	3.669	10.368	<0.001
Site*Treat	6	0.770	1.088	0.380
		Leaf dry weight (g)		
Site	2	9.439	6.901	0.002
Treat	3	40.496	19.738	<0.001
Site*Treat	6	5.847	1.425	0.220
		Root DW/shoot DW (g)		
Site	2	0.034	1.498	0.232
Treat	3	0.313	9.140	<0.001
Site*Treat	6	0.127	1.859	0.103

Table A.3. One-way ANOVA values for the effect of site (1, 2, and 3) on the growth characteristics of red-osier dogwood cuttings after 4 weeks in hydroponics (control cuttings) ($\alpha = 0.05$).

	DF	Mean Square	F Ratio	Prob > F
Root dry weight (g)	2	2.986	6.846	0.008
Shoot dry weight (g)	2	7.629	3.532	0.055
Stem dry weight (g)	2	0.830	3.529	0.055
Leaf dry weight (g)	2	3.899	3.657	0.051
Root DW/Shoot DW ratio	2	0.005	0.726	0.500

Table A.4. Increase in stem length, number of leaves, and NaCl injury of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 6).

Site	NaCl (mM)			
	0	25	50	100
		Increase in stem length (cm)		
1	57.0 ± 16.1	19.1 ± 6.4	14.1 ± 4.6	2.4 ± 0.9
2	31.5 ± 6.3	13.9 ± 6.4	17.2 ± 5.0	3.4 ± 0.8
3	44.3 ± 7.2	23.0 ± 8.1	15.9 ± 4.4	3.0 ± 0.5
		Number of leaves		
1	74.3 ± 12.3	52.2 ± 10.9	42.7 ± 5.8	23.3 ± 1.8
2	58.3 ± 6.0	41.5 ± 4.5	55.1 ± 8.8	23.3 ± 2.0
3	81.6 ± 8.0	56.8 ± 11.5	51.8 ± 6.0	27.3 ± 3.9
		Injury		
1	0.0 ± 0.0	0.4 ± 0.2	1.2 ± 0.3	3.2 ± 0.5
2	0.0 ± 0.0	0.6 ± 0.2	0.9 ± 0.2	2.8 ± 0.3
3	0.0 ± 0.0	0.7 ± 0.2	1.3 ± 0.3	2.9 ± 0.2

Table A.5. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the increase in stem length, number of leaves, and NaCl injury of red-osier dogwood ($\alpha = 0.05$).

Source	DF	Sum of Squares	F Ratio	Prob > F
		Increase in stem length (cm)		
Site	2	578.192	1.224	0.301
Treat	3	16178.263	22.836	<0.001
Site*Treat	6	1652.213	1.166	0.336
		Number of leaves		
Site	2	1184.313	2.053	0.137
Treat	3	19719.694	22.793	<0.001
Site*Treat	6	1822.410	1.053	0.401
		Injury		
Site	2	0.215	0.379	0.686
Treat	3	89.955	105.570	<0.001
Site*Treat	6	0.701	0.412	0.869

Table A.6. ANOVA values for the effect of sites (1, 2, and 3) on the increase in stem length and number of leaves of red-osier dogwood after 4 weeks of 0 mM NaCl ($\alpha = 0.05$).

Growth Parameter	DF	Mean Square	F Ratio	Prob > F
Increase in stem length (cm)	2	975.406	1.656	0.224
Number of leaves	2	854.222	2.037	0.165

Table A.7. Root, shoot, stem, and leaf water content of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 6).

Site	Water content (g g^{-1} DW)			
	NaCl (mM)			
	0	25	50	100
		Root		
1	5.32 ± 0.28	4.75 ± 0.32	4.72 ± 0.32	2.96 ± 0.25
2	5.03 ± 0.22	5.12 ± 0.40	5.42 ± 0.36	3.18 ± 0.14
3	5.13 ± 0.19	4.65 ± 0.47	4.84 ± 0.27	3.19 ± 0.09
		Shoot		
1	1.96 ± 0.10	1.78 ± 0.12	2.04 ± 0.18	1.29 ± 0.13
2	1.80 ± 0.13	1.82 ± 0.16	2.11 ± 0.10	1.36 ± 0.07
3	1.91 ± 0.08	1.80 ± 0.20	2.17 ± 0.19	1.40 ± 0.06
		Stem		
1	2.01 ± 0.10	1.56 ± 0.13	1.42 ± 0.14	1.00 ± 0.06
2	1.76 ± 0.19	1.57 ± 0.16	1.53 ± 0.09	1.04 ± 0.04
3	1.95 ± 0.10	1.58 ± 0.17	2.19 ± 0.77	1.08 ± 0.06
		Leaf		
1	1.95 ± 0.11	1.89 ± 0.11	2.36 ± 0.19	1.55 ± 0.22
2	1.82 ± 0.12	1.92 ± 0.16	2.32 ± 0.11	1.48 ± 0.15
3	1.90 ± 0.08	1.87 ± 0.21	2.29 ± 0.15	1.58 ± 0.07

Table A.8. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the root, shoot, stem, and leaf water of red-osier dogwood ($\alpha = 0.05$).

Source	DF	Sum of Squares	F Ratio	Prob > F
		Root water content (g g^{-1} DW)		
Site	2	0.954	1.091	0.343
Treat	3	49.200	37.502	<0.001
Site*Treat	6	1.954	0.745	0.616
		Shoot water content (g g^{-1} DW)		
Site	2	0.040	0.223	0.801
Treat	3	5.441	19.991	<0.001
Site*Treat	6	0.139	0.255	0.955
		Stem water content (g g^{-1} DW)		
Site	2	0.741	1.164	0.319
Treat	3	7.478	7.838	<0.001
Site*Treat	6	1.550	0.812	0.565
		Leaf water content (g g^{-1} DW)		
Site	2	0.030	0.139	0.871
Treat	3	5.642	17.291	<0.001
Site*Treat	6	0.074	0.114	0.994

Table A.9. ANOVA values for the effect of sites (1, 2, and 3) on the water content of red-osier dogwood tissues after 4 weeks of 0 mM NaCl ($\alpha = 0.05$).

		Water Content (g g^{-1} DW)		
Tissue	DF	Mean Square	F Ratio	Prob > F
Root	2	0.137	0.496	0.618
Shoot	2	0.042	0.750	0.489
Stem	2	0.104	1.137	0.347
Leaf	2	0.025	0.477	0.630

Table A.10. Stomatal conductance of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 6).

Week	Site	Stomatal conductance (mol H_2O m^{-2} s^{-1})			
		NaCl (mM)			
		0	25	50	100
1	1	0.095 ± 0.024	0.062 ± 0.018	0.060 ± 0.005	0.039 ± 0.016
	2	0.048 ± 0.014	0.050 ± 0.014	0.056 ± 0.015	0.029 ± 0.005
	3	0.085 ± 0.012	0.029 ± 0.007	0.060 ± 0.011	0.021 ± 0.004
2	1	0.130 ± 0.028	0.094 ± 0.008	0.080 ± 0.005	0.041 ± 0.011
	2	0.089 ± 0.018	0.085 ± 0.022	0.067 ± 0.011	0.036 ± 0.006
	3	0.130 ± 0.028	0.071 ± 0.020	0.076 ± 0.017	0.028 ± 0.005
3	1	0.168 ± 0.018	0.107 ± 0.019	0.080 ± 0.013	0.038 ± 0.003
	2	0.141 ± 0.024	0.097 ± 0.019	0.098 ± 0.016	0.031 ± 0.007
	3	0.147 ± 0.026	0.107 ± 0.031	0.097 ± 0.012	0.029 ± 0.005
4	1	0.189 ± 0.031	0.132 ±0.018	0.092 ± 0.019	0.034 ± 0.005
	2	0.200 ± 0.036	0.132 ± 0.023	0.087 ± 0.018	0.029 ± 0.004
	3	0.182 ± 0.028	0.150 ± 0.028	0.096 ± 0.007	0.031 ± 0.001

Table A.11. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the stomatal conductance of red-osier dogwood ($\alpha = 0.05$).

Week	Stomatal conductance (mol H_2O m^{-2} s^{-1})				
	Source	DF	Sum of Squares	F Ratio	Prob > F
1	Site	2	0.001	0.156	0.856
	Treat	3	0.131	26.967	<0.001
	Site*Treat	6	0.004	0.404	0.874
2	Site	2	0.004	1.250	0.294
	Treat	3	0.061	14.139	<0.001
	Site*Treat	6	0.006	0.704	0.648
3	Site	2	0.001	0.156	0.856
	Treat	3	0.131	26.967	<0.001
	Site*Treat	6	0.004	0.404	0.874
4	Site	2	0.000	0.027	0.973
	Treat	3	0.248	36.564	<0.001
	Site*Treat	6	0.003	0.185	0.980

Table A.12. ANOVA values for the effect of sites (1, 2, and 3) on the stomatal conductance of red-osier dogwood tissues after 4 weeks of 0 mM NaCl ($\alpha = 0.05$).

Week	Stomatal conductance (mol H_2O m^{-2} s^{-1})			
	DF	Mean Square	F Ratio	Prob > F
1	2	0.004	2.407	0.124
2	2	0.003	1.089	0.362
3	2	0.001	0.491	0.622
4	2	0.001	0.099	0.906

Table A.13. Transpiration rate of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 6).

Week	Site	Transpiration rate (mmol H_2O m^{-2} s^{-1})			
		NaCl (mM)			
		0	25	50	100
1	1	3.06 ± 0.63	2.10 ± 0.43	2.10 ± 0.21	1.38 ± 0.43
	2	1.69 ± 0.37	1.73 ± 0.40	1.89 ± 0.39	1.11 ± 0.20
	3	2.82 ± 0.38	1.16 ± 0.29	2.03 ± 0.28	0.82 ± 0.16
2	1	3.50 ± 0.71	2.74 ± 0.16	2.46 ± 0.22	1.35 ± 0.30
	2	2.60 ± 0.50	2.40 ± 0.52	2.09 ± 0.23	1.15 ± 0.19
	3	3.43 ± 0.62	2.05 ± 0.49	2.27 ± 0.42	0.95 ± 0.15
3	1	4.12 ± 0.45	2.83 ± 0.35	2.21 ± 0.20	1.22 ± 0.08
	2	3.51 ± 0.44	2.71 ± 0.49	2.65 ± 0.28	0.99 ± 0.19
	3	3.79 ± 0.57	2.55 ± 0.56	2.55 ± 0.19	0.97 ± 0.17
4	1	3.79 ± 0.35	2.99 ± 0.28	2.22 ± 0.30	1.04 ± 0.18
	2	4.04 ± 0.53	2.91 ± 0.37	2.13 ± 0.22	0.88 ± 0.07
	3	3.87 ± 0.44	3.19 ± 0.37	2.37 ± 0.19	0.92 ± 0.06

Table A.14. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the transpiration rate of red-osier dogwood ($\alpha = 0.05$).

Week	Transpiration rate (mmol H_2O m^{-2} s^{-1})				
	Source	DF	Sum of Squares	F Ratio	Prob > F
1	Site	2	0.269	0.198	0.821
	Treat	3	68.711	33.765	<0.001
	Site*Treat	6	1.949	0.479	0.821
2	Site	2	2.633	1.507	0.230
	Treat	3	37.589	14.342	<0.001
	Site*Treat	6	2.679	0.511	0.798
3	Site	2	0.269	0.198	0.821
	Treat	3	68.711	33.765	<0.001
	Site*Treat	6	1.949	0.479	0.821
4	Site	2	0.121	0.125	0.883
	Treat	3	84.905	58.576	<0.001
	Site*Treat	6	0.580	0.200	0.975

Table A.15. ANOVA values for the effect of sites (1, 2, and 3) on the transpiration rate of red-osier dogwood tissues after 4 weeks of 0 mM NaCl ($\alpha = 0.05$).

Week	Transpiration rate (mmol H_2O m^{-2} s^{-1})			
	DF	Mean Square	F Ratio	Prob > F
1	2	3.227	2.831	0.091
2	2	1.508	0.795	0.470
3	2	0.547	0.452	0.645
4	2	0.100	0.102	0.904

Table A.16. Photosynthetic rate of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 6).

Week	Site	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})			
		NaCl (mM)			
		0	25	50	100
1	1	7.00 ± 1.32	4.93 ± 0.96	5.93 ± 0.46	2.81 ± 1.11
	2	4.32 ± 1.06	4.00 ± 1.05	4.64 ± 0.79	2.09 ± 0.40
	3	6.45 ± 0.75	2.84 ± 0.68	4.87 ± 0.46	1.52 ± 0.40
2	1	8.20 ± 1.24	6.61 ± 0.25	5.72 ± 0.64	2.93 ± 1.03
	2	6.20 ± 0.85	5.33 ± 1.05	5.30 ± 0.84	2.44 ± 0.35
	3	7.69 ± 1.13	5.88 ± 1.33	5.91 ± 0.55	2.23 ± 0.43
3	1	9.84 ± 0.70	7.91 ± 0.96	7.42 ± 0.78	3.23 ± 0.77
	2	9.05 ± 0.64	7.71 ± 0.86	8.15 ± 0.69	2.35 ± 0.61
	3	8.63 ± 0.78	7.26 ± 1.67	7.42 ± 0.87	2.61 ± 0.46
4	1	10.14 ± 1.02	9.96 ± 0.62	7.74 ± 0.90	3.30 ± 0.83
	2	10.52 ± 1.10	9.36 ± 1.21	7.97 ± 0.81	2.58 ± 0.19
	3	10.40 ± 0.76	9.93 ± 1.31	8.58 ± 0.38	3.05 ± 0.17

Table A.17. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the photosynthetic rate of red-osier dogwood ($\alpha = 0.05$).

Week	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})				
	Source	DF	Sum of Squares	F Ratio	Prob > F
1	Site	2	4.604	0.615	0.544
	Treat	3	425.557	37.896	0.000
	Site*Treat	6	5.864	0.261	0.953
2	Site	2	13.308	1.728	0.186
	Treat	3	223.292	19.333	0.000
	Site*Treat	6	7.350	0.318	0.925
3	Site	2	4.604	0.615	0.544
	Treat	3	425.557	37.896	0.000
	Site*Treat	6	5.864	0.261	0.953
4	Site	2	1.779	0.244	0.785
	Treat	3	605.772	55.277	0.000
	Site*Treat	6	3.971	0.181	0.981

Table A.18. ANOVA values for the effect of sites (1, 2, and 3) on photosynthetic rate of red-osier dogwood tissues after 4 weeks of 0 mM NaCl ($\alpha = 0.05$).

Week	Photosynthetic rate (μmol CO_2 m^{-2} s^{-1})			
	DF	Mean Square	F Ratio	Prob > F
1	2	12.026	2.098	0.157
2	2	6.489	1.096	0.360
3	2	2.276	0.896	0.429
4	2	0.223	0.047	0.954

Table A.19. Water-use efficiency of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately ($n = 6$).

Week	Site	WUE (%)			
		NaCl (mM)			
		0	25	50	100
1	1	0.235 ± 0.027	0.246 ± 0.017	0.283 ± 0.010	0.174 ± 0.023
	2	0.250 ± 0.021	0.231 ± 0.027	0.249 ± 0.012	0.182 ± 0.035
	3	0.243 ± 0.035	0.247 ± 0.020	0.260 ± 0.014	0.160 ± 0.032
2	1	0.248 ± 0.021	0.264 ± 0.015	0.259 ± 0.034	0.206 ± 0.042
	2	0.261 ± 0.021	0.230 ± 0.022	0.254 ± 0.030	0.212 ± 0.027
	3	0.231 ± 0.014	0.281 ± 0.013	0.284 ± 0.029	0.224 ± 0.021
3	1	0.252 ± 0.018	0.283 ± 0.030	0.337 ± 0.017	0.251 ± 0.049
	2	0.290 ± 0.021	0.303 ± 0.030	0.325 ± 0.029	0.229 ± 0.028
	3	0.244 ± 0.023	0.274 ± 0.011	0.305 ± 0.023	0.259 ± 0.007
4	1	0.282 ± 0.016	0.349 ± 0.018	0.361 ± 0.022	0.296 ± 0.044
	2	0.281 ± 0.028	0.336 ± 0.018	0.388 ± 0.018	0.305 ± 0.015
	3	0.281 ± 0.018	0.316 ± 0.014	0.380 ± 0.031	0.334 ± 0.015

Table A.20. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on the water-use efficiency of red-osier dogwood (α = 0.05).

Week	WUE (%)				
	Source	DF	Sum of Squares	F Ratio	Prob > F
1	Site	2	0.003	0.481	0.620
	Treat	3	0.059	5.852	0.001
	Site*Treat	6	0.013	0.640	0.698
2	Site	2	0.003	0.482	0.620
	Treat	3	0.028	2.851	0.045
	Site*Treat	6	0.012	0.605	0.726
3	Site	2	0.003	0.481	0.620
	Treat	3	0.059	5.852	0.001
	Site*Treat	6	0.013	0.640	0.698
4	Site	2	0.000	0.090	0.914
	Treat	3	0.086	10.756	0.000
	Site*Treat	6	0.010	0.615	0.717

Table A.21. ANOVA values for the effect of sites (1, 2, and 3) on the water-use efficiency of red-osier dogwood tissues after 4 weeks of 0 mM NaCl (α = 0.05).

Week	WUE (%)			
	DF	Mean Square	F Ratio	Prob > F
1	2	0.000	0.086	0.918
2	2	0.001	0.759	0.485
3	2	0.004	1.666	0.222
4	2	0.000	0.002	0.998

Table A.22. Chloride, sodium, and potassium content of the roots, stems, and leaves of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (Cl^-, n = 6; Na^+ and K^+, n = 4).

Site	NaCl (mM)			
	0	25	50	100
	Chloride (mg g^{-1})			
Root				
1	0.74 ± 0.21	9.15 ± 1.26	11.58 ± 1.00	8.48 ± 1.47
2	1.39 ± 0.22	12.18 ± 2.51	18.17 ± 3.39	10.65 ± 1.62
3	2.08 ± 1.32	13.11 ± 2.16	18.05 ± 3.55	14.43 ± 1.96
Stem				
1	0.68 ± 0.33	2.85 ± 0.58	3.77 ± 1.11	3.52 ± 1.39
2	1.62 ± 0.48	6.59 ± 1.96	8.87 ± 2.17	6.00 ± 0.88
3	1.36 ± 0.44	5.69 ± 1.21	8.53 ± 1.35	6.05 ± 1.31
Leaf				
1	2.76 ± 0.59	19.00 ± 3.46	51.26 ± 10.44	66.16 ± 10.18
2	1.30 ± 0.47	15.10 ± 3.85	33.36 ± 10.20	32.28 ± 4.15
3	2.96 ± 0.70	21.10 ± 6.13	46.32 ± 7.55	45.89 ± 6.44
	Sodium (mg g^{-1})			
Root				
1	0.11 ± 0.02	4.18 ± 0.49	7.57 ± 0.45	6.99 ± 1.31
2	0.19 ± 0.03	4.33 ± 0.46	8.68 ± 0.68	10.40 ± 0.86
3	0.11 ± 0.02	3.22 ± 0.77	7.01 ± 1.01	8.47 ± 0.77
Stem				
1	0.15 ± 0.02	2.26 ± 0.30	3.04 ± 0.67	1.96 ± 0.64
2	0.07 ± 0.01	3.26 ± 1.07	4.29 ± 0.47	2.82 ± 0.56
3	0.06 ± 0.01	2.45 ± 0.72	4.06 ± 0.73	3.25 ± 0.29
Leaf				
1	0.05 ± 0.02	0.68 ± 0.19	3.53 ± 1.67	9.08 ± 1.80
2	0.04 ± 0.01	2.68 ± 0.91	2.75 ± 0.92	10.69 ± 2.45
3	0.04 ± 0.02	0.94 ± 0.15	4.41 ± 1.02	7.33 ± 1.20
	Potassium (mg g^{-1})			
Root				
1	41.63 ± 3.63	44.78 ± 3.84	33.33 ± 2.22	15.38 ± 5.30
2	33.91 ± 1.43	23.76 ± 1.88	13.56 ± 5.26	24.53 ± 6.03
3	13.86 ± 5.67	12.74 ± 6.41	8.77 ± 0.38	9.01 ± 0.63
Stem				
1	8.32 ± 0.37	6.97 ± 1.19	8.59 ± 1.20	4.65 ± 1.00
2	3.88 ± 0.48	4.33 ± 0.68	3.70 ± 0.44	3.49 ± 0.33
3	3.53 ± 0.25	2.36 ± 0.26	2.44 ± 0.18	2.86 ± 0.25
Leaf				
1	24.15 ± 5.49	16.02 ± 3.63	20.33 ± 4.54	35.41 ± 2.66
2	23.05 ± 5.29	31.91 ± 0.74	46.00 ± 2.23	35.88 ± 2.71
3	36.67 ± 1.76	32.40 ± 4.69	34.29 ± 2.31	36.48 ± 5.24

Table A.23. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on chloride, sodium, potassium content of roots, stems, and leaves of red-osier dogwood ($\alpha = 0.05$).

Tissue	Source	DF	Sum of Squares	F Ratio	Prob > F
			Chloride (mg g^{-1})		
Root					
	Site	2	248.792	6.214	0.004
	Treat	3	2028.948	33.785	<0.001
	Site*Treat	6	87.653	0.730	0.627
Stem					
	Site	2	134.603	8.781	0.001
	Treat	3	323.861	14.086	<0.001
	Site*Treat	6	36.624	0.796	0.576
Leaf					
	Site	2	2480.498	5.978	0.004
	Treat	3	25198.388	40.483	<0.001
	Site*Treat	6	2154.224	1.730	0.129
			Sodium (mg g^{-1})		
Root					
	Site	2	15.183	5.240	0.010
	Treat	3	545.950	125.609	<0.001
	Site*Treat	6	16.937	1.948	0.099
Stem					
	Site	2	5.124	2.732	0.079
	Treat	3	88.521	31.465	<0.001
	Site*Treat	6	4.119	0.732	0.627
Leaf					
	Site	2	6.692	0.822	0.448
	Treat	3	562.064	46.041	<0.001
	Site*Treat	6	30.672	1.256	0.302
			Potassium (mg g^{-1})		
Root					
	Site	2	619.818	6.061	0.005
	Treat	3	5814.766	37.910	<0.001
	Site*Treat	6	700.931	2.285	0.057
Stem					
	Site	2	3.825	1.458	0.246
	Treat	3	199.893	50.811	0.000
	Site*Treat	6	4.084	0.519	0.790
Leaf					
	Site	2	415.004	4.892	0.013
	Treat	3	2425.090	19.056	<0.001
	Site*Treat	6	329.046	1.293	0.285

Table A.24. Sodium/potassium ratio, calcium, and magnesium content of the roots, stems, and leaves of red-osier dogwood from the three selected sites after four weeks of treatment with 0, 25, 50, or 100 mM NaCl. No significant interaction between sites and treatments at $\alpha = 0.05$ with each tissue analyzed separately (n = 4).

Site	NaCl (mM)			
	0	25	50	100
	Sodium/potassium ratio			
Root				
1	0.003 ± 0.000	0.309 ± 0.048	0.711 ± 0.180	0.807 ± 0.264
2	0.004 ± 0.001	0.128 ± 0.014	0.431 ± 0.142	1.182 ± 0.049
3	0.003 ± 0.001	0.133 ± 0.023	0.665 ± 0.202	0.941 ± 0.052
Stem				
1	0.018 ± 0.001	0.514 ± 0.090	0.821 ± 0.144	0.903 ± 0.393
2	0.011 ± 0.003	0.830 ± 0.233	1.268 ± 0.221	1.199 ± 0.309
3	0.008 ± 0.002	0.549 ± 0.092	1.167 ± 0.215	1.157 ± 0.139
Leaf				
1	0.002 ± 0.001	0.019 ± 0.004	0.075 ± 0.033	0.292 ± 0.074
2	0.003 ± 0.001	0.120 ± 0.030	0.075 ± 0.021	0.305 ± 0.053
3	0.002 ± 0.001	0.030 ± 0.004	0.122 ± 0.032	0.201 ± 0.015
	Calcium (mg g^{-1})			
Root				
1	4.95 ± 0.25	6.45 ± 0.39	5.42 ± 0.24	4.15 ± 0.31
2	4.74 ± 0.42	4.97 ± 0.46	4.00 ± 0.29	3.95 ± 0.21
3	4.49 ± 0.30	2.61 ± 0.22	3.02 ± 0.20	3.40 ± 0.30
Stem				
1	9.04 ± 0.58	8.67 ± 0.55	8.23 ± 0.48	8.75 ± 1.18
2	9.03 ± 0.47	8.55 ± 0.82	9.48 ± 0.50	9.52 ± 0.49
3	8.56 ± 0.14	5.32 ± 2.02	7.18 ± 0.52	8.23 ± 0.26
Leaf				
1	19.84 ± 1.33	19.78 ± 1.33	14.65 ± 5.36	19.43 ± 1.50
2	18.04 ± 1.89	15.57 ± 1.52	22.38 ± 2.64	21.12 ± 1.83
3	18.13 ± 2.13	14.54 ± 1.14	13.76 ± 0.61	10.98 ± 1.13
	Magnesium (mg g^{-1})			
Root				
1	3.55 ± 0.19	3.54 ± 0.08	3.44 ± 0.42	3.08 ± 0.33
2	3.28 ± 0.35	3.10 ± 0.53	3.00 ± 0.18	3.38 ± 0.24
3	3.35 ± 0.11	1.92 ± 0.21	2.43 ± 0.35	2.13 ± 0.42
Stem				
1	1.13 ± 0.12	1.04 ± 0.18	1.02 ± 0.13	0.88 ± 0.26
2	0.87 ± 0.14	0.75 ± 0.15	0.79 ± 0.14	0.78 ± 0.08
3	0.79 ± 0.10	0.40 ± 0.12	0.44 ± 0.04	0.47 ± 0.02
Leaf				
1	2.89 ± 0.39	2.79 ± 0.22	2.24 ± 0.75	2.34 ± 0.28
2	2.50 ± 0.33	1.85 ± 0.25	2.61 ± 0.25	2.72 ± 0.21
3	2.07 ±0.31	1.68 ± 0.16	1.79 ± 0.12	1.39 ± 0.12

Table A.25. Two-way ANOVA values for the effect of site (1, 2, and 3) and four weeks of NaCl treatment (0, 25, 50, and 100 mM NaCl) on sodium/potassium ratio, calcium, and magnesium content in roots, stems, and leaves of red-osier dogwood ($\alpha = 0.05$).

Tissue	Source	DF	Sum of Squares	F Ratio	Prob > F
			Sodium/potassium ratio		
Root					
	Site	2	0.005	0.057	0.945
	Treat	3	6.808	53.012	<0.001
	Site*Treat	6	0.550	2.141	0.072
Stem					
	Site	2	0.559	2.451	0.100
	Treat	3	9.298	27.160	<0.001
	Site*Treat	6	0.324	0.473	0.824
Leaf					
	Site	2	0.012	2.012	0.148
	Treat	3	0.469	51.933	0.000
	Site*Treat	6	0.045	2.466	0.042
			Calcium (mg g^{-1})		
Root					
	Site	2	4.197	7.287	0.002
	Treat	3	41.740	48.310	<0.001
	Site*Treat	6	3.912	2.264	0.059
Stem					
	Site	2	1.636	0.405	0.670
	Treat	3	36.453	6.020	0.002
	Site*Treat	6	19.839	1.638	0.165
Leaf					
	Site	2	158.263	5.442	0.009
	Treat	3	347.762	7.971	<0.001
	Site*Treat	6	9.292	0.106	0.995
			Magnesium (mg g^{-1})		
Root					
	Site	2	0.589	1.008	0.375
	Treat	3	12.537	14.294	<0.001
	Site*Treat	6	0.422	0.240	0.960
Stem					
	Site	2	0.016	0.140	0.869
	Treat	3	2.410	14.032	<0.001
	Site*Treat	6	0.065	0.189	0.978
Leaf					
	Site	2	3.001	4.716	0.015
	Treat	3	7.146	7.488	0.001
	Site*Treat	6	0.195	0.102	0.996

Figure A.1. Red-osier dogwood seedlings from Alberta after 2 weeks of treatment with 0 (1), 25 (2), 50 (3), and 100 (4) mM NaCl (A). Roots of red-osier dogwood from British Columbia after 2 weeks of treatment with 0 (1), 25 (2), and 50 (3) mM NaCl (B). Red-osier dogwood seedlings from Alberta (1), British Columbia (2), and New Brunswick (3) after two weeks of treatment of 100 mM NaCl (C). The seedling from British Columbia had increased salinity tolerance and reduced Na^+ uptake. This seedling also had short and thickened roots (C). Close-up of the short and thickened roots of British Columbia seedling (D). (Renault, Personal Communication.) Scale bars: (A – C) = 5 cm; (D) = 0.5 cm.


A
1
2
3
4
B
1
2
3
C
1
2
3
D