EFFECTS OF DORMANT SEASON SOIL FLOODING AND SOIL TEMPERATURE ON PIN OAK (*Quercus palustris*) SEEDLINGS

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Master of Science

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by

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

EFFECTS OF DORMANT SEASON SOIL FLOODING AND SOIL TEMPERATURE ON PIN OAK (*Quercus palustris*) SEEDLINGS

presented by Ian Vaughan,

a candidate for the degree of master of science,

and hereby certify that, in their opinion, it is worth of acceptance.

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ABSTRACT

Bottomland forests are the dominant forest cover along the Mississippi Alluvial Valley. Changes in land use and hydrologic regimes have reduced the area of bottomland forest, putting pressure on the remaining area to meet multiple objectives. To maintain migratory waterfowl habitat, some forests are managed as greentree reservoirs and artificially flooded during the fall and winter. Red oaks are a desirable component of these forests for their acorn production, but oak decline and inadequate recruitment pose problems for maintaining quality habitat. Artificial flooding regimes may be a driving factor in oak decline, as flood timing and duration may be outside of historic conditions. Previous studies have shown growing season floods can inhibit root growth, but the impacts of dormant season flooding are not as well studied.

We conducted a greenhouse study to determine how winter flooding at different soil temperatures affects the growth and development of *Quercus palustris* (pin oak) seedlings. We examined the effects of soil temperature and dormant season flooding on *Q. palustris* seedlings using insulated water baths at 5, 10, and 15°C. Half of the seedlings received soil flooding for 60 days from January to March, after which floodwater was drained for the remainder of the study. Seedlings were harvested before, during, and after flooding. Root length was determined using scanned images and WinRHIZO (Regent Instruments, Inc), after which samples were oven-dried to determine mass.

During soil flooding, root mass was significantly less in flooded seedlings than those that were not flooded. However, following drainage this difference was no longer present. By the end of the experiment, seedlings that received flooding exhibited greater

lateral root mass and length than those that had not been flooded. Flooding had minor positive impacts on aboveground variables, such as increased stem area, but did not significantly impact aboveground seedling biomass. These findings suggest that *Q. palustris* may experience temporary stress during dormant season flooding but can recover without lasting effects. While these results seem promising for current greentree reservoir management practices, care should be taken in applying them to field conditions.

Chapter I: Literature Review

Bottomland Forests

Bottomland forests are forested wetlands occurring on floodplains (Allen et al., 2001). Bottomland forests are some of the most species rich ecosystems in the United States, supporting many tree, bird, mammal, and amphibian species (Kellison & Young, 1997). The Society of American Foresters recognizes 16 forest types within bottomlands (Eyre, 1980). Though hardwoods commonly dominate in many bottomland sites, some bottomlands are dominated by gymnosperms such as *Taxodium distichum* (baldcypress). Common tree species in bottomlands include *Salix nigra* (black willow), *Populus deltoides* (eastern cottonwood), *Betula nigra* (river birch), *T. distichum*, *Nyssa biflora* (swamp tupelo), *Acer negundo* (boxelder), *A. rubrum* (red maple), *Liquidambar styraciflua* (sweetgum), *Fraxinus pennsylvanica* (green ash), *Ulmus americana* (American elm), *Celtis laevigata* (sugarberry), *Carya aquatica* (water hickory), and a variety of oaks including *Quercus lyrata* (overcup oak), *Q. texana* (Nuttall oak), and *Q. palustris* (pin oak) (Hodges, 1994).

Diversity in forest types that occur on bottomland sites can be explained in part by minor changes in topography. While bottomlands are relatively flat compared to uplands, small changes in elevation and sediment deposition patterns from floods affect soil texture, hydrologic regimes, and composition of vegetative communities. Bottomland soils are of alluvial origin. Flooding events result in rivers overflowing their banks and depositing suspended sediment. Heavy coarse sediments like sands are often deposited closest to the banks to form fronts, while finer silts and clays are carried further into flats and sloughs (Hodges, 1997). These alluvial soils are often nutrient rich and high in

organic matter, leading to high forest productivity (Stanturf & Schoenholtz, 1998). The soil parent material in a bottomland may have originated from upland sites many miles upriver. Kellison and Young (1997) recognize multiple bottomland site types, categorized partially by the location from which the alluvium originated. Differing patterns of deposition and erosion produce identifiable features along bottomlands, including bars, fronts, flats, sloughs, ridges, and terraces. Differences in elevation and microtopography associated with these features can influence the depth and duration of flooding, as well as species composition and successional patterns (Hodges, 1994).

Bottomland forests provide regulating ecosystem services, commodities, wildlife habitat, as well as recreation and hunting opportunities. As forested wetlands, bottomland forests help to attenuate flood waters, limit soil erosion, provide opportunities for groundwater recharge, and sequester carbon (Barnett et al., 2016). With their highly productive soils, many bottomland forests are capable of producing valuable timber products. Many oak species are desirable for lumber, with some, such as *Q. pagoda* (cherrybark oak), being highly valued. *T. distichum* and *N. aquatica* (water tupelo) stands can grow at high tree densities, which can both store large amounts of carbon and produce sawlogs (Goelz, 1995). Bottomland forests provide habitat for a variety of mammals, amphibians, birds, and fish (Heitmeyer et al., 2006). These habitats are particularly important to many species of migratory forest-breeding birds and waterfowl (Twedt & Portwood, 1997). *Anas platythynchos* (mallards) consume significantly more food in habitats of shallow flooding, gaining more body mass and increasing likelihood of survival and reproduction (Heitmeyer, 2006). The preferential use of flooded forests in by waterfowl affords opportunities for hunting.

Though once widespread in the southeast, bottomland forest cover in the United States has declined substantially and has become increasingly fragmented. The largest area of bottomland forest is the Lower Mississippi Alluvial Valley (LMAV), which spans seven states: Illinois, Missouri, Kentucky, Tennessee, Arkansas, Mississippi, and Louisiana. Historical estimates of bottomland hardwood cover in the LMAV are as high as 10 million ha (Putnam et al., 1960; Stanturf et al., 2000). Modern determinations of bottomland forest cover in the region are only about a quarter of what was present in the past. Twedt and Loesch (1999) reported only 2.6 million ha of forest cover in the LMAV, with the remaining forest being highly fragmented. Much of the decrease in cover can be attributed to changes in land use, most notably to favor agriculture, following European settlement (MacDonald et al., 1979). Compounding changes in land use, the installation of roads, drainage ditches, and levees that cross topographic features have altered the hydrologic regime of many bottomland forests (Heitmeyer et al., 2006).

Greentree reservoirs (GTRs) are bottomland forests with water control structures that allow for manipulation of flooding and water levels, often in the dormant season to provide winter waterfowl habitat (King & Fredrickson, 1998). Red oaks are an important component of GTRs, as their acorns are a high-value food source for waterfowl (Heitmeyer, 2006). While management activities in GTRs are not known to decrease acorn production, shifts in species composition have been observed (McQuilkin & Musbach, 1977). Changes in the timing and duration of flooding are suspected to be driving species composition away from oaks and toward more flood-tolerant species (King et al., 1998). *A. rubrum* is increasingly dominant in the regeneration layer and

midstory on many flooded sites with a simultaneous decline in overstory oaks and oak recruitment (Heitmeyer et al., 2006).

Root Growth and Functions

While roots remain difficult to study in many circumstances, much is known about their anatomy and structure. In eudicots, a group of plants to which trees belong, the root system generally develops from the radicle into a single dominant taproot from which lateral roots branch (Seago & Fernando, 2013). As the root system develops, the initial taproot may not remain dominant and can give rise to other root formations (Stokes & Mattheck, 1996). The branching structure of root systems and the sizes of individual roots are related to their function (Fitter, 1982). The larger roots that make up a system are consider "coarse" roots and contrast significantly in size and function from smaller "fine" roots. Fine roots are arbitrarily classified as roots with diameters less than 2 mm, though some studies establish a 5 mm threshold (Jackson et al., 1997; Perruchoud, 1999; Pregitzer, 2002). Roots can also be classified by branching hierarchy, with first-order roots being the smallest diameter unbranched roots that branch from higher-order roots (Eissenstat et al., 2000).

The primary functions of coarse roots are to store and transport carbon, water, and nutrients, and to provide anchorage for trees (Pregitzer et al., 1997). Roots can make up between 20-40% of the biomass in a forest, with the proportion in root mass varying with tree age (Brunner & Godbold, 2007; Peichl & Arain, 2007). Coarse roots account for a substantial proportion of total root mass and can accumulate as much as twenty times the amount of carbon as fine roots (Perruchoud, 1999). Carbohydrates stored in roots can be

an important source of energy for new spring growth (Crawford, 1978). Coarse roots in the soil can transfer forces acting on the stem, such as wind, to reduce mechanical stress (Stokes & Mattheck, 1996). Rooting depth is an important factor in anchorage, as deeper roots increase the force required for a tree to be windthrown (Nicoll et al., 2006). Coarse roots also give rise to lateral roots, as they are often high-order roots that the first- and lower-order roots grow from (Eissenstat et al., 2000)

Fine roots are characterized by high surface areas which facilitate water and nutrient uptake from the soil. While fine roots may not account for a large portion of the mass of a root system, they do hold the vast majority of surface area. The ratio of root length to mass in fine roots is indexed as specific root length (SRL) and is inversely related to carbon investment per length of fine roots. Fine root SRLs in tree species vary, with reported values ranging from 23.4 to 72.3 m g^{-1} (Eissenstat et al., 2000; Weemstra et al., 2020). This variation is driven strongly by root diameter and, subsequently, the size classes of roots being examined (Alvarez-Uria & Korner, 2007). The carbon demand of respiring fine roots affects whole-tree carbon balance (Weemstra et al., 2020). Higher SRLs are correlated with increased root respiration rates and shorter root lifespans, suggesting high maintenance costs are worthwhile during periods of high resource availability (Eissenstat et al., 2000).

Unlike the aboveground tissues of temperate deciduous trees, roots are able to grow at any time of year as long as soil temperature and moisture are sufficient (Perry, 1971). Field studies have observed root growth in upland trees continuing into early January, well after leaf senescence (Kuhns et al., 1985; Teskey & Hinckley, 1981). These studies determined root growth was influenced by combinations of soil temperature and

soil water potential, with growth increasing with increased temperature until water availability became limiting. A soil temperature of 4°C has been suggested as a lower limit for root growth to occur, but roots of some species native to colder climates may continue growth at colder temperatures (Alvarez-Uria & Korner, 2007).

Roots are perhaps the most difficult to study and least understood of tissues of trees. Our inability to readily observe and monitor roots pose significant challenges, and studies are often limited to destructive sampling of root biomass and length. Direct measures of root biomass are labor intensive and not feasible for broad studies (Vogt et al., 1998). In controlled experiments, destructive sampling can be used to get accurate measures of root mass within a soil profile (Alvarez-Uria & Korner, 2007). However, destructive sampling by its nature makes repeated measures on individual plants impossible and cannot fully account for the dynamic properties of living roots. Some methods exist that allow for limited study of active root systems. Rhizotrons can be installed into the soil to provide a viewing window for observing root growth (Kuhns et al., 1985; Teskey & Hinckley, 1981). Minirhizotrons, clear tubes through which cameras can be inserted, can be used to sample the spatial distribution of roots (Hendrick & Pregitzer, 1992).

Flooding

Flooding causes shifts in soil chemistry and microbial activity. Oxygen diffuses readily in air and can move easily to regions of low concentrations as it is depleted within soil pores. When soil pores are saturated with flood water oxygen diffusion is significantly slower (Colmer, 2003). The lack oxygen replenishment can result in highly

reduced soil conditions that favor anaerobic bacteria and reactions. Changes in soil chemistry and metabolic processes can also lead to accumulations of compounds toxic to plants (Kozlowski, 1997). Reintroduction of oxygen can cause reactions with accumulated ferric iron or ethanol, potentially damaging plant tissues (Crawford, 2003).

Flooding events, as a common natural disturbance in bottomlands, can perturb plants in a variety of ways. Many species exhibit reduced growth during flooded stress conditions (Frye & Grosse, 1992; Glenz et al., 2006; Gravatt & Kirby, 1998). Decreases in photosynthesis associated with lower stomatal conductance have been observed in oak species under reduced conditions typical of flooded soils (Pezeshki et al., 1996). Non-stomatal limitations to photosynthesis have also been observed, with flooded *Q. pagoda* seedlings exhibiting decreased photosynthetic efficiency (Gardiner & Krauss, 2001). Studies on starch allocation suggest that flooding can disrupt photosynthate translocation from leaves (Gravatt & Kirby, 1998). Nutrient form and uptake by roots, as well as distribution of those nutrients, can be altered by flooded conditions, though the effects appear to vary with species (Harrington, 1987). Reduced stem growth or stem dieback can be caused by flooding events even in flood tolerant species (Kabrick et al., 2012). Flooding is also understood to decrease root growth (Dreyer, 1994; Wang et al., 2016).

Many plants in bottomland forests or other flood-prone environments have adaptations that assist in tolerating flooded conditions. Some species of wetland plants produce highly aerated aerenchyma tissues and leach oxygen into the rhizosphere, maintaining a partially oxygenated soil environment (Colmer, 2003). Adventitious roots, which are produced by some species in response to flooding, have an anatomy that promotes gas exchange and hydraulic conductivity (Calvo-Polanco et al., 2012). These

roots also tend to grow close to the soil surface where oxygen is more abundant (Alves et al., 2013).

Chapter II: Introduction

Bottomland forests are commonly found in the southeastern United States (Allen et al., 2001). Though floodplains are relatively flat, small changes in elevation give rise to different vegetation patterns (Stanturf & Schoenholtz, 1998). Bottomland forests are typically composed of hardwoods but can also include softwood species like *T. distichum*. Species assemblages can vary from cypress and tupelo on the wettest sites to oaks, maples, and elms on better drained sites. The Society of American Foresters recognizes 16 different bottomland forest cover types (Kozlowski, 2002). Collectively, these bottomland forests provide important habitat, particularly for many breeding and migratory birds (Twedt & Portwood, 1997). In addition to habitat, bottomlands sequester carbon, provide natural flood control and water filtration, and offer recreational, economic, and aesthetic values (Barnett et al., 2016).

While once widespread, bottomland forest cover has decreased substantially with increasing human settlement and activity. The broadest area of bottomland forests is the Lower Mississippi Alluvial Valley (LMAV). Spanning across seven states, the LMAV covers an area from southern Illinois to Louisiana. Estimates of historical cover suggest that 10 million ha of bottomland forests covered the LMAV prior to European settlement (Putnam et al., 1960). By 1999, it was reported that only about a quarter of this forest cover remained, and the remainder is highly fragmented (Twedt & Loesch, 1999). Much of the loss in bottomland forests can be attributed to land use changes, specifically conversion to agricultural use (MacDonald et al., 1979). Compounding the loss of forest cover are disruptions to natural hydrologic regimes caused by drainage ditches, impoundments, and roads (Heitmeyer et al., 2006).

To maintain or restore the habitat and functions of lost and increasingly fragmented bottomlands, some forests, such as those in the Mingo National Wildlife Refuge (MNWR), are managed as greentree reservoirs (GTR). These GTRs are intentionally flooded during the fall and winter months to create temporary wetlands. These wetlands are important seasonal habitat for waterfowl. It is common for GTRs to produce high-protein mast since dormant season flooding does not reduce acorn production (McQuilkin & Musbach, 1977). However, oak species in the MNWR are experiencing increased die-back and mortality, and regeneration of competing species is increasingly abundant (Heitmeyer et al., 2006). At similar sites, mortality has been attributed to competition and flood duration (King et al., 1998). Silvicultural treatments can mitigate the effects of competing species, but a more in-depth understanding of the effect of dormant season flooding is needed to guide management decisions.

This study is designed to investigate root development under different soil flooding and temperature conditions in a controlled greenhouse. Our objectives with this study are to determine:

- How soil temperature affects root growth of *Q. palustris* seedlings during the dormant season,
- How dormant season flooding affects the growth of above and belowground tissues,
- Whether negative effects of flooding are exacerbated at higher soil temperatures.

We expect that higher soil temperatures will lead to increased root growth, that flooding will decrease or inhibit root growth, and that flooding effects will be more

severe at higher temperatures. The findings of this study will ideally allow for better informed water management decisions in GTRs.

Chapter III: Methods

Experimental Design and Treatments

This study was conducted at the Center for Bottomland Hardwoods Research, a USDA Forest Service research laboratory in Stoneville, MS (33.42244, -90.90708). Two greenhouses were used in the experiment, one for germinating acorns and growing seedlings and the second for applying experimental treatments. The experiment utilized a randomized complete block factorial design with repeated measures. Treatments were applied randomly to six insulated water baths (tanks) in each of three blocks. Blocks were arranged east to west along a ventilation and airflow gradient in the treatment greenhouse. Treatments consisted of combinations of three soil temperatures (5, 10, and 15°C) and two flood conditions (60-day flood and non-flooded). Temperature and flood conditions were crossed to create a 3 x 2 factorial design, making a total of six experimental treatment combinations.

Seedlings were transferred from the germination greenhouse to the water baths in the treatment greenhouse on December 2, 2020. At this time, temperature in all water baths was 15°C. Seedlings were allowed to acclimate for five days before initiating the temperature treatment. Beginning December 7, water temperatures were incrementally lowered towards assigned temperature levels over the next 30 days. Temperatures reached their treatment levels on January 6. Starting on March 7, we began to raise water temperature incrementally in all baths towards 20°C over 30 days. By April 6, all water baths reached 20°C, which was maintained until the end of the experiment. The 60-day flood was initiated on January 6 and terminated March 7. Treatment conditions throughout the duration of the experiment are summarized in Figure 1.

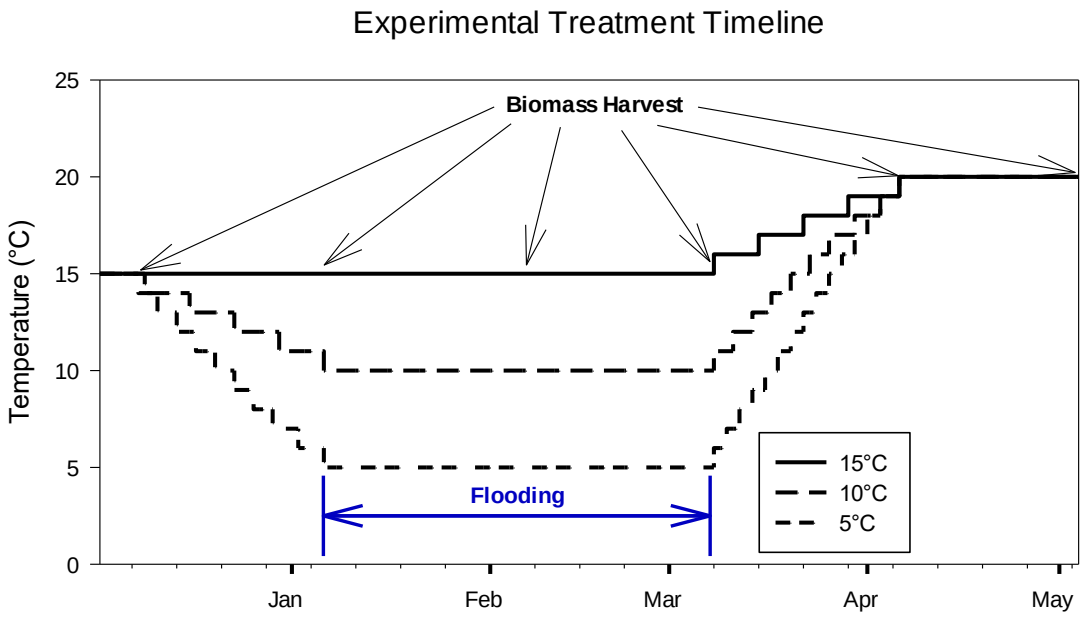

Figure 1: Timeline for the experimental treatments and biomass harvests. Temperature in all treatment levels were maintained at 15°C during the acclimation period before gradually being brought to their treatment temperature level. After the March biomass harvest, all temperature treatment levels were gradually raised to 20°C.

Materials

An estimated 5000 *Q. palustris* acorns were collected from the Mingo National Wildlife Refuge between early December 2019 and January 2020 and refrigerated to maintain dormancy. In the germination greenhouse, 5000 D60L nursery pots (Stuewe & Sons, Inc.) were filled one inch from the top with BM7 Bark Mix (Berger Inc.), a mixture of peat moss (63-69%), pine bark (23-27%), and perlite (8-12%). In late August 2020, the collected acorns were soaked in water for a day before being sown into nursery pots. The acorns were then covered with another inch of potting mix and the nursery pots were placed under 50% shade cloth for acorns to germinate and seedlings to grow. Soil was kept well-watered during this time.

In late November, established seedlings were selected for use in the experiment. Seedlings that showed signs of branching, disease, insect damage, or that had not reached a 2-lag stage of ontogeny (Hanson et al., 1986) were rejected. We selected 1080 viable seedlings and randomly assigned them to experimental units. Nursery pots of selected seedlings were covered by plastic bags secured with rubber bands. The bags extended an inch over the rim of the pot and formed a waterproof seal (Figure 2). Plastic bags surrounding seedlings were punctured to initiate the flooding treatment, allowing water to enter the pots and fully saturate soil. The punctured bags were replaced with intact bags to prevent further entry of water when flooding was terminated.

Figure 2: Photo taken March 9 of seedlings in one of the experimental water baths. The plastic bags surrounding nursery pots allow heat exchange between water and soil while preventing water from entering.

In the treatment greenhouse, 18 insulated water baths were established. Water baths consisted of 350-gallon aluminum tanks surrounded by foam insulation. Each tank was fit with a heavy vinyl liner to limit water contact with aluminum surfaces and filled with reverse osmosis water. A 1.0 horsepower water chiller/heater (Frigid Units, Inc.) was placed on the northeast corner of each tank to maintain water temperature within ±0.5°C of the programmed temperature and keep water in the tank circulating. Mesh filters were placed over PCV piping in the southwest corners of each tank. The piping served as the intake for water circulated by the chiller/heater, and filters were cleaned and replaced as needed during the experiment.

On December 2, the previously selected seedlings were placed into the water baths. Each tank received 90 seedlings. The water level in each tank was kept even with the surface of soil in pots. As water evaporated from tanks, additional water was added to maintain the desired water level. Seedlings were watered as needed, based on visual inspection and feeling of the soil surface. The greenhouse was generally maintained at ambient outdoor temperature but was heated to 1°C if external air temperature fell below 0.5°C.

Data Collection

To determine treatment effects, data were collected on aboveground and belowground seedling tissues, seedling phenology, and soil characteristics. A listing of all variables collected, as well as the frequency of observation for each, are presented later in Table 2.

Leaf Phenology

Leaf phenology was documented each day throughout the experiment. All seedlings in a tank were observed for their degree of color change, amount of leaf fall, and whether they had experienced budburst. Ten phenology codes were created to document seedling progression from fall to spring (Table 1). These codes were assigned based on the proportion of seedlings in a tank exhibiting a given condition, and the proportion accounted for plant numbers changing as biomass harvests progressed. After examining all seedlings in a tank, the tank was assigned a phenology code for the daily observation. Only one code was assigned to a tank per day, and the highest numbered codes were considered first. If a tank met the criteria for both 5 and 7, the code recorded for that day would be 7.

Table 1: Phenology codes for color change, leaf fall, and bud burst.

Phenology Code	Observed Condition
1	>3/4 seedlings have fully green leaves
2	>1/4 seedlings have leaves showing yellow/brown/red colors
3	>1/2 seedlings have leaves showing yellow/brown/red colors
4	>3/4 seedlings have leaves showing yellow/brown/red colors
5	>1/2 seedlings have dropped leaves
6	>3/4 seedlings have dropped leaves
7	At least 1 seedling has broken bud
8	>1/4 seedlings have broken bud
9	>1/2 seedlings have broken bud
10	>3/4 seedlings have broken bud

Biomass Harvests

Biomass harvests were conducted every 30 days, starting on December 7 (Figure 1). The treatment schedule allowed for two biomass harvests to occur prior to reaching temperature targets and soil flooding, two harvests at treatment conditions, and two harvests in post-treatment conditions, making six repeated harvests from each tank. At each biomass sample period, ten seedlings were randomly selected from each tank. Harvested seedlings were dissected into leaf, stem, and root tissues. First, leaves were removed from the stem. The stem and roots were then removed from their nursery pot and washed to remove excess soil. Stems were cut from the roots at the root collar, and root systems were placed in refrigerated storage. If adventitious roots were present, they were also separated and put into refrigerated storage. Stem diameter at the root collar was recorded as the average of two perpendicular caliper measurements, to the nearest 0.1 mm, and diameter was used to calculate stem cross-sectional area. The length of each stem was also recorded by straightening stems along a ruler. Distance from the root collar to the terminus of each flush of growth was recorded to the nearest 0.5 cm, with distance to the terminus of the final flush equaling total stem length. After measuring length, stems were placed in paper bags and put in a 70°C drying oven. Leaves were dried using the same procedure. When oven-dried, stems and leaves were weighed to the nearest 0.002 g.

While randomly sampling for biomass harvests, seedlings were rejected if they exhibited any branching or split in the stem, showed damage not related to experimental treatment, or if there was no clearly defined dominant tap root. Roughly 10% of seedling were rejected, with the most common cause being tap root defects. Rejected seedlings

were replaced by additional randomly selected seedlings until ten suitable seedlings had been harvested from each tank.

Root Processing

Previously harvested root systems were taken out of cold storage for further processing. Roots were washed a second time to ensure they were free of soil and debris. The tap root was isolated from the rest of the root system by cutting off lateral roots at their connection to the tap root. The tap root was then further separated into three tissue categories: suberized, unsuberized, and dead. Unsuberized tissue was distinguished by creamy white coloration, while dead tissue appeared black or partially decayed. Unsuberized and dead tissues were cut away from the rest of the suberized tap root. Lateral roots were separated into the same three tissue categories. Because of the abundance of smaller, unsuberized hairlike roots, only unsuberized roots with a diameter of 0.5 mm or greater were cut off and separated. This process partitioned the original root system into seven components: adventitious roots, suberized tap, unsuberized tap, dead tap, suberized laterals, unsuberized laterals, and dead lateral roots.

Working with one root component at a time, roots were placed in a shallow tray of water and spread so that root branching was clearly visible. The tray of water was placed on an Epson Expression 12000XL scanner to produce a scanned image of the roots against a white background at 300 DPI. After scanning, roots were removed from the water tray and placed in an oven at 70°C until dry. Once oven-dried, each component of the root system was weighed in the same manner as the stems and leaves, with mass recorded to the nearest 0.002 g. In some samples, a component of the root system was

observed but mass was too light for the scale to detect. In these cases, a weight of 0.000g was recorded, while if a root component was not observed it was recorded as NA.

Scanned images of roots were processed with the root analysis software WinRHIZO Basic 2017a (Regent Instruments, Inc.). This program distinguishes root material from the background in a scanned image, and when calibrated to the size of the scan area can determine the total length of roots in the image. Lengths are given to the nearest 0.001 cm. Fine root specific root lengths (SRL) were calculated as the ratio of lateral root length to lateral root mass (cm g^{-1}). Tap root lengths were often overestimated when using the scanned images because small protrusions branching off the tap root were incorrectly added to the total length. To get an accurate measure of the tap root length, images were edited so these protrusions were "painted over" using the background color. This was done until the analysis output showed only two root tips and zero branches, ensuring that the total length reported was accurate (Figure 3).

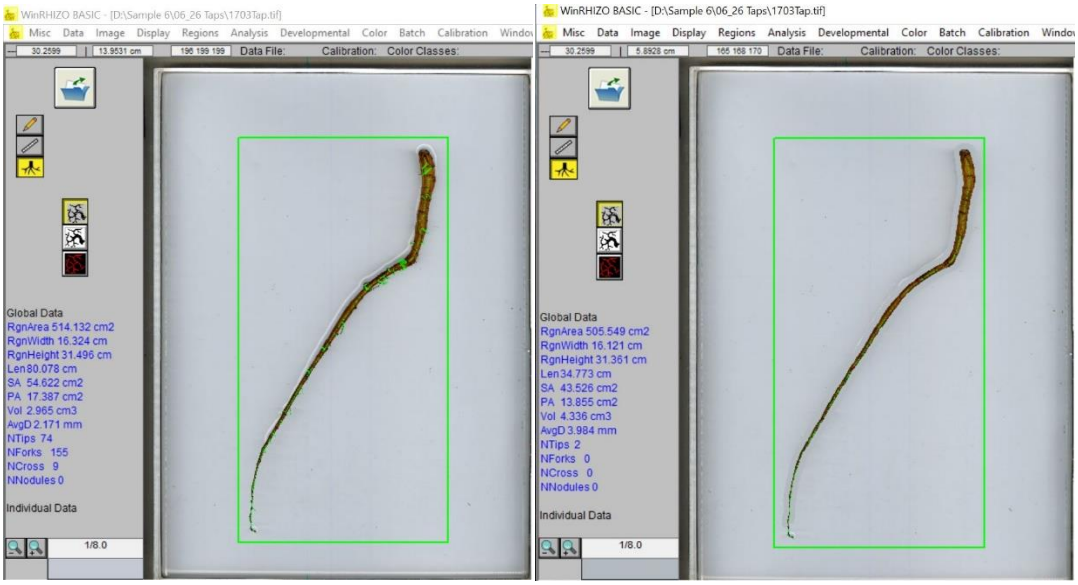

Figure 3: A side-by-side example of a tap root scan before (left) and after (right) editing. Root protrusions were edited out of the image until the number of root tips (NTips) was 2 and the number of forks (NForks) was 0. The final edited image was saved and the length (Len) was recorded.

pH and Oxidation Reduction Potential

After 60 days of flooding (March 6), measurements of soil pH and oxidation reduction potential (ORP) were recorded before terminating the flood treatment. Measurements in each flooded tank were taken in the soil surrounding four randomly selected seedlings. The temperature, pH, and ORP were measured using an EcoSense pH100A Meter (YSI, Inc.). The meter and probes were calibrated using pH 7.00 and 4.01 buffer solutions for pH and Zobell solution for ORP. Probes were inserted about 10 cm into the soil and their reading recorded after stabilizing. The instrument is accurate within ±0.3°C, ±0.03 pH units, and ±0.1% mV, with resolution to the nearest 0.1 °C, 0.01 pH units, and 1 mV.

Data Analysis

Linear models were fit for leaf phenology observations. Response variables were the number of days until reaching each phenology stage, with temperature, flooding, their interaction, and block included as fixed effects. We used ANOVA for a 3 x 2 factorial, randomized complete block design to test for significant effects. For most response variables from the biomass harvests and root processing, linear mixed effects models were fit. These models included temperature, flooding, time, and all their interactions as fixed effects and block as a random effect. For proportional response variables, such as proportion of suberized root mass, generalized linear mixed effects models were fit using a binomial distribution. These models were tested using repeated measures ANOVA for a 3 x 2 factorial randomized complete block design, with the experimental unit (tank) being the repeated measured subject. Results of these statistical models are provided in

Appendices 1-3. In all models, if a significant interaction was detected we tested for significant effects of one factor within all levels of the other factor(s). Pairwise comparisons with a Tukey's Honest Significant Difference adjustment were used to determine which groups of means differed from each other. Statistical differences were determined using an $\alpha = 0.05$.

All analysis was conducted using R studio 4.0.2 (R Core Team, 2020). Models were constructed using the *lmer* function from the *lme4* package (Bates et al., 2015) for most response variables from biomass harvests and root processing. The *lme* function in the *nlme* package (Pinherio et al., 2021) was used for leaf phenology, and the *glmmTMB* function from the *glmmTMB* package (Brooks et al., 2017) was used for proportional variables with non-normal distributions. The *emmeans* function from the *emmeans* package (Length, 2021) and *cld* function from the *multcomp* package (Hothorn et al., 2008) were used for conducting and displaying pairwise comparisons, and the *joint_tests* function from *emmeans* was used for testing significant effects within levels of an interacting factor.

Table 2: Summary of response variables measured. Models refer to the type of linear model used: linear models (LM), linear mixed effects models (LMM), generalized linear mixed effects models (GLMM). NA indicates that no statistical models were made.

Response Variable	Frequency	Units	Model
Aboveground			
Leaf Phenology	Daily	# of days	LM
Leaf Mass	30-days	g	LMM
Stem Mass	30-days	g	LMM
Stem Cross-section	30-days	mm^2	LMM
Stem Length	30-days	cm	LMM
Belowground			
Tap Root Mass	30-days	g	LMM
Suberized Tap Root Mass	30-days	g	LMM
New Tap Root Mass	30-days	g	LMM
Dead Tap Root Mass	30-days	g	LMM
Lateral Root Mass	30-days	g	LMM
Suberized Lateral Root Mass	30-days	g	LMM
New Lateral Root Mass	30-days	g	LMM
Dead Lateral Root Mass	30-days	g	LMM
Total Root Mass	30-days	g	LMM
Suberized Root Mass	30-days	g	LMM
New Root Mass	30-days	g	LMM
Dead Root Mass	30-days	g	LMM
Tap Root Length	30-days	cm	LMM
Suberized Tap Root Length	30-days	cm	LMM
New Tap Root Length	30-days	cm	LMM
Dead Tap Root Length	30-days	cm	LMM
Lateral Root Length	30-days	cm	LMM
Suberized Lateral Root Length	30-days	cm	LMM
New Lateral Root Length	30-days	cm	LMM
Dead Length Root Length	30-days	cm	LMM
Total Root Length	30-days	cm	LMM
Suberized Root Length	30-days	cm	LMM
New Root Length	30-days	cm	LMM
Dead Root Length	30-days	cm	LMM
New Root Tips	30-days	# of tips	LMM
Specific Root Length	30-days	$cm\ g^{-1}$	LMM
Proportion of New Lateral Root Mass	30-days	$g\ g^{-1}$	GLMM
Proportion of New Lateral Root Length	30-days	$cm\ cm^{-1}$	GLMM
Soil Factors			
pH	One-time	pH units	LMM
Oxidation Reduction Potential	One-time	mV	NA

Chapter IV: Results

Belowground

Root Mass

Generally, total root mass increased or remained unchanged over the duration of the experiment, but some treatment combinations showed a decrease in root mass between April and May. A significant interaction was found between flooding and time ($p = 0.004$) on total root mass. Time was significant within both the flooded ($p < 0.001$) and non-flooded treatment levels ($p < 0.001$). Seedlings in the flooded treatment level had significantly lower total root mass than those in the non-flooded treatment in February ($p = 0.011$), March ($p = 0.001$), and April ($p = 0.001$). By May, the two flooding treatments were not significantly different from each other ($p = 0.720$) (Figure 4A). In the non-flooded treatment, increases over time were statistically significant. Total root mass among non-flooded seedlings were greater in March and April than in January ($p = 0.013$ and 0.010). In May, total root mass observed in non-flooded seedlings decreased significantly ($p = 0.006$), while in the flooded treatment level root mass remained unchanged ($p = 0.999$).

Total root mass was also affected by the interaction of temperature and time ($p = 0.020$). Time was a significant factor within all levels of temperature ($p = 0.001$). Within time, temperature was only a significant factor in March ($p = 0.017$) and May ($p = 0.002$). In March, seedlings in the 15°C treatment level were observed to have greater total root mass than those in the 5°C treatments ($p = 0.014$). In May, seedlings from 15°C treatment level had higher total root mass than those from both the 5 ($p = 0.004$) and 10°C treatments ($p = 0.004$) (Figure 4B). Within temperature treatments, total root mass

was lowest in December, being significantly lower than all other months for all treatment combinations ($p < 0.05$). Total root mass within a given temperature treatment remained relatively similar between January and April. May observations of total root mass in the 5°C treatment level was nominally lower than those in April but were not significantly different ($p = 0.343$) and mass in the 15°C level remained unchanged ($p = 0.999$). In the 10°C level, however, total root mass in May was significantly lower than in April ($p = 0.017$).

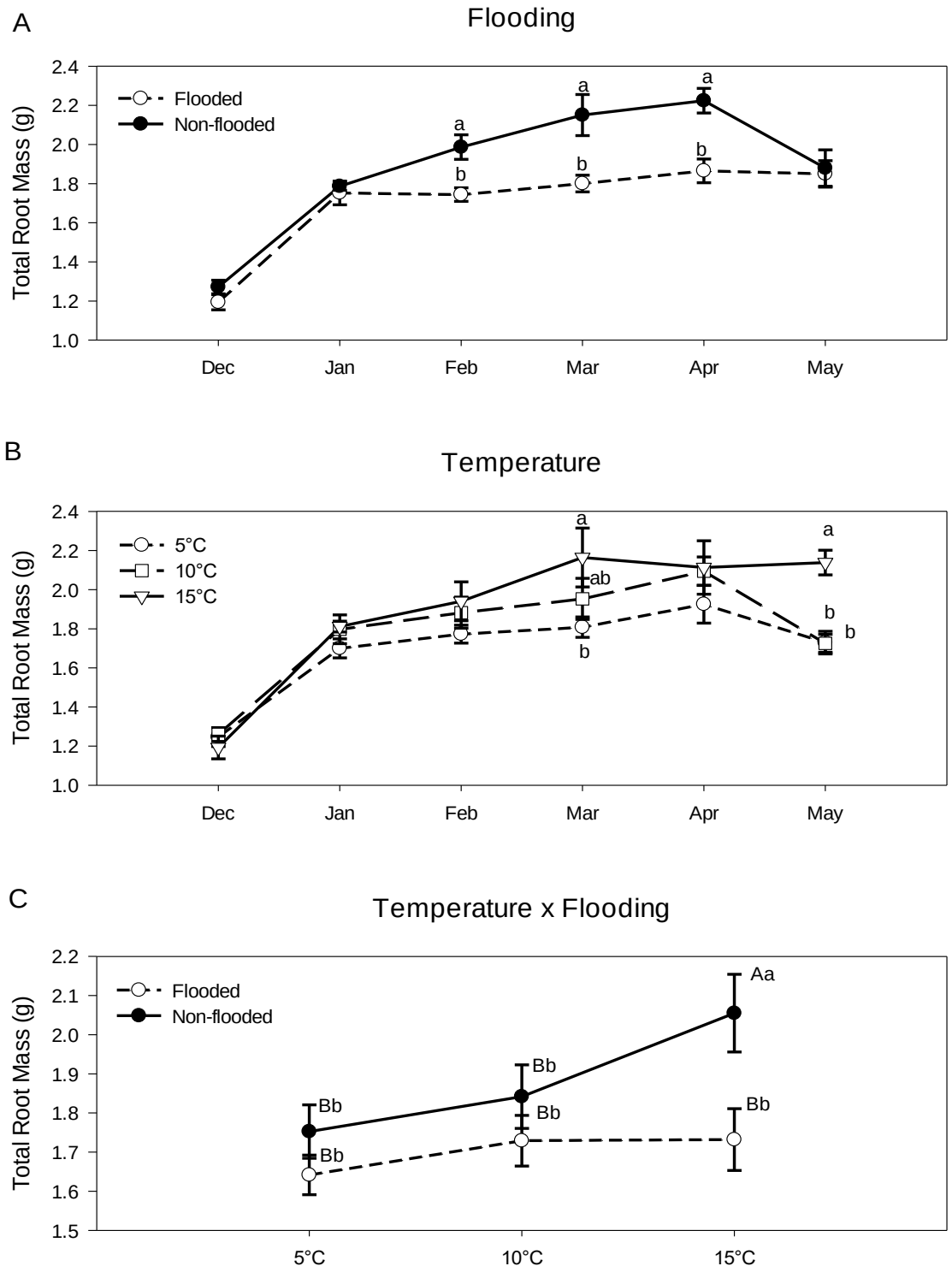

Figure 4: Panel A shows mean total root mass by flooding treatment through time. B shows average total root mass by temperature treatment through time. C shows the average total root mass for each temperature and flooding treatment combination averaged across time. Lower case letters indicate significant differences between flooding levels (A), temperature levels (B), and temperature levels within a flooding level (C), while upper case letters indicate significant differences between flooding levels within a temperature level (C). Error bars show mean ±1 SE.

A significant interaction was also seen between temperature and flooding ($p = 0.050$). Within flooding treatments, temperature was only significant in the non-flooded seedlings ($p = 0.002$). Seedlings in the 15°C treatment level had greater total root mass than non-flooded seedlings in the 5 and 10°C levels ($p = 0.001$ and 0.017). Within levels of temperature, flooding was only significant at 15°C ($p = 0.001$), with greater total root mass in the non-flooded level than in the flooded level (Figure 4C).

Total root mass can be broken into broad categories of tap root and lateral root masses. Tap root mass makes up a large portion of the total root mass and shows a similar pattern to total root mass. Significant interactions were detected between flooding and time ($p = 0.013$) and flooding and temperature ($p = 0.040$). However, the interaction between temperature and time observed in total root mass was not significant for tap root mass ($p = 0.052$). Within the flooded and non-flooded treatment levels, time was a significant factor ($p < 0.001$), with patterns similar to that described for total root mass. Within time, flooding was significant in February ($p = 0.010$), March ($p = 0.002$), and April ($p = 0.001$). In all three months, non-flooded seedlings had greater tap root mass than flooded seedlings (Figure 5). Within levels of temperature, flooding treatments were only significantly different from each other at 15°C ($p = 0.001$). Within levels of flooding, temperature affected tap root mass of non-flooded seedlings ($p = 0.004$), with non-flooded seedlings at 15°C having greater tap root mass than those at 5°C ($p = 0.004$) and 10°C ($p = 0.026$).

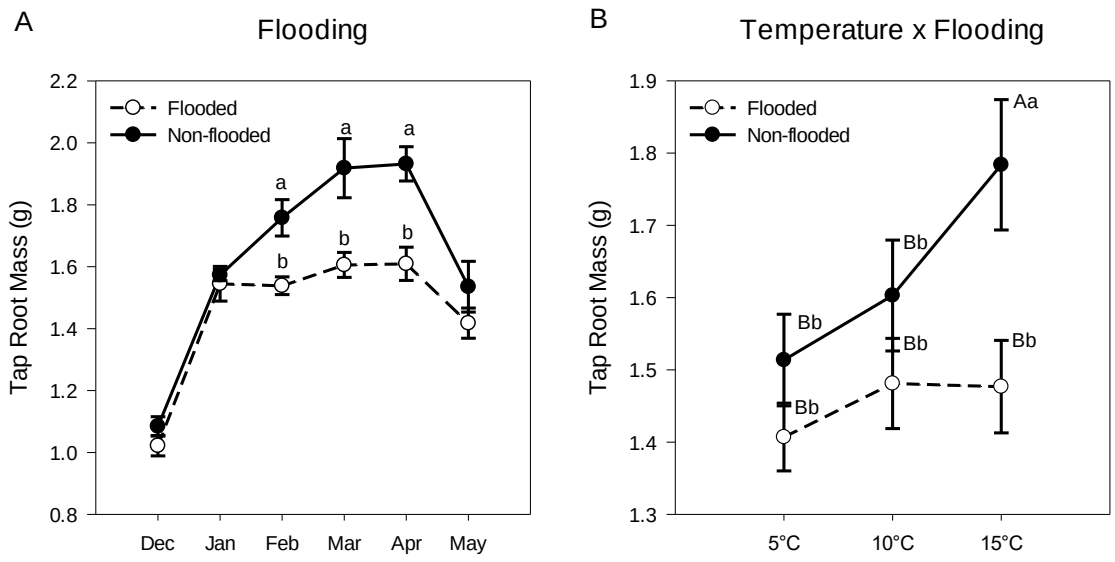

Figure 5: Panel A shows mean tap root mass by flooding treatment through time, while B shows tap root mass averaged across time for each temperature and flooding treatment combination. Lowercase letters indicate significant differences between flooding levels (A) and temperature levels within a flooding level (B), while uppercase letters indicate significant differences between flooding levels within a temperature level (B). Errors bars show mean ±1 SE.

When examining lateral root mass, a significant interaction between flooding and time was observed ($p = 0.001$). Time was significant in flooded and non-flooded treatment levels ($p = 0.001$). Seedlings in the flooded treatment level had greater root mass than those in the non-flooded treatment level in May ($p = 0.003$) (Figure 6). Similar to total root mass, lateral root mass generally increased with time, although the temporal pattern differed from that of tap root mass. Lateral root mass in May was greater than in April in flooded seedlings ($p < 0.001$) but not in non-flooded seedlings ($p = 0.163$).

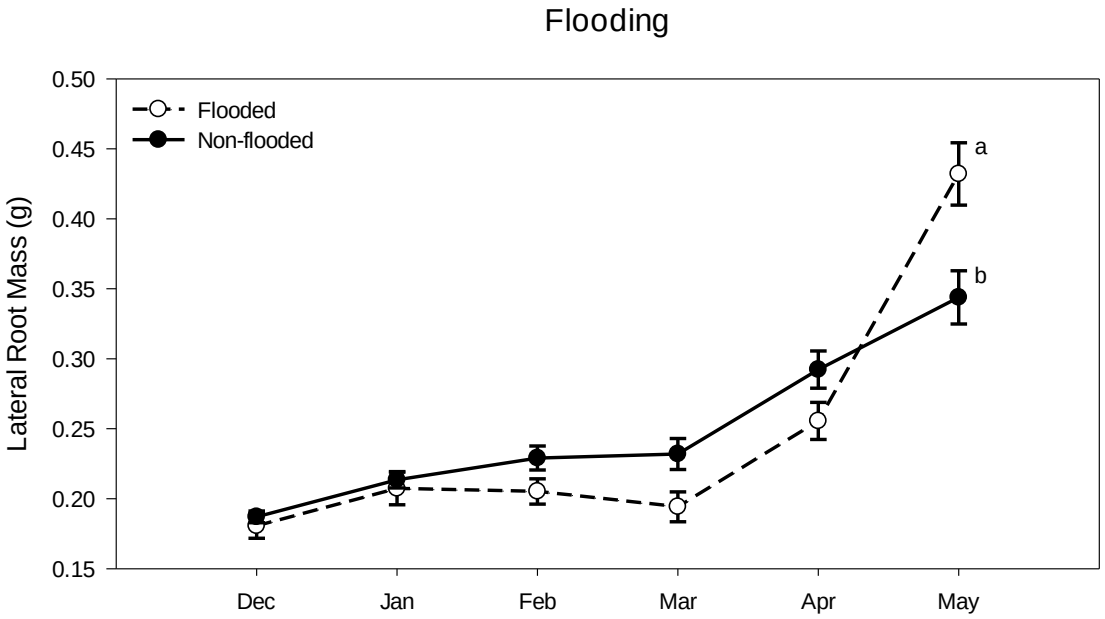

Figure 6: Mean lateral root mass by flooding treatment level through time. Letters indicate significant difference between flooding levels. Error bars show mean ±1 SE.

Root Length and SRL

Lateral root length was significantly affected by an interaction of flooding and time ($p = 0.007$). Time affected root length within the flooded and non-flooded levels ($p < 0.001$ and $p = 0.001$, respectively). Within time, the flood effect was significant only in May ($p = 0.002$), with seedlings from the flooded level having greater lateral root length than those from the non-flooded level (Figure 7). Within flooding, lateral root length generally increased with time. If seedlings received flooding, root lengths in April were greater than in all previous month ($p < 0.028$), and the same response was observed in May ($p < 0.001$). For seedlings raised in the absence of soil flooding, root lengths in April were greater than in the previous four months ($p < 0.002$). However, their root lengths did not differ between April and May ($p = 0.068$).

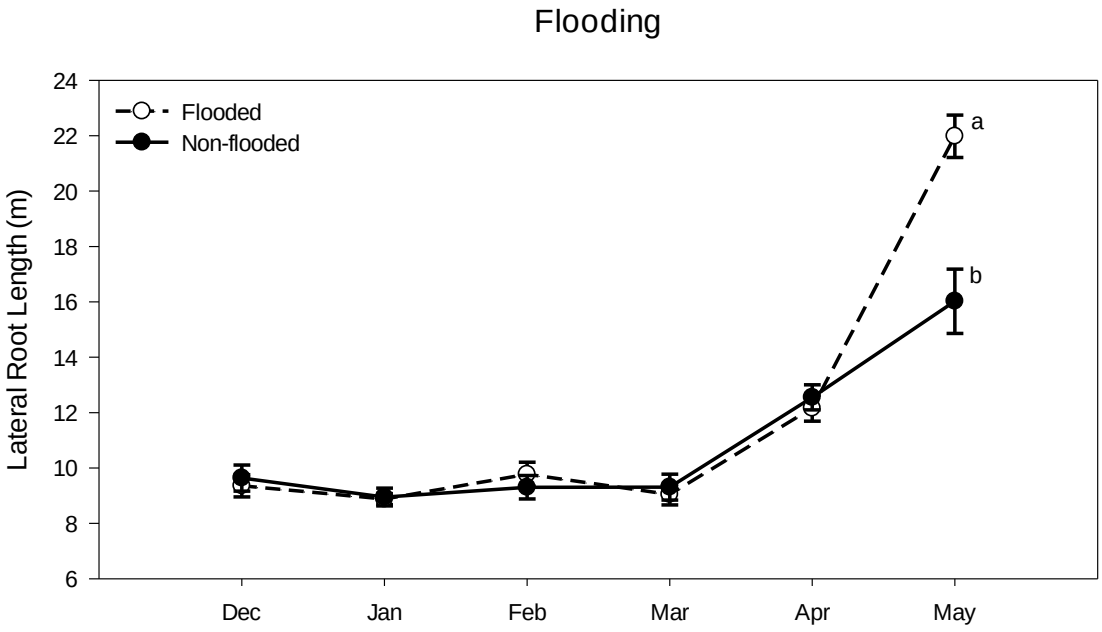

Figure 7: Mean length of lateral roots through time by flooding treatment. Letters indicate significant difference between flooding treatments levels. Error bars show mean ±1 SE.

Tap root length was not significantly affected by temperature, flooding, or their interaction. It was, however, affected by time ($p = 0.001$). Tap root lengths in May were greater than in all months except April ($p = 0.369$ for April, $p < 0.05$ other months). Numerically, differences were minor, with roots averaging 2.3 cm longer at the end of the experiment than at the beginning.

Fine root SRL was significantly affected by flooding treatments ($p = 0.014$) and time ($p = 0.001$). When averaged across time and temperature, seedlings in the flooded treatment level had a higher SRL than non-flooded seedlings ($p = 0.016$) (Figure 8A). Through time, fine root SRL was greatest in December. December SRLs were statistically greater than in the next four months ($p < 0.049$). In May, fine root SRL was nominally greater than in the previous four months and was not statistically different from SRLs observed in December ($p = 0.276$) (Figure 8B).

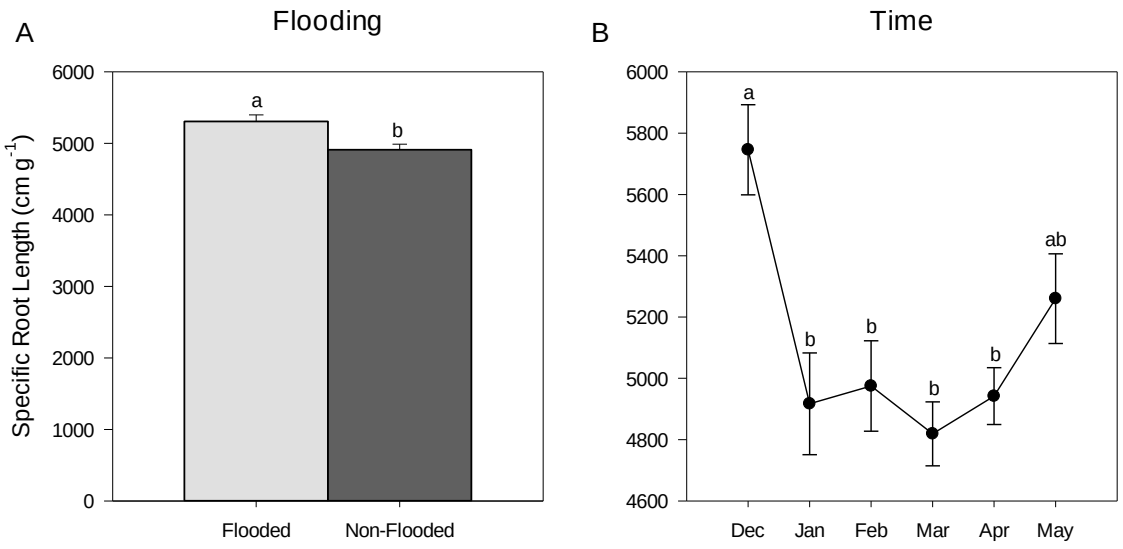

Figure 8: Panel A shows mean specific root length by flooding treatment and B shows mean specific root length through time. Letters indicate significant differences between flooding levels (A) and between sample periods (B). Error bars show mean ±1 SE.

New Roots and Tissue Proportions

There was a significant interaction between flooding and time on the number of new lateral root tips ($p = 0.029$). Time was a significant factor within the flooded level ($p = 0.025$) but not in the non-flooded level ($p = 0.553$). If seedlings experienced soil flooding, the number of new lateral roots was least in March and greatest in May ($p = 0.030$) but was not different between other months. Within time, the effect of flooding treatments was only significant in March ($p = 0.044$). In March, non-flooded seedlings were observed to have more new lateral root tips than seedlings that received flooding ($p = 0.044$) (Figure 9A).

New root mass was affected by the interaction of flooding and time ($p = 0.021$). Time influenced new root mass of seedlings in the flooded ($p = 0.042$) and non-flooded treatment levels ($p = 0.018$). Despite time being significant within the flooding treatment level, the Tukey adjusted pairwise comparisons showed no significant differences in new

lateral root mass between months. Among the non-flooded seedlings, new lateral root mass was greater in March than in December ($p = 0.012$) and January ($p = 0.037$). Within time, flooding affected new lateral root mass in March ($p = 0.008$), April ($p = 0.031$), and May ($p = 0.010$). In March, flooding led to seedlings with less new root mass than those that had not received flooding. But, sampling in April and May revealed that seedlings assigned soil flooding had greater new root mass than those assigned no flooding (Figure 9B).

Similar to mass, the length of new lateral roots was affected by the interaction of flooding and time ($p = 0.003$). Time was a significant factor within the flooded treatment ($p = 0.003$) but not in the non-flooded treatment ($p = 0.415$). Within the flooded treatment, new lateral root length in April was greater than in December ($p = 0.029$), February ($p = 0.016$), and March ($p = 0.016$), but not January ($p = 0.059$). New lateral root length of seedlings that received flooding was greater in May than in all other months except for April ($p < 0.05$, $p = 1.000$ for April). Within time, flooding impacted new lateral root length in March ($p = 0.008$), April ($p = 0.028$), and in May ($p = 0.010$). In March, new lateral root length was lowest for seedlings assigned soil flooding ($p = 0.008$). However, in April and May the opposite effect was observed – new lateral root length was greater for seedlings assigned soil flooding ($p = 0.028$ and 0.010, respectively) (Figure 9C).

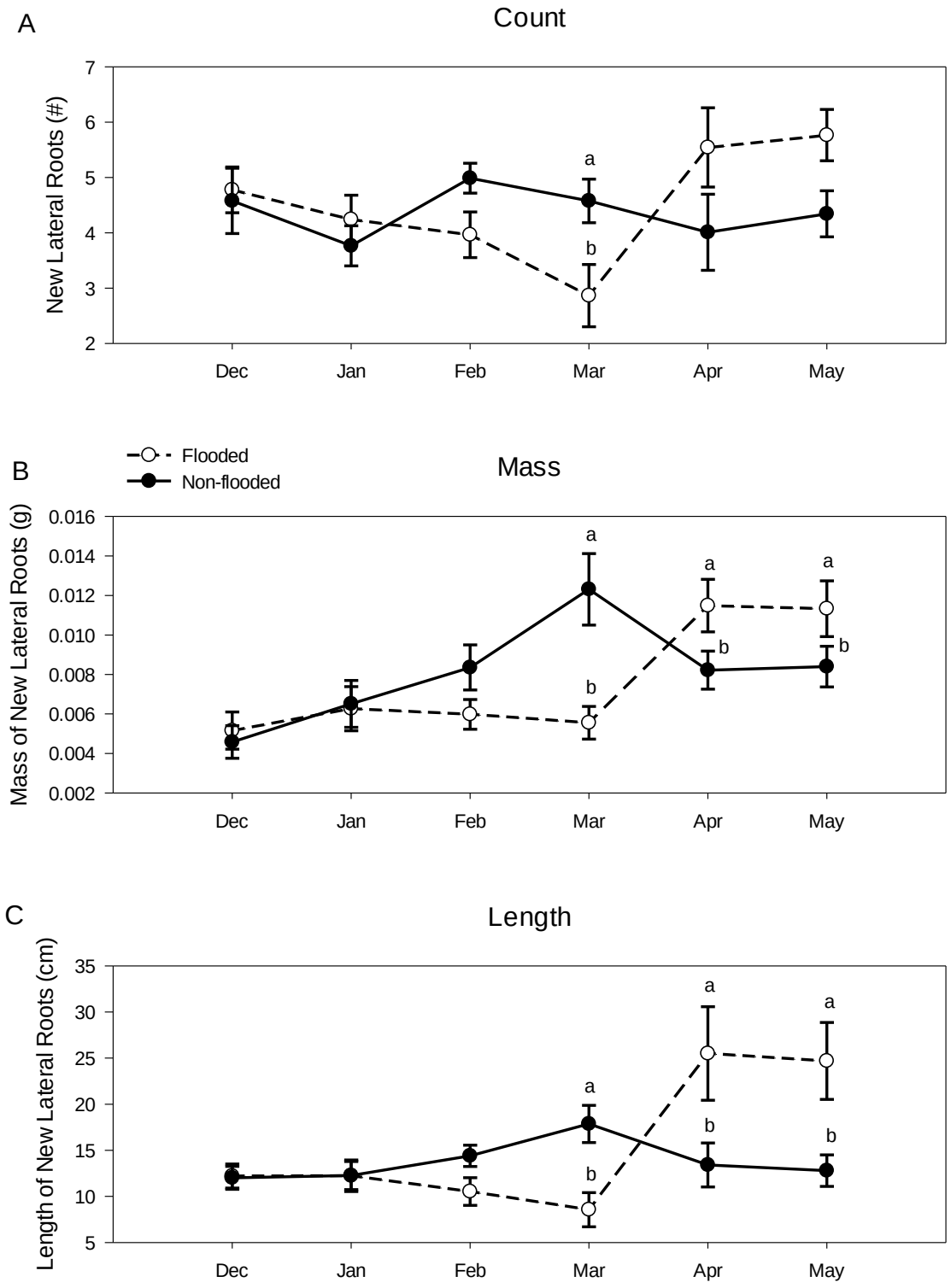

Figure 9: Panel A shows mean number of new lateral roots by flooding treatment through time. B shows mean mass of new lateral roots by flood treatment through time. C shows mean total length of lateral roots by flooding treatment through time. Letters indicate significant differences between flooding treatments. All error bars show mean ±1 SE.

Treatment effects were not detected for the proportion of lateral roots that had unsuberized tissues. Likewise, treatment effects were not apparent for proportions of suberized tissue or dead root tissue. These findings are consistent for proportional length and proportional mass in each tissue category.

Adventitious Roots

Only one sampled seedling produced an adventitious root. The seedling was assigned to the 15°C flooded treatment combination, and the root was observed during the March biomass harvest after soils had been flooded for 60-days. Statistical tests could not be conducted for the presence of adventitious roots.

Oxidation Reduction Potential and pH

Our measures of ORP were unreliable and ultimately discarded. Recorded values differed by as much 400 mV within a tank, and multiple observations taken in the same nursery pot were inconsistent. Measures of pH were more reliable. pH of flooded soil ranged from 4.74 to 6.31, with an average of 5.98. Numerically, average pH in 5°C soil was slightly lower than in other treatment levels, but no difference between temperature levels was detected ($p = 0.076$).

Aboveground

Leaf and Stem Mass

Neither temperature nor flooding treatment had a detectable effect on above ground biomass. Total aboveground biomass was affected by time ($p < 0.001$). Total

aboveground biomass was not significantly different between December and January ($p = 0.890$). Mass from February to April did not differ from each other but were lower than those observed in December, January, and May ($p < 0.05$). Biomass in May was greater than in all other months ($p < 0.001$) (Figure 10).

Aboveground biomass is comprised of stem and leaf tissues. Seedling stem mass was affected by time ($p < 0.001$). Unlike total aboveground biomass, stem mass increased steadily with time. Stem mass in February and March was significantly greater than in December ($p = 0.028$ and 0.002 respectively). In April, stem mass was greater than in December, January, and February ($p = 0.001$, 0.007, 0.012, respectively). Stem mass in May was greater than in all previous months except April ($p < 0.001$) (Figure 10).

Time also influenced leaf biomass ($p < 0.001$). Unlike stem mass, leaf mass did not have a single trend through time but instead followed the pattern of total aboveground biomass. Leaf mass declined from December to February, then increased between April and May. Leaf mass in December was greater than in January ($p = 0.010$), which was greater than in the next three months ($p < 0.001$). Leaf mass in May was greater than in all other months ($p < 0.001$) (Figure 10).

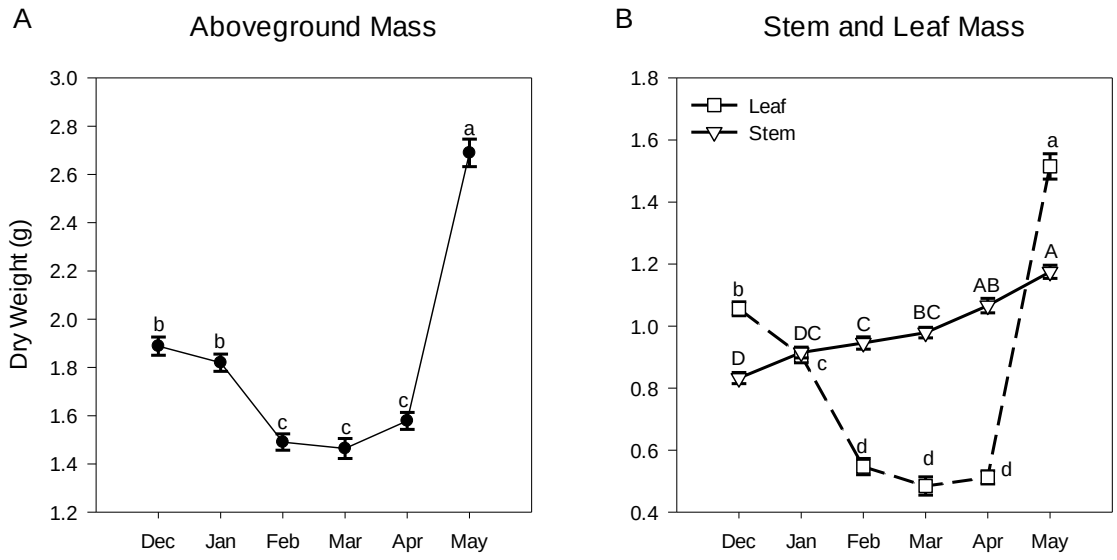

Figure 10: Panel A shows mean total aboveground mass through time. Panel B shows mean stem and leaf masses through time. Lower case letters indicate significant differences between aboveground mass (A) and leaf mass (B), and uppercase letters indicate significant difference between stem mass (B). Error bars show mean ±1 SE.

Stem Length and Cross-Sectional Area

Stem length was not affected by temperature or flooding treatment but was affected by time ($p < 0.001$). Seedling stem length remained unchanged from December to March, then increased in April and May. Stem length in April was greater than all previous months ($p < 0.001$), and in May was greater than in April ($p < 0.001$) (Figure 11).

Cross-sectional stem area was affected by the interaction of flooding and time ($p = 0.016$). Time was significant within both levels of flooding ($p > 0.001$), while flooding was significant within time only during May ($p = 0.005$). From December to March, stem area did not differ between months or between flood levels. Stem area in April was observed to be greater than in March in both the flooded ($p = 0.007$) and the non-flooded treatment levels ($p = 0.002$). Seedlings that received soil flooding had greater stem area in May than in April ($p = 0.001$), while seedlings assigned to the non-flooded level did not

increase in stem area during this time (p = 0.552). In May, seedlings from the flooded level had greater stem area than those in the non-flooded level (p = 0.005) (Figure 11).

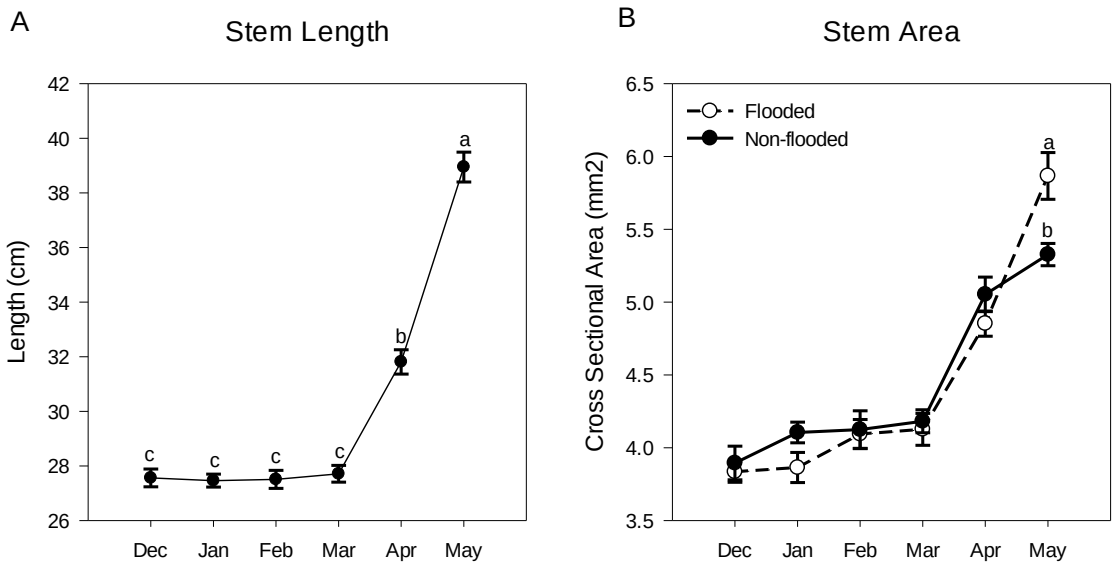

Figure 11: Panel A shows mean length of seedling stems through time. Panel B shows mean cross sectional stem area by flooding treatment through time. Letters indicate significant differences in stem length (A) and stem area (B). Error bars show mean ±1 SE.

Leaf Senescence and Bud Burst

Neither soil temperature nor flooding impacted leaf fall, as determined from phenology observations. We did detect a block effect within the greenhouse on the number of days it took seedlings to reach code 5 (p = 0.005). Seedlings from block 3 lost over half of their leaves between 3 and 4 days earlier than those from blocks 1 and 2 (p = 0.004 and 0.042, respectively).

More variation was observed in spring bud burst than in leaf senescence, and at each stage one or more factors were observed to have a significant effect. Block was a significant factor in the number of days taken to reach all spring phenology codes: 7 (p = 0.010), 8 (p = 0.003), 9 (p = 0.002), and 10 (p = 0.002). On average, seedlings in block 1

experienced bud burst earliest, while bud burst for seedlings in block 3 was slightly delayed (Figure 12A).

The interaction of temperature and flooding influenced the number of days to reach stage 7 ($p = 0.005$) and stage 10 ($p = 0.012$), but not stages 8 and 9 ($p = 0.346$ and 0.561, respectively). For stage 7, flooding was significant within the 15°C level ($p = 0.001$), and temperature was significant within the flooded level ($p = 0.001$). The 15°C flooded treatment combination reached stage 7 earlier than all other treatment combinations (Figure 12B). For stage 10, a similar trend was observed. Flooding was significant within the 15°C level ($p = 0.001$), and temperature was significant within the flooded level ($p < 0.001$). Within the flooded level, seedlings assigned the 10°C level reached stage 10 sooner than those in the 5°C level ($p = 0.038$), and seedlings in the 15°C level reached stage 10 sooner than those in the 10°C level ($p = 0.002$). In the 15°C level, seedlings in the flooded level reached stage 10 earlier than the non-flooded level ($p = 0.001$).

Where interactions were not significant, soil temperature had a significant effect on days to stages 8 ($p < 0.001$) and 9 ($p = 0.004$). Seedlings in the 10°C and 15°C levels reached stage 8 earlier than those in the 5°C level ($p = 0.009$ and < 0.001, respectively) (Figure 12C). For number of days to reach stage 9, seedlings in 5°C soil took longer to reach stage 9 than those in 15°C soil ($p = 0.003$) but not 10°C soil ($p = 0.113$). Flooding also significantly impacted number of days to reach stage 9 ($p = 0.041$). Seedlings in the flooded level reached stage 9 earlier than those in non-flooded level ($p = 0.041$) (Figure 12D).

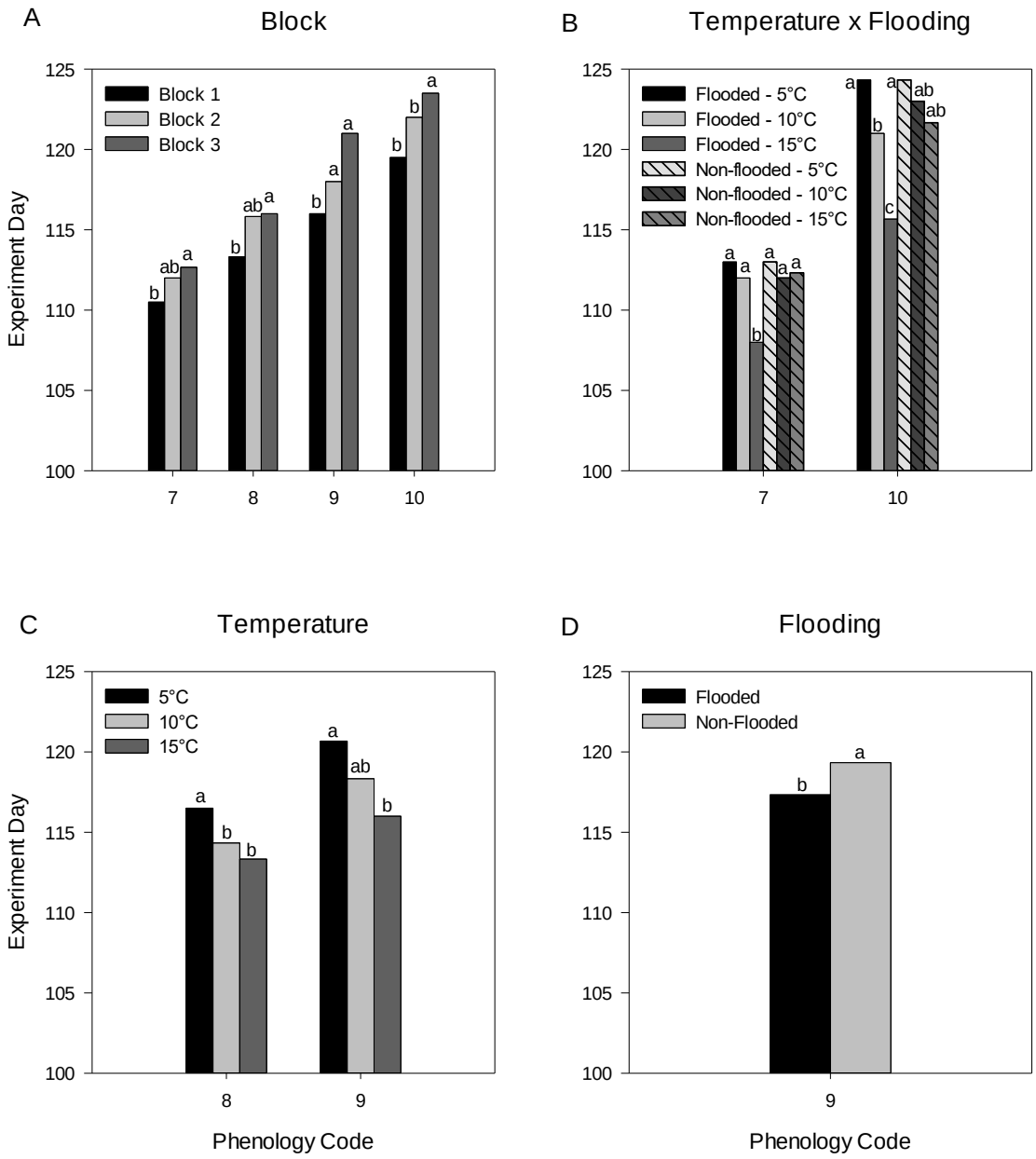

Figure 12: Average number of days to reach phenology codes by block (A), combinations of temperature and flood (B), temperature (C), and flood (D). Letters indicate significant differences between blocks (A), treatment combinations (B), soil temperature (C), and flooding (D) within a phenology code. Experiment Day 100 corresponds with March 13, 2021.

Chapter V: Discussion

Flooding Decreased Root Growth

Soil flooding during the dormant season reduced root growth in *Q. palustris* seedlings. In the months after flooding treatments were initiated, seedlings grown in flooded conditions had lower tap root mass than those in non-flooded conditions. This apparently arose from inhibited growth rather than from loss of existing root mass. Tap root mass increased between January and March in non-flooded seedlings, while flooded seedlings maintained an unchanged tap root mass. There was comparatively less growth in lateral roots than tap roots between January and March. Lateral root mass remained relatively constant in non-flooded seedlings and flooded seedlings during this time, and no difference in lateral root mass between flood levels was detected in these months. Flooded seedlings averaged higher specific fine root lengths than non-flooded seedlings, suggesting either less carbon investment per length of root or higher carbon costs to maintaining roots.

Differences in root mass between flooding treatments indicate that dormant season flooding may negatively impact functional processes in *Q. palustris*. Flooding has previously been shown to decrease photosynthesis and disrupt translocation of sugars in oak seedlings (Gardiner & Krauss, 2001; Gravatt & Kirby, 1998). Given no difference in tap root length, the effect on tap root mass was likely driven by carbohydrate storage. Decreased carbohydrate allocation to roots, whether due to production, translocation, or demand, would explain why root mass in the flooded soil was lower than in the non-flooded soil. We did not measure photosynthesis, leaf starch concentrations, or root starch concentrations, so we cannot determine the degree that these processes may have

been disrupted. As root mass in flooded soil was being maintained, tap roots were likely receiving some photosynthate from aboveground. Root metabolism and respiration may have decreased in response to flooding, complicating the relationship between root mass dynamics and root processes (Crawford, 2003).

Soil Temperature and Flooding

The relationship between soil temperature and root growth may be partially influenced by flooding. For seedlings that did not experience soil flooding, root biomass was positively associated with soil temperature, with an increase in root mass at 15°C. The observation of increased root growth with temperature is supported by previous studies for the range of temperatures we studied, but available results varied (Alvarez-Uria & Korner, 2007; Kuhns et al., 1985; Pregitzer et al., 2000). Alvarez-Uria and Korner (2007) reported a greater difference between 6° and 9°C than we observed between 5° and 10°C, but this greater response may have been driven by species, as their study examined several alpine species. Our results more closely followed those observed by Kuhns et al. (1985), who observed a rapid increase in root growth between 13° and 15°C in *Juglans nigra* (black walnut).

In contrast, seedlings that experienced two months of soil flooding did not clearly display a positive relationship between soil temperature and root growth. Though we did detect a difference in root mass between the flooding levels at 15°C, we do not know the cause of this difference. It may indicate that in flooded soils seedlings cannot fully exploit the benefits of warmer soil environments. It is also possible the stress of flooding is exacerbated at higher soil temperatures. Assuming root respiration increases with soil

temperature, available oxygen may be consumed faster, and this could accelerate flooding stress. Regardless of the mechanism, soil flooding appears to have a more negative effect on *Q. palustris* root growth when soil temperature is relatively high.

Aboveground Growth

Aboveground responses of *Q. palustris* to soil flooding in winter were minimal. We did not detect treatment effects on leaf mass, stem mass, or stem length. Though previous flooding research reported stem dieback of *Q. palustris* seedlings that had been inundated with standing water (Kabrick et al., 2012), soil flooding in our study did not produce this effect. Stem area in May was greatest for seedlings that experienced winter flooding, but the magnitude of this increase was small. Flooding also affected how quickly seedlings leafed out in the spring, but again the magnitude was small and unlikely to have an impact. Earlier leaf-out in spring may increase vulnerability to spring frosts, but the few days of difference observed between flooding levels makes this an unlikely occurrence.

While we observed flooding to initiate few aboveground differences, past research suggests other effects that could have occurred. Foliar nitrogen concentration in leaves may have differed between treatment levels even if leaf mass remained similar (Gardiner & Krauss, 2001). Leaf starch concentration may have differed as well if flooding disrupted photosynthate translocation of photosynthetically active seedlings. Changes in leaf starch concentrations have been observed when seedlings were subjected to flooding, but responses varied by species (Gravatt & Kirby, 1998).

Post-drainage Responses

The effects of flooding on root growth appear to be temporary. Initial observations indicated that root mass in seedlings exposed to soil flooding was lower than in seedlings raised free of flooding. This effect persisted into April after flooded soils were drained. By the end of the study in May, total root mass did not differ between the treatment levels. This was due largely to decreases in total root mass in non-flooded seedlings rather than increased growth in the previously flooded seedlings. Tap root mass decreased under both treatment levels between April and May, with a larger decreased observed in seedlings assigned no soil flooding. The decrease in tap root mass could be associated with the utilization of stored carbohydrates for above ground growth (Crawford, 1978). Despite the smaller decrease in root mass in previously flooded seedlings versus non-flooded seedlings, aboveground growth was not affected. With no lasting difference in belowground or aboveground biomass, the effects of winter flooding on *Q. palustris* seedlings did not seem to persist into the growing season.

The response of *Q. palustris* to winter flooding aligns with the current understanding of flooding and seedling flood tolerance. Findings support the consensus that dormant season floods are less stressful to woody plants than growing season floods, because there were no lasting negative effects (Kozlowski, 1997). *Q. palustris* is often considered moderately flood tolerant, though flood tolerance can be difficult to quantify (Kabrick et al., 2012). This classification is supported by the recovery of seedlings that received soil flooding to a similar condition as those that received no flooding. We did observe negative effects while soil was flooded, and this was not unexpected because seedlings are more susceptible to flood stress than older trees (Kennedy & Krinard,

1974). We might expect mature trees would fare just as well or better, but this does not account for the possibility of cumulative stress from repeated flooding events. Acclimation from exposure to flooding may increase tolerance to future flood events, but support for this idea is somewhat limited (Coutts & Philipson, 1978).

Possible Benefits of Flooding

The response of some variables following drainage suggest dormant season flooding may be somewhat beneficial for *Q. palustris*. For seedlings that received winter soil flooding, lateral root growth increased after floodwater was drained. Root growth is expected to occur as seedlings enter the growing season, but seedlings assigned the flooded treatment level had greater new root mass than seedlings of the non-flooded level. Though our methods for quantifying new root growth were limited, this trend is reflected in total lateral root mass and length. At the end of the experiment, stem area was also higher in flood treated seedlings than in non-flooded seedlings, though the magnitude of this observation is small and may not be meaningful. These observations of improved post-flood growth in May suggest flooding may have benefited the seedlings as proposed by Broadfoot (1967), who attributed increased growth following dormant season flooding to high soil moisture availability.

Growth patterns during the dormant and growing seasons can vary, so extrapolation from our measurements should be made with caution. Both previously flooded and non-flooded soil was kept well-watered in the spring, so increased soil moisture may not explain differences in root growth. The growth responses may be temporary, either compensating for decreased root growth during the dormant season or

as a response to no longer being in a waterlogged environment. More research is needed to determine if effects of dormant season flooding may impose long-term impact to seedlings.

Chapter VI: Conclusion

This experiment was conducted to determine how dormant season soil flooding and soil temperature affect *Q. palustris* seedlings and to identify if there is an interaction between these factors. Repeated destructive samples of seedlings were used to detect how roots grew during the winter and how root growth may have been affected by the treatments. We found that soil flooding and soil temperature affected seedling root mass over the duration of the experiment. Flooding reduced root mass while temperature increased root mass. We also detected an interaction between soil flooding and temperature in which the effect of soil flooding was more severe at high soil temperatures.

This study also allowed for observation of general trends in winter root growth. Root mass increased early in the winter, with a large increase between December and January, before slowing substantially until spring. The tap root portion of the root system primarily drove this trend. In the spring, however, different trends emerged between tap and lateral roots. Tap root mass decreased during the spring growth flush, while lateral root mass increased.

Our data show that soil flooding during the dormant season affected root system growth of *Q. palustris*. Root growth between sample periods was minor during flooding, although roots continued to grow rather than lose mass. Flooding did limit root growth during the winter, but this effect did not persist into the growing season. A positive effect of soil flooding was seen in the early growing season after floodwater was drained. Lateral root mass increased rapidly during the spring growth flush in previously flooded seedlings, resulting in greater lateral root mass compared to non-flooded seedlings.

Soil temperature affected root growth, but the effect interacted with soil flooding. Generally, soil temperature was positively associated with root growth, but differences between temperature levels were only occasionally observed. Total root mass and tap root mass reflected this trend, but temperature did not affect lateral root mass. In soil that was not flooded, root mass was greatest at 15°C. However, seedlings did not exhibit this increase in root mass when 15°C soil was flooded, instead mass remained similar to that observed at colder temperatures.

Soil flooding during the winter had a negligible impact on seedling aboveground biomass. Negative effects were not detected during flooding or following drainage. Stem cross-sectional area of flooded seedlings was slightly greater in the spring, but differences were small. We also found small differences in the timing of budburst and leaf-out in the spring, but these differences were small being on the order of a few days. It is not unexpected that there was little effect on aboveground tissues of seedlings, as our flooding treatment was limited to soil flooding rather than inundation.

Management Implications

Artificial flooding during the dormant season in GTRs is likely to affect oaks to some degree. Seedlings exhibited decreased root growth under all temperature levels while soil was flooded. However, seedlings appeared to fully recover following drainage. Our findings suggest that dormant season flooding, if applied when the majority of root growth has stopped and if drained adequately before spring, does not significantly reduce the growth or survival of young *Q. palustris*. We observed a large increase in root growth between December and January that occurred before we initiated the flooding levels. If

flooding is conducted during this early winter period of active root growth, it could lead to more substantial negative effects than what we observed, because flooding is expected to be more stressful when roots are actively growing.

Flooding when soils are warm may exacerbate the negative effects on oak seedlings. Negative flooding effects on root growth were more pronounced at higher soil temperatures. This indicates that flooding when soils are warm, such as late fall or early winter, may be more harmful to seedlings. Where possible, delaying flooding further into the winter when soil temperatures have cooled is recommended. Although this study only examined dormant season flooding, it could be speculated that flood water retained into the spring when soils begin to warm would become more harmful to seedlings. If this is true, ensuring flood water is adequately drained before soils warm may be just as important as waiting to initiate flooding. Though the resolution is coarse, this study suggests flooding at soil temperatures of 10°C or below appears to have had only a small impact on seedlings.

Despite the observed negative effects caused by soil flooding during the dormant season, *Q. palustris* seedlings appeared to fully recover following drainage. Following drainage and the first flush of spring growth, root mass for seedlings that received flooding was similar to mass of those that did not receive flooding. This trend was observed at higher soil temperatures where flooding effects were more pronounced. Additionally, there were no negative affects observed on the aboveground tissues of seedlings. While it remains possible that there were treatment effects that could not be detected, dormant season flooding does not seem to have a lasting effect on *Q. palustris* seedlings.

There is still much we do not know about the effects of water management on bottomland oaks. Though seedlings appeared to recover after flooding, it is possible that repeated flooding may reduce vigor or prolong recovery across multiple years. It is also important to note that this study utilized soil flooding rather than partial or complete inundation as may be expected in a managed GTR. Inundation could be expected to have more significant effects on seedlings, though to what degree is beyond the scope of this study. Care should be taken when associating soil temperature with seasonality. Though we utilized three different soil temperatures that could be expected to occur naturally at different points in the year, all flooding treatments were applied at the same time. Other environmental cues and drivers besides soil temperature, such as photoperiod and air temperature, may influence seedling growth patterns and stress responses that were not capture in this study. Finally, potting soil and controlled greenhouse conditions may not accurately represent field soil conditions. Temperature gradients associated with soil depth may produce more variability in root responses than the uniform temperature in our nursery pots.

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Appendix: Statistical Output

*Appendix 1: ANOVA tables for linear mixed effects models. Degrees of freedom (Den DF) determined using Satterthwaite's method. P values less than 0.05 noted by *.*

Response	*Factor*	*DF*	*Den DF*	*F value*	*P value*
Total root mass	Time	5	43.13	59.137	<0.0001*
	Flood	1	13.62	24.361	0.0002*
	Temp	2	13.62	9.5721	0.0025*
	Time×Flood	5	43.13	4.0784	0.0040*
	Time×Temp	10	43.13	2.4519	0.0203*
	Flood×Temp	2	13.62	3.7653	0.0499*
	Time×Flood×Temp	10	43.13	0.8922	0.5479
Tap root mass	Time	5	36.830	66.356	<0.0001*
	Flood	1	14.209	30.086	<0.0001*
	Temp	2	14.209	9.2318	0.0027*
	Time×Flood	5	36.830	3.3783	0.0130*
	Time×Temp	10	36.830	2.0863	0.0515
	Flood×Temp	2	14.209	4.0569	0.0404*
	Time×Flood×Temp	10	36.830	1.1152	0.3773
Lateral root mass	Time	5	46.249	44.645	<0.0001*
	Flood	1	14.299	0.2111	0.6528
	Temp	2	14.299	3.7554	0.0489*
	Time×Flood	5	46.249	4.7757	0.0013*
	Time×Temp	10	46.249	1.6291	0.1281
	Flood×Temp	2	14.299	0.7943	0.4709
	Time×Flood×Temp	10	46.249	0.5549	0.8414
Lateral root length	Time	5	21.142	42.702	<0.0001*
	Flood	1	9.1307	6.4671	0.0312*
	Temp	2	9.1307	2.4584	0.1399
	Time×Flood	5	21.142	4.3620	0.0070*
	Time×Temp	10	21.142	1.0368	0.4479
	Flood×Temp	2	9.1307	0.5900	0.5742
	Time×Flood×Temp	10	21.142	0.6052	0.7923
Tap root length	Time	5	19.731	6.7846	0.0008*
	Flood	1	13.822	0.0064	0.9376
	Temp	2	13.822	0.5077	0.6127
	Time×Flood	5	19.731	1.2922	0.3070
	Time×Temp	10	19.731	0.9961	0.4787
	Flood×Temp	2	13.822	0.1580	0.8553
	Time×Flood×Temp	10	19.731	0.8508	0.5894

Response	*Factor*	*DF*	*Den DF*	*F value*	*P value*
Fine specific root length	Time	5	16.451	8.0321	0.0005*
	Flood	1	12.262	8.2544	0.0137*
	Temp	2	12.262	0.0163	0.9839
	Time×Flood	5	16.541	0.7065	0.6267
	Time×Temp	10	16.541	1.4953	0.2257
	Flood×Temp	2	12.262	0.0078	0.9922
	Time×Flood×Temp	10	16.541	0.3185	0.9646
Number of new lateral roots	Time	5	25.277	1.7132	0.1679
	Flood	1	19.073	0.2312	0.6361
	Temp	2	19.073	1.0677	0.6345
	Time×Flood	5	25.277	3.0052	0.0291*
	Time×Temp	10	25.277	0.6364	0.7692
	Flood×Temp	2	19.073	1.8934	0.1778
	Time×Flood×Temp	10	25.277	0.5880	0.8085
New lateral root mass	Time	5	26.454	5.3799	0.0015*
	Flood	1	12.845	2.2395	0.1464
	Temp	2	12.845	0.3122	0.5860
	Time×Flood	5	26.545	1.2538	0.3045
	Time×Temp	10	26.545	3.2158	0.0212*
	Flood×Temp	2	12.845	0.9022	0.4299
	Time×Flood×Temp	10	26.545	1.5514	0.1764
New lateral root length	Time	5	26.784	3.1900	0.0218*
	Flood	1	15.170	1.6233	0.2297
	Temp	2	15.170	1.7628	0.2039
	Time×Flood	5	26.785	1.3376	0.2615
	Time×Temp	10	26.785	4.7722	0.0030*
	Flood×Temp	2	15.170	0.6687	0.5269
	Time×Flood×Temp	10	26.785	1.5450	0.1780
pH	Temp	2	9.7655	3.4726	0.0726
Aboveground biomass	Time	5	26.541	79.456	<0.0001*
	Flood	1	82.244	0.8379	0.3627
	Temp	2	82.244	0.6278	0.5363
	Time×Flood	5	26.541	0.7947	0.5631
	Time×Temp	10	26.541	0.5314	0.8524
	Flood×Temp	2	82.244	2.2891	0.1078
	Time×Flood×Temp	10	26.541	0.9939	0.4728

Response	*Factor*	*DF*	*Den DF*	*F value*	*P value*
Stem mass	Time	5	29.862	34.356	<0.0001*
	Flood	1	43.027	0.0012	0.9729
	Temp	2	43.027	0.0292	0.9712
	Time×Flood	5	29.862	1.2023	0.3320
	Time×Temp	10	29.862	0.6611	0.7502
	Flood×Temp	2	43.027	1.0087	0.3732
	Time×Flood×Temp	10	29.862	0.5681	0.8263
Leaf mass	Time	5	28.267	153.76	<0.0001*
	Flood	1	32.042	1.9650	0.1706
	Temp	2	32.042	1.2853	0.2904
	Time×Flood	5	28.267	0.3627	0.8697
	Time×Temp	10	28.267	0.8923	0.5518
	Flood×Temp	2	32.042	2.2598	0.1208
	Time×Flood×Temp	10	28.267	1.0741	0.4131
Stem length	Time	5	18.803	98.256	<0.0001*
	Flood	1	11.424	0.8555	0.3741
	Temp	2	11.424	1.0145	0.3931
	Time×Flood	5	18.803	0.6962	0.6329
	Time×Temp	10	18.803	0.7381	0.6818
	Flood×Temp	2	11.424	1.1819	0.3416
	Time×Flood×Temp	10	18.803	0.5670	0.8203
Stem cross-sectional area	Time	5	29.717	78.576	<0.0001*
	Flood	1	41.536	0.0145	0.9046
	Temp	2	41.536	1.8927	0.1634
	Time×Flood	5	29.717	3.3587	0.0160*
	Time×Temp	10	29.717	1.1700	0.3485
	Flood×Temp	2	41.536	0.3082	0.7365
	Time×Flood×Temp	10	29.717	1.4135	0.2224

Appendix 2: Chi-Square tests for generalized linear mixed effects models.

Response	*Factor*	*DF*	X^2	*P value*
Proportion of suberized root mass	Time	5	0.3267	0.9971
	Flood	1	0.0010	0.9754
	Temp	2	0.1959	0.9067
	Time×Flood	5	0.3488	0.9966
	Time×Temp	10	1.1493	0.9997
	Flood×Temp	2	0.2075	0.9014
	Time×Flood×Temp	10	0.8682	0.9999
Proportion of new root mass	Time	5	0.1511	0.9996
	Flood	1	0.0001	0.9936
	Temp	2	0.0191	0.9905
	Time×Flood	5	0.1426	0.9996
	Time×Temp	10	0.4363	1.0000
	Flood×Temp	2	0.0518	0.9744
	Time×Flood×Temp	10	0.3429	1.0000
Proportion of dead root mass	Time	5	0.1403	0.9996
	Flood	1	0.0114	0.9152
	Temp	2	0.0516	0.9746
	Time×Flood	5	0.0296	1.0000
	Time×Temp	10	0.1359	1.0000
	Flood×Temp	2	0.0015	0.9993
	Time×Flood×Temp	10	0.0671	1.0000
Proportion of suberized root length	Time	5	0.1246	0.9997
	Flood	1	0.0014	0.9702
	Temp	2	0.0349	0.9827
	Time×Flood	5	0.1093	0.9998
	Time×Temp	10	0.4398	1.0000
	Flood×Temp	2	0.0370	0.9816
	Time×Flood×Temp	10	0.3194	1.0000
Proportion of new root length	Time	5	0.1511	0.9996
	Flood	1	0.0001	0.9936
	Temp	2	0.0191	0.9905
	Time×Flood	5	0.1426	0.9996
	Time×Temp	10	0.4363	1.0000
	Flood×Temp	2	0.0518	0.9744
	Time×Flood×Temp	10	0.3429	1.0000

Response	*Factor*	*DF*	X^2	*P value*
Proportion of dead root length	Time	5	0.2836	0.9979
	Flood	1	0.0023	0.9617
	Temp	2	0.0156	0.9922
	Time×Flood	5	0.0035	1.0000
	Time×Temp	10	0.0606	1.0000
	Flood×Temp	2	0.0077	0.9962
	Time×Flood×Temp	10	0.0153	1.0000

*Appendix 3: ANOVA tables for linear models. P values less than 0.05 noted by *.*

Response	*Factor*	*DF*	*Den DF*	*F value*	*P value*
Days to reach phenology code 2	Block	2	10	0.4930	0.6249
	Temp	2	10	1.3380	0.3055
	Flood	1	10	0.2817	0.6072
	Temp×Flood	2	10	0.4930	0.6249
Days to reach phenology code 3	Block	2	10	1	0.4019
	Temp	2	10	1	0.4019
	Flood	1	10	1	0.3409
	Temp×Flood	2	10	1	0.4019
Days to reach phenology code 4	Block	2	10	2.500	0.1317
	Temp	2	10	0.625	0.5549
	Flood	1	10	2.500	0.1449
	Temp×Flood	2	10	0.625	0.5549
Days to reach phenology code 5	Block	2	10	9.4915	0.0049*
	Temp	2	10	3.1356	0.0877
	Flood	1	10	0.5297	0.4834
	Temp×Flood	2	10	0.5932	0.5709
Days to reach phenology code 7	Block	2	10	7.4719	0.0104*
	Temp	2	10	12.528	0.0019*
	Flood	1	10	9.4944	0.0116*
	Temp×Flood	2	10	9.4944	0.0049*
Days to reach phenology code 8	Block	2	10	10.843	0.0031*
	Temp	2	10	15.899	0.0008*
	Flood	1	10	4.5506	0.0587
	Temp×Flood	2	10	1.1798	0.3467
Days to reach phenology code 9	Block	2	10	11.633	0.0025*
	Temp	2	10	10.000	0.0041*
	Flood	1	10	5.5120	0.0408*
	Temp×Flood	2	10	0.6122	0.5613
Days to reach phenology code 10	Block	2	10	12.458	0.0019*
	Temp	2	10	24.746	0.0001*
	Flood	1	10	16.271	0.0024*
	Temp×Flood	2	10	7.1186	0.0120*