THE EFFECTS OF RHIZOSPHERE INUNDATION ON THE GROWTH AND PHYSIOLOGY OF RED MAPLE (*Acer rubrum* L.) SEEDLINGS DERIVED FROM WET AND DRY SITES

by

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(ABSTRACT)

Red maple seedlings grown from fruits collected from matched wet and dry sites from three physiographic regions of Virginia were flooded to test whether red maple seedlings derived from wet sites are affected differently by flooding than are seedlings derived from dry sites.

Thirteen weeks of soil inundation on seedling growth found no interactions between flooding and maternal hydrologic condition. However, flooding significantly decreased leaf, stem, and root dry matter accumulation as well as height growth, leaf area growth, root to shoot ratio, mean relative growth rate, net assimilation rate, and mean leaf area ratio.

Thirteen days of rhizosphere inundation as well as six days of recovery on seedling gas exchange determined that flooding significantly decreased photosynthetic rate and leaf conductance. A larger decrease in photosynthetic rate than in leaf conductance resulted in decreased water use efficiency and leaf limitation. There were no interactions between flooding and maternal hydrologic condition.

Fourteen days of flooding decreased root aerobic respiratory capacity and root ethylene evolution, and caused shoot water potential to be less negative. As in the previous studies, no interaction between flooding and maternal hydrologic condition existed.

Although rhizosphere inundation negatively affected the growth and physiology of red maple seedlings, there does not appear to exist any genetic differentiation between wet site and dry site populations affording either of the populations enhanced flood tolerance. Rather, red maple appear to have the species wide phenotypic plasticity to survive flooded conditions.

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INTRODUCTION AND JUSTIFICATION

In any habitat, water availability is one of the main factors that determines a plant's ability to survive and reproduce. Although much has been published concerning the effects of drought on plants, comparatively little emphasis has been placed on a plant's response to flooding (Kozlowski 1984). At present, most work dealing with woody plants and flooding examines either an individual species response to soil waterlogging or the relative flood tolerance of several different species. Few studies that address possible intraspecific differences in response to rhizosphere inundation.

Red maple (*Acer rubrum* L.) has the largest distribution of any eastern hardwood and thrives under the widest spectrum of site characteristics (Walters and Yawney 1990). Much of the phenotypic variation displayed by red maple has been shown to be under genetic control. Previous studies have shown significant differences between red maple seed sources grown under identical conditions for drought tolerance (Townsend and Roberts 1973), ozone tolerance (Townsend and Dochinger 1974), verticillium wilt tolerance (Townsend and Hock 1973), height, diameter, autumn color, winter injury, date of flushing and growth cessation (Townsend 1977), winter chilling requirement (Perry and Wu 1960), temperature optima (Perry 1962) and beaded rootlet formation (Medve 1970). Since red maple flourishes on sites ranging from swamps to dry mountain ridges (Walters and Yawney 1990), this species provides a unique opportunity to study the extent to which genetic differentiation contributes to the survival of red maple in very wet locations.

At present, little research links intraspecific variation to differences in flood tolerance among populations. By comparing flooded red maple seedlings derived from wet site and dry site maternal parents, not only can the effect of flooding upon red maple be determined, but also, the differences in flood response between wet and dry site populations can be studied. Studying the results of waterlogged wet site and dry site derived seedlings will determine

whether ecotypic differentiation or species-wide phenotypic plasticity enables red maple to survive where flooding occurs for extended periods during the growing season.

OBJECTIVES

The specific objectives of this project were:

(1) to determine the effect of flooding on growth and physiology of red maple seedlings grown from fruits obtained from three physiographic regions in Virginia,

(2) to determine the differences in growth and physiology between red maple seedlings derived from wet and dry site maternal parents,

(3) to determine the extent to which changes in growth and physiology in response to rhizosphere inundation depend on the native water regime of the maternal parent,

(4) to calculate the genetic similarity among red maple half-sib families.

The objectives were met by:

(1) measuring the effect of four, nine, and thirteen weeks of continuous soil inundation on the growth of wet and dry site derived red maple seedlings,

(2) measuring the effect that one, three, five, nine, and thirteen days of soil inundation as well as two, four, and six days of recovery has on the gas exchange of wet and dry site red maple seedlings,

(3) measuring the effect that one, three, five, and twelve days of soil inundation as well as one and seven days of recovery has on shoot water potential, root ethylene production, and root aerobic respiratory capacity of wet and dry site derived red maple seedlings.

LITERATURE REVIEW

Flooding and woody plants

Soil inundation is deleterious to plant growth because it causes rhizosphere anoxia. Upon flooding, air filled soil pores are displaced with water, and dissolved oxygen is rapidly consumed by microbial organisms (Kozlowski et al. 1991a). Oxygen is not replenished because the molecular diffusion rate of oxygen is 10^4 times slower in water than in air (Ponnamperuma 1984). Flood induced soil anaerobiosis harms plants indirectly by altering soil chemistry and directly by curtailing root aerobic processes.

Soil chemistry changes that occur after several days of inundation include reduced soil redox potential, altered soil pH, and the accumulation of phytotoxic compounds that are produced by anaerobic organisms. Redox potential indicates the oxidation status of a soil. As oxygen is depleted, anaerobic bacteria reduce inorganic soil components such as Fe, Mn and S. When the effect of decreased redox potential was isolated from the other effects of rhizosphere inundation, root growth was hindered even for the very flood tolerant *Taxodium distichum* L. (Pezeshki 1991). Soil pH also is altered by waterlogging. Flooding increases the pH of acidic soils due to the reduction of Fe^{3+} to Fe^{2+} and decreases the pH of alkaline soils due to the accumulation of carbon dioxide and its subsequent conversion to carbonic acid (Kozlowski et al. 1991a).

Cessation of root aerobic processes affects plant growth and physiology in various complex ways involving water, mineral, carbohydrate, and growth regulator relations (Kozlowski et al. 1991a). The extent that flooding is detrimental to tree growth depends on species, time of flooding, and duration of flooding. Evidence suggests rhizosphere anaerobiosis is more harmful to plants than are soil chemistry changes (Sanderson and Armstrong 1980).

In general, young trees are more susceptible to flooding than older trees (Kennedy and Krinard 1973, Broadfoot and Williston 1973, Newsome et al. 1982, Kozlowski 1984, Peterson and Bazzaz 1984, and Yamamoto and Kozlowski 1987a). Furthermore, stagnant, warm water has more of an adverse effect than does flowing, cool water (Kozlowski 1984, Harms 1973) because moving, cooler water contains higher O_2 and lower CO_2 concentrations (Harms 1973).

Effect of flooding on woody plant growth

Anaerobic soil conditions resulting from flooding affect shoot growth by "inhibiting internode elongation and formation and expansion of leaves, and by inducing chlorosis, premature senescence, and abscission" (Kozlowski et al. 1991a). Smit et al. (1989) found retarded leaf growth of *Populus trichocarpa x deltoides* was due to a reduction in cell division and decreased cell wall extensibility. Cambial growth has been shown to either increase or decrease upon soil inundation (Kozlowski et al. 1991a). However, any accelerated cambial expansion which occurs is temporary (Broadfoot and Williston 1973). Diameter growth is not indicative of cambial expansion because often the diameter increase due to flooding can be attributed to additional phloem production as well as to the formation of intercellular spaces (Yamamoto and Kozlowski 1987a)

Flooding reduces root system growth and facilitates decay of the preexisting root system through increased activity of *Phytophthora* spp. fungi. *Phytophthora* spp. tolerate low oxygen levels and take advantage of decreased root system vigor (Kozlowski et al. 1991a). However, many flood tolerant species produce adventitious roots when subjected to flooding. Although the importance of adventitious roots has not been clarified, they are generally thicker, containing larger cells and more intercellular space (Kozlowski et al. 1991a). Work done by Sena Gomes and Kozlowski (1980) found that adventitious root formation coincided

with increased water absorption and stomatal reopening in *Fraxinus pennsylvanica* Marsh. However, other researchers, including Tripepi and Mitchell (1984), found no apparent loss of flood tolerance when adventitious roots were removed or inhibited. Adventitious roots are thought to be induced by increasing concentrations of apically produced auxin (Yamamoto and Kozlowski 1987b) and by the reduction of root produced cytokinins and gibberillins (Reid and Bradford 1984).

Besides the formation of adventitious roots, flooding can induce other morphological changes in woody plants. Development of intercellular spaces and hypertrophied lenticels are two morphological changes that are thought to aid the aeration of submerged plant portions (Kozlowski 1984). Intercellular spaces develop due to cellulase activity in the cortex and occasionally in the xylem of woody plants (Kozlowski 1984). Hypertrophied lenticels form on the stem below and just above the water level. Lenticel hypertrophy results from increased production of phellem tissue that contains more intercellular space (Larson et al. 1991).

Effects of flooding on the physiology of woody plants

Flooding causes many changes in woody plant metabolism and physiology. Probably the most immediate plant response to soil inundation is stomatal closure (Kozlowski and Pallardy 1984). Closure results from rising levels of abscisic acid in the leaves causing an exodus of K^+ ions from the guard cells and therefore, a subsequent loss of turgor (Jackson and Hall 1987). Stomatal closure due to flooding has been found in association with both the presence and absence of leaf dehydration (Kozlowski and Pallardy 1984). When leaf dehydration does occur, it is because water absorption is decreased due to increasing root resistance which results from low oxygen and high carbon dioxide levels in the flooded soil water (Harrington 1987, Anderson et al. 1984, and Smit and Stachowiak 1988). However, most work with woody plants has shown that stomata close although no water deficiency develops. In experiments

involving a wide variety of woody species, water potentials of the flooded trees were the same as or less negative than unflooded controls (Smit et al. 1989, Smith and Agar 1988, Pezeshki and Chambers 1986, Tang and Kozlowski 1984a, Tang and Kozlowski 1982, and Pereira and Kozlowski 1977).

Another physiological effect of flooding is a rapid reduction in photosynthetic rate. This reduction is a result of decreased CO_2 uptake due to increasing stomatal resistance as well as a subsequent decrease in photosynthetic capacity (Kozlowski et al. 1991a). Within three days, soil inundation decreased both photosynthesis and transpiration of *Acer saccharinum* L. (Peterson and Bazzaz 1984).

Waterlogging causes a decrease in root aerobic respiratory capacity. This decreased capacity is presumably due to the deterioration of mitochondrial membranes caused by the prolonged absence of oxygen (Carpenter and Mitchell 1980). When drained, the ability of roots to resume aerobic processes is essential to a plant's recovery. Therefore, it is thought that "root respiration capacity of flooded plants can be used as an indicator of flood tolerance, root viability, and integrity of the respiratory apparatus" (Tripepi and Mitchell 1984).

Flooding influences the nutrient status of a plant by altering the plant's ability to acquire minerals, as well as by changing the nutrient's bioavailability. Uptake of N, P, K, and to a lesser extent Ca and Mg is inhibited by anaerobic soil (Kozlowski and Pallardy 1984). Additionally, when the soil becomes anaerobic, Fe and Mn change to their reduced, bioavailable forms. This increase in available Fe and Mn ions, as well as increases in other compounds such as sulfides and CO_2, can have acute phytotoxic effects (Kozlowski and Pallardy 1984). Furthermore, in the absence of O_2, roots undergo glycolysis, which results in phytotoxic end products including ethanol, acetaldehyde, and cyanogenic compounds (Kozlowski 1985).

Growth regulators and flooding

Flooding alters growth regulator balance. Cytokinins and gibberellins are normally produced by oxygenated roots. Therefore, flood induced rhizosphere anaerobiosis leads to decreased cytokinin and gibberellin production and transport. Decreasing cytokinin concentrations attributable to flooding are thought to hasten senescence. Decreases in gibberellin levels also hasten leaf senescence and are linked to retarded stem extension. Furthermore, flooding may disrupt the normal distribution of the shoot produced growth regulators auxin and abscisic acid (Jackson 1990).

Increasing ethylene concentrations have long been known to be involved with plant responses to flooding (Jackson 1988). Increased ethylene levels are most likely caused by the increased production and translocation of the ethylene precursor, ACC, from the anoxic roots to the emerged, aerated shoot. Then in the presence of oxygen, ACC is converted to ethylene (Jackson 1990). Reid and Bradford (1984) suggested that the production of ethylene by anoxic roots was over emphasized due to experimental error resulting from the conversion af ACC to ethylene in samples subjected to oxygen during or before testing. Despite this potential effect, the measured ethylene levels using techniques that incubate previously anoxic roots in the presence of oxygen represent the potential ethylene production upon its transport to the stem (Donovan et al. 1989).

Increased ethylene due to flooding has been proven to cause leaf epinasty and stem hypertrophy and is thought to retard leaf growth (Jackson 1990). Yamamoto and Kozlowski (1987b) determined that ethylene played a major role in regulating leaf epinasty, leaf senescence, leaf abscission, and hypertrophied lenticel production in *Acer negundo* L. In *Pinus serotina* Michx., exogenously applied ethylene to aerobically grown seedlings resulted in aerenchyma formation similar to that induced by flooding (Topa and McLeod 1988).

Flood tolerance of red maple

The range of red maple (*Acer rubrum* L.) is vast, encompassing a wide spectrum of habitats throughout eastern North America. Red maple is commonly found where moisture conditions are extreme (Walters and Yawney 1990). In the southern United States, Putnum et al. (1960) describe red maple as scattered widely, living everywhere except high ridges and deep swamps . However, other sources list red maple as common vegetation in southeastern deepwater swamps (Mitsch and Gosselink 1986).

Numerous studies have demonstrated that red maple is flood tolerant. Healthy red maple stems were observed on land that had been flooded an average of 34.6% of the growing season for eight consecutive years (Hall and Smith 1955). Of the 38 other species on the site, only *Nyssa aquatica* L., *Fraxinus* sp., *Quercus palustris* Muenchh., *Cephalanthus occidentalis* L., *Quercus lyrata* Walt., *Salix nigra* Marsh., and *Planara aquatica* Walt. survived when flooded for an equivalent or greater percentage of the time (Hall and Smith 1955). Elsewhere, twelve years of annual flooding between mid March and late June resulted in an increase in the frequency of red maple (Malecki et al. 1983). Furthermore, red maple has been documented as surviving continuous flooding for two growing seasons (Hall et al. 1946 as found in Broadfoot and Williston 1973). The relative flood tolerance of red maple is cited many places, including McKnight et al. 1981, and Harms et al. 1980.

Effects of flooding on red maple

Limited research has been conducted to determine the effects of flooding upon red maple seeds or mature red maple stems. Hosner (1957) soaked seeds of *Acer rubrum* var *drummondii* for 32 days. The seeds failed to germinate in water. However, once removed, the seeds germinated with the same frequency as control seeds (Hosner 1957). A decrease in mean annual diameter increment was determined for flooded, mature red maples (Malecki et al.

1983). The presence of adventitious roots on adult trees has also been noted (Harms et al. 1980)

The bulk of the research pertaining to red maple and flooding has been done with seedlings. In general, rhizosphere inundation adversely impacts red maple seedling growth. Red maple seedlings with an initial height of 10 cm survived between 10 and 20 days of complete inundation with stagnant, turbid water. In comparison to the thirteen other bottomland species tested, when removed, red maple seedlings recovered at a rapid rate after 5 days of inundation, and at a moderate rate after 10 days (Hosner 1960).

McDermott (1954) placed red maple seedlings 5 cm in height in flooded tanks with a water level 0.65 cm below the soil surface. At the end of 32 days, the study showed that flooding significantly reduced shoot elongation even in treatments flooded for a single day. The amount of growth reduction was generally proportional to the duration of flooding, with the greatest reduction in height growth observed on plants flooded for the entire 32 days. At the end of 20 days of recovery, the 1 and 2 day flooded treatments showed no significant differences in height compared to the control, and those flooded for 32 days were growing at a faster rate than the controls. Because of this compensatory growth, McDermott considered red maple to recover very rapidly from flooding.

Red maple seedlings with an initial height of 39.3 cm were inundated with stagnant water 2.5 cm above the soil line for 60 days (Hosner and Boyce 1962). No seedling mortality occurred. A nonsignificant reduction in height growth was measured. Some adventitious roots were noted after 15 days and many after 30 days. Hosner and Boyce inferred that the nonadventitious root systems at the end of the treatment were dormant, yet quickly resumed normal growth.

Hosner and Leaf (1962) flooded another group of current year red maple seedlings 2.5 cm above the soil line for 60 days. At the end of treatment, shoots were weighed and analyzed

for nutrient content. Flooding resulted in increased shoot dry weight, ash weight, nitrogen and phosphorus, as well as decreases in shoot potassium, calcium, and magnesium. Even though total shoot N and P increased, shoot N and P concentrations declined. Once again the root system was classified as dormant with many flood induced adventitious roots.

Day (1987) looked at the effect of intermittent and continuous flooding as well as N and P enrichment upon two-year old red maple seedling biomass and N and P absorption. From April to September, second year seedlings were flooded 5 cm above the soil. The intermittent flood treatment alternated weeks between waterlogged and drained. At the end of the treatment, continuous flooding, more so than periodic, reduced biomass accumulation in leaves, stems, and roots. After an initial increase in root:shoot ratio from April to June, the ratio decreased during the remainder of the study. Nutrient enrichment had the greatest effect on the flooded treatment and appeared to ameliorate the deleterious effect of flooding on above-ground biomass. Nitrogen and phosphorus concentrations decreased in shoots of flooded seedlings. Morphological changes resulting from flooding included senescence, hypertrophied lenticels, and adventitious roots.

Carpenter and Mitchell (1980) flooded 2-year old red maple seedlings for 22 days, determining aerobic root respiratory capacity of excised root tips after 0, 4, 8, and 22 days. The rate at which oxygen was utilized by the excised tips was proportionate to the oxygen concentrations in which the tips were incubated. When incubated at 21% and 5% oxygen, consumption by the excised root tips decreased gradually with increasing duration of flooding. When incubated at 0.5% oxygen, oxygen consumption increased after 4 and 8 days of flooding after which it decreased. The effect of flooding was not as severe as that seen on *Acer saccharum* Marsh. The difference suggested that red maple utilizes an avoidance or escape mechanism to maintain aerobic root respiratory capacity under anaerobic conditions (Carpenter and Mitchell 1980).

To determine the role of morphological adaptations in sustaining aerobic root respiratory capacity during flooding, Tripepi and Mitchell (1984) inhibited the formation of hypertrophied lenticels and adventitious roots by maintaining anaerobic conditions around the emerged stem. Oxygen consumption of excised root tips was measured at 11, 24, and 29 days. Adventitious roots respired at rates three times faster than pre-treatment fibrous roots of the same plant and faster than fibrous roots of nonflooded plants. When flooded, the inhibition of lenticel intumescences and adventitious roots did not further reduce the respiratory capacity of the fibrous root system or result in any mortality. Because the prevention of hypertrophied lenticels and adventitious roots did not adversely affect root respiration, Tripepi and Mitchell (1984) suggested that it was the ability of roots to shift to anaerobic respiration that allowed them to maintain aerobic respiratory capacity.

Genotypic Comparisons

Within a species, there is some evidence linking maternal site hydrologic conditions with progeny flood tolerance. When current year *Populus deltoides* Marsh. clones from a sand dune, strip mine, and floodplain site were flooded, photosynthesis and transpiration declined at rates dependent on population source (McGee et al. 1981). When flooded, trees derived from the drier sand dune environment delayed stomatal closure. Because the waterlogged sand dune clones kept their stomata open, they were able to maintain their preflooded photosynthetic rates longer than the other clones (McGee et al. 1981).

Keeley (1979) grew *Nyssa sylvatica* Marsh. seedlings obtained from upland sites, floodplains, and swamps under flooded conditions. The results conclusively showed that genetic variation in flood tolerance corresponded to the water regime from which the seeds were obtained. For the flooded upland plants, survival was poor, growth stunted, root systems deteriorated, and root respiratory rates decreased. When flooded, the swamp plants showed

increased survival, increased root respiration, increased anaerobic respiration, and development of soil water roots. The floodplain plants displayed a response intermediate between the upland and swamp plants.

In contrast, other studies have shown no connection between maternal site water regime and offspring flood tolerance. Smit (1988) found no differences in flood tolerance between *Populus trichocarpa* seedlings obtained from five populations growing under varying moisture conditions. Rather, effects of flooding on growth and stomatal conductance were highly variable within populations. Similarly, the water relations of flooded wet site and xeric site derived *Thuja occidentalis* L. seedlings showed no differences (Collier and Boyer 1989).

Some studies, although not attempting to link maternal site moisture availability with offspring flood tolerance, have found intraspecific genetic differences in the effect of flooding on plant growth and physiology. Hook and Stubbs (1967) determined that both *Nyssa sylvatica* and *Nyssa aquatica* seedlings exhibit seed source variation in height growth and dry weight accumulation under differing water regimes. When *Pinus taeda* L. progeny derived from different seed sources were flooded, differences in flood tolerance, height growth, and root carbon dioxide production were found to vary with seed source (Hook and Shear 1987). When current year clones of *Hevea brasiliensis* Muell. Arg. clones from different sources were flooded, dry weight increment, diameter growth, and adventitious root production were found to be dependent on clone type (Sena Gomes and Kozlowski 1988).

METHODS

Site descriptions

In order to obtain a representation of red maple in Virginia, fruits were collected from a wet and dry site on the Coastal Plain, Piedmont, and Ridge and Valley physiographic regions.

Within a given physiographic region, wet and dry sites were chosen within four kilometers of each other to reduce seedling variation due to factors other than maternal water regime.

Coastal Plain wet site: Fruits were collected from six trees along the Chickahominy River in New Kent County, Virginia. Approximate latitude, longitude, and elevation of the site are 37°26' North, 77°43' West, and 1.5 meters. The soil on the site has a water table that fluctuates annually between 0.6 meters above the soil surface and 0.15 meters below the soil surface (USDA 1983).

Coastal Plain dry site: Fruits were collected from six trees in New Kent County, Virginia along a narrow upland ridge 30 meters in elevation. Approximate latitude and longitude are 37°27' North and 77°42' West. The soil series on the site has a water table deeper than 2 meters (USDA 1983).

Piedmont wet site: Fruits were collected from five trees along Fish Pond Creek on the Appomattox-Buckingham State Forest in Appomattox County, Virginia. The creek bottom is 185 meters above sea level and has a latitude and longitude of 37°25' North and 78°16' West.

Piedmont dry site: Fruits were obtained from six trees on a west facing slope 210 meters in elevation on the Appomattox-Buckingham State Forest in Appomattox County, Virginia. Approximate latitude and longitude are 37°25' North and 78°15' West.

Ridge and Valley wet site: Fruits were collected from a red spruce bog several kilometers north of the University of Virginia Mountain Lake Biological Station in Giles County, Virginia. Approximate latitude, longitude, and elevation are 37°23' North, 81°09' West, and 1190 meters.

Ridge and Valley Dry site: Fruits were collected from five trees on a ridge top along the Appalachian Trail in Giles County, Virginia. Approximate latitude, longitude, and elevation are 37°25' North, 81°10' West, and 1250 meters.

Seed collection and pre-experiment handling

All seeds were collected in the spring of 1991 prior to seed fall by clipping fruit bearing branchlets with a pole pruner. Fruits from the Coastal Plain wet and dry sites were collected on April 19th. All Piedmont fruits were obtained on April 26th. Fruits from the Ridge and Valley wet site were obtained on May 17th and from the dry site on May 22nd.

All fruits were air dried at room temperature for at least one week. After drying, fruits were stored at 2° C. On May 29th, seeds were imbibed with tap water for three hours. After soaking, seeds were drained, put in polyethylene freezer bags, and placed in stratification at 2° C. Seeds were inspected weekly during stratification.

On July 17th, some seeds had germinated during stratification, particularly those from the Coastal Plain dry site and the Piedmont wet site. On July 18th, the germinated seeds were planted. The remaining ungerminated seeds were placed in flats filled with Promix BX (Hummert Seed Co., MO, U.S.A.) to germinate at 19.5° C. Seeds were considered germinated when the radicle exceeded one cm in length. All seeds were planted prior to July 26th in 166 cm^3 Leach tubes (Ray Leach Corp., OR, U.S.A.) filled with a 25:75 v/v ratio of sand and ProMix BX.

Seedlings were grown in a greenhouse where the ambient temperature ranged from 20°C to 35°C. Throughout the experiments, sodium vapor lamps were used to maintain a 16 hour photoperiod. Prior to initiating treatments, the plants were watered as needed and fertilized weekly using 10 ml of a solution containing 200 *ug*/g nitrogen, 87 *ug*/g phosphorus, and 166 *ug*/g potassium (supplied as Peters General Purpose 20-20-20, Fogelsville PA,

U.S.A.) Seedlings were fertilized on September 4th with the Peters Soluble Trace Element Mix (Fogelsville PA, U.S.A.) at the concentration prescribed on the label for a one time application.

Experiment 1 - Periodic Harvest

The general null hypotheses tested in this experiment were:

Ho_1: Height, diameter, biomass accumulation, biomass partitioning, leaf area, and allometric growth of current year red maple seedlings are not affected by flooding.

Ho_2: Height, diameter, biomass accumulation, biomass partitioning, leaf area, and allometric growth do not differ between current year red maple seedlings derived from wet site maternal parents and dry site maternal parents.

Ho_3: Changes in height, diameter, biomass accumulation, biomass partitioning, leaf area, and allometric growth due to flooding do not differ between current year red maple seedlings derived from wet site maternal parents and dry site maternal parents.

Ho_4: There are no significant differences in height, diameter, biomass accumulation, biomass partitioning, leaf area, and allometric growth between red maple half-sib families.

Statistical Design

The three physiographic regions across the state of Virginia served as the blocking variable. By using physiographic regions as blocks, the results of the experiment represent the response of flooded red maple throughout the state. Within each block (physiographic region), three subsamples of each treatment combination were measured. Seedlings were randomized within blocks and subsamples. The treatment combinations consisted of wet maternal site-flooded, wet maternal site-unflooded, dry maternal site-flooded, and dry maternal site-unflooded. To obtain a representation of the populations from each site, one seedling from each of three half-sib families per site comprised each subsample (with the exception of the Coastal Plain wet site where only two half-sib families were used along with a third group of

seedlings derived from four different seed sources). Enough seedlings were grown to carry out four harvests. At each harvest, the average of the three half-sib families across the three subsamples served as the experimental unit. Seedlings from all four harvests comprising a particular subsample x treatment combination were placed together in treatment groups (Figure 1).

Experimental procedure

On September 4th, each treatment group was placed into an 11.6 liter plastic bucket filled with washed sand. In addition to holding the seedlings in place, the sand served to reduce nutrient dilution of the flooded treatments by decreasing the volume of water needed for inundation. Furthermore, the sand served to maintain root temperatures of the control seedlings at the same level as root systems of the flooded seedlings. A layer of silk screen cloth placed at the bottom of each Leach tube prevented roots from growing into the sand.

On September 5th, the buckets assigned the waterlogging treatment were flooded with tap water to a level 1 cm above the soil surface. Water was not changed during the experiment, but rather added daily to maintain the water level above the soil surface.

Soil redox potential and pH were periodically measured with a redox-pH meter (Markson-41064, U.S.A.). All seedlings were fertilized on October 25th and November 13th with 10 ml of solution containing 200 *ug*/g nitrogen, 87 *ug*/g phosphorus, and 166 *ug*/g potassium (supplied as Peters General Purpose 20-20-20, PA, U.S.A.). Seedlings were harvested prior to flooding on September 4th, after four weeks of flooding on October 4th, after nine weeks of flooding on November 11th, and after thirteen weeks of flooding on December 6th.

At harvest, seedling heights were measured to the nearest mm and seedling diameters measured with calipers to the nearest 0.05 mm. Seedlings were then removed from the Leach tubes and their roots washed free of the potting mixture. Stems, and roots, and water roots

(thicker, more succulent roots originating from the stem and main root of the flooded seedlings) were separated and placed in an oven maintained at 60° C to dry. Leaves were kept under refrigeration in sealed polyethylene bags until their areas were measured. Immediately after measuring, leaves also were placed in the oven. Leaf area was measured to the nearest 0.01 centimeter with a portable area meter (LI-3000, LI-COR, Inc., Lincoln, NE, U.S.A.). After drying, leaves, stems, and roots were weighed to the nearest 0.001 gram

Relative growth rates (RGR), net assimilation rates (NAR), leaf area ratios (LAR), root to shoot ratios (R:S), and specific leaf areas (SPLA) were calculated (Radford 1967) where:

$$RGR = (\log_e d.wt._a - \log_e d.wt._{a-1})(1/t)$$

$$NAR = \frac{(d.wt._a - d.wt._{a-1})(\log_e A_a - \log_e A_{a-1})}{t(A_a - A_{a-1})}$$

$$LAR = \frac{(A_a - A_{a-1})(\log_e d.wt._a - \log_e d.wt._{a-1})}{(\log_e A_a - \log_e A_{a-1})(d.wt._a - d.wt._{a-1})}$$

$$R:S = \frac{root\ d.wt._a}{shoot\ d.wt._a}$$

$$SPLA = \frac{A_a}{leaf\ d.wt._a}$$

where:

a = harvest number
t = number of days between a and a-1
A = leaf area

Statistical analysis

Data were $\log_{10}$ transformed to eliminate heterosciasticity then analyzed with a two-way ANOVA where the main effects of water treatment (flooded vs. control) and maternal hydrologic condition (wet site vs. dry site) as well as the interaction between water treatment and maternal hydrologic condition were tested using the following df:

Source	df
Region	2
Water treatment	1
Maternal hydrologic condition	1
Wat. tr. x Mat. hydr. con.	1
Error	6
Total	11

Genetic analysis

An ANOVA was used to determine the effect of half-sib relationship on seedling growth. For each harvest, water treatments (flooded and control) were analyzed separately. The ANOVA used the following df:

Source	df
Family	16
Error	35
Total	51

To obtain a measurement of how strong the genetic control exerted by family was, one-fourth of the additive genetic variance was divided by the total phenotypic variance to obtain family correlations. This calculation was conducted only for those growth parameter estimates significantly affected by family relations.

Experiment 2 - Gas exchange

The general null hypotheses tested in this experiment were:

Ho_1: Photosynthetic rate, leaf conductance, water use efficiency, and stomatal limitation of current year red maple seedlings are not affected by flooding.

Ho_2: Photosynthetic rate, leaf conductance, water use efficiency, and stomatal limitation do not differ between current year red maple seedlings derived from wet site maternal parents and dry site maternal parents.

Ho_3: Flood induced changes in photosynthetic rate, leaf conductance, water use efficiency, and stomatal limitation do not differ between current year red maple seedlings derived from wet site maternal parents and dry site maternal parents.

Statistical Design

The gas exchange study used the same blocking variable (physiographic region) and treatment combinations (wet maternal site-flooded, wet maternal site-unflooded, dry maternal site-flooded, dry maternal site-unflooded) as did the periodic harvest experiment. Five single seedling subsamples per block were measured on each sampling date. The average of the five subsamples per block served as the experimental unit. Seedlings were randomized within blocks and subsamples. One half-sib family per site was used (Figure 2).

Experimental procedure

On October 9th, seedlings were transplanted into one liter, clear plastic pots (diamter = 10 cm, height = 13 cm). Between the time of transplant and the initiation of the experimental treatments, pots were fertilized weekly with 50 ml of a solution containing 200 *ug*/g nitrogen, 87 *ug*/g phosphorus, and 166 *ug*/g potassium (supplied as Peters General Purpose 20-20-20, PA, U.S.A.) Aluminum foil was wrapped around the pots to keep the root systems in darkness. On December 7th, pots assigned the waterlogging treatment were flooded with tap water 1-2 cm above the soil surface. Water was added daily to maintain the water level above the soil surface. Flooded seedlings were drained on December 20th.

While flooded, leaf gas exchange was measured on December 8th, 10th, 12th, 16th, and 20th. Gas exchange was measured after draining on December 22nd, 24th, and 26th. Measurements were made using a 1/4 liter cuvette attached to a portable photosynthesis

system (LI-6250, LI-COR, Inc., Lincoln, NE, U.S.A.). Measurements were taken under a 400 watt sodium vapor lamp to ensure a photon flux density above 990uMm^{-2}s^{-1}. Heat from the lamp was dissipated by a water bath placed between the lamp and the cuvette. Between days, relative humidity inside the cuvette ranged from 22% and 43% (mean = 28.6, s.d. = 6.95), temperature inside the cuvette ranged from 24° C and 32° C (mean = 27.8, s.d. = 1.89), and CO_2 inside the cuvette ranged from 354 ppm to 358 ppm (mean = 356.0, s.d. = 1.38). Within sampling days relative humidity was held as constant as possible inside the cuvette (average s.d. within sampling days = 2.95). For each plant, the same leaf was measured on each sampling day.

Water potential estimates were attempted on each of the sampling dates using a pressure chamber (PMS Corvallis OR, U.S.A.). After the gas exchange measurements, a fully expanded leaf was detached and put in the chamber. However, phloem bubbling obscured the end point, making an accurate measurement impossible (Bahari et al. 1985). All measurements were taken between 0830 and 1530 hours.

After the last sampling date, the leaf segments that were used to take gas exchange measurements were excised, their area measured to the nearest 0.01 cm^2 (LI-COR-3000), dried, and weighed to the nearest 0.0001 gram. Photosynthetic rate, transpiration rate and leaf conductance were calculated on a per area basis. Water use efficiency (WUE) and stomatal limitation (SL) were calculated where:

$$WUE = PSA/TSA$$

$$SL = (CA-CI)/CA$$

where:

PSA = Photosynthetic rate

TSA = Transpiration rate

CA = Ambient carbon dioxide

CI = Leaf internal carbon dioxide

Statistical analysis

Data were $\log_e$ transformed to eliminate heterosciasticity then analyzed with a two-way ANOVA where the main effects of water treatment (flooded vs. control) and maternal hydrologic condition (wet site vs. dry site) as well as the interaction between water treatment and maternal hydrologic condition were tested using the following df:

Source	df
Region	2
Water treatment	1
Maternal hydrologic condition	1
Wat. tr. x Mat. hydr. con.	1
Error	6
Total	11

Experiment 3 - Root physiology

The general null hypotheses tested in this experiment were:

Ho_1: Shoot water potential, root ethylene production, and the root aerobic respiratory capacity of current year red maple are not affected by flooding.

Ho_2: Shoot water potential, root ethylene production, and the root aerobic respiratory capacity do not differ between current year red maple seedlings derived from wet site maternal parents and dry site maternal parents.

Ho_3: Changes in shoot water potential, root ethylene production, and the root aerobic respiratory capacity due to flooding do not differ between current year red maple seedlings derived from wet site maternal parents and dry site maternal parents.

Statistical Design

The root physiology study used the same blocking variable (physiographic region) and treatment combinations (wet maternal site-flooded, wet maternal site-unflooded, dry maternal site-flooded, dry maternal site-unflooded) as did the previous experiments. Enough seedlings

were grown to harvest and measure four subsamples per block on each of six sampling dates. The average of the four subsamples per block served as the experimental unit. Seedlings were randomized within blocks and subsamples. One half-sib family per site was used. Seedlings from all six harvests comprising a particular subsample x treatment combination were placed together in treatment groups (Figure 3).

Experimental procedure

On January 25th, treatment groups were placed into an 11.6 liter plastic buckets filled with washed sand. Sand and silk-screen cloth were used for the reasons described in the periodic harvest experiment. Each bucket contained two treatment groups.

Buckets assigned the flooded treatment were inundated to at least one centimeter above the soil surface on January 26th. Water was added daily to maintain the water level above the soil surface. Measurements were taken after one day of flooding on January 27th, after three days of flooding on January 29th, after five days of flooding on February 1st, and after twelve days of flooding on February 8th. Measurements also were made one day after draining on February 9th and seven days after draining on February 15th.

One seedling per treatment group was randomly chosen and measured on each sampling date. Water potentials of cut shoots were measured with a pressure chamber. Seedlings then were carefully removed from the Leach tubes and their roots gently washed in a bucket of water maintained at ambient temperature. The lower portion of the root system, approximately 6 cm, was excised, placed in a 15 ml test tube, and capped with a septum stopper. Test tubes were placed in the dark to incubate. Between days, incubation temperature ranged from 22° C to 25° C, but was kept as constant as possible within a sampling day.

After approximately 30 and 150 minutes, 100 microliters of air was drawn from the test tube with an air tight syringe. Carbon dioxide contained in the air samples was measured with

the a portable photosynthesis system (LI-6250, LI-COR, Inc., Lincoln, NE, U.S.A.). A length of tubing containing a septa was inserted in place of the cuvette so that the LI-COR could receive the gas sample. After incubating approximately 300 minutes, 250 microliter air samples were drawn from the test tubes. The air samples were injected into the gas chromatograph (Varian, CA , U.S.A.) to measure ethylene concentrations. The chromatograph set up was the same as used by Seiler and Johnson (1984) which consisted of a six foot column filled with activated alumina to separate the sample constituents and flame ionization to detect the samples. After the root samples were oven dried at 60° C, they were weighed to the nearest 0.0001 gram, to allow the expression of carbon dioxide and ethylene evolution on a minute^{-1} gram^{-1} basis

Statistical analysis

Data were $\log_e$ transformed to eliminate heterosciasticity then analyzed with a two-way ANOVA where the main effects of water treatment (flooded vs. control) and maternal hydrologic condition (wet site vs. dry site) as well as the interaction between water treatment and maternal hydrologic condition were tested using the following df:

Source	df
Region	2
Water treatment	1
Maternal hydrologic condition	1
Wat. tr. x Mat. hydr. con.	1
Error	6
Total	11

RESULTS AND DISCUSSION

Experiment 1 - Periodic Harvest

Soil pH and redox potential

Soil pH and redox potential were impacted by rhizosphere inundation. The soil of the flooded treatments was more alkaline than the soil of the control treatments (Figure 4). Because the potting mixture used in this experiment was slightly acidic, a flood induced pH increase due to the reduction of Fe^{3+} to Fe^{2+} was expected (Ponnamperuma 1984). The general trend of the control treatment pH can be in part explained by the fertilization regime. The application of nitrogen causes a decrease in pH (Tisdale et al. 1985). Thus, the rising pH of the control treatment soil during the first part of the experiment may have been due to the cessation of fertilization. In the same way, the decrease in pH of the control treatment soil during the latter stages of the experiment may have been a result of fertilization on October 25th and November 13th. Anomalous readings for the control treatment soil on September 9th were excluded from the data set.

Because the biological effect of redox potential is pH dependent, redox potential was normalized to a pH of 6.0. The redox potential of the flooded treatments decreased considerably, stabilizing near -150 mV after two weeks of waterlogging (Figure 5). The large difference between the redox potential of the control and flooded treatments indicates both the severity with which soil inundation affected rhizosphere oxygen availability and the status of rhizosphere compounds. Soil redox potential decreases when flooded because when oxygen is depleted, facultative anaerobes use oxidized soil compounds as respiratory electron acceptors.

Interaction between maternal site hydrologic condition and rhizosphere inundation

For all parameters measured, no interactions were found between maternal hydrologic condition (wet and dry) and rhizosphere inundation treatment (waterlogged and control) (Table1). This lack of interaction indicates that growth of flooded red maple seedlings from Virginia is not dependant on maternal site hydrologic condition. Therefore, because red maple derived from dry sites are impacted by waterlogging to the same degree as red maple derived from wet sites, there does not appear to be any genetic differentiation among red maple affording some populations enhanced flood tolerance. Most likely, phenotypic plasticity enables red maple to survive and grow in very wet areas.

The remote potential existed for pollen flow between wet and dry site trees. This might have resulted in genetic mixing and consequent masking of a potential adaptation to flooding. However, even if pollen flow had occurred, the majority of fruits likely would have been fertilized by pollen from neighboring, same site trees. Thus, it is unlikely that the lack of evidence indicating ecotypic differentiation is due to pollen mixture.

The lack of genetic differentiation found in the growth rate of these flooded red maple seedling populations was similar to the findings of Smit (1988) who worked with *Populus trichocarpa*. The only previous studies that provide evidence of intraspecific flood tolerance differences examined *Nyssa* sp. (Hook and Stubbs 1967 and Keeley 1979) and *Pinus taeda* (Hook and Shear 1987). In contrast to the red maple study, Keeley used different varieties of *Nyssa sylvatica* to represent different populations and Hook and Shear (1987) examined half-sib family responses to flooding rather than population responses. However, just as flood tolerance differs among species, no doubt, so does the degree to which a species genetically differentiates in response to the stresses imposed by flooding.

The effect of rhizosphere inundation on seedling growth

Throughout the experiment, no flood induced mortality occurred. However, growth was retarded by rhizosphere inundation. By the second harvest (four weeks of flooding), rhizosphere inundation had significantly reduced ($p<.05$) root biomass production (Table 2). The difference between the root weight of the flooded seedlings and the root weight of the control seedlings continued to increase throughout the experiment. After the second harvest (four weeks of flooding), the flooded seedlings ceased height growth for the remainder of the experiment. Differences between the height of the flooded seedlings and the height of the control seedlings were significant on the third and fourth harvests (nine and thirteen weeks), as were differences between stem weight of the flooded and control seedlings (Table 2). Due to a decrease between harvests three and four, leaf area and leaf weight of the flooded seedlings were significantly less than those of the control seedlings (Table 2). These decreases in leaf area and leaf weight were due in part to leaf abscission. Seedling diameter was not affected by waterlogging (Table 2).

The response to flooding of most plants is to reduce height growth, leaf growth, and root growth (Kozlowski et al. 1991a). Results of this study confirm these universal responses as do previous works examining flooded red maple. Previous studies of red maple found that rhizosphere inundation decreased height growth (McDermott 1954, and Hosner and Boyce 1962) and biomass production (Day 1987). Root mass production was most severely reduced by flooding (Day 1987).

Although the height and stem weight growth of the flooded seedlings were reduced, the diameter growth of the flooded seedlings was not. Disproportional diameter growth of flooded seedlings is a common response to flooding. This accelerated diameter growth is caused by the production of lower density, larger cells (Kozlowski 1984). Rhizosphere inundation also induced lenticel hypertrophy and adventitious root formation. No significant differences in

adventitious root production between wet and dry site derived seedlings existed. Therefore, the weights of adventitious and nonadventitious roots were summed and analyzed.

The effect of rhizosphere inundation on allometric growth

Specific leaf area was not affected by flooding. The lack of flood induced alteration of specific leaf area indicates that waterlogging did not affect leaf density (Table 2). However, flooding did impact root to shoot ratios. Root to shoot ratio of the control seedlings increased over the duration of the experiment. On the other hand, root to shoot ratio of the flooded seedlings decreased after four weeks of flooding, increased to preflooded levels between four and nine weeks of flooding, and then decreased between nine and thirteen weeks of flooding (Table 2). The discrepancy between the ratios of flooded and control seedling resulted in a significant flood induced reduction of root to shoot ratio from the second harvest through the end of the experiment. This response is typical of flooded woody plants because root growth usually is affected by flooding more than shoot growth (Kozlowski 1984).

Flooding significantly ($p<0.05$) reduced mean relative growth rate for the intervals between harvests one and two, and two and three (Table 3). Mean relative growth rate between harvests three and four was reduced ($p=0.06$) (Table 3). Waterlogging also significantly decreased mean net assimilation rate between all harvest intervals (Table 3). This flood induced reduction of net assimilation rate indicates that the waterlogged seedlings were adding less biomass per unit of leaf area than the control seedlings. One may infer from these results that the reduction in growth of the flooded seedlings was caused not by a lack of photosynthetic surface, but rather by a disruption of plant function.

The mean leaf area ratio of the flooded seedlings was significantly greater than those of the control seedlings for all harvest intervals. Thus, the waterlogged seedlings had a greater leaf area per unit biomass (Table 3). The increase of the flooded seedling leaf area ratio

occurred because root weight was reduced by flooding to a much greater extent than was leaf area.

The effect of maternal site hydrologic condition on seedling growth

Of all the growth parameters, only height at nine and thirteen weeks, stem weight at thirteen weeks, and root to shoot ratio at nine and thirteen weeks were significantly different between the wet site and dry site derived seedlings regardless of flooding treatment (Table 4). The stems of the wet site derived seedlings were taller and heavier. The heavier stems, hence heavier shoots, of the wet site derived seedlings resulted in the wet site seedlings having significantly lower root to shoot ratios than the dry site seedlings. No significant differences existed between wet and dry site seedling allometric growth (Table 5).

Even though the seeds obtained from the wet sites were significantly heavier than those from the dry sites (Will, unpublished data), seed weight did not result in a discrepancy between the wet and dry site seedlings at the beginning of the experiment. Because the discrepancy developed later in the experiment, the wet site derived seedlings probably have the genetic ability to grow in height faster than the dry site derived seedlings. Although this study showed that wet site and dry site derived seedlings were affected the same way by waterlogging, perhaps the ability of frequently flooded seedlings to grow tall quicker allows them to keep their shoots above water during future flooding episodes.

Genetic analysis

Soil waterlogging increased half-sib family differences (Table 6). These results suggest that under the stress of flooding, growth is more rigidly controlled by genetics. With the exception of the seedlings inundated for nine weeks, leaf area and leaf weight did not exhibit significant differences among families. The heights of flooded and control seedlings were

under strong genetic control (Table 6), verifying the genetic basis for the significant differences in height growth between wet and dry site derived seedlings. Stem weight, a growth parameter related to shoot height, did not show as strong a half-sib family effect as height. Significant half-sib family effects existed for the stem weight of the flooded seedling after four, nine, and thirteen weeks of flooding and for the control seedlings initially and after thirteen weeks (Table 6).

The only significant diameter family effects were for the flooded seedlings after nine and thirteen weeks of waterlogging (Table 6). The presence of significant family differences between the flooded seedling diameters only, suggests that under flooded conditions, diameter growth is more strongly controlled by genetics. Initially the root weights of the flooded and control seedlings showed significant differences between families. After the initial measurements, only the root weight of the flooded seedlings remained under tight genetic control. Therefore, it appears that flood imposed stress maintains some family genetic differences existing early in seedling development.

Growth parameters that were significantly influenced by family relations had high family correlations (Table 6). High family correlations indicate a large contribution of genetic variance to the overall phenotypic variance. Therefore, to get an accurate representation of a population, several families should be tested to account for family differences.

Experiment 2 - Gas Exchange

Interaction between maternal site hydrologic condition and rhizosphere inundation

As was the case for red maple seedling growth, no significant gas exchange interactions between red maple maternal site hydrologic conditions and waterlogging existed (Table 7). The lack of an interaction indicates an absence of genetic differentiation in photosynthetic rate

or leaf conductance of wet site and dry site populations that may have afforded either population an adaptation to flooding.

The lack of a differential gas exchange response to flooding between red maple seedling populations supports the results of Smit (1988) who studied *Populus trichocarpa* seedlings, but differs from the results of McGee et al. (1981). McGee et al. found that although the photosynthetic rate of flooded *Populus deltoides* did not depend on population source, their transpiration rate did. However, it should be noted that McGee et al. used *Populus* clones rather than seedlings. Clonal differences indicate individual differences rather than ecotypic differentiation.

Effect of rhizosphere inundation on seedling gas exchange

The photosynthetic rate of the flooded seedlings was significantly ($p < 0.05$) reduced after the third day of waterlogging and remained depressed until four days after draining (Table 8). The photosynthetic rate of the flooded seedlings was two-thirds that of the control seedlings after one day of waterlogging and dropped to below half the rate of the control seedlings for the remainder of the waterlogging treatment (Table 8). A four day lag time between draining the pots and photosynthetic rate recovery occurred (Table 8).

Rhizosphere inundation usually causes a severe and immediate reduction in photosynthetic rate (Kozlowski et al. 1991a). Although no work has been done detailing the effect of flooding on red maple physiology, Peterson and Bazzaz (1983) found that the photosynthetic rate of flooded *Acer saccharinum* decreased. Unfortunately, comparison between this red maple study and the silver maple study is difficult because the measurements in the silver maple study were taken on a weekly basis. A reduction in the photosynthetic rate of the flooded red maple seedlings was apparent shortly after the initiation of the waterlogging treatment. A rapid decrease in the photosynthetic rate of flooded woody plants is usually

caused by increasing stomatal resistance reducing the plant's carbon dioxide uptake ability (Kozlowski et al. 1991a).

The leaf conductance of the flooded red maple seedlings was significantly reduced after three and five days of rhizosphere inundation (Table 8). After one day of waterlogging, the conductance of the flooded seedlings was seventy percent of the control seedlings and decreased to forty-nine percent after three days of flooding (Table 8). By the ninth day of waterlogging, the conductance of the flooded seedling rebounded to approximately seventy percent of the controls, resulting in nonsignificant differences between the treatments. Draining resulted in a steady recovery of leaf conductance.

Although it was impossible to take water potential readings during the gas exchange experiment due to phloem bubbling, results from the root physiology study suggest that flooded red maple water potentials do not become more negative than the controls. The lack of a flood induced water potential decrease indicates that stomatal closure was not induced by a leaf water deficit. A large portion of the decrease in the photosynthetic rate of the flooded seedlings can be attributed to stomatal closure. However, photosynthetic rate of the flooded seedlings remained more depressed than did their leaf conductance. Therefore, the photosynthetic rate of flooded seedlings may have been affected by factors other than stomatal closure alone.

The water use efficiency of the flooded seedlings remained approximately ninety percent of the control seedlings until after nine and thirteen days of flooding at which time it decreased to fifty-nine and forty-nine percent respectively (Table 9). These large decreases arose because the photosynthetic rate of the flooded seedling remained depressed while leaf conductance, hence transpiration rates rebounded. However, due to a large variance, these decreases in water use efficiency were significant only at a $p < 0.10$ (Table 9). A similar

decrease in the water use efficiency of flooded *Acer saccharinum* was noted by Peterson and Bazzaz (1983).

Stomatal limitation, a measurement indicating the extent to which stomatal aperture limits photosynthetic rate, followed a trend similar to water use efficiency (Table 9). When compared to the controls, after nine and thirteen days of waterlogging, the stomatal limitation of the flooded seedlings was greatly reduced. This reduction in stomatal limitation during the later stages of the waterlogging treatment suggests that as the duration of flooding lengthened, a factor besides stomatal closure was contributing to retard photosynthetic rate. The greater reduction in photosynthetic rate of the flooded seedlings than in their leaf conductances, as quantified by decreasing water use efficiencies, further verified the action of a limiting agent beyond leaf conductance. However, if nonuniform stomatal closure occurs, stomatal limitation is underestimated and the degree of nonstomatal photosynthetic inhibition is overestimated (Kozlowski et al. 1991b)).

Both water use efficiency and stomatal limitation recovered more quickly after draining than photosynthetic rate or leaf conductance. Therefore,because the lingering reduction in the photosynthetic rate of the drained seedlings was caused mainly by stomatal aperture, the other factors that contributed in decreasing photosynthetic rate during flooding were quickly alleviated by draining.

Effect of maternal site hydrologic condition on seedling gas exchange

No significant differences between the gas exchange parameters of the wet site and dry site derived seedlings existed (Tables 10 and 11). The wet site seedlings tended to have a slightly lower photosynthetic rate and greater leaf conductance than the dry site seedlings (Table 10). These differences usually resulted in the wet site seedlings having a lower water

use efficiency and stomatal limitation (Table 11). On dry sites, enhanced water use efficiency may be an important adaptation to minimize water loss.

Experiment 3 - Root physiology

Interaction between maternal site hydrologic condition and rhizosphere inundation

As was the case for the growth and gas exchange of red maple seedlings, no significant interactions in root physiology between red maple maternal site hydrologic condition and waterlogging existed (Table 12). The lack of an interaction indicates that there is no genetic differentiation between wet and dry site seedling shoot water potentials, root carbon dioxide evolution, or root ethylene evolution that could have afforded one of the populations an adaptation to better withstand flooding stress.

Two previous studies of different species did find intraspecific differences in the root physiology of flooded woody plants (Keeley 1979, and Shear and Hook 1987). Although Keeley (1979) found significant root aerobic respiration differences between upland, floodplain, and swamp populations of *Nyssa sylvatica*, significant differences did not develop until after one month of waterlogging, a time period longer than the red maple study was conducted. Also, unlike the red maple root physiology study, Keeley used different varieties of *Nyssa sylvatica*, (var. *sylvatica* for the upland population and var. *biflora* for the swamp population). Shear and Hook (1987) found significant differences in anaerobic root carbon dioxide production for flooded half-sib *Pinus taeda* families. However, differences in *Pinus taeda* flood tolerance were not examined at the population level.

Results similar to this red maple study were found for *Thuja occidentalis* seedlings (Collier and Boyer 1989). As was the case for flooded wet site and dry site derived red maple

seedlings, there were no differences between the water potentials of flooded xeric upland and flooded swampy floodplain *Thuja occidentalis* seedlings.

Effect of rhizosphere inundation on root physiology

The water potential of the control seedlings was significantly more negative than those of the flooded seedlings ($p<0.05$) after the fifth day of waterlogging. The treatment induced water potential differences continued until at least one day after draining (Table 13). The less negative water potential of the flooded seedlings indicate that the flooded seedlings were less water stressed than were the control seedlings. Because readings were taken during the afternoon, the control seedlings were likely experiencing normal midday water deficits. The flooded seedlings did not experience these midday deficits because, as was learned in the gas exchange study, flooded red maple seedlings decreased leaf conductance within the first day of rhizosphere inundation. The decreased leaf conductance of the flooded seedlings decreased the quantity of water lost through transpiration enough to offset any flood induced decreases in root water absorption.

The lack of leaf water deficit driven stomatal closure implies that some chemical messenger acts to increase stomatal resistance of flooded red maple seedlings. As more research is completed on the cause of flood induced stomatal closure in woody plants, the evidence is accumulating that, as in this experiment, leaf water deficits are not the controlling factor (Smit and Stachowiak 1988, Smith and Agar 1988, Pezeshki and Chambers 1986, Tang and Kozlowski 1984a, Tang and Kozlowski 1982, and Pereira and Kozlowski 1977).

Soil waterlogging drastically reduced the potential of red maple roots to aerobically respire (Table 14). After one day of inundation and throughout the remainder of the experiment, the flooded seedlings produced less carbon dioxide during incubation than the controls (Table

14). Flooded seedling root carbon dioxide production partially recovered after seven days of drained conditions.

Previous studies involving the aerobic respiratory capacity of flooded red maple produced similar results. Eight days of flooding reduced two-year old red maple sapling root carbon dioxide evolution by forty percent compared to preflooded levels (Carpenter and Mitchell 1980). Eleven days of flooding reduced red maple seedling oxygen consumption to sixty-four percent of the control seedlings (Tripepi and Mitchell 1984). The degree by which red maple aerobic respiration capacity was decreased by flooding in previous studies is similar to the reductions induced by flooding in this study (Table 14).

One and three days after the initiation of waterlogging, the flooded seedlings had a slightly higher root ethylene evolution rate (Table 15). However, contrary to previous studies examining the effects of soil inundation on the ethylene production of woody plants, after five days, the flooded red maple seedlings produced ethylene at a significantly slower rate than the control seedlings (Table 15). Most previous studies measured the ethylene production of stem segments or the accumulation of ACC in roots (Yamamoto and Kozlowski 1987a, Yamamoto and Kozlowski 1987b, Yamamoto and Kozlowski 1987c, Yamamoto and Kozlowski 1987d, Tang and Kozlowski 1984a, Tang and Kozlowski 1984b). However, incubating root tissue under aerobic conditions, as was done for this red maple study, allows the conversion of ACC to ethylene, therefore providing an estimate of root ethylene production potential (Donovan et al. 1989).

Ethylene is thought to be active in causing flood induced morphological changes including leaf epinasty, leaf senescence and abscission, lenticel hypertrophy, and adventitious root formation (Tang and Kozlowski 1984a). Because the red maple seedlings in the periodic harvest experiment experienced these flood induced changes and because increased ethylene production is the usual response to flooding, the failure to find increased

ethylene evolution in the flooded red maple root systems was probably a result of sampling the wrong tissue.

The decrease in ethylene production rates of the flooded red maple root systems was probably due to the rapid decay of physiologically active fine roots and the suppression of new fine root formation. Flood induced root injury was reflected by the drastic decrease in root aerobic respiration capacity. Although Donovan et al. (1989) found increased ethylene production in flooded *Nyssa aquatica* and *Taxodium distichum* root tissue, they did not find a corresponding decrease in root aerobic respiration capacity. Therefore, a future study involving ethylene production of flooded red maple should use either stem segments or root tissue nearer the soil surface.

Effect of maternal site hydrologic condition on seedling root physiology

The wet site derived seedlings a had more negative water potential on all sampling dates. The difference was significant after twelve days of waterlogging and after one and seven days of drained conditions (Table 16). The more negative water potentials of the wet site derived seedlings were consistent with the finding from the gas exchange study that the wet site seedlings had a greater leaf conductance than the dry site seedlings. Greater conductance would result in greater midday water deficits, hence more negative water potentials. No significant differences between wet site and dry site seedling carbon dioxide or ethylene evolution rates existed (Tables 17 and 18).

CONCLUSIONS

Although red maple seedling growth, gas exchange, and root physiology were adversely impacted by rhizosphere inundation, no significant differences existed in the degree to which wet and dry site derived seedlings were hindered. Because there were several genetic

differences between the growth and physiology of wet and dry site derived populations, the lack of a differential response to waterlogging by the genetically distinct populations indicates that red maple does not exhibit ecotypic differences in flood tolerance. Rather, these results imply that red maple is a habitat generalist with the ability to acclimate in response to habitat variation.

The factors enabling a species or population to flourish under particular environmental influences are complex, diverse, and dynamic. This experiment isolated flood tolerance. Quite possibly red maple populations are ecotypically distinct in respect to another variable such as drought tolerance or in respect to an interaction of variables such as flooding and temperature. Furthermore, the waterlogging stress of this experiment was severe. Seedlings, the most flood sensitive life stage, were subjected to rhizosphere inundation with stagnant water. The stress applied in this experiment may have been too severe to allow the expression of a possible adaptation to flooding.

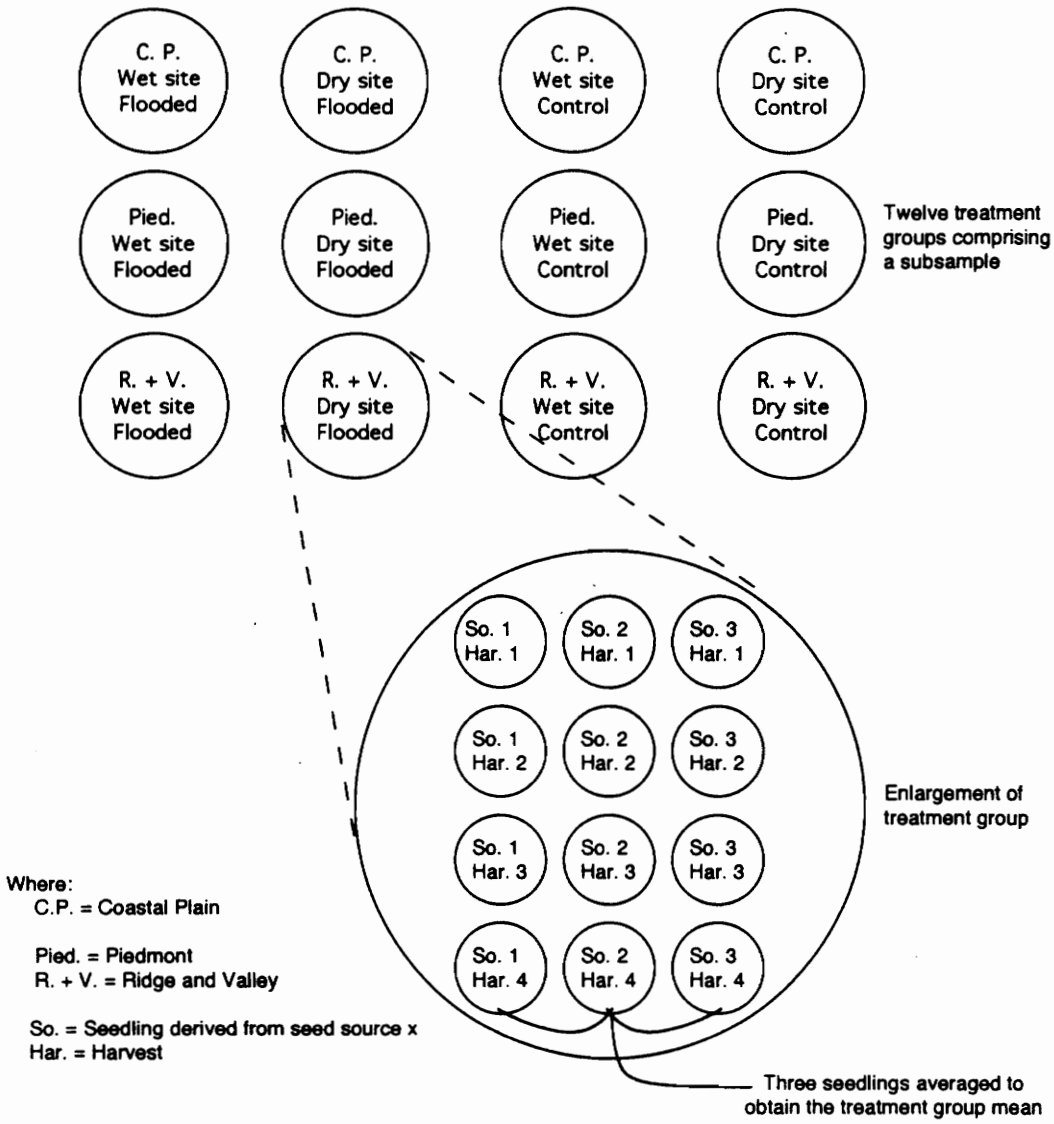
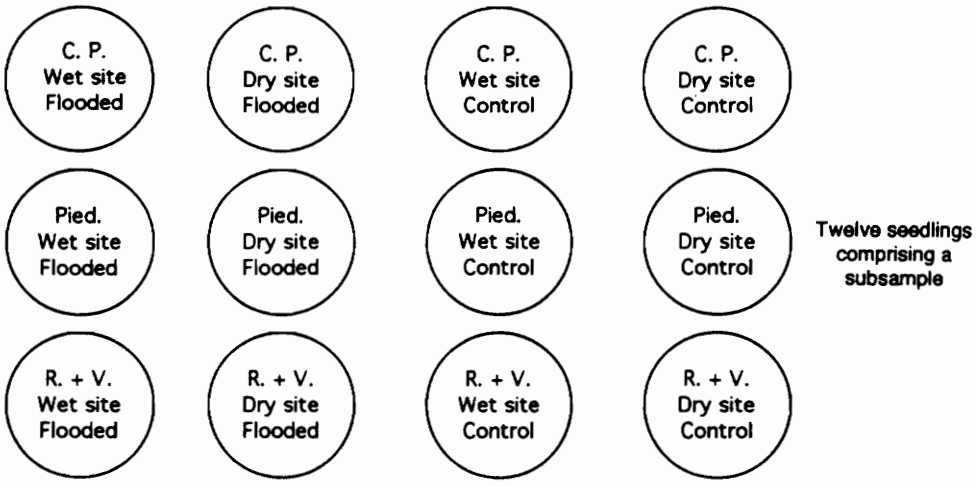


Figure 1. Experimental design of the periodic harvest experiment. Shown above is one of the three subsamples as well as an enlargement of one treatment group. Treatment groups were randomized within subsamples as were seedlings within treatment groups. At each harvest, the measurements of the three seedlings from a treatment group were averaged. Later, treatment group means of the same physiographic region x treatement combinations were averaged across the three subsamples to obtain the experimental unit.



Where:
C.P. = Coastal Plain

Pied. = Piedmont
R. + V. = Ridge and Valley

Figure 2. The experimental design of the gas exchange experiment. Shown above is one of the five subsamples. Seedlings were randomized within blocks and subsamples. Each seedling was measured on each sampling date. The seedling measurements from similar phsysiographic region x treatment combinations were averaged across the five blocks to obtain the experimental unit.

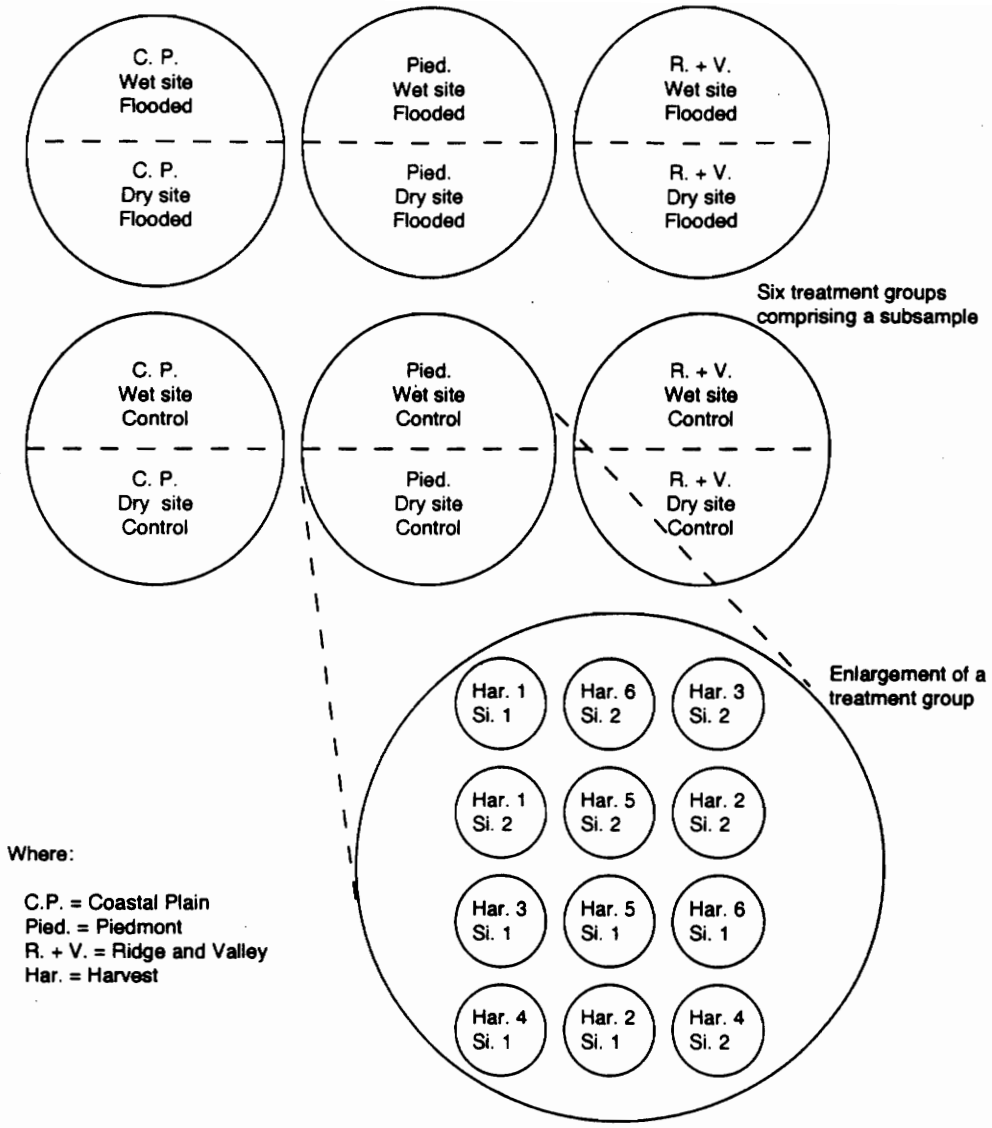


Figure 3. Experimental design of the root physiology experiment. Shown above is one of the four subsamples as well as an enlargement of one treatment group. Treatment groups were randomized within blocks as were seedlings within treatment groups. At each harvest, the measurements from similar physiographic region x treatment combinations were averaged across the four subsamples to obtain the experimental unit.

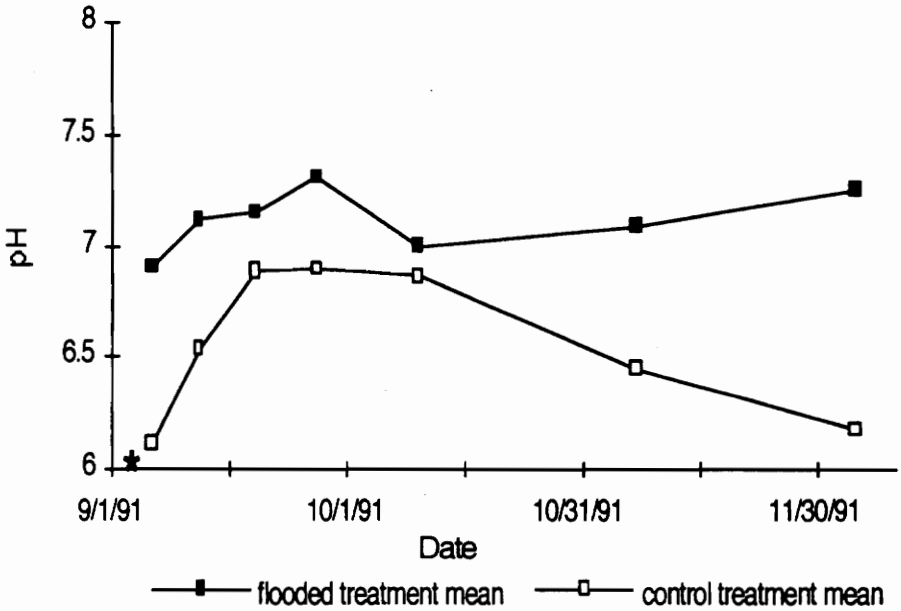


Figure 4. The soil pH of the flooded and unflooded treatments from the periodic harvest study. The asterisck (*) on the x-axis indicates the date the flooding treatment was imposed.

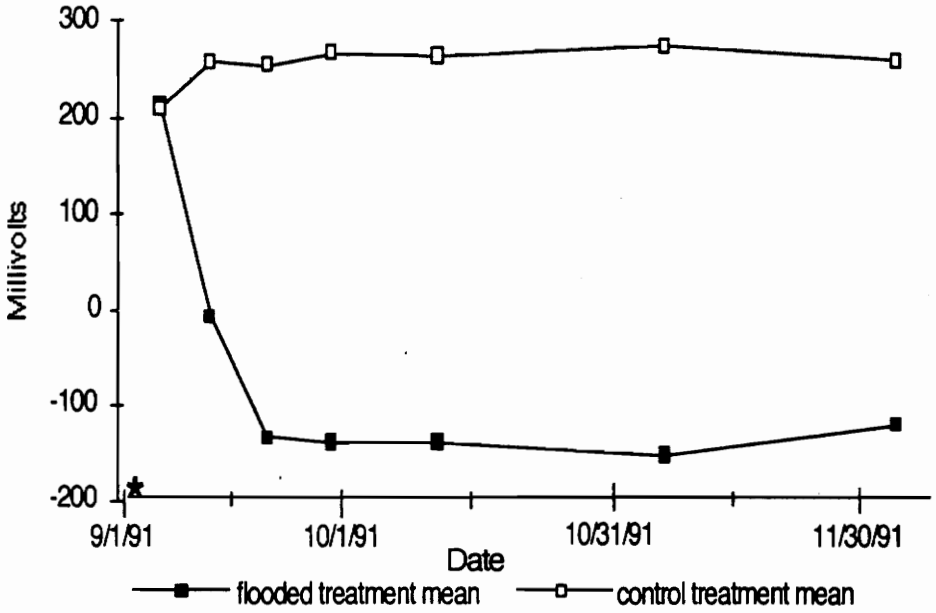


Figure 5. The soil redox potential (adjusted to a pH of 6.0) of the flooded and unflooded treatments from the periodic harvest study. The asterisck (*) on the x-axis indicates the date the flooding treatments were imposed.

Table 1. P values associated with the interaction between maternal hydrologic condition and flooding treatment from the periodic harvest study.

Duration of flooding	Diameter p value 1	Height p value	Leaf Area p value	Leaf Weight p value	Stem Weight p value	Root Weight p value
Initial	0.10	0.72	0.43	0.32	0.28	0.75
Four weeks	0.98	0.93	0.80	0.94	0.73	0.93
Nine weeks	0.85	0.73	0.91	0.88	0.44	0.63
Thirteen weeks	0.62	0.95	0.67	0.67	0.91	0.72

1 -The probability of a type I error

Table 2. The means, ratios, and p values associated with the flooded and unflooded treatment growth parameter measurements from the periodic harvest study. Ratios were calculated by dividing the flooded treatment mean by the unflooded treatment mean. For all means, n is six.

		Duration of flooding			
		Initial	Four weeks	Nine weeks	Thirteen weeks
Diameter (mm)	Flooded [1]	1.88	3.22	3.83	4.27
	Control [2]	1.82	3:11	3.88	4.40
	Flooded/control [3]	1.03	1.04	0.99	0.97
	p value [4]	0.01	0.22	0.67	0.42
Height (mm)	Flooded	86.83	95.56	94.93	92.20
	Control	86.01	103.24	105.30	112.72
	Flooded/control	1.01	0.93	0.90	0.82
	p value	0.82	0.07	< 0.01	< 0.01
Leaf area (sq cm)	Flooded	38.77	49.03	53.08	46.92
	Control	36.21	54.16	56.87	67.71
	Flooded/control	1.07	0.91	0.93	0.69
	p value	0.36	0.28	0.20	< 0.01
Leaf weight (g)	Flooded	0.128	0.206	0.233	0.212
	Control	0.132	0.225	0.250	0.303
	Flooded/control	0.97	0.92	0.93	0.70
	p value	0.43	0.23	0.23	< 0.01
Stem weight (g)	Flooded	0.038	0.127	0.149	0.217
	Control	0.038	0.128	0.187	0.253
	Flooded/control	1.00	0.99	0.80	0.86
	p value	0.44	0.76	0.02	0.05
Root weight (g)	Flooded	0.052	0.094	0.178	0.209
	Control	0.050	0.212	0.480	0.660
	Flooded/control	1.04	0.44	0.37	0.32
	p value	0.43	< 0.01	< 0.01	< 0.01
Root to Shoot ratio	Flooded	1.51	0.95	1.51	1.17
	Control	1.44	1.92	2.87	2.97
	Flooded/control	1.05	0.49	0.53	0.39
	p value	0.51	< 0.01	< 0.01	< 0.01
Specific leaf area (sq cm/g)	Flooded	293	240	229	223
	Control	283	240	228	225
	Flooded/control	1.04	1.00	1.00	0.99
	p value	0.36	0.99	0.83	0.56

1 - The mean of the flooded seedling measurements

2 - The mean of the control seedling measurements

3 - The mean of the flooded seedlings divided by the mean of the control seedlings

4 - The probability of a type I error

Table 3. The means, ratios, and p values associated with the flooded and unflooded treatment mean relative growth rate, mean net assimilation rate, and mean leaf area ratio measurements from the periodic harvest study. Ratios were calculated by dividing the flooded treatment mean by the unflooded treatment mean.

		Flooding interval		
		Initial - four weeks	Four weeks - nine weeks	Nine weeks - thirteen weeks
Mean relative growth rate (gram/gram*day)	Flooded [1]	0.0230	0.0075	0.0043
	Control [2]	0.0343	0.0139	0.0098
	Flooded/control [3]	0.67	0.54	0.44
	p value [4]	< 0.01	0.01	0.06
Mean net assimilation rate (gram/sq. cm.*day)	Flooded	1.67 x 10-4	0.75 x 10-4	0.54 x 10-4
	Control	2.82 x 10-4	1.84 x 10-4	1.68 x 10-4
	Flooded/control	0.59	0.41	0.32
	p value	< 0.01	< 0.01	0.01
Mean leaf area ratio (sq. cm./gram)	Flooded	140	106	83
	Control	123	76	59
	Flooded/control	1.14	1.39	1.41
	p value	0.03	< 0.01	< 0.01

1 - The mean of the flooded seedling measurements
2 - The mean of the control seedling measurements
3 - The mean of the flooded seedlings divided by the mean of the control seedlings
4 - The probability of a type I error

Table 4. The means, ratios, and p values associated with the wet site and dry site derived seedling growth parameter measurements from the periodic harvest study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

		Duration of growth			
		Initial	Four weeks	Nine weeks	Thirteen weeks
Diameter (mm)	Wet site [1]	1.86	3.16	3.83	4.38
	Dry site [2]	1.84	3.18	3.87	4.29
	Wet site/dry site [3]	1.01	0.99	0.99	1.02
	p value [4]	0.85	0.80	0.84	0.51
Height (mm)	Wet site	89.69	101.70	106.56	111.72
	Dry site	83.15	97.07	93.67	93.20
	Wet site/dry site	1.08	1.05	1.14	1.20
	p value	0.18	0.41	< 0.01	0.01
Leaf area (sq cm)	Wet site	36.69	51.08	55.03	60.92
	Dry site	38.69	52.11	54.92	53.70
	Wet site/dry site	0.95	0.98	1.00	1.13
	p value	0.23	0.59	0.69	0.10
Leaf weight (g)	Wet site	0.130	0.213	0.243	0.276
	Dry site	0.130	0.218	0.240	0.240
	Wet site/dry site	1.00	0.98	1.01	1.15
	p value	0.51	0.64	0.84	0.09
Stem weight (g)	Wet site	0.040	0.128	0.181	0.256
	Dry site	0.036	0.128	0.155	0.214
	Wet site/dry site	1.11	1.00	1.17	1.20
	p value	0.24	0.83	0.10	0.05
Root weight (g)	Wet site	0.054	0.153	0.328	0.442
	Dry site	0.048	0.153	0.330	0.428
	Wet site/dry site	1.13	1.00	0.99	1.03
	p value	0.61	0.97	0.99	0.53
Root to Shoot ratio	Wet site	1.47	1.43	2.01	1.96
	Dry site	1.47	1.44	2.33	2.18
	Wet site/dry site	1.00	0.99	0.86	0.90
	p value	0.96	0.85	0.02	0.02
Specific leaf area (sq cm/g)	Wet site	279	239	227	224
	Dry site	298	241	230	225
	Wet site/dry site	0.94	0.99	0.99	1.00
	p value	0.12	0.81	0.63	0.71

1 - The mean of the wet site seedling measurements
2 - The mean of the dry site seedling measurements
3 - The mean of the wets site seedlings divided by the mean of the dry site seedlings
4 - The probability of a type I error

Table 5. The means, ratios, and p values associated with the wet site and dry site derived seedling mean relative growth rate, mean net assimilation rate, and mean leaf area ratio measurements from the periodic harvest study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

		Growth interval		
		Initial - four weeks	Four weeks - nine weeks	Nine weeks - thirteen weeks
Mean relative growth rate (gram/gram*day)	Wet site [1]	0.0275	0.0115	0.0082
	Dry site [2]	0.0298	0.0098	0.0059
	Wet site/dry site [3]	0.92	1.17	1.39
	p value [4]	0.41	0.37	0.37
Mean net assimilation rate (gram/sq. cm.*day)	Wet site	2.22 x 10-4	1.40 x 10-4	1.26 x 10-4
	Dry site	2.26 x 10-4	1.19 x 10-4	0.96 x 10-4
	Wet site/dry site	0.98	1.18	1.31
	p value	0.89	0.35	0.32
Mean leaf area ratio (sq. cm./gram)	Wet site	128	90	72
	Dry site	136	93	73
	Wet site/dry site	0.94	0.97	0.99
	p value	0.28	0.57	0.77

1 - The mean of the wet site seedling measurements
2 - The means of the dry site seedling measurements
3 - The mean of the wet site seedlings divided by the mean of the dry site seedlings
4 - The probability of a type I error

Table 6. P values associated with growth differences between the red maple families used for the periodic harvest study as well as the calculated family correlations.

			Duration of flooding			
			Initial	Four weeks	Nine weeks	Thirteen weeks
Diameter	Flooded	p value [1]	N.S.	N.S.	< 0.01	< 0.01
		Correlation [2]	N.A.	N.A.	0.47	0.41
	Control	p value	N.S.	N.S.	N.S.	N.S.
		Correlation	N.A.	N.A.	N.A.	N.A.
Height	Flooded	p value	< 0.01	< 0.01	< 0.01	< 0.01
		Correlation	0.49	0.40	0.71	0.52
	Control	p value	< 0.01	0.01	< 0.01	< 0.01
		Correlation	0.50	0.36	0.49	0.62
Leaf area	Flooded	p value	N.S.	N.S.	0.03	N.S.
		Correlation	N.A.	N.A.	0.26	N.A.
	Control	p value	N.S.	N.S.	N.S.	N.S.
		Correlation	N.A.	N.A.	N.A.	N.A.
Stem weight	Flooded	p value	N.S.	0.02	< 0.01	< 0.01
		Correlation	N.A.	0.30	0.61	0.46
	Control	p value	< 0.01	N.S.	N.S.	0.04
		Correlation	0.39	N.A.	N.A.	0.15
Root weight	Flooded	p value	0.01	< 0.01	< 0.01	< 0.01
		Correlation	0.34	0.61	0.58	0.47
	Control	p value	0.04	N.S.	N.S.	N.S.
		Correlation	0.26	N.A.	N.A.	N.A.

1 - The probability of a type I error

2 - The family correlations which were calculated by dividing one-fourth of the additive genetic variance by the total phenotypic variance

Table 7. P values associated with the interaction between maternal hydrologic condition and flooding treatment from the gas exchange study.

	Photosynthetic rate	Stomatal conductance	Water use efficiency	Stomatal limitation
[Duration of flooding]	p value [1]	p value	p value	p value
One day	0.62	0.81	0.56	0.55
Three days	0.94	0.96	0.46	0.73
Five days	0.65	0.92	0.32	0.24
Nine days	0.58	0.58	0.11	0.44
Thirteen days	0.81	0.97	0.68	0.66
[Days since draining]				
Two days	0.54	0.42	0.58	0.86
Four days	0.62	0.81	0.56	0.55
Six days	0.67	0.80	0.29	0.33

1 - The probability of a type I error

Table 8. The means, ratios, and p values associated with the flooded and unflooded treatment photosynthetic rate and leaf conductance measurements from the gas exchange study. Ratios were calculated by dividing the flooded mean by the unflooded mean. For all means, n is six.

	Photosynthetic rate (nanoMoles/sq cm*second)				Leaf conductance (nanoMoles/sq cm*second)			
[Duration of flooding]	Flooded [1]	Control [2]	Flooded/control [3]	p value [4]	Flooded	Control	Flooded/control	p value
One day	0.183	0.275	0.67	0.07	2.32	3.33	0.70	0.12
Three days	0.133	0.283	0.47	< 0.01	1.67	3.42	0.49	< 0.01
Five days	0.123	0.246	0.50	0.01	2.03	3.49	0.58	0.02
Nine days	0.111	0.245	0.45	0.02	2.07	2.91	0.71	0.19
Thirteen days	0.090	0.196	0.46	0.03	1.91	2.47	0.77	0.24
[Days since draining]								
Two days	0.097	0.200	0.49	0.02	1.67	2.61	0.64	0.09
Four days	0.183	0.275	0.67	0.07	2.32	3.33	0.70	0.12
Six days	0.159	0.223	0.71	0.21	2.58	3.21	0.80	0.26

1 - The mean of the flooded seedling measurements

2 - The mean of the unflooded seedling measurements

3 - The flooded seedling mean divided by the unflooded seedling mean

4 - The probability of a type I error

Table 9. The means, ratios, and p values associated with the flooded and unflooded treatment water use efficiency and stomatal limitation measurements from the gas exchange study. Ratios were calculated by dividing the flooded mean by the unflooded mean. For all means, n is six.

	Water use efficiency (microMoles carbon dioxide/milliMoles water)				Stomatal limitation (percent)			
[Duration of flooding]	Flooded [1]	Control [2]	Flooded/control [3]	p value [4]	Flooded	Control	Flooded/control	p value
One day	3.23	3.90	0.83	0.42	0.386	0.442	0.87	0.48
Three days	3.21	3.44	0.93	0.57	0.393	0.467	0.84	0.15
Five days	4.24	4.69	0.90	0.45	0.334	0.38	0.88	0.35
Nine days	1.73	2.94	0.59	0.07	0.224	0.448	0.50	0.18
Thirteen days	1.60	3.25	0.49	0.07	0.223	0.432	0.52	0.07
[Days since draining]								
Two days	2.35	2.44	0.96	0.83	0.359	0.403	0.89	0.27
Four days	3.23	3.90	0.83	0.42	0.386	0.442	0.87	0.48
Six days	2.05	2.23	0.92	0.56	0.339	0.378	0.90	0.33

1 - The mean of the flooded seedling measurements

2 - The mean of the unflooded seedling measurements

3 - The flooded seedling mean divided by the unflooded seedling mean

4 - The probability of a type I error

Table 10. The means, ratios, and p values associated with the wet site and dry site derived seedling photosynthetic rate and leaf conductance measurements from the gas exchange study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

	Photosynthetic rate (nanoMoles/sq cm*second)				Leaf conductance (nanoMoles/sq cm*second)			
[Duration of flooding]	Wet site [1]	Dry site [2]	Wet site/dry site [3]	p value [4]	Wet site	Dry site	Wet site/dry site	p value
One day	0.220	0.238	0.92	0.69	2.97	2.68	1.11	0.63
Three days	0.204	0.213	0.96	0.80	2.65	2.44	1.09	0.62
Five days	0.179	0.191	0.94	0.71	3.08	2.45	1.26	0.23
Nine days	0.178	0.178	1.00	0.94	2.81	2.17	1.29	0.31
Thirteen days	0.138	0.147	0.94	0.80	2.50	1.89	1.32	0.21
[Days since draining]								
Two days	0.142	0.154	0.92	0.71	2.27	2.02	1.12	0.61
Four days	0.220	0.238	0.92	0.69	2.97	2.68	1.11	0.63
Six days	0.190	0.192	0.99	0.95	3.22	2.58	1.25	0.26

1 - The mean of the wet site derived seedling measurements
2 - The mean of the dry site derived seedling measurements
3 - The mean of the wet site seedlings divided by the mean of the dry site seedlings
4 - The probability of a type I error

Table 11. The means, ratios, and p values associated with the wet site and dry site derived seedling water use efficiency and stomatal limitation measurements from the gas exchange study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

	Water use efficiency (microMoles carbon dioxide/milliMoles water)				Stomatal limitation (percent)			
[Duration of flooding]	Wet site [1]	Dry site [2]	Wet site/dry site [3]	p value [4]	Wet site	Dry site	Wet site/dry site	p value
One day	3.31	3.82	0.87	0.54	0.383	0.445	0.86	0.44
Three days	3.10	3.54	0.88	0.36	0.409	0.450	0.91	0.43
Five days	3.58	5.36	0.67	0.09	0.302	0.412	0.73	0.10
Nine days	2.48	2.20	1.13	0.32	0.364	0.307	1.19	0.74
Thirteen days	2.15	2.71	0.79	0.60	0.289	0.366	0.79	0.58
[Days since draining]								
Two days	2.10	2.70	0.78	0.17	0.352	0.410	0.86	0.35
Four days	3.31	3.82	0.87	0.54	0.383	0.445	0.86	0.57
Six days	1.92	2.36	0.81	0.17	0.325	0.392	0.83	0.11

1 - The mean of the wet site derived seedling measurements
2 - The mean of the dry site derived seedling measurements
3 - The mean of the wet site seedlings divided by the mean of the dry site seedlings
4 - The probability of a type I error

Table 12. P values associated with the interaction between maternal hydrologic condition and flooding treatment from the root physiology study.

	Shoot water potential	Root carbon dioxide evolution	Root ethylene evolution
[Duration of flooding]	p value [1]	p value	p value
One day	0.18	0.95	0.94
Three days	0.16	0.19	0.47
Five days	0.97	0.57	0.99
Twelve days	0.53	0.81	0.90
[Days since draining]			
One day	0.51	0.95	0.98
Seven days	0.18	0.39	0.60

1 - The probability of a type I error

Table 13. The means, ratios, and p values associated with of the flooded and unflooded treatment water potential measurements from the root physiology study. Ratios were calculated by dividing the flooded mean by the unflooded mean. For all means, n is six.

	Shoot water potential (MPa)			
[Duration of flooding]	Flooded [1]	Control [2]	Flooded/control [3]	p value [4]
One day	-0.528	-0.490	1.08	0.21
Three days	-0.560	-0.585	0.96	0.48
Five days	-0.534	-0.603	0.89	0.02
Twelve days	-0.558	-0.635	0.88	< 0.01
[Days since draining]				
One day	-0.428	-0.538	0.80	< 0.01
Seven days	-0.443	-0.463	0.96	0.24

1 - The mean of the flooded seedling measurements

2- The mean of the unflooded seedling measurements

3 - The flooded seedling mean divided by the unflooded seedling mean

4 - The probability of a type I error

Table 14. The means, ratios, and p values associated with of the flooded and unflooded treatment root carbon dioxide evolution rate measurements from the root physiology study. Ratios were calculated by dividing the flooded mean by the unflooded mean. For all means, n is six.

	Root carbon dioxide evolution rate (micoMoles/gram*minute)			
[Duration of flooding]	Flooded [1]	Control [2]	Flooded/control [3]	p value [4]
One day	0.311	0.438	0.71	0.06
Three days	0.411	0.652	0.63	< 0.01
Five days	0.245	0.519	0.47	< 0.01
Twelve days	0.342	0.594	0.58	< 0.01
[Days since draining]				
One day	0.413	0.722	0.57	< 0.01
Seven days	0.444	0.500	0.89	0.08

1 - The mean of the flooded seedling measurements

2- The mean of the unflooded seedling measurements

3 - The flooded seedling mean divided by the unflooded seedling mean

4 - The probability of a type I error

Table 15. The means, ratios, and p values associated with of the flooded and unflooded treatment root ethylene evolution measurements from the root physiology study. Ratios were calculated by dividing the flooded mean by the unflooded mean. For all means, n is six.

	Root ethylene evolution (nanoMoles/gram*minute)			
[Duration of flooding]	Flooded [1]	Control [2]	Flooded/control [3]	p value [4]
One day	0.0104	0.0100	1.04	0.74
Three days	0.0128	0.0112	1.14	0.27
Five days	0.0080	0.0096	0.83	0.05
Twelve days	0.0082	0.0107	0.77	0.07
[Days since draining]				
One day	0.0119	0.0121	0.98	0.77
Seven days	0.0108	0.0146	0.74	0.09

1 - The mean of the flooded seedling measurements

2- The mean of the unflooded seedling measurements

3 - The flooded seedling mean divided by the unflooded seedling mean

4 - The probability of a type I error

Table 16. The means, ratios, and p values associated with the wet site and dry site derived seedling water potential measurements from the root physiology study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

	Shoot water potential (MPa)			
[Duration of flooding]	Wet site [1]	Dry site [2]	Wet site/dry site [3]	p value [4]
One day	-0.525	-0.494	1.06	0.29
Three days	-0.614	-0.531	1.16	0.07
Five days	-0.579	-0.558	1.04	0.30
Twelve days	-0.622	-0.571	1.09	0.01
[Days since draining]				
One day	-0.514	-0.452	1.14	0.01
Seven days	-0.466	-0.440	1.06	0.05

1 - The mean of the wet site derived seedling measurements

2- The mean of the dry site derived seedling measurements

3 - The mean of the wet site derived seedlings divided by the mean of the dry site derived seedlings

4 - The probability that of a type I error

Table 17. The means, ratios, and p values associated with the wet site and dry site derived seedling root carbon dioxide evolution measurements from the root physiology study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

	Root carbon dioxide evolution (microMoles/gram*minute)			
[Duration of flooding]	Wet site [1]	Dry site [2]	Wet site/dry site [3]	p value [4]
One day	0.384	0.366	1.05	0.73
Three days	0.521	0.542	0.96	0.31
Five days	0.369	0.395	0.93	0.29
Twelve days	0.447	0.488	0.92	0.69
[Days since draining]				
One day	0.565	0.570	0.99	0.84
Seven days	0.464	0.480	0.97	0.39

1 - The mean of the wet site derived seedling measurements

2- The mean of the dry site derived seedling measurements

3 - The mean of the wet site derived seedlings divided by the mean of the dry site derived seedlings

4 - The probability that of a type I error

Table 18. The means, ratios, and p values associated with the wet site and dry site derived seedling root ethylene evolution measurements from the root physiology study. Ratios were calculated by dividing the wet site mean by the dry site mean. For all means, n is six.

Root ethylene evolution
(nanoMoles/gram*minute)

[Duration of flooding]	Wet site [1]	Dry site [2]	Wet site/dry site [3]	p value [4]
One day	0.0116	0.0088	1.32	0.30
Three days	0.0127	0.0113	1.12	0.14
Five days	0.0089	0.0086	1.03	0.81
Twelve days	0.0101	0.0088	1.15	0.29
[Days since draining]				
One day	0.0127	0.0113	1.12	0.42
Seven days	0.0130	0.0125	1.04	0.60

1 - The mean of the wet site derived seedling measurements

2- The mean of the dry site derived seedling measurements

3 - The mean of the wet site derived seedlings divided by the mean of the dry site derived seedlings

4 - The probability that of a type I error

Appendix A. Sample calculations for CO_2 determination.

CO_2 determination:

Li-Cor volume

An injection of 40 μliters of pure CO_2 resulted in a 723.5 ppm CO_2 incrase.

$$1\,\text{ppm} = \frac{1\ \mu\text{liter}}{\text{liter}} \therefore \frac{40\ \mu\text{liters}}{723.5\ \text{ppm}} = 0.0553\ \text{liters}$$

Detemination of an unknown CO_2 concentration

After incubating 113 minutes, a 100 μliter injection of sample contained 38 ppm CO_2 more than an initial 100 μliter injection. The incubated tissue weighed 0.2621 grams and was contained in a 15 milliler test tube.

$$\therefore \frac{38\ \mu\text{liters}\ CO_2}{\text{liter}} \times 0.0553\,\text{liters} = 2.10\ \mu\text{liters}\ CO_2$$

1 mole of gas = 22.4 liters

$$\therefore 2.10\ \mu\text{liters}\ CO_2 \times \frac{\mu\text{mole}}{22.4\ \mu\text{liters}} = 0.0938\ \mu\text{moles}\ CO_2$$

$$\frac{0.0938\ \mu\text{moles}\ CO_2}{0.2621\,\text{grams} \times 113\ \text{minutes}} = \frac{0.00317\ \mu\text{moles}\ CO_2}{\text{gram} \times \text{minute}}$$

100 μliters is $\frac{1}{150}$ of the total test tube volume

$$\therefore \frac{0.00317\ \mu\text{moles}\ CO_2}{\text{gram} \times \text{minute}} \times 150 = \frac{0.4755\ \mu\text{moles}\ CO_2}{\text{gram} \times \text{minute}}$$

Appendix B Sample calculations for ethylene determination.

Ethylene determination

Standard calculation

A 4 μliter injection of standard containing 94.5 ppm ethylene resulted in a peak of 54 millimeters in height.

$$\therefore \frac{94.5\mu\text{liters}}{\text{liter}} \times \frac{1\mu\text{mole}}{22.4\mu\text{liters}} \times 4\mu\text{liters} \times \frac{10^{-6}\text{liters}}{\mu\text{liter}} \times \frac{1}{54\text{ mm}} = \frac{3.15 \times 10^{-7}\mu\text{moles}}{\text{millimeter}}$$

Ethylene calcualtion

After incubating 313 minutes, a 250 μliter injection of sample resulted in a peak of 46 millimeters. The incubated tissue weighed 0.2621grams and was contained in a 15 milliliter test tube.

$$\therefore 46\text{millimeters} \times \frac{3.15 \times 10^{-7}\ \mu\text{moles}}{\text{millimeter}} = 1.44 \times 10^{-5}\ \mu\text{moles ethylene}$$

$$\frac{1.44 \times 10^{-5}\mu\text{moles}}{313\text{ minutes} \times 0.2621\text{grams}} = \frac{1.76 \times 10^{-7}\ \mu\text{moles ethylene}}{\text{gram} \times \text{minute}}$$

250 μliters is $\frac{1}{60}$ of the total test tube volume

$$\therefore \frac{1.76 \times 10^{-7}\ \mu\text{moles ethylene}}{\text{gram} \times \text{minute}} \times 60 \times \frac{1000\ \eta\text{moles}}{\mu\text{mole}} = \frac{0.0106\ \eta\text{moles ethylene}}{\text{gram} \times \text{minute}}$$

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