This is to certify that the

thesis entitled

EFFECTS OF FLOODING DURATION ON THE GROWTH, PHYSIOLOGY, AND STARCH RESERVES OF BLACK ASH (FRAXINUS NIGRA MARSH.), GREEN ASH (FRAXINUS PENNSYLVANICA MARSH.), AND WHITE ASH (FRAXINUS AMERICANA L.) SEEDLINGS.

presented by

Renee Rose Cloutier

has been accepted towards fulfillment
of the requirements for

M. S. degree in Horticulture

Major professor

Date May 12, 1999

EFFECTS OF FLOODING DURATION ON THE GROWTH, PHYSIOLOGY, AND STARCH RESERVES OF BLACK ASH (FRAXINUS NIGRA MARSH.), GREEN ASH (FRAXINUS PENNSYLVANICA MARSH.), AND WHITE ASH (FRAXINUS AMERICANA L.) SEEDLINGS.

By

Renee Rose Cloutier

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Horticulture

1999

ABSTRACT

EFFECTS OF FLOODING DURATION ON THE GROWTH, PHYSIOLOGY, AND STARCH RESERVES OF BLACK ASH (FRAXINUS NIGRA MARSH.), GREEN ASH (FRAXINUS PENNSYLVANICA MARSH.), AND WHITE ASH (FRAXINUS AMERICANA L.) SEEDLINGS.

By

Renee Rose Cloutier

The effect of flood duration on growth, gas exchange, and starch content of bare root black ash (*Fraxinus nigra* Marsh.), green ash (*F. pennsylvanica* Marsh.), and white ash (*F. americana* L.) seedlings was studied to characterize their flood response and compare their flooding tolerance. Seedlings were flooded for 0, 4, 8, 16, and 32 days with the recovery interval extended to day 60. Green ash seedlings grew throughout the study however flooding reduced stem height, leaf number, and leaf area of this species. In contrast, black and white ash seedlings did not grow during flood stress. After 32 days of flooding, root starch concentrations were lower in flooded seedlings than in the control seedlings. Flooded green ash seedlings had no reduction in root biomass when compared to control seedlings. Root biomass was reduced in both black and white ash seedlings in response to flood. Flooding also decreased stomatal conductance followed by a reduction in assimilation for black, white, and green ash seedlings, respectively. Green ash seedlings maintained a higher assimilation rate than black and white ash seedlings throughout the 32- day flood. Hence, green ash seedlings recovered earlier from flood stress and is considered more flood tolerant than both black and white ash seedlings.

Pour Rose-Helene

ACKNOWLEDGMENTS

I am grateful to my major advisor, Dr. R.E. Schutzki, for his patience and support during the course of my graduate studies. I also want to thank my committee members, Drs. J.A. Flore and F. Ewers for their encouragement and helpful criticisms. A special thanks to Drs. A. Janoudi, and F. Dennis for their review of the manuscripts, to Drs. W. Loescher and Z. Gao for their helpful advice on a method to determine starch and to Drs. G. Hosfield, M. Nair, and J. Hancock for the use of their laboratory equipment. Many others have contributed to this work and also deserve mention such as Susan Gruber, Dr. Shirley Owens, Dave Freville, Bill Chase and the staff of the MSU Horticulture Teaching and Research Center, Jerome Kosman, Burke Jenkins, Katie Wilkinson, Mike Meyers, Jamie Mulvaney, Carrie May, Sarah Breitzkreut, and Leo Lombardini for their assistance in the field and laboratory. I appreciate your time, effort, and most of all your friendship. Finally, I want to thank my parents, Yvon and Gaetane Cloutier, and my husband, Clifford Beninger, for their love and support.

TABLE OF CONTENTS

LIST OF TABLES

Table		Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

General and physiological terms

A . . . . carbon assimilation

ca. . . . . circa (approximately)

E . . . . transpiration

Eh . . . . soil redox potential

g_s . . . . stomatal conductance

O_2 . . . . oxygen gas

Rubisco . . . . ribulose-1,5- biphosphate

WUE . . . . water use efficiency

INTRODUCTION

Woody plants have different strategies to deal with environmental stresses such as flooding, drought, soil compaction, extremes in temperature, and high salinity. These vary according to species and include both morphological and physiological adaptations. Even closely related species will differ in flood tolerance, as seen by Norby and Kozlowski (1983) for *Betula papyrifera* and *B. nigra*. In a 60 day study, flooding reduced growth of *B. nigra*; but, not to the extent seen in *B. papyrifera* where leaf area was drastically reduced. Inhibition of new leaves and senescence decreased growth and for many seedlings, flooding lead to death. In contrast, *B. nigra* seedlings produced hypertrophied lenticels and adventitious roots. Both responses are viewed as adaptations to flood stress that enhance gas exchange and help eliminate the buildup of toxic metabolites whereas other metabolic processes help to tolerate anoxia. Consequently, each trait can contribute to a species' plasticity and ecological success. Unfortunately, criteria used to determine flooding tolerance differ with each investigation. Changes in growth, gas exchange, injury, and survival are common parameters whereas some studies include hormone or enzyme activity. For example in Phung and Knipling (1976), photosynthesis rates for four citrus rootstocks were 80 to 90 % lower within ten days of flooding. Clemens et al. (1978) determined this flood tolerance order with *Eucalyptus grandis* > *E. robusta* > *E. saligna* was related to the formation of adventitious roots. Anella and Whitlow (1999) ranked seven red maple cultivars by characterizing photosynthesis, lenticel intumescence, and survival in order to make cultivar recommendations for use in urban landscapes. In addition, Tang and Kozlowski (1983) reported that flooding reduced the height growth for *Pinus banksiana* and *P. resinosa*. Each study contributes to our knowledge of woody species in response to flooding stress.

Although characteristics of the flood water, seasonality, and duration of flooding all affect response, such responses vary with the age of plant, seedlings being more susceptible than mature trees. Initially, flooding reduces the oxygen content of the soil and this causes variable and complex changes in plant-water relations including transient wilting, reduced transpiration, and a decline in root conductivity (Kozlowski et al. 1991; Reid et al. 1991). Roots are sensitive to oxygen deficiency and no records of sustained growth by anoxic roots has been reported (Armstrong et al. 1994). Under hypoxic or anoxic conditions, seedling survival by anaerobic metabolism is dependant on the availability of carbohydrate reserves and temperature, which controls respiration rate (Jackson and Drew 1984). In addition, the elimination of toxic metabolites such as ethanol, acetaldehyde, or ethylene may also be critical for the root system (Chirkova and Gutman 1972). Not surprisingly, flooding adversely affects stem, leaf, and cambial growth as well as reproductive growth of trees (Kozlowski 1985). Many studies have indicated that above-ground processes such as photosynthesis and stomatal conductance also decrease. For example, Kozlowski and Pallardy (1979) observed that flooding induced stomatal closure in 5 month old green ash seedlings with a gradual reopening. Pezeshki and Chambers (1986) showed that flooding green ash and baldcypress did not cause a significant decrease in photosynthesis despite a 50 % decrease in stomatal conductance. In another study, Pezeshki et al. (1996) reported a reduction in stomatal conductance of *Quercus lyrata* and *Q. falcata* but not in *Taxodium distichum* under low soil redox conditions. Reduced net photosynthesis, root and leaf dry weights were also observed for these three species.

In North America, 16 native species belong to the genus *Fraxinus*. The ashes represent a unique group of trees because many species have markedly different native habitats. Two trees commonly planted in urban landscapes, green (*Fraxinus pennsylvanica* Marsh.) and white ash (*Fraxinus americana* L.), differ in their ability to tolerate flooding stress. Green ash is one of the most adaptable trees, with a wide geographic distribution. Green ash is a highly flood-tolerant species that can grow on land subjected to periodic flooding and display vigorous growth even when flooded for much of the growing season (Tang and Kozlowski 1984). For example, Broadfoot and Williston (1973) showed that green ash had a 50 to 100 % increase in radial growth during flood years. In contrast, white ash, which usually appears on deep, well-drained upland soils, is a flood-intolerant species (Hall and Smith 1955; Kozlowski et al. 1991). Very little information is available for white ash and its response to flood stress. Black ash (*Fraxinus nigra* L.) is an extremely hardy species common to low woodlands, swamps, and periodically inundated river bottoms. Often referred to as swamp or hoop ash, black ash can tolerate standing water for many weeks usually when spring or early summer flooding occurs. Black ash is a determinant species meaning that the next season's growth is determined by climatic conditions during the previous season (Lechowicz 1984; Tardiff and Bergeron 1993).

Comparison of the morphological and physiological response of three Fraxinus species will add to our knowledge of flood tolerance mechanisms of woody species. In addition, it can indicate why ashes occupy such broad ecological niches. Practical benefits of this research can also be applied in plant selection process and identifying species able to tolerate flooding and soil compaction common in urban landscapes. For these reasons, observation of growth, gas exchange, and starch reserves will be critical for determining the flood tolerance of each species. Little research has been done on how closely related species in the genus *Fraxinus* cope with environmental stresses such as flooding. On a broader scale, information on how flood duration changes carbon partitioning is needed especially since late-season stresses that affect root starch reserves can severely impact future growth and survival (Loescher et al. 1990). Therefore, our objectives were to characterize and compare the growth, physiology, and starch content of black, green, and white ash seedlings exposed to increasing durations of flooding stress during the summer.

LITERATURE CITED

Anella L.B. and T.H. Whitlow 1999. Flood-tolerance ranking of red and Freeman maple cultivars. J. of Arboriculture 25: 31-36.

Armstrong W., R. Brandle and M.B. Jackson 1994. Mechanisms of flood tolerance in plants. Acta Bot. Neerl 43: 307-358.

Broadfoot W.M. and H.L. Williston 1973. Flooding effects in southern forest. J. For. 71: 584-587.

Chirkova T.V. and T.S. Gutman 1972. Physiological role of branch lenticels in willow and poplar under conditions of root anaerobiosis. Sov. Plant Phys. 19: 289-295.

Clemens J., A.M. Kirk and P.D. Mills 1978. The resistance to waterlogging of three Eucalyptus species, effect of flooding and of ethylene releasing growth substances on *E. robusta*, *E. grandis* and *E. saligna*. Oecologia 34: 125-131.

Dirr M.A. 1990. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. (4th Ed.) Stipes Publishing Co., 10-12 Chester St., Champaign, IL 61820. 1007 p.

Hall T.F. and G.E. Smith 1955. Effects of flooding on woody plants, West Sandy dewatering project, Kentucky Reservoir, J. For. 53: 281-285.

Jackson M.B. and M.C. Drew 1984. Effects of flooding on growth and metabolism of herbaceous plants. In Flooding and Plant Growth (T.T. Kozlowski, Ed.) Academic Press, Orlando, FL, pp. 47-128.

Kozlowski T.T. and S.G. Pallardy. 1979. Stomatal responses of *Fraxinus pennsylvanica* seedlings during and after flooding. Physiol. Plant. 46: 155-158.

Kozlowski T.T. 1984. Plant responses to flooding of soil. BioScience 34: 162-167.

Kozlowski T.T. 1984. Flooding and plant growth. Academic Press, Inc. Orlando, FL, 356 p.

Kozlowski T.T. 1985. Soil aeration, flooding, and tree growth. J. Arboriculture 11: 85-96.

Kozlowski T.T., P.J. Kramer, and S.G. Pallardy 1991. The physiological ecology of woody plants. Academic Press, Inc., San Diego, CA, USA.

Lechowicz M.J. 1984. Why do temperate deciduous trees leaf out at different times? adaptation and ecology of forest communities. Am. Nat. 124: 821-842.

Loescher W.H. ,T. McCamant, and J.D. Keller 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience 25: 274-281.

Norby R.J. and T.T. Kozlowski 1983. Flooding and SO_2 stress interaction in *Betula papyrifera* and *B. nigra* seedlings. For. Sci. 29:

Pezeshki S.R. and J.L. Chambers 1986. Variations in flood-induced stomatal and photosynthetic responses of three bottom-land tree species. For. Sci. 32: 914-923.

Pezeshki S.R., J.H. Pardue, and R.D. DeLaune. 1996. Leaf gas exchange and growth of flood-tolerant and flood-sensitive tree species under low soil redox conditions. Tree Physiology 16: 453-458.

Phung H.T. and E.B. Knipling 1976. Photosynthesis and transpiration of citrus seedlings under flooded conditions. HortScience 11: 131-133.

Reid D.M., F.D. Beall, and R.P. Pharis 1991. Environmental cues in plant growth and development. In: Plant Physiology, A Treatise Vol X: Growth and Development

Chapter 2, Academic Press, Inc. pp.65-181.

Tang Z.C. and T.T. Kozlowski 1984 Water relations, ethylene production, and morphological adaptation of *Fraxinus pennsylvanica* seedlings to flooding. Plant Soil 77: 183-192.

Tardiff J. and Y. Bergeron 1993. Radial growth of *Fraxinus nigra* in a Canadian boreal floodplain in response to climatic and hydrological fluctuations. J. Veg. Sci. 4: 751-758.

CHAPTER 1

EFFECTS OF FLOODING DURATION ON THE GROWTH AND PHYSIOLOGY OF BLACK ASH (FRAXINUS NIGRA MARSH.), GREEN ASH (FRAXINUS PENNSYLVANICA MARSH.), AND WHITE ASH (FRAXINUS AMERICANA L.) SEEDLINGS.

ABSTRACT

Bare root black ash (*Fraxinus nigra* Marsh.), green ash (*Fraxinus pennsylvanica* Marsh.), and white ash (*Fraxinus americana* L.) 2-1 seedlings 30.5 to 45.7 cm in height were planted into 19 liter plastic containers filled with a sterilized 2:1 (v/v) sand:loam mixture. Flood treatments were imposed for 0, 4, 8, 16, and 32 days with the recovery interval extended to day 60. Minimal growth was observed for black and white ash seedlings. For these two species, flooding for 32 days reduced dry weight biomass 46 % in roots. Differences in biomass were not observed for green ash seedlings regardless of treatment, but stem height, leaf number, and leaf area were reduced by flooding for 8, 16, and 32 days. Green ash seedlings were slower to react to flooding stress than were black and white ash seedlings. Flooded black, white, and green ash seedlings had reductions in stomatal conductance on days 2, 4, and 8, respectively. Lower assimilation rates were observed by day 4, 8, and 11. During recovery, opposite results were observed with green, black, and finally white ash seedlings reopening their stomata and resuming photosynthesis. Physiological recovery for each species was modified by the plant response and duration of the flood. Once a reduction in stomatal conductance occurred, *Fraxinus* seedlings required a 12 to 16 day period to recover. As the flood duration increased to 32 days, a shorter recovery time of 8 days was observed. Thereafter, the ability of black and white ash seedlings to recover increased to 12 days compared to green ash seedlings which recovered after 8 days.

INTRODUCTION

Responses of woody plants to environmental stresses are known to vary appreciably. For example, Ceulemans et al. (1984) reported that variations in gas exchange rates among different plant species (or cultivars) are determined by both environmental factors and plant characteristics. Consequently, woody plants respond to stresses, such as flooding, drought, soil compaction, wind, and extremes in temperatures, with different morphological and physiological adaptations. Many of these adaptations are related to a species' plasticity and ecological success.

Two trees commonly seen in urban landscapes are green ash (*Fraxinus pennsylvanica* Marsh.) and white ash (*Fraxinus americana* L.). Green ash is one of the most adaptable trees, with a wide geographic distribution. Green ash is a highly flood-tolerant species that can grow on land subjected to periodic flooding and display vigorous growth even when flooded for much of the growing season (Tang and Kozlowski 1984). In contrast, white ash, which usually appears on deep, well-drained upland soils, is a flood-intolerant species (Hall and Smith 1955; Kozlowski et al. 1991). Black ash (*Fraxinus nigra* L.) is an extremely hardy species common to low woodlands, swamps, and periodically inundated river bottoms. Often referred to as swamp or hoop ash, black ash can tolerate standing water for many weeks. Black ash is also a determinant species meaning that the next season's growth is determined by climatic conditions during the previous season (Lechowicz 1984; Tardiff and Bergeron 1993).

Conditions of flood water, seasonality, and duration are factors that determine a species' response. The response also changes with the age of the plant, with seedlings more susceptible than mature trees. Initially, flooding reduces the oxygen content of the soil (Kozlowski et al. 1991). This affects many aspects of growth and development (Kozlowski ed. 1984). For example, flooding of roots causes variable and complex changes in plant-water relations including transient wilting, reduced transpiration, and a decline in root conductivity. Some researchers find no water deficit, while others describe symptoms of long-term water deficit (Reid et al. 1991). In 1979, Kozlowski and Pallardy observed that flooding induced stomatal closure in 5 month old green ash seedlings with a gradual reopening. Pezeshki and Chambers (1986) showed that flooding green ash and baldcypress did not cause a significant decrease in photosynthesis despite a 50 % decrease in stomatal conductance. The physiological changes caused by flooding stress is critical to the plant's survival, growth, and future development.

The objectives of this study were to characterize and compare the growth and physiology of black, green, and white ash seedlings exposed to increasing durations of flooding stress during the summer. Determination of these variables may help to explain differences in physiological and ecological responses among these three species with respect to flood tolerance.

MATERIALS AND METHODS

Plant materials

Bare root black ash, green ash, and white ash seedlings (2-1; 30.5 to 45.7 cm in height) were obtained from Lawyers' Nursery in Plains, Montana, USA, and stored in a cold room at 2-3 °C until potted. Seedlings were ranked based on uniform caliper size taken at the root collar. Seedling roots were pruned to 18 to 20 cm prior to planting into 19-liter plastic containers (Classic 1200, Mollema, 34 cm width x 38 cm height) lined at the bottom with a layer of cheese cloth and filled with a sterilized 2:1 (v/v) sand:loam mixture. Lateral branches were pruned and one terminal shoot was allowed to develop for each seedling. Seedlings were placed in an uncovered frame house at the Michigan State University Horticulture Teaching and Research Center, Lansing, MI, USA (42.8°N, 84.5°W) where they were kept well watered until the beginning of the flood treatment. Seedlings were allowed to grow 4 weeks prior to the onset of flood duration treatments. The flood treatment began on the 24th of June 1998, and continued for 32 days. The recovery interval began upon release from flood for each treatment and lasted until August 23rd, 1998 on day 60. Thus, seedlings flooded for 4, 8, 16 and 32 days recovered for a total of 56, 52, 44, and 28 days, respectively. Throughout the recovery period, seedlings were fertilized once a week with 200 ppm of nitrogen from a 20:10:20 NPK soluble fertilizer using a HOZON applicator.

Experimental Design

This factorial experiment was arranged in a completely randomized design with a total of

264 seedlings assigned to one of two groups. In the first group, eight replicate seedlings of each species (black, green, and white ash) were flooded for durations of 0 (control), 4, 8, 16, or 32 days and monitored for growth and gas exchange. Control seedlings were watered regularly to maintain soil moisture below or near field capacity. In the second group, eight replicate seedlings of each species were flooded for durations of 0 (control), 8, 16, and 32 days. These seedlings were used to determine the effect of flooding on biomass and carbohydrate reserves (Chapter 2). To ensure uniformity in soil moisture, soil moisture data was recorded every 4 days on the control and drained seedlings using a Theta Meter type HH1 (Delta-T Devices, Cambridge, England) equipped with a Theta Probe type ML1. The flood treatments were imposed by inserting the 19-liter container inside a larger container lined with a reinforced plastic bag. The water level was maintained near the rim of the container using a drip irrigation system controlled by a time clock (Model LX-12, Rainbird, USA), so as to mimic standing water. Plants released from the flood treatment were allowed to drain and were maintained similar to the control seedlings.

Growth parameters

Growth measurements were taken every 4 days on 120 seedlings. Current stem lengths were measured using a ruler to the nearest mm. The current stem lengths were measured from the terminal bud scar to just below the apical meristem. The average of two stem diameter measurements (45 to 90° angles from each other) were taken using a Max Series Electronic Digital Calipers (Fred V. Fowler Company Inc., Japan) to the nearest 0.1 mm at 2 cm above the container rim. Leaf number was determined by counting. Data is reported as the increase in leaves from the onset of flooding. Leaflet area was measured by taking the length and width of the most recently expanding terminal leaflet and multiplying these by a correction coefficient previously estimated by measuring the lamina length and width of individual terminal leaflets and determining their area using a Delta-T Area Measurement System (Delta-T Devices Ltd., Cambridge, England). Correction coefficients for black, green, and white ash terminal leaflets were 0.6129, 0.7154, and 0.6823. These were determined from 20 terminal leaflets for green ash and 24 terminal leaflets for black and white ash. Regressions between the estimated leaflet area and measured area were highly correlated (r^2= 0.98) for each species (Figure 1.1). Separate t-tests comparing the estimated and measured areas were not different, indicating the accuracy of the correction coefficient (black ash F=1.07, P=0.8682; green ash F=1.08, P=0.8654; white ash F=1.14, P=0.7570).

After 8, 16, and 32 days of flood stress, control and flooded seedlings from the second group were harvested to determine the dry weight for each plant component. Leaves, old and current stems, and roots were washed, separated, bagged, and kept in coolers until processed in the laboratory. Fresh and dry weights were determined using an analytical balance (Mettler PM460, Mettler Instruments Corp., Hightstown, NJ, USA) to an accuracy of 0.001 g. Prior to lyophilization (Virtis, Genesis 12EL, Gardiner, NY, USA), samples stored in a -30 °C freezer were transferred to a -80 °C freezer (Fisher Scientific, Isotemp Freezer, USA). This prevented thawing of the samples during the freeze-drying process.

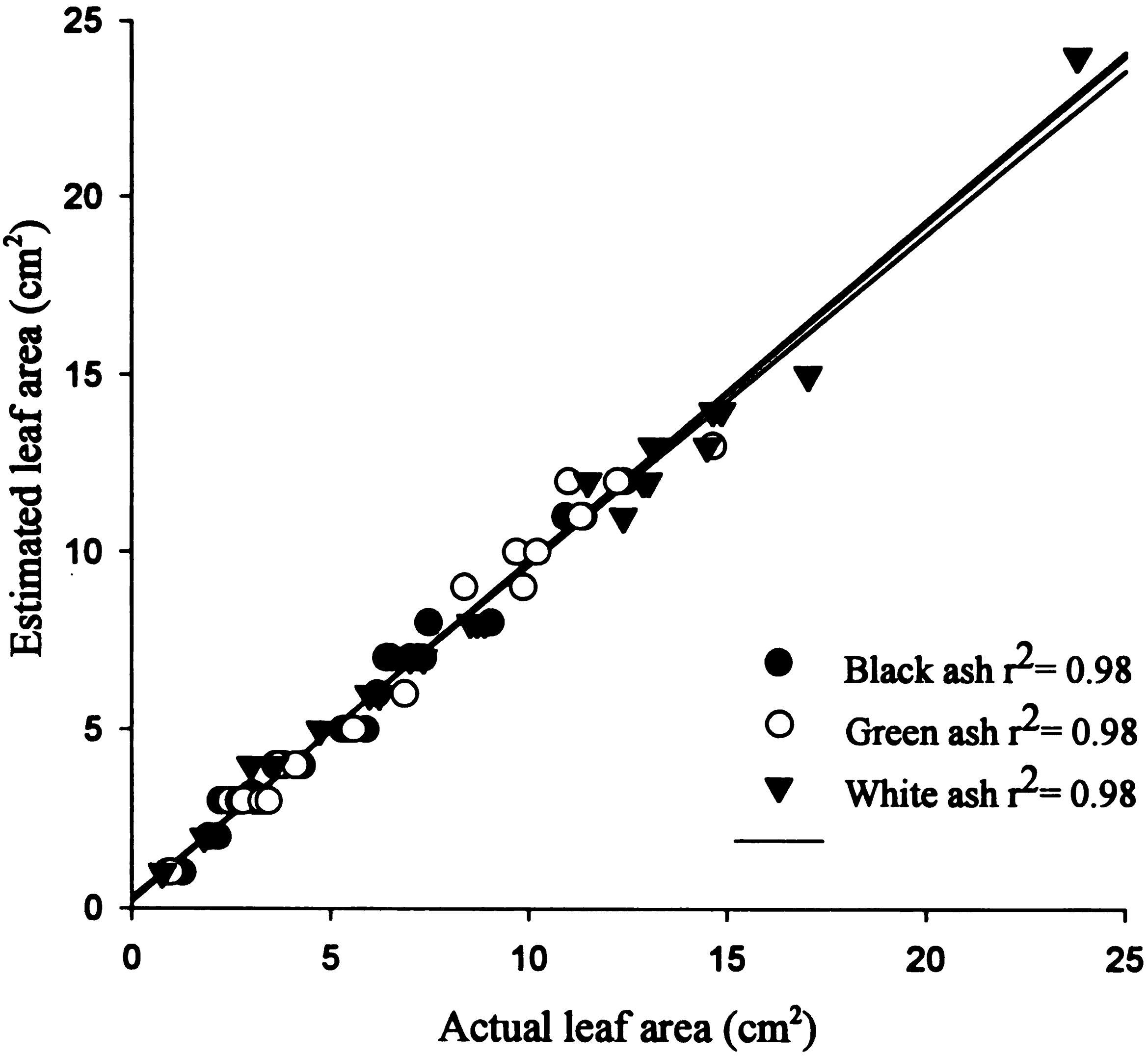
Gas exchange

Carbon assimilation (A), stomatal conductance (g_s), transpiration (E), and water use efficiency (WUE) were determined using a CIRAS-1 photosynthesis system (PP Systems, Haverhill, MA, USA) equipped with a CIRAS-1 broad Parkinson leaf cuvette (PP Systems, Haverhill, MA, USA). Gas exchange measurements were taken on a 4-day interval, or when weather permitted between 9:30 and 1:30 HR on the terminal leaflet of a mature fully expanded leaf. Control and 32-day flooded seedlings were measured throughout the study with seedlings flooded for 4, 8, and 16 days added after release from their respective flood treatments until the end of the experiment.

Statistical analyses

Growth and gas exchange parameters were analysed for each flood treatment interval. Growth in caliper, current stem height, and leaf number were calculated by subtracting initial measurements on day 0 from the data measured on day 60. Leaf area expansion (terminal leaflet) was calculated by subtracting initial measurements on day 0 from data collected on day 32 because leaf expansion had ceased. Regressions of the estimated and measured leaflet areas were conducted followed by a t-test to ensure the accuracy of the correction coefficient to estimate the leaflet area for each species. Physiological data were pooled to compare species and flood duration for the entire study. Analysis of variance was conducted using PROC GLM and regression analysis using the PROC REG procedure in SAS/PC software (SAS Institute Inc. 1985). Comparisons were tested using a Duncan's multiple range test. Probabilities less than or equal to 0.05 were regarded as significant.

Figure 1.1. Regression of estimated terminal leaflet area over the actual leaflet area measured from three ash species. Estimated leaflet area was calculated by length x width x correction coefficient. Correction coefficients for black, green, and white ash terminal leaflets are 0.6129, 0.7154, and 0.6823. Regression line for each species was based on 20-24 terminal leaflets.


Estimated leaf area (cm²)
Actual leaf area (cm²)
0
5
10
15
20
25
Black ash r²= 0.98
Green ash r²= 0.98
White ash r²= 0.98

RESULTS

Growth parameters

Changes in caliper and leaf area had significant species and duration main effects (Table 1.1). Green ash seedlings accrued a greater caliper and leaf area compared to black and white ash seedlings. In addition, white ash seedlings had a greater leaf area when compared to black ash seedlings. As the flood duration increased, stem caliper growth increased proportionally whereas the leaf area decreased. In general, ash seedlings flooded for 32 days had a greater increase in caliper (1.98 mm) than seedlings flooded for the other durations. Eight and sixteen day seedling calipers (1.39 and 1.52 mm) exceeded caliper for seedlings flooded for 0 and 4 days (1.09 and 1.02 mm). Increases in the leaf area of the terminal leaflet were greater (ca. 2 fold difference) for controls with a value of 15.72 cm^2 when compared to seedlings flooded for 4, 8, 16, and 32 days with values ranging between 7.13 and 7.55 cm^2.

Current stem height and leaf number had a significant species x flood duration interaction (Table 1.2). Green ash control and 4-day flooded seedlings had greater stem growth (7.65 and 6.60 cm) compared to seedlings flooded for 8,16, and 32 days (2.06, 1.41, and 3.08 cm). Minor current stem growth of 0.45 to 0.70 cm were observed for black and white ash seedlings, respectively. Leaf numbers were also greater in green ash seedlings compared to black and white ash counterparts, in which there was no increase and even a decrease in leaf number. Green ash control and 4-day flooded seedlings had a similar increase in leaf number (6.63 and 5.63). These values were greater than 8-day flooded green ash with a value of 3.75, which was greater than 16 and 32-day flooded green ash seedlings

Table 1.1. Mean increase in caliper and leaf area for black, green, and white ash seedlings flooded for 0, 4, 8, 16, and 32 days. The changes in caliper were presented for the entire study (0 to 60 days) whereas leaf area were presented during the flood (0 to 32 days) period. Means, followed by the same letter within a column for each main effect, do not differ significantly (Duncan, $P \leq 0.05$).

	Growth parameters	
Main effects	Caliper (mm)	Leaf area (cm^2)
Species		
Black ash	1.18b	2.69c
Green ash	1.84a	17.78a
White ash	1.19b	6.49b
Duration		
Day 0	1.09cd	15.72a
Day 4	1.02d	7.55b
Day 8	1.39bc	7.54b
Day 16	1.52b	7.21b
Day 32	1.98a	7.13b

Table 1.2. Mean current stem height growth and increase in leaf number for black, green, and white ash seedlings flooded for 0, 4, 8, 16, and 32 days. Stem height and leaf number were presented for the entire study (0 to 60 days). Means, followed by the same letter within a column for each species x flood duration interaction, do not differ significantly (Duncan, n=8, $P \leq 0.05$). Negative values in the leaf number indicate net loss of leaves.

Species x Duration of flooding (d)	Stem height (cm)	Leaf number
Black ash Day 0	0.46c	0.00de
Day 4	0.53c	0.00de
Day 8	0.56c	-0.25de
Day 16	0.63c	-0.38e
Day 32	0.59c	-0.25de
Green ash Day 0	7.65a	6.63a
Day 4	6.60a	5.63a
Day 8	2.06bc	3.75b
Day 16	1.41bc	2.13c
Day 32	3.08b	1.50c
White ash Day 0	0.64c	0.00de
Day 4	0.59c	-0.25d
Day 8	0.70c	-0.13de
Day 16	0.54c	0.00de
Day 32	0.45c	-0.50e

with a leaf number increase of 2.13 and 1.50. Overall, green ash seedlings increased in height and produced leaves after black and white ash seedlings ceased.

Depending on the harvest interval, dry weights for each plant component differed with species regardless of the flood treatments (Table 1.3). White ash seedlings harvested after 8 days of flooding had a greater leaf, current stem, old stem, and root dry weight compared to black ash seedlings. Green ash seedlings had similar dry weights to black ash seedlings with two exceptions. First, green ash seedling had a current stem dry weight that was not different from either white or black ash seedlings. Secondly, green ash seedlings old stem dry weights were greater than those for black ash seedlings. The significant species difference for old stem dry weight for each harvest is due to initial size differences where smaller black ash seedlings are compared with larger green and white ash seedlings. After 16 days of flood stress, no differences were observed for leaf and root dry weights. Current stem samples were not available. After 32 days of flood stress, leaf and current stem dry weights were similar for control and flooded seedlings of each species (Table 1.3). Flooded black ash seedlings, however, had a lower leaf dry weight than control and flooded green and white ash seedlings. In addition, flooded black ash seedlings had a lower current stem dry weight than control and flooded green ash and control white ash seedlings. Root dry weight had a significant species x flood treatment interaction after 32 days of flood stress (Table 1.3). Control black and white seedlings had greater root dry weight compared to their flooded counterparts. Dry weights were reduced by 46 % for roots in flooded black and white ash seedlings. In contrast, control and flooded green ash seedlings had no root dry weight differences indicating that flooding did not restrict root growth.

Table 1.3. Leaf, current stem, old stem, and root dry weights for black, green, and white ash seedlings harvested after 8, 16, and 32 days of flood stress. Means, followed by the same letter within a column for each separate harvest, do not differ significantly (Duncan, n=8, $P \leq 0.05$). Current stems harvested on day 16 were not available (N/A).

Harvests	Dry weight biomass for each plant component			
Day 8	Leaf (g)	Current stem (g)	Old stem (g)	Roots (g)
Black control	7.51b	0.86c	11.30b	23.01b
flooded	8.68ab	1.57abc	19.36b	28.34ab
Green control	9.52ab	2.31ab	33.33a	31.82ab
flooded	8.50b	1.39bc	28.61a	22.37b
White control	11.83a	2.34ab	33.82a	33.50a
flooded	10.50ab	2.67a	34.60a	34.36a
Day 16				
Black control	9.39	N/A	14.09b	34.92
flooded	8.69	N/A	21.34b	26.54
Green control	10.78	N/A	41.78a	31.37
flooded	9.09	N/A	38.97a	26.90
White control	11.15	N/A	36.24a	36.64
flooded	8.69	N/A	33.93a	28.64
Day 32				
Black control	9.91ab	1.64ab	17.41c	48.20a
flooded	7.64b	1.35b	17.01c	22.30b
Green control	12.27a	3.11a	37.90b	33.83b
flooded	11.41a	3.20a	51.35a	31.86b
White control	11.47a	2.96a	41.58ab	54.53a
flooded	11.45a	2.66ab	41.89ab	24.83b

Physiological parameters

Carbon assimilation (A), stomatal conductance (g_s), and transpiration (E) rates showed significant species x flood duration interactions (Table 1.4). Water use efficiency (WUE), however, only had a significant species main effect wherein black ash seedlings had greater values compared to both white and green ash seedlings (data not shown). In Table 4, means pooled for the entire study showed that 16-day flooded green ash seedlings had higher A rates (9.94 umol m^{-2} s^{-1}) than control and 8-day flooded green ash seedlings with A rates of 9.80 and 9.20 umol m^{-2} s^{-1}, respectively. Carbon assimilation of the 8-day flooded green ash was not different from 4 and 32-day flooded green ash, control and 4-day flooded white ash, and control black ash seedlings. Carbon assimilation rates for these seedlings ranged between 8.91 and 8.59 umol m^{-2} s^{-1} during the entire study. White ash seedlings flooded for 8 days had an intermediate A rate (8.18 umol m^{-2} s^{-1}) that did not differ from the latter group; but, was also similar to rates seen in 16-day flooded white ash and 4, 8, and 16-day flooded black ash seedlings. Finally, 32-day flooded white and black ash seedlings had lower A rates (5.99 and 4.92 umol m^{-2} s^{-1}), which were also different. Overall, control green ash had higher g_s and E rates followed by either 4-day flooded green ash or control white ash seedlings when evaluating either g_s or E rates. Both g_s, and E rates decreased consistently with an increase in flood duration except for 4-day black ash seedlings, which had a slightly lower g_s, and E rates than its' 8 and 16-day counterparts (Table 1.4). Again, reductions in A, g_s, and E rates were most pronounced in 32-day flooded seedlings for black and white ash seedlings. In white ash, seedlings flooded for 8, 16, and 32 days had a proportional and gradual decrease in A, g_s, and E rates (Table 1.4). In contrast, black ash seedlings flooded for 4, 8, and 16 days

Table 1.4. Mean assimilation, stomatal conductance, and transpiration for black, green, and white ash seedlings flooded for 0, 4, 8, 16, and 32 days. Values presented are for the entire study including the both the flood and the recovery intervals. Means, followed by the same letter within a column for each species x flood duration interaction in the experiment, do not differ significantly (Duncan, $P \leq 0.05$).

Species x Duration of flooding (d)	Assimilation (umol m^{-2} s^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})	Transpiration (mmol m^{-2} s^{-1})
Black ash Day 0	8.65cd	109.75de	1.77cde
Day 4	7.58e	94.35fg	1.51f
Day 8	7.83e	101.34ef	1.61ef
Day 16	7.60e	99.19ef	1.52f
Day 32	4.92g	62.06h	1.03h
Green ash Day 0	9.80b	150.11a	2.26a
Day 4	8.90cd	136.95ab	2.02b
Day 8	9.20bc	132.52b	2.04b
Day 16	9.94a	129.42bc	1.88bcd
Day 32	8.91cd	132.13b	1.89bcd
White ash Day 0	8.97c	131.45b	2.08ab
Day 4	8.59cd	126.01bc	1.94bc
Day 8	8.18de	116.14cd	1.79cde
Day 16	7.80e	116.46cd	1.70def
Day 32	5.99f	81.45g	1.25g

had similar A, g_s, and E rates which were greater than values in their 32-day flooded counterpart. The 32-day flooded green ash seedlings maintained similar A and g_s rates to other flooded green ash seedlings.

The above trends in the gas exchange parameters are present in data taken at each measurement interval. After 4 days of flooding, control black ash seedlings had a greater A rate than flooded black ash seedlings (Figure 1.2a). By day 8, white control seedlings had greater A rates than their flooded counterparts. Carbon assimilation rates of control and flooded green ash were different on day 11. Thereafter, seedlings flooded for 32 days had lower A rates from controls with one exception. Control and flooded green ash seedlings had similar A rates on day 28 (Figure 1.2b).

The variation in A rates follow a similar pattern found for both g_s and E rates (Figure 1.3-1.4). Two days after the onset of the flood, control black ash seedlings had higher g_s and E rates compared to flooded black ash seedlings. Differences in g_s and E rates for white and green ash seedlings occurred after 4 and 11 days of flooding, respectively (Figure 1.3-1.4c). Thereafter, control seedlings had greater g_s and E rates compared to flooded seedlings until seedlings recovered (Figure 1.3-1.4).

Once released from the 32-day flood, A rates returned to control levels for green, black, and white ash seedlings by day 39, 40, and 44, respectively (Figure 1.2abc). During recovery, A rates for flooded green ash seedlings exceeded those of control seedlings on days 48 and 52 (Figure 1.2b). Green ash seedlings had similar g_s and E values by day 39 compared to both black and white ash seedlings that recovered by day 44 (Figure 1.3-1.4). On day 52, g_s and E rates for green flooded seedlings exceeded those found for green control seedlings.

Figure 1.2. Mean assimilation ($\pm$SE) of black ash, *Fraxinus nigra* L. (a), green ash, *Fraxinus pennsylvanica* Marsh. (b), and white ash, *Fraxinus americana* L. (c), seedlings flooded for 0, 4, 8, 16, and 32 days. Seedlings flooded for 0 and 32 days were measured throughout the study whereas seedlings flooded for 4, 8, and 16 days were added when released to monitor recovery. (Duncan, n=8, $P \leq 0.05$)

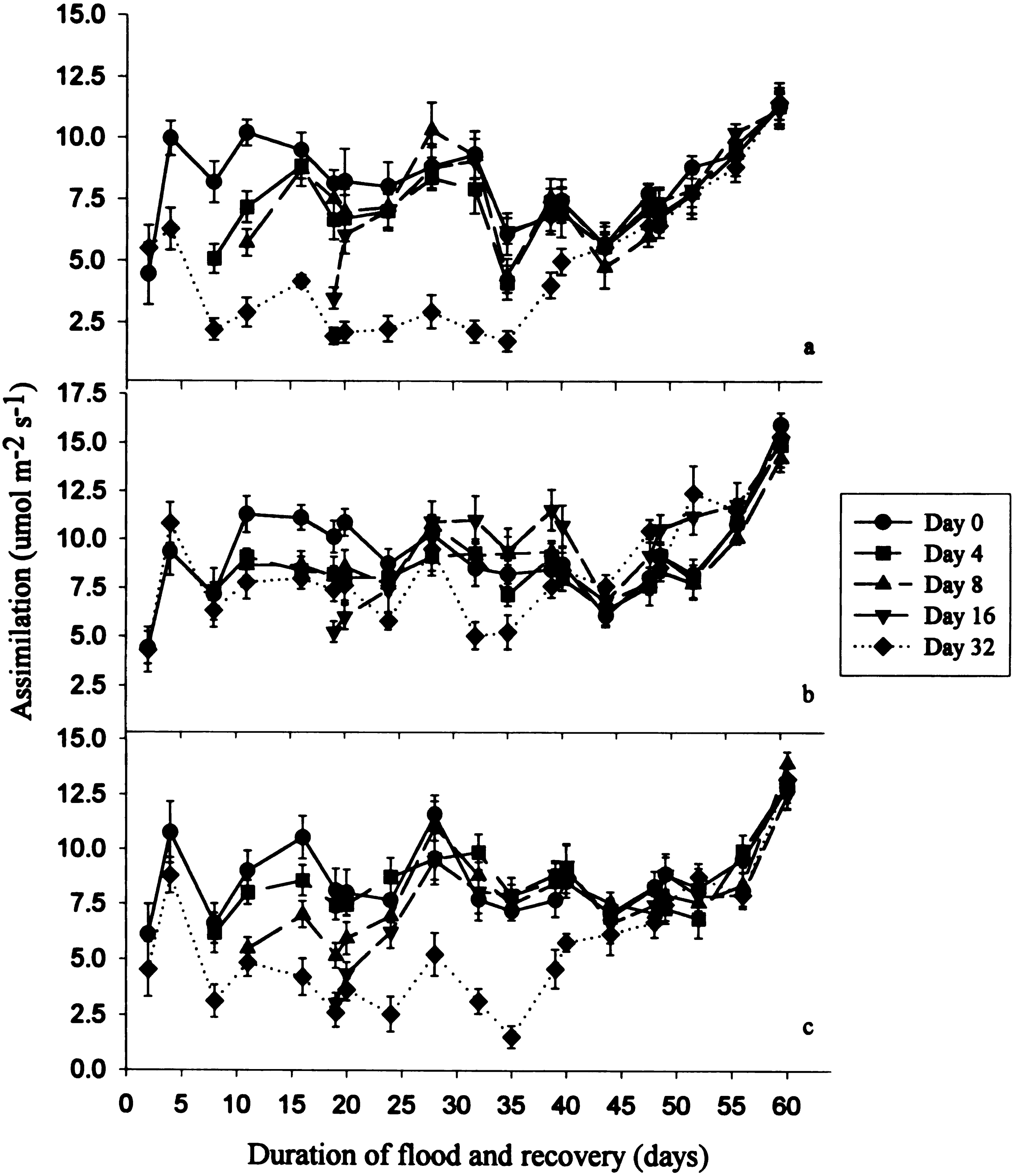

15.0
12.5
10.0
7.5
5.0
2.5
a
17.5
15.0
12.5
10.0
7.5
5.0
2.5
b
15.0
12.5
10.0
7.5
5.0
2.5
0.0
c
Assimilation (umol m-2 s-1)
0 5 10 15 20 25 30 35 40 45 50 55 60
Duration of flood and recovery (days)
Day 0
Day 4
Day 8
Day 16
Day 32

Figure 1.3. Mean stomatal conductance (±SE) of black ash, *Fraxinus nigra* L. (a), green ash, *Fraxinus pennsylvanica* Marsh. (b), and white ash, *Fraxinus americana* L. (c), seedlings flooded for 0, 4, 8, 16, and 32 days. Seedlings flooded for 0 and 32 days were measured throughout the study whereas seedlings flooded for 4, 8, and 16 days were added when released to monitor recovery. (Duncan, n=8, $P \leq 0.05$)

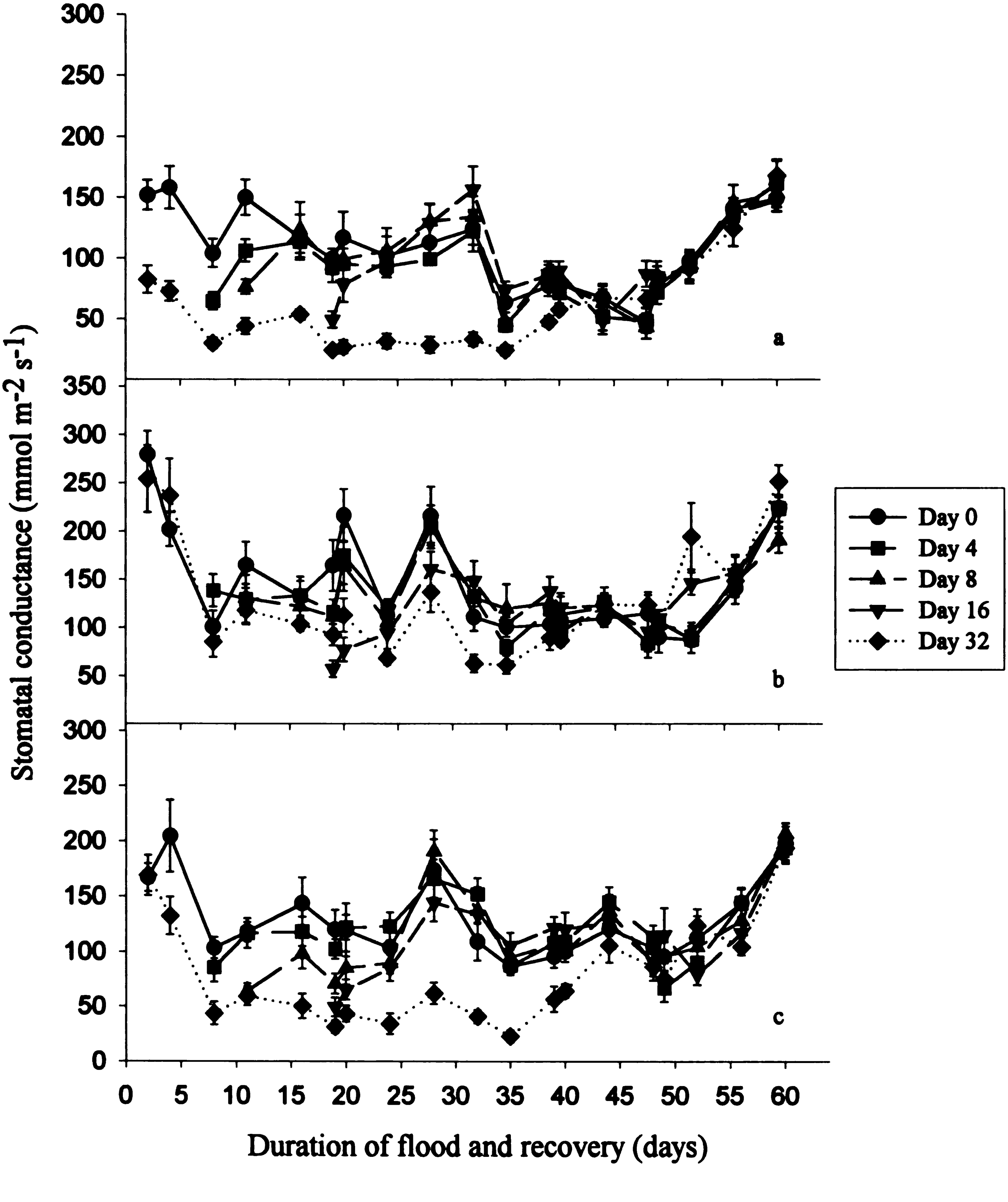
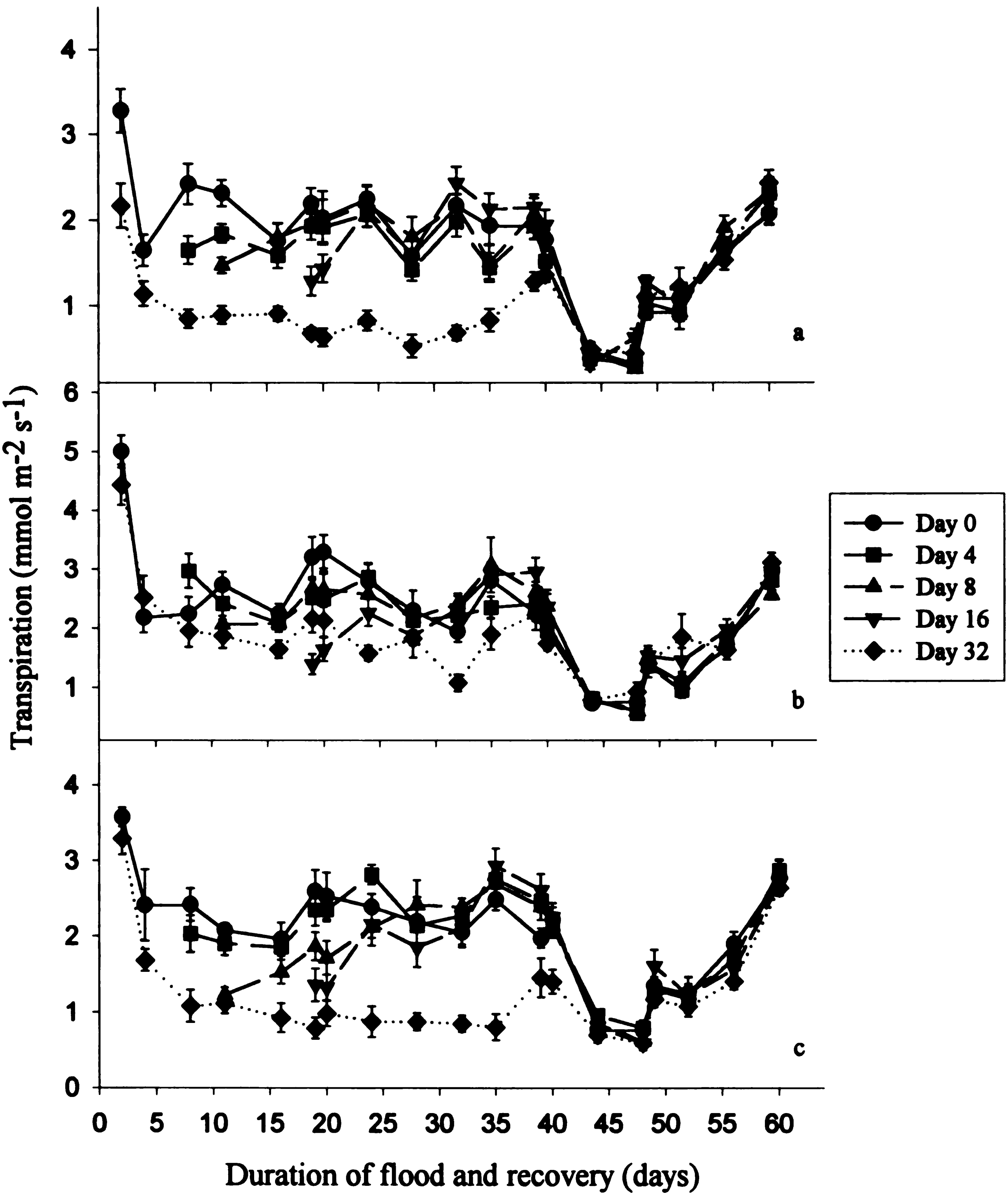

Stomatal conductance (mmol m-2 s-1)
Duration of flood and recovery (days)
Day 0
Day 4
Day 8
Day 16
Day 32
a
b
c

Figure 1.4. Mean transpiration (±SE) of black ash, *Fraxinus nigra* L. (a), green ash, *Fraxinus pennsylvanica* Marsh. (b), and white ash, *Fraxinus americana* L. (c), seedlings flooded for 0, 4, 8, 16, and 32 days. Seedlings flooded for 0 and 32 days were measured throughout the study whereas seedlings flooded for 4, 8, and 16 days were added when released to monitor recovery. (Duncan, n=8, $P \leq 0.05$)


Transpiration (mmol m-2 s-1)
Duration of flood and recovery (days)
Day 0
Day 4
Day 8
Day 16
Day 32
a
b
c

Seedling recovery for 32-day flooded seedlings was faster or similar to seedlings flooded for 4, 8, and 16 days. For example, 4-day flooded black ash seedlings, with lower A rates than control seedlings, recovered 12 days after release from the flood stress on day 16. As the flood duration increased to 8 and 16 days, carbon assimilation rates of black ash seedlings recovered within 8 and 4 days, respectively. Thereafter, A rates of black ash seedlings flooded for 32 days recovered after 12 days. Similar recovery pattern and times were observed for flooded white and green ash seedlings which had lower A rates after 8 and 11 days of flooding, respectively. Four day flooded seedlings recovered 16 days after release. Again, a shorter recovery period of 8 days was observed for flooded green ash seedlings as the flood duration increased to 16 and 32 days. In contrast, A rates for white ash seedlings flooded for 16 and 32 days recovered within 8 and 12 days, respectively.

Growth, gas exchange, and dry weights were summarized in Table 1.5 to clearly indicate when differences occurred. Caliper, stem height, and leaf number were not different for white ash and black ash except for the caliper width of flooded black ash seedlings on day 32. White ash seedlings had a significant change in leaf area throughout the study whereas black ash seedlings had none. Root dry weights for these two species was also different on day 32. Earlier changes in gas exchange for black and white ash were seen and maintained compared to the green ash seedlings. For example, the g_s of black and white ash seedlings was different on day 2 and 4 followed by a difference in A rates on day 4 and 8, respectively. In contrast, A and E rates of green ash seedlings were different on day 16 followed by differences in all three gas exchange parameters (A, g_s, and E) on day 32. Green ash seedling growth was also influenced by flooding with differences in the leaf area throughout the study. Leaf numbers were different by day 8 whereas stem height was influenced on day 16. The caliper widths of green ash was different only on days 4 and 32 (Table 1.5).

Table 1.5. Summary of growth, gas exchange, and plant component dry weights for black, green, and white ash seedlings exposed flood after 2, 4, 8, 16, and 32 days. Differences between control and flooded seedlings at the $P \leq 0.05$ or $P \leq 0.01$ level are indicated as * and ** , respectively (Duncan, n=8). (Blanks were included where no determination was made whereas N/A was noted when the data was not available.)

	Duration of flood stress in days														
Species	Black ash					Green ash					White ash				
Parameters	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32
Caliper		NS	NS	NS	*		**	NS	NS	**		NS	NS	NS	NS
Stem height		NS	NS	NS	NS		NS	NS	*	**		NS	NS	NS	NS
Leaf number		NS	NS	NS	NS		NS	**	**	**		NS	NS	NS	NS
Leaf area		NS	NS	NS	NS		**	**	**	**		*	*	*	*
Assimilation	NS	*	**	**	**	NS	NS	NS	**	**	NS	NS	**	**	**
Stomatal conductance	*	*	**	**	**	NS	NS	NS	NS	*	NS	*	**	**	**
Transpiration	**	NS	**	**	**	NS	NS	NS	*	**	NS	NS	**	**	**
Leaf biomass			NS	NS	NS			NS	NS	NS			NS	NS	NS
Current stem biomass			NS	N/A	NS			NS	N/A	NS			NS	N/A	NS
Old stem biomass			NS	NS	NS			NS	NS	*			NS	NS	NS
Root biomass			NS	NS	**			*	NS	NS			NS	NS	**

DISCUSSION

Flooding black ash (*Fraxinus nigra*), green ash (*Fraxinus pennsylvanica*), and white ash (*Fraxinus americana*) seedlings during the growing season stimulated different morphological and physiological responses. Changes in current stem height, leaf number, and leaf area expansion were negligible when comparing control and flooded black or white ash seedlings (Table 2). In contrast, green ash seedlings continued growing throughout the study. Although flooding began June 24th 1998, it is likely that both black and white ash seedlings had completed the majority of their growth for the season. For example, white ash saplings grown in Ontario are known to develop their canopy in early May (Brundrett and Kendrick 1988). In addition to phenological and developmental characteristics which vary with tree size (Fredericksen et al. 1996), transplant shock and pruning are two additional factors that need careful consideration when dealing with bare root seedlings (Schutzki pers. com.). Planting shock is known to reduce seedling performance because of the mechanical and physiological stresses that accompany the seedlings through lifting, processing, storage, and subsequent planting (Coutts 1980; Sutton 1980). Unfortunately, very little information is available for black and white ash seedlings. One study on green ash seedlings, however, determined that root pruning had no sustained effect on growth because root regenerated quickly (Arnold and Struve 1989).

Flooding adversely affects stem, leaf, and cambial growth as well as reproductive growth of trees (Kozlowski 1985). Stem swelling due to increased stem hydration commonly occurs during flooding. In this study, caliper widths for all three flooded species were greater than their control counterparts. Changes in current stem height, leaf number, and leaflet area for black and white ash seedlings were small compared to green ash seedlings. Cessation of growth was evident with no change or a fewer number of leaves and a small expansion in leaflet area. Black and white ash leaflets increased 2.69 and 6.49 cm^2 compared to green ash leaflets with a mean increase of 17.78 cm^2 (Table 1.1).Consequently, flooding stress, regardless of duration, reduced leaf expansion with control seedlings gaining twice as much leaf area. Flooding experiments on different species have shown that flooding impedes leaf initiation and expansion and in severe cases causes leaf senescence (Kozlowski 1985). Caliper growth changes induced by flooding green ash seedlings were greater in magnitude and different from control seedlings. Flooded green ash had a greater caliper increase overall whereas current stem height, leaf number, and leaf expansion were greater for the control seedlings (Table 1.2).

Seedling dry weights harvested after the 32 day flood also highlight species and flood induced differences. Leaf and current stem dry weights were not different except for flooded black ash seedlings had lower leaf and current stem dry weights (Table 1.3). A proportionally smaller size for black ash seedlings may account for its lower accrued growth and an increased susceptibility to flood stress. This is important since roots are sensitive to oxygen deficiency and that no records of sustained growth by anoxic roots has been reported (Armstrong et al. 1994). Consequently, seedling survival by anaerobic metabolism is dependant on the availability of substrates present in the roots for respiration (Jackson and Drew 1984). In this study, flooded black and white ash seedlings had a 46 % reduction in root dry weights compared to their control counterparts. Similar results by Smith and Bourne

(1989) had 50 and 48 % reduction in root mass for 28 day flooded pecan seedlings when compared to controls during bud break or active growth, respectively. Flooded pecan roots were also brown to black compared to control seedlings with light yellow roots. During the harvest, flooded roots were partly yellow with brown and black discolorations of varying degrees mainly at the tips. Root discoloration was most pronounced in black ash seedlings and least in green ash seedlings. Generally, reductions in root dry weights exhibit both a decrease in root mass due to root death and inhibited root growth (Smith and Bourne 1989). Studies on various species have outlined the inhibition of root growth in response to flood stress conditions (Pezeshki and DeLaune 1990; Liu and Dickmann 1992; Topa and Cheeseman 1992). For green ash seedlings, however, control and flooded root dry weights were similar, clearly showing a greater flood tolerance. These findings support a study by Good and Patrick (1987) where root aeration and anaerobic respiration contributed to the survival of green ash seedlings flooded for a period of 9.5 months (mid-July to May). Other studies on green ash seedlings (70 to 150 days old) with shorter flood duration (28 to 30 days) reported reductions in dry weight increment of roots, stems, and leaves combined with the formation of hypertrophied lenticels and adventitious roots (Sena Gomes and Kozlowski 1980; Tang and Kozlowski 1984). Although the leaf, current and old stem, and root dry weights for control and flooded green ash seedlings did not differ, significant growth differences were observed during the flood. In addition, hypertrophied lenticels were observed for all three *Fraxinus* species exposed to flooding stress. Black and green ash seedlings developed numerous hypertrophied lenticels on their submerged stems within 8 to 12 days whereas those for white ash developed later as the flood progressed. By the end of the 32-day flood, raised lenticels covered most of the submerged stems but no apparent adventitious roots developed contrary to results noted in Sena Gomes and Kozlowski (1980). Among woody plants, Hook (1984) noted that the development of prominent lenticels at the stem base and on older roots contributes to improved gas exchange. It is thought to enhance O_2 diffusion to roots (Kozlowski 1984) or eliminate potentially toxic metabolites such as ethanol, acetaldehyde, or ethylene (Chirkova and Gutman 1972). The development of hypertrophied lenticels, however, does not necessarily confer flood-tolerance (Larson and Schaffer 1991).

Other processes, especially those that are metabolic or physiological in nature, may be critical in determining flood tolerance. For example, many flood responses help minimize transpirational loss of water by either leaf shedding, stomatal closure, leaf epinasty, or slowing of growth. Everard and Drew (1987, 1989) emphasized that minimizing these losses may originate from the low conductivity of oxygen-deficient roots to water. Not surprisingly, woody plants often exhibit a decrease in A and g_s within 1 to 3 days after flooding, although longer flooding durations are required for reductions in growth (Andersen et al 1984; Crane and Davies 1989). Sena Gomes and Kozlowski (1980) reported that stomatal closure was one of the earliest responses to flooding green ash seedlings. In comparing the three *Fraxinus* species, stomatal closure occurred after 2 days of flooding for black ash. Lower g_s for white ash seedlings were observed after day 4 whereas green ash seedlings responded after 11 days. Similarly, reductions in A rate were observed in black, white, and green ash seedlings after 4, 8, and 11 days. The time required for stomatal closure to inhibit A rates may be attributed to a species sensitivity to anaerobic soil conditions. Hypoxic conditions within the roots may adversely affect photosynthetic processes (Bradford (1983). For example, flooded soils with low soil redox potential (Eh) could cause a reduction in net photosynthesis with either decreased leaf water potential, reduced Rubisco activity (Vu and Yelenosky 1992; Pezeshki 1994), disruption in photosynthate transport, alteration in source-sink relationships, or reduced sink demand (Wample and Thornton 1984; Drew 1990). A flood-resistant plant can avoid oxygen deficiency either by transporting O_2 from the leaves internally to its' root system (Armstrong 1978) or by sustaining and tolerating anaerobic metabolism (Crawford 1982; Barclay and Crawford 1982). During flooding, A rates for flooded black and white seedlings remained lower primarily because g_s were no greater than 68 mmol m^{-2} s^{-1} (Figure 1.3). In contrast, stomatal conductances for flooded green ash seedlings were two folds higher with a mean of 126 mmol m^{-2} s^{-1}. The ability of flooded green ash to maintain open stomata may indicate an osmotic adjustment to flooding stress. Daily fluctuations in g_s during the flood displays a control and capacity to optimize A while adjusting for water loss to the environment. These two physiological processes may explain why green ash biomass was not as affected by flood stress as the other two species (Figure 1.3-1.4, Table 1.3).

Duration of the flood stress influenced seedling recovery time for each species. The capability to return to normal levels of photosynthesis following a period of stress is an important factor in the fitness of a plant (Bacone et al. 1976; Ormsbee et al. 1976; McGee et al. 1981; Peterson and Bazzaz 1984). Seedling response to flooding is undoubtedly mediated by various plant hormones which attempt to optimize A and growth. Theoretically, Jackson (1993) advises that changes in hormones between roots and shoots can involve 'positive', 'negative', 'accumulative', and 'debit' messages (Armstrong et al. 1994). This topic is beyond the scope of this study.

In summary, the mechanisms whereby flood tolerance is attributed to a species involves a combination of both physiological and morphological adaptations. Within the genus *Fraxinus,* species from markedly different native habitats demonstrated different growth and flood responses. Overall, green ash seedlings had greater A, g_s, and E rates compared to black and white ash seedlings. In addition, green ash dry weights were not influenced by the flood. Both black and white ash seedlings, however, had minimal incremental growth, a proportional reduction in root dry weights, and a slow post-flood recovery from reduced g_s and A rates. These results make it difficult to discern which of these two species (black or white ash) is more flood tolerant especially since the initial seedling size differed. Nonetheless, black and white ash possess certain adaptations to flooded soil conditions but are not as highly flood-tolerant as green ash seedlings.

LITERATURE CITED

Andersen P.C., P.B. Lombard, and M.N. Westwood 1984. Leaf conductance, growth, and survival of willow and deciduous fruit tree species under flooded soil conditions. J. Amer. Soc. Hort. Sci. 109: 132-138.

Armstrong W. 1978. Root aeration in the wetland condition. In: Plant Life in Anaerobic Environments (D.D. Hook and R.M.M. Crawford, Eds.). Ann Arbor Science Publishers, Ann Arbor, MI, pp. 269-297.

Armstrong W., R. Brandle and M.B. Jackson 1994. Mechanisms of flood tolerance in plants. Acta Bot. Neerl 43: 307-358.

Arnold M.A. and D.K. Struve 1989. Green ash establishment following transplant. J. Amer. Soc. Hort. Sci. 114 (4): 591-595.

Bacone J., F.A. Bazzaz, and W.R. Boggess 1976. Correlated photosynthetic responses and habitat factors of two successional tree species. Oecologia 23: 63-74.

Barclay H.M. and R.M.M. Crawford 1982. Plant growth and survival under strict anaerobiosis. J. Exp. Bot. 22: 541-549.

Bradford K.J.1983a. Effects of soil flooding on leaf gas exchange of tomato plants. Plant Physiol. 73: 475-479.

Bradford K.J.1983b. Involvement of plant growth substances in the alteration of leaf gas exchange of flooded tomato plants. Plant Physiol. 73: 480-483.

Brundrett M.C. and B. Kendrick 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can. J. Bot. 66: 1153-1173.

Ceulemans R., I. Impens, and V. Steenackers 1984. Stomatal and anatomical leaf characteristics of 10 *Populus* clones. Can. J. Bot. 62: 513-518.

Chirkova T.V. and T.S. Gutman 1972. Physiological role of branch lenticels in willow and poplar under conditions of root anaerobiosis. Sov. Plant Phys. 19: 289-295.

Coutts M.P. 1980. Control of water loss by actively growing sitka spruce seedlings after transplanting. J. Exp. Bot. 31: 1587-1597.

Crane J.H. and F.S. Davies 1989. Flooding responses of *Vaccinium* species. HortSci. 24: 203-210.

Crawford R.M.M. 1982. Physiological responses to flooding. In Enclyclopedia of Plant Physiology, Vol. 12B, Physiological Plant Ecology (O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler, Eds.). Springer-Verlag, Berlin, pp.453-477.

Dirr M.A. 1990. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. (4^{th} Ed.) Stipes Publishing Co., 10-12 Chester St., Champaign, IL 61820. 1007 p.

Drew M.C. 1990. Sensing soil oxygen. Plant Cell & Environ. 13: 681-693.

Everard J.D. and M.C. Drew 1987. Mechanisms of inhibition of water movement in anaerobically treated roots of *Zea mays* L.. J. Exp. Bot. 38: 1154-1165.

Everard J.D. and M.C. Drew 1989. Water relations of sunflower (*Helianthus annuus*) shoots during exposure of the root system to oxygen deficiency. J. Exp. Bot. 40: 1255-1264.

Flint H.L. 1983. Landscape plants for Eastern North America. Wiley-Interscience, New York, USA, 667 pp.

Fredericksen T.S., K.C. Steiner, J.M. Skelly, B.J. Joyce, T.E. Kolb, K.B. Kouterick, and J.A.

Ferdinand 1996. Diel and seasonal patterns of leaf gas exchange and xylem water potentials of different-sized *Prunus serotina* Ehrh. Trees. For. Sci. 42: 359-365.

Good B.J. and W.H. Jr. Patrick. 1987. Gas composition and respiration of water oak (*Quercus nigra* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) roots after prolonged flooding. Plant and Soil 97: 419-427.

Hall T.F. and G.E. Smith 1955. Effects of flooding on woody plants, West Sandy dewatering project, Kentucky Reservoir, J. For. 53: 281-285.

Hook D.D. 1984. Adaptations to flooding with fresh water. In: Kozlowski, T.T. (Ed.) Flooding and Plant growth. Academic Press, London pp. 265-294.

Jackson M.B. 1993. Are plant hormones involved in root to shoot communication? Adv. Bot. Res. 19: 104-187.

Jackson M.B. and M.C. Drew 1984. Effects of flooding on growth and metabolism of herbaceous plants. In Flooding and Plant Growth (T.T. Kozlowski, Ed.) Academic Press, Orlando, FL, pp. 47-128.

Kozlowski T.T. and S.G. Pallardy. 1979. Stomatal responses of *Fraxinus pennsylvanica* seedlings during and after flooding. Physiol. Plant. 46: 155-158.

Kozlowski T.T. 1984. Plant responses to flooding of soil. BioScience 34: 162-167.

Kozlowski T.T. 1984. Flooding and plant growth. Academic Press, Inc. Orlando, FL, 356 p.

Kozlowski T.T. 1985. Soil aeration, flooding, and tree growth. J. Arboricult. 11: 85-96.

Kozlowski T.T., P.J. Kramer, and S.G. Pallardy 1991. The physiological ecology of woody plants. Academic Press, Inc., San Diego, CA, USA.

Larson K.D., B. Schaffer, and F.S. Davies 1991. Flooding, leaf gas exchange and growth of mango in containers. J. Am. Soc. Hort. Sci. 116: 156-160.

Lechowicz M.J. 1984. Why do temperate deciduous trees leaf out at different times? adaptation and ecology of forest communities. Am. Nat. 124: 821-842.

Liu Z. and D.I. Dickmann 1992. Responses of two hybrid *Populus* clones to flooding, drought, and nitrogen availability. I. Morphology and growth. Can. J. Bot. 70: 2265-2270.

McGee A.B., M.R. Schmierbach, and F.A. Bazzaz 1981. Photosynthesis and growth in populations of *Populus deltoides* from contrasting habitats. Am. Midl. Nat. 105: 305-311.

Ormsbee P., F.A. Bazzaz, and W.R. Boggess 1976. Physiological ecology of *Juniperus virginiana* in oldfields. Oecologia 23: 75-82.

Peterson D.L. and F.A. Bazzaz 1984. Photosynthetic and growth responses of silver maple (*Acer saccharinum* L.) seedlings to flooding. Am. Midl. Nat. 112: 261-272.

Pezeshki S.R. and J.L. Chambers 1986. Variations in flood-induced stomatal and photosynthetic responses of three bottom-land tree species. For. Sci. 32: 914-923.

Pezeshki S.R. and R.D. DeLaune 1990. Influence of sediment oxidation-reduction potential on root elongation in *Spartina patens*. Acta. Oecol. 11: 377-383.

Pezeshki S.R. 1994. Responses of baldcypress seedlings to hypoxia: leaf protein content, ribulose-1,5-bisphosphate carboxylase/oxygenase activity and photosynthesis. Photosynthetica 30:59-68.

Reid D.M., F.D. Beall, and R.P. Pharis 1991. Environmental cues in plant growth and development. In: Plant Physiology, A Treatise Vol X: Growth and Development Chapter 2, Academic Press, Inc. pp.65-181.

SAS Institute Inc. 1988. SAS/STAT user's guide, version 6.03 Edition, Cary, NC., 1028 pp.

Sena Gomes A.R and T.T. Kozlowski 1980. Growth responses and adaptations of *Fraxinus pennsylvanica* seedlings to flooding. Plant Physiol. 66: 267-271.

Smith M.W. and R.D. Bourne 1989. Seasonal effects of flooding on greenhouse-grown seedling pecan trees. HortScience 24: 81-83.

Sutton R.F. 1980. Techniques for evaluating planting stock quality. For. Chron. 56: 116-120.

Tang Z.C. and T.T. Kozlowski 1984 Water relations, ethylene production, and morphological adaptation of *Fraxinus pennsylvanica* seedlings to flooding. Plant Soil 77: 183-192.

Tardiff J. and Y. Bergeron 1993. Radial growth of *Fraxinus nigra* in a Canadian boreal floodplain in response to climatic and hydrological fluctuations. J. Veg. Sci. 4: 751-758.

Topa M.A. and J.M. Cheeseman 1992. Effects of root hypoxia and a low P supply on relative growth, carbon dioxide exchange rates and carbon partitioning in *Pinus serotina* seedlings. Physiol. Plant. 86: 136-144.

Vu J.C.V. and G. Yelenosky 1992. Photosynthetic responses of rough lemon and sour orange to soil flooding, chilling and short-term temperature fluctuation during growth. Environ. Exp. Bot. 32: 471-477.

Wample R.L. and R.K. Thornton 1984. Differences in the response of sunflower (*Helanthus annuus*) subjected to flooding and drought stress. Physiol. Plant. 61: 611-616.

CHAPTER 2

EFFECTS OF FLOODING DURATION ON THE BIOMASS AND STARCH CONTENT BLACK ASH (FRAXINUS NIGRA MARSH.), GREEN ASH (FRAXINUS PENNSYLVANICA MARSH.), AND WHITE ASH (FRAXINUS AMERICANA L.) SEEDLINGS.

ABSTRACT

Bare root black ash (*Fraxinus nigra* Marsh.), green ash (*Fraxinus pennsylvanica* Marsh.), and white ash (*Fraxinus americana* L.) 2-1 seedlings 30.5 to 45.7 cm in height were planted into 19-liter plastic containers filled with a sterilized 2:1 (v/v) sand:loam mixture. Flood treatments were imposed for 0 (control), 8, 16, and 32 days. Minimal biomass growth was observed for black and white ash seedlings. For these two species, flooding for 32 days reduced root dry weight biomass by 46 %. Differences in biomass were not observed for green ash seedlings regardless of treatment. Starch content varied with tissue type, flood duration and species. A species x duration interaction was present only for the starch concentrations in fine roots on day 32. On days 8 and 16, flooded seedlings had higher leaf starch than control seedlings, while there were no differences in leaf starch concentration on day 32. On day 16 and 32, white ash leaves had 32 and 54 % less starch compared to the other species. On day 8, starch in current and old stems was greater for black ash than in green ash seedlings. This difference did not persist as starch concentrations increased during the study. Stem starch ranged from 28.76 to 45.81 mg/g DW on day 8 and from 61.48 to 76.72 mg/g DW on day 32. After 32 days, fine and coarse roots of flooded seedlings had 84 to 88 % and 60 % less starch, respectively, when compared to control seedlings. Green ash seedlings, however, had higher coarse root starch throughout the study. Consequently, green ash seedlings were more tolerant to increased duration of flood stress compared to black and white ash seedlings where root biomass and starch reserves were reduced.

INTRODUCTION

Woody plants have different strategies to deal with environmental stresses such as flooding, drought, and high salinity. Morphological and physiological adaptations can assure survival of an individual in a species if growth and partitioning of reserves become limited. In woody species, starch is the main insoluble storage carbohydrate present in most above-ground tissues (Loescher et al. 1990). High concentrations of starch can also be stored in the roots. These reserves fluctuate seasonally to protect the tree during winter and insure renewed growth in the spring. Little research has been done on how environmental stresses impact carbon reserves in roots during the growing season. In a review of carbohydrate reserves in roots, Loescher et al. (1990) note that late-season stresses impact root reserves and adversely affect plant yield, flowering, and subsequent fruiting.

More information, however, is available on how environmental stresses influence photosynthesis in tree species, especially fruit crops of economic value. Starch synthesis within the chloroplasts is highly coordinated with sucrose synthesis in the cytosol (Taiz and Zeiger 1991). Source-sink relationships play an important role in determining how carbon is fixed and the regulation of photosynthesis (Guinn and Mauney 1980; Daie 1985; Roper et al. 1988). For example, high leaf starch concentrations lead to the formation of additional soluble carbohydrates, which in turn decrease the net photosynthetic rate (Mauney et al. 1979; Luxmoore 1991). In some species, sorbitol or mannitol (nonreducing sugars) can replace sucrose as the principal photosynthetic product. Some Rosaceae species accumulate sorbitol, whereas some species of Oleaceae, Apiaceae, and Scrophulariaceae accumulate mannitol (Loescher et al. 1990). The three species studied in this paper are from the genus *Fraxinus* in the family Oleaceae. How production of mannitol alters starch synthesis in the chloroplasts is not known. Detailed studies are needed to show how the type and ratio of soluble carbohydrates influence carbon fixation. This topic is beyond the scope of this study where the effect of flooding stress on growth and starch reserves at the whole plant level will be examined.

Trees commonly planted in urban landscapes, such as green (*Fraxinus pennsylvanica* Marsh.) and white ash (*Fraxinus americana* L.), differ in their ability to tolerate flooding stress. Green ash is one of the most adaptable trees, with a wide geographic distribution. Green ash is also a highly flood-tolerant species that can grow on land subjected to periodic flooding and displays vigorous growth even when flooded for much of the growing season (Tang and Kozlowski 1984). In contrast, white ash, which usually occurs on deep, well-drained upland soils, is a flood-intolerant species (Hall and Smith 1955; Kozlowski et al. 1991). Black ash (*Fraxinus nigra* L.) is an extremely hardy species common to low woodlands, swamps, and periodically inundated river bottoms. Often referred to as swamp or hoop ash, black ash can tolerate standing water for many weeks. Black ash is a determinant species, meaning that the next season's growth is determined by climatic conditions during the previous season (Lechowicz 1984; Tardiff and Bergeron 1993).

Although characteristics of the flood water, seasonality, and duration of flooding all affect response, such responses vary with the age of plant, seedlings being more susceptible than mature trees. Initially, flooding reduces the oxygen content of the soil (Kozlowski et al. 1991). Under these conditions, root respiration decreases and anaerobic metabolism compensates for the reduction in ATP formation. Survival in hypoxic or anoxic conditions then depends on availability of carbohydrate reserves and temperature controlling respiration rate (Jackson and Drew 1984). The elimination of toxic metabolites such as ethanol, acetaldehyde, or ethylene may also be critical for the root system (Chirkova and Gutman 1972). Above-ground processes are altered by reduced water and nutrient uptake by roots. Many studies report lower photosynthetic and stomatal conductances (Jackson and Drew 1984; Reid et al. 1991) wherein decreased growth is observed after a longer period. Good et al. (1986) showed that green ash was able to survive long periods of flood stress by oxidizing the rhizosphere and maintaining anaerobic respiration. To what extent the duration of flood stress changes carbon partitioning, future growth, and if it predisposes a tree to other environmental stresses is not known especially with respect to root reserves.

The objectives of this study were to characterize and compare the biomass and starch content in several tissues of black, green, and white ash seedlings exposed to increasing durations of flooding stress during the summer. These data might help to explain differences in physiological and ecological responses among these three species with respect to flood tolerance.

MATERIALS AND METHODS

Plant materials

Bare root black ash, green ash, white ash seedlings (2-1; 30.5 to 45.7 cm in height) were obtained from Lawyers' Nursery in Plains, Montana, USA, and stored in a cold room at 2-3 °C until potted. Seedlings were ranked based on uniform caliper size taken at the root collar. Seedling roots were pruned to 18 to 20 cm prior to planting into 19-liter plastic containers (Classic 1200, Mollema, 34 cm width x 38 cm height) lined at the bottom with a layer of cheese cloth and filled with a sterilized 2:1 (v/v) sand:loam mixture. Lateral branches were pruned and one terminal shoot was allowed to develop for each seedling. Seedlings were placed in an uncovered frame house at the Michigan State University Horticulture Teaching and Research Center, Lansing, MI, USA (42.8°N, 84.5°W) where they were kept well watered until the beginning of the flood treatment. Seedlings were allowed to grow 4 weeks prior to the onset of flood duration treatments. The flood treatment began on the 24th of June 1998, and continued for 32 days.

Experimental Design

This factorial experiment was arranged in a completely randomized design with a total of 264 seedlings assigned to one of two groups. In the first group, eight replicate seedlings of each species (black, green, and white ash) were flooded for durations of 8, 16 or 32 days with an equivalent number of control seedlings reserved to compare biomass and starch reserves. Control seedlings were watered regularly to maintain soil moisture below or near field capacity. In the second group, eight replicate seedlings of each species were flooded for durations of 0 (control), 4, 8, 16, or 32 days. Growth and gas exchange was monitored on this second group of seedlings during and after the flood treatment (Chapter 1). To ensure uniformity in soil moisture, soil moisture data was recorded every 4 days on the control seedlings using a Theta Meter type HH1 (Delta-T Devices, Cambridge, England) equipped with a Theta Probe type ML1. The flood treatments were imposed by inserting the 19-liter container inside a larger container lined with a reinforced plastic bag. The water level was maintained near the rim of the container using a drip irrigation system controlled by a time-clock (Model LX-12, Rainbird, USA) so as to mimic standing water. Plants released from the flood treatment were allowed to drain and were maintained similar to the control seedlings.

Biomass and carbohydrate determination

After 8, 16, and 32 days of flood stress, control and flooded seedlings were harvested to determine the dry weight for each plant component. Leaves, old and current stems, and roots were separated, washed (roots only), bagged, and kept in coolers until processed in the laboratory. Fresh and dry weights were determined using an analytical balance (Mettler PM460 ,Mettler Instrument Corp., Highstown, NJ, USA) to an accuracy of 0.001 g. Prior to lyophilization (Virtis, Genesis 12EL, Gardiner, NY), samples were then stored in a -30 °C freezer and transferred a -80 °C freezer (Isotemp, Fisher Scientific, USA). This prevented thawing of samples and minimized enzymatic reactions during lyophilization. Once weighed, samples were stored in a -30 °C freezer in sealed plastic bags with drierite as a dessicant.

Dried root tissues were then separated into fine and coarse roots, respectively. Fine roots were defined as extremity or peripheral segments (≤ 1 mm thickness) whereas coarse roots were thicker (≥ 1-3 mm) and bore the fine roots. Dried tissues were ground with a Wiley mill to pass a 40-mesh screen (Thomas Scientific Co., Swedesboro, NJ, USA) except for old stems which were ground to pass through a 1 mm mesh screen.

A total of 100 mg was weighed into labelled 16 x125 mm test tubes for carbohydrate extraction. Soluble carbohydrates were extracted with 3.5 ml of 80% ethanol for 20 minutes three times. Between each extraction, samples were centrifuged in a Sorvall rotor at 1879 g for 6 minutes and the supernatants collected and dried separately. The remaining starch pellets were dried in a Savant Speedvac SC200 fitted with a refrigerated condensation trap RT4104 at -100 °C (Savant Instruments, Inc., Farmingdale, NY, USA). Dried samples were kept in a freezer with drierite dessicant. After adding 5 ml of 0.1 M acetate buffer (pH 5.0), each test tube was covered with a plastic cap, autoclaved for 30 minutes (121 °C, 19 PSI) (Amsco, Eagle Series 2021 Gravity) and cooled prior to adding the recommended 12 units of amyloglucosidase (Boehringer Mannheim, Indianapolis, IN, USA). Samples were digested for16 hours at 48 °C in a water bath and the resulting glucose was determined colorimetrically using a glucose assay kit (Glucose (Trinder); Procedure No. 315, Sigma Diagnostics). Absorbance of glucose standard and samples were read at 505 nm with a Hitachi U-3110 spectrophotometer (Hitachi Ltd., Tokyo, Japan).

Statistical analyses

Biomass and starch content was analysed for each measurement interval to determine differences over time between green, white, and black ash seedlings in response to flooding stress. Analysis of variance were conducted using PROC GLM in SAS/PC software (SAS Institute Inc. 1985) to determine differences between duration and species. Comparisons between treatment means were tested with a Duncan test. Probabilities less than or equal to 0.05 were regarded as significant.

RESULTS

Dry weights of each plant component had significant species main effects averaged across the flood treatments depending on the harvest interval (Table 2.1). White ash seedlings harvested after 8 days of flooding had a greater leaf, current stem, old stem, and root dry weight compared to black ash seedlings. Green ash seedlings had similar dry weights to black ash seedlings with two exceptions. First, green ash seedling had a current stem dry weight that was not different from both white or black ash seedlings. Second, green ash seedlings old stem dry weights were greater than those for black ash seedlings. The significant species difference for old stem dry weight for each harvest is due to initial size differences where smaller black ash seedlings are compared with larger green and white ash seedlings. After 16 days of flood stress, no differences were observed for leaf and root dry weights. Current stem samples were not available. After 32 days of flood stress, leaf and current stem dry weights were similar for control and flooded seedlings of each species (Table 2.1). Flooded black ash seedlings, however, had a lower leaf dry weight than control and flooded green and white ash seedlings. In addition, flooded black ash seedlings had a lower current stem dry weight than control and flooded green ash and control white ash seedlings. Root dry weight had a significant species x flood treatment interaction after 32 days of flood stress (Table 2.1). Control black and white seedlings had greater root dry weight compared to their flooded counterparts. Dry weights were reduced by 46 % for roots in flooded black and white ash seedlings. In contrast, green ash seedlings had no treatment differences in root dry weight indicating that flooding did not restrict root growth.

Table 2.1. Leaf, current stem, old stem, and root dry weight means for black, green, and white ash seedlings harvested after 8, 16, and 32 days of flood stress. Means, followed by the same letter within a column for each separate harvest, do not differ significantly (Duncan, n=8, $P \leq 0.05$). Current stems harvested on day 16 were not available (N/A).

Harvests	Dry weight biomass for each plant component			
Day 8	Leaf (g)	Current stem (g)	Old stem (g)	Roots (g)
Black control	7.51b	0.86c	11.30b	23.01b
flooded	8.68ab	1.57abc	19.36b	28.34ab
Green control	9.52ab	2.31ab	33.33a	31.82ab
flooded	8.50b	1.39bc	28.61a	22.37b
White control	11.83a	2.34ab	33.82a	33.50a
flooded	10.50ab	2.67a	34.60a	34.36a
Day 16				
Black control	9.39	N/A	14.09b	34.92
flooded	8.69	N/A	21.34b	26.54
Green control	10.78	N/A	41.78a	31.37
flooded	9.09	N/A	38.97a	26.90
White control	11.15	N/A	36.24a	36.64
flooded	8.69	N/A	33.93a	28.64
Day 32				
Black control	9.91ab	1.64ab	17.41c	48.20a
flooded	7.64b	1.35b	17.01c	22.30b
Green control	12.27a	3.11a	37.90b	33.83b
flooded	11.41a	3.20a	51.35a	31.86b
White control	11.47a	2.96a	41.58ab	54.53a
flooded	11.45a	2.66ab	41.89ab	24.83b

Leaf, current stem, old stem, and coarse root starch content had a significant species and duration main effects (Table 2.2). A species x duration interaction has present, however, for starch concentrations in fine roots on day 32 (Table 2.3). Changes in starch content varied with tissue type. On days 8 and 16, flooded seedlings had higher leaf starch than control seedlings, while there were no differences in leaf starch concentration on day 32. On day 16 and 32, white ash leaves had 32 and 54 % less starch compared to the other species. (Table 2.2). The amount of starch in current and old stems were similar and increased slightly during the study. For example, stem starch concentrations ranged from 28.76 to 45.81 mg/g DW on day 8 and from 61.48 to 76.72 mg/g DW on day 32. Flooded stem tissues had 19 to 28 % more starch than control seedlings during each harvest interval. Black ash seedlings had more starch present in both current and old stems than green ash seedlings on day 8. Similar results were obtained on day 16 for the old stems with white ash seedlings having intermediate values, which did not differ from the other two species. By day 32, starch concentrations in the stems of black and green ash seedlings were no longer different. However, white ash seedlings now had a lower current stem starch concentration than green ash seedlings. Flooding also reduced the starch concentrations of below ground tissues. On day 8, fine root starch concentrations ranged from 4.57 to 12.13 mg/g DW. During this harvest, flooded seedlings consistently had lower starch concentrations (Table 2.3). The same was observed on day 16 except that differences were greater between flooded and control black and white ash seedlings and starch concentrations ranged from 4.65 to 29.86 mg/g DW. Overall, flooded black and white ash seedlings had 65 and 76 % less starch than the control seedlings. At 32 days, fine and coarse roots of flooded seedlings had 84 to 88 % and

60 % less starch, respectively, when compared to control seedlings. Fine roots of flooded seedlings had lower starch concentrations with 2.95, 3.05, and 5.98 mg/g DW for white, black, and green ash seedlings, respectively (Table 2.3). For this harvest, starch fine root concentrations ranged from 2.95 to 38.63 mg/g DW. In comparison, green ash seedlings had higher coarse root starch than the other species throughout the study.

Table 2.2. Mean starch concentration of leaf, current and old stem, and coarse roots for control and flooded black, green, and white ash seedlings harvested after 8, 16, and 32 days of flood stress. Current stems harvested on day 16 were not available (N/A). Means, followed by the same letter within a column for each separate harvest, do not differ significantly (Duncan, $P \leq 0.05$).

Main effects		Glucose content in mg per g DW for each plant component			
Day 8 Harvest		Leaf	Current stem	Old stem	Coarse root
Species	Black ash	50.85	40.63a	45.81a	27.87b
	Green ash	54.61	28.76b	38.30b	47.75a
	White ash	38.88	30.44b	42.34ab	32.43b
Treatment	Control	40.81b	28.05b	35.25b	44.00a
	Flooded	55.41a	38.51a	49.05a	27.88b
Day 16 Harvest					
Species	Black ash	75.79a	N/A	59.70a	36.72b
	Green ash	88.13a	N/A	49.96b	61.86a
	White ash	55.41b	N/A	56.59ab	38.63b
Treatment	Control	66.18b	N/A	49.57b	51.05
	Flooded	80.04a	N/A	61.26a	40.76
Day 32 Harvest					
Species	Black ash	59.95b	65.83ab	74.54	33.27b
	Green ash	102.97a	76.72a	74.10	57.32a
	White ash	34.68c	61.48b	69.41	38.65b
Treatment	Control	68.16	58.45b	64.95b	61.57a
	Flooded	63.57	77.57a	80.42a	24.60b

Table 2.3. Mean starch concentration of fine roots for control and flooded black, green, and white ash seedlings harvested after 8, 16, and 32 days of flood stress. Means, followed by the same letter within a column for each species x treatment interaction, do not differ significantly (Duncan, n=8, $P \leq 0.05$).

Species x Treatment	Glucose content in mg per g DW for fine root tissue		
Harvest Time	Day 8	Day 16	Day 32
Black ash control	9.81ab	13.08ab	24.75b
flooded	6.89ab	4.65b	3.05c
Green ash control	12.13a	24.43ab	38.63a
flooded	8.32ab	20.04ab	5.98c
White ash control	8.84ab	29.86a	18.11b
flooded	4.57b	7.22b	2.95c

DISCUSSION

Flooding black ash (*Fraxinus nigra*), green ash (*Fraxinus pennsylvanica*), and white ash (*Fraxinus americana*) seedlings during the growing season altered the biomass and starch concentrations in specific tissues. After the 32 day flood, dry weights from seedlings highlighted species and flood induced differences. Leaf and current stem dry weights were not different except for flooded black ash seedlings, which had lower dry weights (Table 2.1). Black ash seedlings were also shorter in height compared to the green and white ash seedlings (data not shown). This is evident when observing the old stem dry weight of black ash seedlings, which are considerably lower when compared to green and white ash seedlings. The smaller size of each black ash seedling may account for lower accrued growth and an increased susceptibility to flood stress. This is important since roots are sensitive to oxygen deficiency and that no records of sustained growth by anoxic roots has been reported (Armstrong et al. 1994). Under hypoxic or anoxic conditions, seedling survival by anaerobic metabolism is dependant on the availability of substrates present in the roots for respiration (Jackson and Drew 1984).

Root starch concentrations were negatively impacted by imposing flood stress (Table 2.2 and 2.3). After 32 days, flooded ash seedlings had approximately 60 % less starch in its' coarse roots than control seedlings. In comparison, fine roots suffered more drastic reductions, with approximately 84 to 88 % less starch compared to control seedlings. Between day 8 and 16, coarse root starch for all seedlings increased whereas no change occurred between day 16 and 32. Throughout the study, green ash coarse roots had higher starch than both black and white ash seedlings. These absolute values may indicate that more starch is present or required in the root system of green ash. Changes in the fine roots indicate that green ash accumulated starch as flooding proceeded through day 16, then declined by day 32. This starch may have been used by these roots to survive the flooding. White ash also did the same to a much lesser extent and, thus, was less able to tolerate the hypoxic conditions. In contrast, black ash did not accumulate starch in the fine roots during flooding.

This information may suggest that anaerobic fermentation took place and that starch was utilized for maintenance of roots when exposed to low oxygen levels for an extended period (32 days). Another hypothesis to consider is that cell membrane injury occurs after a certain amount of time in anoxic conditions resulting in the cell contents leaking out. Both of these events can occur in response to flood stress. Not surprisingly, these costly processes can drastically reduced root dry weights. During each harvest, we observed that flooded roots were partly yellow with brown and black discolorations of varying degrees mainly at the tips. Root discoloration was most pronounced in black ash seedlings and least in green ash seedlings. Hence, flooded black and white ash seedlings had a 46 % reduction in root dry weight compared to their control counterparts. Similar results by Smith and Bourne (1989) had 50 and 48 % reduction in root mass for 28 day flooded pecan seedlings when compared to controls during bud break or active growth, respectively. For green ash seedlings, however, root dry weights were similar regardless of the flood treatment. Green ash seedlings were clearly more tolerant to flooding than black and white ash seedlings. Research by Good and Patrick (1987) noted that root aeration and anaerobic respiration contributed to the survival of green ash seedlings flooded for a period of 9.5 months (mid-July to May). This adaptive response to flooding may explain why green ash coarse roots had more starch and why root dry weight was not different from control seedlings.

In general, studies on various species have outlined the inhibition of root growth in response to flood stress conditions (Pezeshki and DeLaune 1990; Liu and Dickmann 1992; Topa and Cheeseman 1992). Reductions in root dry weights can be attributed to the combined effects of root death and inhibited root growth (Smith and Bourne 1989). Inasmuch as the root system becomes compromised during flooding, limited water and nutrient uptake subsequently impede above-ground growth and development. Both leaves and stems starch concentrations were influenced by flooding stress. Starch concentrations in current and old stems were similar and increased throughout the study. Black ash seedlings had a greater starch concentration in stem tissue on days 8 and 16. These values were not different for old stem starch observed in white ash seedlings. In comparison, green ash seedlings had slightly higher starch content than the other two species for the final harvest. These findings may suggest that starch allocation to the stem occurs at different rates or times in these three species. In general terms, stems are viewed as a storage compartment where reserves accumulate mostly in the absence of competition by vegetative or reproductive sinks (Goldschmidt and Koch 1996; Loescher et al. 1990). Surprisingly, stems of flooded seedlings had 19 to 28 % more starch than control seedlings regardless of the species. Possibly indicating that flooding increases starch allocation to stems because growth is inhibited and translocation of carbohydrates to the roots may be too costly or wasteful. Additional research is needed to collaborate these findings and it's significance at the whole plant level.

Starch allocation to the stem, however, must be linked to photosynthesis. Therefore, starch accumulation in the leaves may also reveal how flooding stress modifies above-ground processes. For example, high leaf starch concentrations were observed on day 8 and 16 in flooded seedlings; but not on day 32. White ash seedlings had much lower starch concentrations on day 16. By day 32, white ash leaves had a lower starch content than black ash leaves with an intermediate value, which was also lower when compared to starch in the leaves of green ash seedlings. This suggests that flooding may increase leaf starch synthesis initially; but, not after a longer period of time. High leaf starch concentrations, however, can lead to a decrease in net photosynthetic rate. This occurs through feedback inhibition where high leaf starch concentrations lead to the formation of additional soluble carbohydrates, which in turn decrease photosynthesis (Mauney et al. 1979; Luxmoore 1991).

Ultimately, the changes caused by flooding will control the quantity of reserves that is stored prior to the end of the growing season. Our study has shown that black and white ash seedlings had a sizeable reduction in root biomass and starch. In contrast, green ash seedlings had no change in root biomass and a higher concentration of starch in its' coarse roots. Fine root starch concentrations, however, were similar to values in the other two Fraxinus species. The ability of a seedlings to tolerate flooding and recover depends on environmental conditions, time or stage of development, and seedling health. Additional research will be needed to improve our understanding of how and to what extent flood stress influences stored carbohydrates at the whole plant level. This is especially important with respect to root reserves, which act as an important and perhaps the major source of substrates for the subsequent year's early respiration, growth, and development (Loescher et al. 1990).

LITERATURE CITED

Armstrong W., R. Brandle and M.B. Jackson 1994. Mechanisms of flood tolerance in plants. Acta Bot. Neerl 43: 307-358.

Chirkova T.V. and T.S. Gutman 1972. Physiological role of branch lenticels in willow and poplar under conditions of root anaerobiosis. Sov. Plant Phys. 19: 289-295.

Daie J. 1985. Carbohydrate partitioning and metabolism in crops. Hortic. Rev. 7: 69-108.

Dirr M.A. 1990. Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses. (4^{th} Ed.) Stipes Publishing Co., 10-12 Chester St., Champaign, IL 61820. 1007 p.

Drew M.C. 1990. Sensing soil oxygen. Plant Cell & Environ. 13: 681-693.

Everard J.D. and M.C. Drew 1989. Water relations of sunflower (*Helianthus annuus*) shoots during exposure of the root system to oxygen deficiency. J. Exp. Bot. 40: 1255-1264.

Everard J.D., R. Gucci, S.C. Kann, J.A. Flore, and W.H. Loescher 1994. Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. Plant Physiol. 106: 281-292.

Flint H.L. 1983. Landscape plants for Eastern North America. Wiley-Interscience, New York, USA, 667 pp.

Good B.J. and W.H. Jr. Patrick. 1987. Gas composition and respiration of water oak (*Quercus nigra* L.) and green ash (*Fraxinus pennsylvanica* Marsh.) roots after prolonged flooding. Plant and Soil 97: 419-427.

Goldschmidt E.E. and K.E. Koch 1996. Chapter 34. Citrus. In Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships (E. Zamski and A.A. Schaffer Eds.). Marcel Dekker, Inc., New York, USA, pp 797-824.

Guinn G. and J.R. Mauney 1980. Analysis of CO_2 exchange assumptions: feedback control. In: Predicting photosynthesis for ecosystem models. (J.D. Hesketh and J.W. Jones, Eds.) Vol. 2, pp.1-16, CRC Press, Boca Raton, FL, USA.

Hall T.F. and G.E. Smith 1955. Effects of flooding on woody plants, West Sandy dewatering project, Kentucky Reservoir, J. For. 53: 281-285.

Jackson M.B. and M.C. Drew 1984. Effects of flooding on growth and metabolism of herbaceous plants. In Flooding and Plant Growth (T.T. Kozlowski, Ed.) Academic Press, Orlando, FL, pp. 47-128.

Kozlowski T.T. 1984. Plant responses to flooding of soil. BioScience 34: 162-167.

Kozlowski T.T. 1984. Flooding and plant growth. Academic Press, Inc. Orlando, FL, 356 p.

Kozlowski T.T., P.J. Kramer, and S.G. Pallardy 1991. The physiological ecology of woody plants. Academic Press, Inc., San Diego, CA, USA.

Lechowicz M.J. 1984. Why do temperate deciduous trees leaf out at different times? adaptation and ecology of forest communities. Am. Nat. 124: 821-842.

Liu Z. and D.I. Dickmann 1992. Responses of two hybrid *Populus* clones to flooding, drought, and nitrogen availability. I. Morphology and growth. Can. J. Bot. 70: 2265-2270.

Loescher W.H., G.C. Marlow, and R.A. Kennedy 1982. Sorbitol metabolism and sink-source interconversions in developing apple leaves. Plant Physiol. 70: 335-339.

Loescher W.H., T. McCamant, and J.D. Keller 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. HortScience 25: 274-281.

Luxmoore R.J. 1991. A source-sink framework for coupling water, carbon, and nutrient dynamics of vegetation. Tree Physiol. 9: 267-280.

Mauney J.R., G. Guinn, K.E. Fry, and J.D. Hesketh 1979. Correlation of photosynthetic CO_2 uptake and carbohydrate accumulation in cotton, soybean, sunflower and sorghum. Photosynthetica 13: 260-266.

Pezeshki S.R. and J.L. Chambers 1986. Variations in flood-induced stomatal and photosynthetic responses of three bottom-land tree species. For. Sci. 32: 914-923.

Pezeshki S.R. and R.D. DeLaune 1990. Influence of sediment oxidation-reduction potential on root elongation in *Spartina patens*. Acta. Oecol. 11: 377-383.

Reid D.M., F.D. Beall, and R.P. Pharis 1991. Environmental cues in plant growth and development. In: Plant Physiology, A Treatise Vol X: Growth and Development Chapter 2, Academic Press, Inc. pp.65-181.

Roper T.R., J.D. Keller, W.H. Loescher and C.R. Rom 1988. Photosynthesis and carbohydrate partitioning in sweet cherry: Fruiting effects. Physiol. Plant. 72: 42-47.

SAS Institute Inc. 1988. SAS/STAT user's guide, version 6.03 Edition, Cary, NC., 1028 pp.

Sena Gomes A.R and Kozlowski, T.T. 1980. Growth responses and adaptations of *Fraxinus pennsylvanica* seedlings to flooding. Plant Physiol. 66: 267-271.

Smith M.W. and R.D. Bourne 1989. Seasonal effects of flooding on greenhouse-grown seedling pecan trees. HortScience 24: 81-83.

Tang Z.C. and T.T. Kozlowski 1984 Water relations, ethylene production, and morphological adaptation of *Fraxinus pennsylvanica* seedlings to flooding. Plant Soil 77: 183-192.

Taiz L. and E. Zeiger 1991. Plant Physiology. The Benjamin/Cummings Publishing Company, Inc. Redwood City, CA, USA.

Tardiff J. and Y. Bergeron 1993. Radial growth of *Fraxinus nigra* in a Canadian boreal floodplain in response to climatic and hydrological fluctuations. J. Veg. Sci. 4: 751-758.

Topa M.A. and J.M. Cheeseman 1992. Effects of root hypoxia and a low P supply on relative growth, carbon dioxide exchange rates and carbon partitioning in *Pinus serotina* seedlings. Physiol. Plant. 86: 136-144.

SUMMARY CONCLUSION

In summary, flooding black, green, and white ash seedlings for a period of 32 days induced various changes in growth, gas exchange, and starch content. Recognition of each response and to what extent it varies during the course of flooding can indicate how seedlings from the genus *Fraxinus* differ in their ability to tolerate flood stress. Differences in above and below-ground processes were observed. During flooding, a reduction in stomatal conductances occurred on days 2, 4, and 8, followed by lower assimilation rates by day 4, 8, and 11 for black, white, and green ash seedlings, respectively. These findings indicate that black ash seedlings are more sensitive to the low soil oxygen levels that accompany flood stress whereas green ash seedlings were not as sensitive. Further investigation is needed to ascertain the factors mediating this reduction in stomatal conductance.

Growth responses also varied according to species and duration of flood. Minimal biomass growth and minor changes in leaf number, stem height, and caliper were observed for black and white ash seedlings. Caliper growth of flooded black ash seedlings was greater after 32 days of flood stress compared to no change for the white ash seedlings. Leaf area expansion of white ash seedlings was reduced during the entire flood compared no change for black ash seedlings. Prolonged flood stress (ie. 32 days) decreased root dry weights in both black and white ash seedlings by 46 %. These findings demonstrate that as flood duration increases root death and inhibition of growth result. In contrast, differences in biomass were not observed for green ash seedlings regardless of treatment, but stem height, leaf number, and leaf area were reduced by flooding for 8, 16, and 32 days. The ability of green ash seedlings to actively grow throughout the 32 day flood suggests that other factors contribute to its' flood tolerance. Besides observations that carbon assimilation and stomatal conductances were higher during the flood than for the other two species, hypertrophied lenticels, which are thought to improve gas exchange, were observed on the submerged stems for all three species. Many were observed in black and green ash seedlings within 8 to 12 days whereas those for white ash developed later as the flood progressed.

At the whole plant level, variation in growth and gas exchange must also influence the allocation of starch reserves. During the study, starch content varied with tissue type, flood duration, and species. Root starch concentrations were negatively impacted by imposing flood stress. After 32 days, flooded ash seedlings had approximately 60 % less starch in its' coarse roots than control seedlings. In comparison, fine roots suffered more drastic reductions, with approximately 84 to 88 % less starch compared to control seedlings. Changes in the fine roots indicate that green ash accumulated starch as flooding proceeded through day 16, then declined by day 32. This starch may have been used by these roots to survive the flooding. White ash also did the same to a much lesser extent and, thus, was less able to tolerate the hypoxic conditions. In contrast, black ash did not accumulate starch in the fine roots during flooding. This information is pertinent and can tentatively explain why root dry weights for control and flooded green ash seedlings were not different. Differences in starch at the leaf and stem level were more difficult to explain over time. Our interpretation for these differences (without enumeration of each change) suggests that carbon is allocated to the stems at different times or rates for each species. In addition when flooding stress reduced leaf and stem growth, it promotes starch allocation to the stem because it may be too costly for the plants to translocate carbohydrate to the roots. Moreover, roots may not act as a resource sink when anoxic conditions are present in the soil. More research is needed to improve our understanding of how and to what extent flood stress influences stored carbohydrates at the whole plant level.

Ultimately, the quantity of starch stored and allocation plays an important role in seedling survival. It can maintain growth during periods of environmental stresses and ensure a faster recovery. Physiological recovery for each species was modified by duration of the flood. Once a reduction in stomatal conductance occurred, all three species required a 12 to 16 day period to recover. As the flood duration increased to 32 days, black and white ash seedlings recovered in 12 days compared to green ash seedlings which recovered after 8 days. Consequently, green ash seedlings were more tolerant to increased duration of flood stress compared to black and white ash seedlings where root biomass and starch reserves were reduced.

The significance of this work was to characterize the flood tolerance of three different *Fraxinus* species during the summer. Comparison of the morphological and physiological response will add to our knowledge of flood tolerance mechanisms of woody species. In addition, it can indicate why ashes occupy such broad ecological niches. Little research has been done on how closely related species in the genus *Fraxinus* cope with environmental stresses such as flooding. Practical benefits of this research can also be applied in plant selection process and identifying species able to tolerate flooding and soil compaction common in urban landscapes. For example, our findings indicate that green ash is more flood tolerant than black and white ash.