Physiological Responses of Himalayan Blackberry (*Rubus armeniacus* Focke) to Flooding and Implications for Wetland Restoration in the Pacific Northwest

David L. Hays

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2012

Committee:

Dr. Kern Ewing

Dr. James Fridley

Dr. Soo-Hyung Kim

Program Authorized to Offer Degree:

School of Environmental and Forest Sciences
College of the Environment

University of Washington
Graduate School

This is to certify that I have examined this copy of a master's thesis by

David L. Hays

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

Kern Ewing

James Fridley

Soo-Hyung Kim

Date: 30 July, 2012

In presenting this thesis in partial fulfillment of the requirements for a master's degree at the University of Washington, I agree that the Library shall make its copies freely available for inspection. I further agree that extensive copying of this thesis is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright Law. Any other reproduction for any purposes or by any means shall not be allowed without my written permission.

Signature ______________________________

July 30, 2012

Date __________________________________

University of Washington

Abstract

Physiological Responses of Himalayan Blackberry (*Rubus armeniacus* Focke) to Flooding and Implications for Wetland Restoration in the Pacific Northwest

David L. Hays

Chair of the Supervisory Committee:
Professor Kern Ewing
School of Environmental and Forest Sciences

Himalayan blackberry (*Rubus armeniacus* Focke) is a common invader of anthropogenically disturbed wetlands in the Pacific Northwest. Understanding of the plant's physiological response to inundation and associated anoxic conditions is necessary to correctly restore and direct *R. armeniacus*-infested wetlands towards autogenic repair. In a greenhouse experiment, *R. armeniacus* was subjected to partial and complete inundation, as well as treatments involving shading and/or clipping of above-ground biomass. Results indicate that *R. armeniacus* is highly tolerant of anoxic conditions and can induce morphological changes in its roots and stems to tolerate long periods of anaerobic respiration or facilitate gas exchange. Flooding was the most powerful influence on plant health, with cutting treatments increasing mortality rates. In some treatment combinations, shade improved plant health, but the harshest combination of shade, cutting, and flooding proved most stressful. Fully submerged plants were able to survive more than 40 days underwater in laboratory conditions, and initiated rapid shoot growth after two weeks of submergence. This information will better inform restorers as to what kinds of site planning or pre-treatments are required before restoring *R. armeniacus*-infested wetlands.

Table of Contents

Page

List of Figures

Figure Number Page

List of Tables

Table Number Page

Acknowledgments

Crafting this document has been both collaborative and solitary. I thank my chairperson, Dr. Kern Ewing, for his patient and steadfast support, and the help and encouragement of my committee members Dr. James Fridley and Dr. Soo-Hyung Kim. Amanda Davis provided guidance throughout my tenure in the U.W. School of Environmental and Forest Sciences, and professors Dr. Jon Bakker, Dr. Sarah Reichard, and Dr. Greg Ettl provided knowledge and inspiration.

I could not have completed graduate school without financial support, and I have many to thank for that. The Northwest Horticultural Society blessed me with a substantial scholarship, and the National Park Service allowed me to continue intermittent work while I concurrently pursued my education. I am grateful for the home I found in the Biology Department, where John Parks, Eileen O’Connor, and Dr. Scott Freedman provided a stable, fulfilling, and eye-opening chance to participate in higher education to an extent far greater than I had dreamed my return to academia would have permitted.

Lastly, my father provided his invaluable proofreading services on what, in parts, is a very dry read. I also thank my peers in the Forestry School such as Katie Murphy, Tristan Nunez, Krissy Mazur, and Kelsey Ketcheson, who made this research possible as much as any other aid. Mark Beggs collected data for many laborious days of my experiment despite the irritating thorns of my plants and the muggy warmth of the greenhouse. And Lloyd Nackley dutifully kept an eye on my plants and warned me when my experiment verged on unleashing an aphid-Armageddon into the greenhouse.

This page left intentionally blank

1. Introduction & Objectives

Wetlands of the Pacific Northwest are recognized as vital to the integrity of ecological systems, provide valuable ecosystem services such as water quality improvement and shoreline stabilization, and have innate cultural significance (Stevens, 1993). Degradation of freshwater wetlands due to anthropogenic disturbance, such as invasive weed introduction, is a problem common in most settled areas west of the Cascades. Here these wetlands appeal to residential, agricultural, commercial, and industrial development for their aesthetics, irrigation water, recreational opportunities, and access to shipping lanes. Where development alters the landscape, introduced invasive species are likely to follow (Chaloupka & Domm, 1986).

Restoration of weed-invaded wetlands is a goal of many public and private land managers, ecologists, as well as concerned citizens; restoration techniques have been investigated (Fierke & Kauffman, 2006; Kauffman, Beschta, Otting, & Lytjen, 1997; Roni et al., 2002), and restoration projects undertaken, with varying degrees of success (Bennett, 2004, 2006; Daar, 1983; Groves, Williams, & Corey, 1998; Kauffman et al., 1997).

A primary goal of wetland restoration is the establishment of appropriate plant communities in a restoration site, as vegetation provides the foundation of the ecological community being restored (Stevens, 1993). Restoration of disturbed wetlands often involves removal of invasive weeds, planting of native species, and re-engineering impaired hydrology to restore permanent or seasonal flooding of ditched, leveed, or otherwise drained areas. In the Pacific Northwest, these areas often include *Rubus armeniacus* (Himalayan blackberry) as a dominant invasive species (Fierke & Kauffman, 2006; Gray, 2005). Introduced from Western Europe more than a century ago, it spreads both by seed and vegetatively at a prodigious rate, and demonstrates substantial phenotypic plasticity in the sites it invades as well as the environmental conditions it can tolerate (Caplan & Yeakley, 2006; Soll, 2004). It forms large impenetrable brambles that thrive in full sunlight and persist in shade, completely replace native vegetation, and require substantial investments of time and labor to remove (Amor, 1973; Bennett, 2004).

All plant species of the freshwater wetlands of the Pacific Northwest, whether native or recently introduced, must contend with seasonally variable stream flows, surface runoff, and elevated groundwater levels. These generally occur between the heavy winter precipitation and dry summers (Miles, Snover, Hamlet, Callahan, & Fluharty, 2000) of this region's mix of Mediterranean and maritime climates (Kottek, Grieser, Beck, Rudolf, & Rubel, 2006). These seasonal events expose wetland plants to conditions ranging from having their roots periodically experience anaerobic hydric soils to complete submergence. The level of tolerance to the stresses caused by these conditions are what stratify plant species along both temporal (Fierke & Kauffman, 2006), and hydrological gradients in wetland sites (Blom & Voesenek, 1996). Where *R. armeniacus* fits into these gradients is unclear, though it is often observed growing to the edge of standing water in hydric soils saturated for long periods of time. While research has investigated control and management techniques for removing the plant from infested areas, as well as the biological mechanisms the plant employs to be a successful invasive species (Caplan & Yeakley, 2010; McDowell & Turner, 2002), questions about the specific physiological adaptations that allow *R. armeniacus* to respond to flood stresses and be an effective wetland invader have not been addressed.

1.1 Purpose & Need

It is imperative to understand how *R. armeniacus* will behave in restored wetland areas subjected to permanent or seasonal flooding and related changes in soil water table levels in order to efficiently and effectively plan restoration activities. A restorationist should ask several relevant questions before trying to restore an infested wetland: Will the weed flourish with periodic flooding, and will autogenic recovery bring back native wetland plants after its removal? Are wetland infestations of *R. armeniacus* more easily controlled if they are under closed canopy cover? What if they are mechanically treated before seasonal flooding begins? Existing published literature about *R. armeniacus* does not answer these questions.

To address this issue, I implemented two experiments, the first on a large scale in a greenhouse and the second in a small submersion tank in a laboratory, at the University of Washington's Center for Urban Horticulture.

The greenhouse experiment involved propagation of 120 *R. armeniacus* cane-tip cuttings of equivalent size. These plants were then split into eight treatment groups, as determined by the experiment's 2x2x2 factorial design with partial flooding, shading, and stem cutting as possible treatments. A control group was subjected to none of these factors, with seven groups then representing all other possible factorial combinations of treatments. With five replicates, the experiment ran for 90 days and measured stress levels with periodic assessments of leaf chlorophyll levels, growth rate, subjective health scoring, and mortality rates. Morphological changes in stem length, stem count per plant, adventitious root growth, and dry biomass were recorded.

1.2 Objectives: Greenhouse Experiment

The objectives of this experiment were to:

I. Observe the degree of stress exhibited by mature *R. armeniacus* plants while partially submerged and add further stresses from shading and/or biomass removal (stem cutting) to detect potential interactions among stressors;

II. Observe physiological and anatomical changes during and after plants are subjected to these stress treatments;

III. Test for correlations between these observed stress levels and physiological/anatomical changes.

To achieve these objectives I experimentally tested the following hypotheses:

1.2.1 Hypothesis 1: Plants subjected to multiple stresses will exhibit the greatest degree of physiological stress, as expressed by changes in (1) growth rate; (2) chlorophyll density in foliage; and (3) ranked vigor. Flooding will be the most important stressor, but cutting and shading will also be significant.

Flooding has well-documented negative impacts on plants not well adapted to anaerobic conditions, and these effects should be amplified when combined with the lower rate of photosynthate production from shading and removal of emergent biomass, thus starving the plant of the energy necessary to affect physiological and anatomical adaptations to anoxia (Larcher, 2003).

1.2.2 Hypothesis 2: Mortality rates of flooded plants under the various experimental treatments can be established to model the tolerance R. armeniacus has to partial inundation.

Assuming the plant cannot tolerate indefinite flooding, as is the case when it is observed in natural settings, it should be possible to experimentally determine the duration of flooding that the plant can tolerate both in shaded and full-sunlight settings, and if canes have been cut.

1.2.3 Hypothesis 3: Flooded plants that exhibit lower stress levels will be more likely to exhibit anatomical or morphological adaptations.

Plants that are able to invoke these changes will thereby better avoid flood stress. These adaptations include changes in leaf chlorophyll levels, hypertrophic stem-swelling to accommodate aerenchyma, and the growth of adventitious roots into surrounding waters to facilitate increased gas exchange between emergent and submerged tissues.

1.3 Objectives: Laboratory Experiment

This second experiment involved fully submerging *R. armeniacus* seedlings in a glass-walled tank and using time-lapse photography to monitor them for up to 40 days. The objectives of this experiment were to:

1. **I.** Observe fully submerged plants to detect morphological or anatomical changes typical of submergence-adapted plants;
2. **II.** Determine the duration of complete submergence fatal to young plants.

As no similar experiments with members of the genus *Rubus* have been performed in the past, any hypothesis as to the expected outcome of this experiment is largely speculative. Nevertheless, I propose:

1.3.1 Hypothesis 1: R. armeniacus will not evoke any morphological or anatomical changes while submerged.

Only specific wetland plants are known to have evolved mechanism like petiole extension (to raise leaves above the surface of flood waters to facilitate gas change) or rapid shoot extension (achieving the same goal) (Jackson, Ishizawa, & Ito, 2008; Larcher, 2003).

1.3.2 Hypothesis 2: R. armeniacus plants will wither and appear visibly dead after 14 days of submergence.

With gas exchange impeded by submersion of the entire plant, accumulation of ethanol and other metabolic waste products in the plant's tissues should lead to metabolic difficulties and eventual cellular and tissue death, leading to mortality within two weeks.

Results of these experiments should inform hydrologists and ecological restorers as to how long or how often *R. armeniacus* must be completely submerged for flooding to be an effective control method, or to determine if mechanical and/or chemical control of *R. armeniacus* is required before an infested wetland site is restored. I shall relate these findings to wetland habitat restoration, weed control, and the potential use of managed flooding as a restoration tool in wetlands prone to *R. armeniacus* infestation.

2. Literature Review

Examining the physiological responses of *Rubus armeniacus* to wetland conditions requires an understanding of a variety of ecological and biological themes. These range from the composition and ecological role of riparian and wetland areas in the Pacific Northwest to the challenges and techniques involved in their restoration, the natural history of *Rubus armeniacus* itself, and the difficulties associated with the management of invasive species on wetland sites. Review of these topics places my research goals in context within the discipline of ecological restoration and will inform the discussion of my experimental results.

2.1 Pacific Northwest Wetlands & Their Restoration

The lands west of the Cascades from British Columbia south to Northern California are renowned for their abundant rainfall and productive ecosystems. Yet rapid European settlement and industrial development have had a significant impact on this landscape (National Research Council [US], 1996). This should come as no surprise, with an estimated population of 180,000 indigenous inhabitants of the Pacific Northwest (Boyd, 1999) being supplanted in 150 years by, as an example, the roughly 3,400,000 residents counted by the 2010 U.S. census in the Seattle metropolitan area alone. The impacts of population growth and land development are many, and their remedies as varied; the formal discipline of Ecological Restoration has developed to address these problems and scientifically assess restoration techniques (Kauffman et al., 1997).

Land use changes can affect wetland biodiversity, ecological functions, and fisheries stock by disrupting processes such as the supply and movement of sediment from upland sources, water temperature and shading, and riparian forest development. For example, timber harvest and agricultural development have converted many riparian areas in the coastal Pacific Northwest from conifer-dominated to hardwood-dominated forests (Roni et al., 2002). These disturbed woodlands are more likely to be invaded by exotic species of plants such as *R. armeniacus*, which can persist in riparian areas and impede the natural progression of vegetative succession (Fierke & Kauffman, 2006; Kauffman et al., 1997; Ringold, Magee, & Peck, 2008). Because of the great ecological and economic value of the Pacific Northwest's wetland ecosystems in particular (Stevens, 1993), efforts to remove invasive weeds from wetlands sites have been further accelerated to reverse troubling declines in native *salmonid* populations (National Research Council [US], 1996).

Modern ecological restoration philosophy expounds the primacy of *passive restoration*, where anthropogenic influences causing detrimental effects on, or disruptions to, natural processes are removed, and restoration sites are subsequently allowed to autogenously restore themselves (Roni et al., 2002). However, in the case of wetlands infested with invasive plants, *active restoration* must take place, at a minimum, to remove the non-native vegetation and prevent its return until natural processes have had time to restore native riparian vegetation the site (Stevens, 1993). Effectively implementing active restoration of riparian communities in this manner requires an understanding of the unique adaptations of wetland plants.

2.2 The Physiological Characteristics of Wetland Plants

Flooding, along with drought, salinity, and temperature are major factors influencing the geographic distribution of plants (Visser, 2003). Plants growing in areas that experience flooding are subjected to stressors related to submergence, physical damage or uprooting, sediment deposition that alters root conditions or buries the plant, and saturated hydric soils. Stress due to inundation can indirectly yet profoundly impact the respiration rate and overall metabolism of a plant (Larcher, 2003). In many plants, flood stress is more profoundly detrimental than drought stress of a much longer duration (Nash & Graves, 1993). Understanding the causes and effects of these impacts, and how wetland plants have adapted to them, is important when investigating the responses of a specific species to these conditions (Jackson et al., 2008).

Partial flooding creates hypoxic to anoxic soil conditions, and complete submersion creates anoxic conditions for all plant tissues. In both cases, the ultimate cause of flood stress in effected plant tissues is the poor rate of diffusion water imposes on CO_2 or O_2 exchange, a two-orders-of-magnitude decrease, between ambient extracellular levels and plant tissues (Larcher, 2003; Visser, 2003). After cellular metabolism has consumed all available intracellular O_2 or available dissolved O_2 in surrounding water, the resulting low oxygen conditions impede normal respiration in submerged tissues and can cause cell, tissue, or whole-plant mortality, depending on the depth and duration of flooding. The proximate cause of cell death is the change in chemical composition in the cell and its extracellular area. The accumulation of CO_2 and waste metabolites of soil microbes (e.g.

Mn^{2+}, Fe^{2+}, S^{2-},H_2S and carboxylic acids) (Jackson, 2005) within the cell leads to progressive acidification of cytoplasm pH, which in turn causes essential enzymes to denature or lose function (Jackson et al., 2008). Concurrently and during re-oxygenation after anoxic periods, anoxia-intolerant plant species show an unchecked increase in hydrogen peroxide (H_2O_2) in their cell's plasma membrane and apoplast, one of several reactive oxygen species (ROS) that can readily cause oxidative stress, i.e membrane damage through lipid peroxidation of bi-lipid cell membranes (Blokhina, Chirkova, & Fagerstedt, 2001). It should therefore be no surprise that waste metabolites created during anaerobic respiration, such as ethylene and ROS like H_2O_2, have also been shown to participate in signaling pathways that activate a plant's physiological response to anoxia (Jackson, 2005).

Because of these detrimental intra- and extra-cellular chemical processes, wetland plants must tolerate, adapt to, or avoid these conditions through either cellular biochemical or whole-plant physiological responses (Blom & Voesenek, 1996; Jackson, 2005; Larcher, 2003). At the cellular level, enzymatic or compensating anti-oxidant production may buffer cell cytoplasm against pH changes and ROS activity, while also down-regulating metabolic processes to make flood-stressed tissues completely dormant until anoxic conditions abate (Mommer & Visser, 2005). Flood-adapted plants have several physiological mechanisms to tolerate partial flooding, which may include the development of gas-filled aerenchyma vessels within hypertrophic stems, or epicormic root growth above the level of flooding or soil saturation. These structures facilitate gas exchange between submerged and emergent parts of the plant, and diffuse the buildup of ethylene produced by plant tissues in low-oxygen environments as they employ a fermentation-based metabolic pathway in submerged tissues (Jackson et al., 2008; Visser, 2003). These adaptations may be either constitutive or induced, with some plants able to rapidly grow new aerial roots or roots near the more oxygenated surface of saturated soils, initiate rapid shoot extension to restore contact with open air, or change petiole angles to elevate leaf tips towards the water surface (Blom & Voesenek, 1996; Visser, 2003). Fully submerged plants adapted to wetland conditions make use of several strategies to endure or avoid the stress of submersion. Some members of the genus *Rumex* are known to invoke ethylene-mediated acceleration of petiole elongation, causing leaves to bend upward in an attempt to breach the surface of floodwaters. Facilitated by aerenchyma in these leaves, the plants can then exchange gasses with the atmosphere and, to some extent, avoid anaerobic fermentation metabolism (Jackson et al., 2008). Other plants can tolerate flooding for shorter durations without changes in architecture through acclimation, usually by down-regulating metabolic rates until submergence subsides. Yet other plants may have no tolerance for flooding when mature, but time their life-cycle events (seed dispersal, germination, etc.) around seasonal flooding to avoid its impacts (Blom & Voesenek, 1996).

With an understanding of wetland plant adaptations to flooding stress, we can then examine the natural history and ecological characteristics of *Rubus armeniacus* and the management of invasive weeds in wetland settings.

2.3 *Rubus armeniacus*, the “Himalayan Giant”

For more than half a century *Rubus armeniacus* has been the most ubiquitous and commonly recognized blackberry of urban and disturbed sites in the Pacific Northwest

(Bailey, 1945). Since its arrival around 1890, it has spread to most hydric and mesic counties of the Pacific coast and inland to Idaho and elsewhere. While it successfully invades a wide variety of disturbed areas (Groves et al., 1998; Oregon State University Extension Service, 2008; Ringold et al., 2008) and is tolerant of warmer, droughtier sites with poor soils (Caplan & Yeakley, 2006), it is also recognized as a highly detrimental riparian and wetland invader (Bennett, 2004; Ringold et al., 2008). Its taxonomic relationship to other *Rubi* is still being established, but its natural history, including many traits that seem to enable its prodigious growth and invasive behavior, is well defined. Furthermore, control methods have been established to manage or remove infestations.

2.3.1 History of Introduction

The historical facts surrounding the introduction of an invasive species can range from precisely recorded to uninvestigated or unnoticed; those regarding *Rubus armeniacus* are a thoroughly muddled combination of all three. Yet the history of a species' introduction is important to habitat restorers and ecologists working to manage exotic weeds, as their historical path to establishment and invasion can lend clues to further controlling or mitigating their spread. Most accounts state that *R. armeniacus* was introduced to the United States by prolific horticulturalist and fruit cultivar developer Luther Burbank as the "Himalaya Giant," (U.S. Department of Agriculture, 1913; Williams, 1915) to which we can credit "Himalayan blackberry," the more commonly used name during the last several decades (Bailey, 1945). Although Burbank undoubtedly possessed, selectively bred, and distributed the plant from his gardens in Santa Rosa, California, his claim that the seeds had been “brought out of the Himalayas by a British expedition” (Markarian & Olmo, 1959) cannot be verified[1]. In fact, Rolfe (1919) stated that J.S. Gamble, while collecting and studying the *Rubi* of the Himalayan region, discovered no *R. armeniacus*-like blackberry there (Mallah, 1954). Furthermore, consensus among many botanists is that *R. armeniacus* is identical to a plant grown from seeds collected in a neighbor’s garden in 1889 by *Garteninspector* Theodore Reimers of Hamburg, Germany. The Theodore Reimers variety, especially plants established in England, are almost indistinguishable from the *R. armeniacus* found in North America today (Mallah, 1954). Therefore an Englishman may well have sent Burbank his seeds, but they were of Western European, not Himalayan, origin.

2.3.2 Distribution

Burbank selected and vegetatively propagated for distribution a particularly vigorous F2-generation plant from the initial seeds he received. This “Himalaya Giant” was offered in his General Catalog of 1893, and he further promoted it in 1894 with a special circular mailed to customers on the Pacific Coast and in the South, where Burbank believed it would be most successful. By 1905 the U.S. Department of Agriculture acquired 200 plants after

---

[1] Burbank received the seeds around 1890, and in truth, the “Great Game” was at its height in Central Asia (Kulke & Rothermund, 2004) and British expeditions of many kinds, from British Army maneuvers to Royal Geographical Society explorers, were plentiful in the Himalayan region. Accordingly, the popular press was full of accounts romanticizing the grandeur and mystery of the Himalayas from reports sent directly to the Times of London from writer-alpinists like William Conway (Molenaar, 2010) and Lawrence Waddell (Waddell, 1899), and the name may have been applied to invoke popular perceptions of the Himalayas when Burbank brought the blackberry to market.

learning it was flourishing in the Puget Sound region of Washington State.[2] The USDA's Office of Foreign Seed and Plant Introduction distributed plants to ten states east of the Rockies where *Rubus armeniacus* proved far from a commercial success; the traits that make it an infamous invasive weed spelled its doom in farms and gardens. Recipients of the plant reported that it was "[the] most troublesome berry plant in the South to control," "...resistant to drought; unmanageable," with some canes growing to 26 feet in length (U.S. Department of Agriculture, 1913).[3] By the middle of the 20th century, Baily (1945) reports the plant's range in North America had reached approximately where it is found today (Figure 1), though infestations have undoubtedly followed the growth of urban and agricultural development in these areas, and even crept into the fringes of wilderness areas (Moore, 2009). Internationally, the plant is also a significant weed in Chile, New Zealand, and Australia, where it may have been introduced as early as 1842 and was subsequently declared a noxious weed in 1894 (Groves et al., 1998).

---

[2] The redistribution of these Puget Sound plants may have inspired Santa Rosa nurseryman Alfred Mitting, contemporary and competitor to Luther Burbank, to claim *R. armeniacus* as having been introduced directly to Seattle from seeds retrieved by British expeditions without Burbank as an intermediary (Markarian & Olmo, 1959); as of 1915 Mitting was a prominent supplier of the plant to farms and nurseries, along with his own hybrids derived by crossing Himalaya Giant with other *Rubus spp*. stock (*Poultry Success,* Volume 26, 1915).

[3] In 1906 an Austin, Texas nurseryman wrote most eloquently: "I bought some plants of this at 50 cents or $1.50 apiece when first introduced, and I now have a row of them 200 yards long, 5 feet deep and 20 feet broad. We can not [*sic*] get at the roots of them to cut them off; they would turn a mad bull or a scared cat… I sold a few plants, but I have apologized to all who bought them and gave them something else."

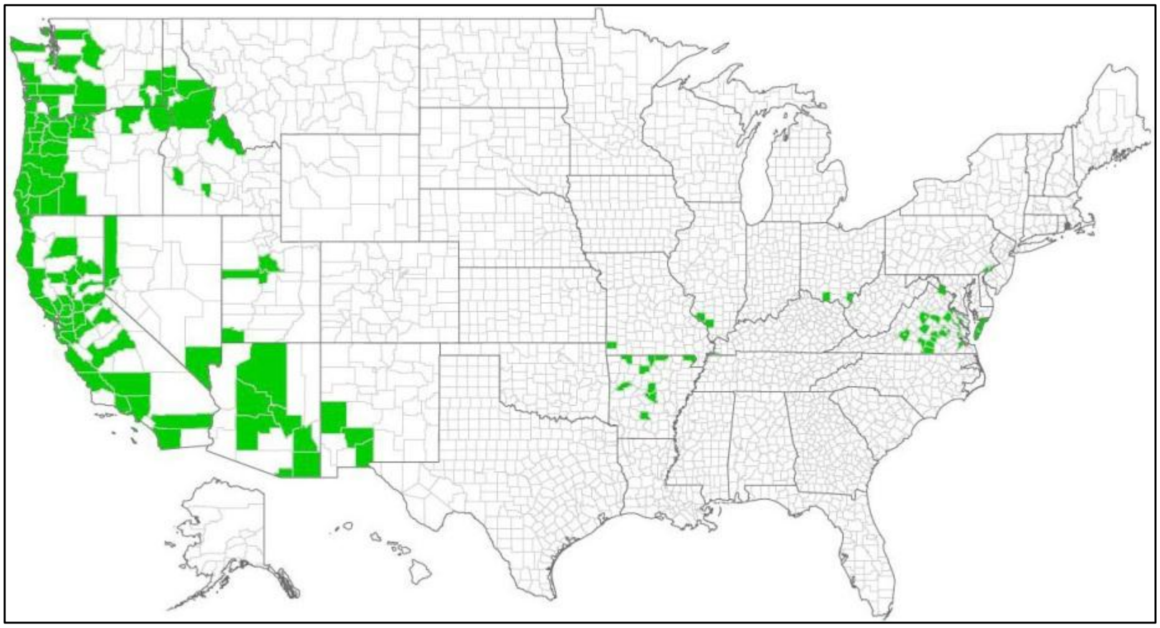


Figure 1. Distribution of Rubus armeniacus in the U.S. Source: USDA Plants database, queried August, 2011. Mapping is notably incomplete in Washington State.

2.3.3 Taxonomy & Genetics

The genus *Rubus* is one of about 100 genera in the family *Rosaceae* (Robertson, 1974) and includes an estimated 1000 species distributed globally (Thompson, 1997). There are more than 200 native species of *Rubus* in North America, some of considerable commercial value such as *R. strigosus* (American raspberry), and at least 15 invasive species[4], not counting introduced but non-invasive cultivated species and varietals (USDA NRCS, 2011). The species names *R. discolor* Weihe & Nees and *R. procerus* auct. non P.J. Müll. ex Genev are older, superseded names now considered synonymous with *R. armeniacus* Focke (USDA NRCS, 2011).

---

[4] To add confusion to the discussion of this species, two invasive *Rubus* species common in the Southeast (but not found in the Pacific Northwest) include *R. bifrons* and *R. geniculatus*, commonly "Himalayan berry" and "false Himalayan berry," names presumably derived from their resemblance to *R. armeniacus*.

Rubus armeniacus is placed in the subgenus *Rubus* (formerly *Eubatus*) and section *Rubus,* where it joins other tetraploid European blackberries with 7 chromosomes (3n=21), more commonly referred to as the *Rubus fruticosus* L. aggregate. Subgenus *Rubus* is well supported as a distinct clade by DNA analysis, but the split between North American sect. *Rubus* and the European *Rubus fruticosus* L. agg. is less distinct (Alice & Campbell, 1999). Hybridization is common even among presumably distant *Rubus fruticosus* L. agg. species, often forming triploid, tetraploid, and pentaploid plants that also greatly confuse taxonomic study. Furthermore, DNA sequencing has also shown homoplasy in key morphological characters, such as leaf type and stem armature, making traditional botanical identification techniques inadequate for mapping this taxon (Alice, Riksson, Riksen, & Campbell, 2001). Therefore, *R. fruticosus* agg. includes several hundred named species of dubious status, many considered redundant misidentifications of others (Alice & Campbell, 1999; Amor, 1973; Thompson, 1997). *Rubus armeniacus* and others of *Rubus fruticosus* L. agg. commonly exhibit apomixis / agamospermy (Alice & Campbell 1999), and some species like *R. armeniacus* almost exclusively produce seeds through pseudogamous apomixis where pollination is necessary to initiate development of the embryo and endosperm, but the embryo is never fertilized and is solely maternal in origin. This asexual seed production, along with its tendency to spread vegetatively, allows for a rapid dispersal of a single genotype (Thompson, 1997); DNA sequencing of Australian populations of *R. armeniacus* found an astonishing lack of genetic variation from 50 plants sampled from 29 distant locations throughout Australia (Evans, et al 1998), effectively identifying the entire *R. armeniacus* infestation of Eastern Australia as a single clone.

2.3.4 Natural History

A Flora of California (Munz & Keck, 1973) presents the following botanical description of mature *Rubus armeniacus,* under the synonym *Rubus procerus*:

> "*Rubus* [*armeniacus*] is a robust, sprawling, more or less evergreen, glandless shrub, appearing as "great mounds or banks" (Bailey 1945), with some of the canes standing up to 3 meters tall. Other canes are decumbent, trailing or scandent up to 20-40 feet long (Bailey 1923), frequently taking root at the tips. The primocanes are pilose-pubescent, becoming nearly glabrous with age. These are strongly angled and furrowed, bearing well-spaced, heavy, broad-based, straight or somewhat curved prickles 6-10 mm long. Primocane leaves are 5-foliate, glabrous above when mature and cano-pubescent to cano-tomentose beneath. There are hooked prickles on the petioles and petiolules. The leaflets are large and broad with the terminal leaflet roundish to broad oblong. Leaflets are abruptly narrowed at the apex, unequally and coarsely serrate-dentate. Floricane leaflets are 3-5 foliate and smaller than on the primocanes. The inflorescence is a large terminal cluster with branches in the lower axils. The peduncles and pedicels are cano-tomentose and prickly. The flowers are white or rose colored, 2-2.5 cm across, with broad petals. Sepals are broad, cano-tomentose, conspicuously pointed and soon reflexed, approximately 7-8 mm long. The roundish fruit is black and shiny, up to 2 cm long, with large succulent drupelets. The fruit ripens late compared with native blackberries and over a considerable interval (Bailey 1945), from midsummer to autumn (Bailey 1923)."

Rubus armeniacus effectively employs both seed production and vegetative cloning as reproductive strategies. Through the apomixic mechanisms described earlier, *R. armeniacus* is capable of producing 7,000 - 13,000 seeds m^{-2} in optimal locations, though plants seldom produce seed when fully shaded. Seeds sewn directly from collected fruits are 7-10% viable and usually germinate after two years (Amor, 1974a; Northcroft, 1927), theoretically allowing each square meter of bramble to directly produce up to approximately 500-1,300 new plants (Amor, 1974a). When in their highly palatable mature drupes (blackberries), seeds are easily consumed and spread by wildlife, and birds have been specifically studied as agents of long-distance dispersal. Northcroft (1927) determined that seeds passed though the guts of various species of birds appear to have an enhanced germination rate[5], up to %17, thereby nearly doubling the plant's seed-derived reproductive potential to an approximate 700-2,200 plants m^{-2}. However, seedlings often fail to establish in areas with less than 44% sunlight and are commonly found at very low densities in or near established infestations (Amor, 1974a). The plant seems to rely on sunny, recently disturbed sites away from established infestations to establish from seed, and although seedlings grown in laboratory settings grow vigorously, seedlings in the field do not always compete well with other weedy species and are easily overtaken by conspecific competitors spreading vegetatively (Hoshovsky, 2000).

---

[5] Brunner, et. al. (1976) studied fecal material from introduced foxes and native emus, two suspected vectors for the plant's spread in Australia, and found the extracted *R. armeniacus* seeds had up to %30 germination in the laboratory. However, Amor (1974), Northcroft (1927), and my own propagation attempts (unpublished) have shown that the seeds are more resistant to germination in field conditions and susceptible to fungal infection while being cold-stratified in the laboratory.

The flower-bearing floricanes of *R. armeniacus* initiate growth from the root crown in the spring of the plant's second year and mature by mid-summer; growth of the larger primocanes that enable vegetative spread begin growing later in the spring or early summer, may accelerate their growth after the summer equinox (Heslop-Harrison, 1959), and contribute greatly to the plant's invasiveness (Amor, 1973). Growing vigorously, they begin to arch over after reaching as little as 40 cm in height and quickly spread on runners up to 6-12 meters in length (Amor, 1974a; Bailey, 1945). Primocane tip apices readily root when they reach soil or leaf detritus, with rooting triggered by low light levels sensed by the apical 25 cm of cane (Heslop-Harrison, 1959), though cane tip rooting only occurs on first-year canes (Amor, 1974a). Vegetative reproduction through this method is very common for *R. armeniacus*; Amor (1974) determined that ninety-six percent of 50 observed first-year canes rooted at their tips, an average distance of 3.3 meters from their parent plants. As *R. armeniacus* infestations grow, lignified and heavily prickled canes older than two years quickly senesce and die in the interior of the bramble, admitting younger primocanes from more exterior daughter plants to re-infest the interior of the bramble with younger plants (See Table 1). This growth pattern creates an ever-increasingly dense and impenetrable mesh of old and young canes that typify established infestations, with a potential density of up to 525 canes m^{-2} (Hoshovsky, 2000).

Table 1. ***R. armeniacus*** **infestation origin and related annual growth pattern**

Plant Origin	*Year 0*	*Year 1*	*Year 2*	*Year 3*
Seed:	Flowers on floricanes form drupes & seed; seeds dispersed; remain dormant first summer.	Few (up to 5% of seeds) germinate.	~10-17% seeds germinate (up to %30 if passed through a animals' gut). First non-flowering canes establish for F0 plant.	Floricanes form and seeds produced; primocanes initiate vegetative spread (See next row).
Vegetative:	Root establish-ment & first non-flowering canes grow at rooted cane tip of parent plant (F0).	Floricanes form and seeds produced by F0, F1 plants; primocanes initiate vegetative spread and start new F2 daughter plants at cane tips.	F2 daughter plants form first floricanes and primocanes. Parent F1 plant begins to senesce. Most F0 plant dead.	Third generation of daughter plants begin cane formation; most F1 dead; F2 and F3 daughter plants re-infest interior of bramble with F4 plants forming at cane tips. 4-years of canes cover 113 m^2 (Amor, 1974a).

Well-established plants develop a large root crown growing irregularly at the base of their canes, up to 20 cm in diameter. Roots have been recorded to a length of nearly 10 meters, growing up to 90 cm deep, and adventitious suckering from roots has also been observed (Northcroft, 1927), though usually occurring close to the root crown proper. Propagation using a single root cutting produced a thicket 5 meters in diameter after two years (Amor, 1973).

Naturally occurring herbivory by browsing mammals has been observed on established *Rubus armeniacus* plants and insects such as *Lepidoptera* larvae and leaf-miners can damage the plant's foliage, by the extent to which this influences the health and reproduction of the plant has not been well established and may not be significant. Herbivory on young, un-lignified shoots and leaves occurs, but is generally well discouraged by the plant's large prickles and spiny, tomentose leaves (Caplan & Yeakley, 2006). Fruits are readily eaten by birds, deer, rodents, foxes, bears, and other wildlife (Brunner, Harris, & Amor, 1976; Hoshovsky, 2000; McDowell & Turner, 2002; Soll, 2004) but small seeds pass though these organisms unharmed, and as discussed, their germination rate is improved.

2.3.5 Management & Control

Significant research has already been conducted on methods to manage and control *Rubus armeniacus* (Amor, 1973, 1974b; Bennett, 2006; Groves et al., 1998; Hobbs & Humphries, 1995; Hoshovsky, 2000; Jones, 2004; Oregon State University Extension Service, 2008; Soll, 2004). The consensus is that small-scale infestations can be controlled by manual excavation of the root-crown of mature plants, but management of large-scale infestations is among the most complex, costly, and difficult tasks for weed managers in the Pacific Northwest.

Physical treatment to remove most above-ground biomass and facilitate access to the infested area is commonly the first step in controlling and managing an infestation, and it is usually followed by an application of chemical herbicide. Physical control may involve mechanical treatments including mowing, hand slashing and digging, or goat grazing (Ingham, 2008), the latter of which shows the least utility in most cases without other treatments (Bennett, 2004). Mowing is effective both as an initial removal of impenetrable brambles, and in areas intended to be prairie-like habitat, grassland, or lawn, continued mowing is sufficient for long-term management of *R. armeniacus* and favors grasses and forbs (Bennett, 2006; Oregon State University Extension Service, 2008). In wetlands where hydric soils will not support wheeled tractors or mowing equipment without damage to the wetland and/or the equipment, physical treatment with handheld brush cutters, weed-eaters, power saws, axes, machetes, loppers and clippers, or other tools may be more appropriate, with commensurate increases in time and labor costs over mechanized approaches (Soll, 2004).

Grazing by goats[6] has also been used to remove above-ground biomass, especially where goats can be penned in infested areas too steep or rocky for safe or easy access. Goats are often preferred over sheep, as sheep are more selective in their browsing and may cause soil compaction and other issues. Furthermore, goats preferentially browse on woody vegetation and readily consume live blackberry, but they do not eat dead portions of blackberry brambles. Besides this limitation, the effects of grazing are similar to mowing; regular grazing can be used to manage infestations (Hoshovsky, 2000), but grazers as a surrogate for other permanent control methods have proven ineffective as the plants readily resprout and re-infest areas after grazing is stopped (Ingham, 2008).

Land managers in recent decades have increasingly seen wildland fire as a tool to be used in ecological restoration and ecosystem management (Brown, Agee, & Franklin, 2004). In mesic or ephemeral wetlands areas where *R. armeniacus* is present, fire can effectively remove above-ground biomass under appropriately dry conditions. However, fire-effects research finds that *R. armeniacus* quickly and vigorously resprouts new canes from its root crown or major root nodes post-burn (Ferguson, 1981). More generally, this weedy plant responds as positively to fires as it does to many other type of disturbance, both by

---

[6] The goat chosen makes a difference: small Angora goats seem to be more practical than larger Spanish goats, and dairy goats are reported to be “goof-offs” (Daar, 1983).

resprouting[7] or through germination of a dormant seedbank that sprouts in response to post-fire changes in soil temperatures or other environmental cues (Tirmenstein, 1989).

Once access to the infested area is provided by physical treatment, the labor-intensive work of excavating and removing the root crowns of individual plants can be attempted. At scales beyond a few acres or in areas sensitive to soil disturbance or the trampling of untrained laborers, excavation may be impractical, especially at sites far from inexpensive labor (i.e. students on school campuses or concerned volunteers at popular parks). The remaining options for control are either biological or chemical.

Biological agents that include native fungal and bacterial pathogens that infect both native and invasive *Rubi* are being tested in Canada. However, these agents are being developed for non-selective control of all weedy species of *Rubus* within the context of use in the timber industry. The intent is to inhibit the weedy growth of native shrubs to reduce competition and improve conditions for commercial timber plantings (Nutt, Oleskevich, Shamoun, & Punja, 1996). However, in Chile, Australia, and New Zealand, strains of the European fungal leaf rust *Phragmidium violaceum* have been introduced as biological control agents, and they have been effective in slowing growth and fruit production of *R. armeniacus* in the areas of western Australia (Groves et al., 1998). Though the mechanism of its arrival is unknown, in 2005, the rust was observed for the first time in North America on the west coast of Oregon (Osterbauer, Trippe, French, & Butler, 2005). Research is now trying to determine how the rust may affect the spread of invasive *R. armeniacus* and *R.*

---

[7] Personal observations of burned areas in the University of Washington's Union Bay Natural Area infested with *R. armeniacus* confirms this; at many sites fire may complicate restoration efforts by indiscriminately removing all vegetation, including desired species, and promoting invasive weeds.

laciniatus plants and address how to manage the disease's potential impacts on commercially important cultivated varieties.

Until the *P. violaceum* rust is better understood or other biological control agents are developed, chemical control remains the most common and effective method of treatment for many large-scale infestations, either in non-selective broadcast spraying or selective spot treatments. The choice of these two approaches is predicated upon the density, scale, and restoration goals of specific sites (Hoshovsky, 2000). Non-selective spraying kills all vegetation on a site, causing a disturbance that may promote re-infestation of undesirable species; may foster herbicide-resistant strains of weeds after repeated application (Oregon State University Extension Service, 2008); may potentially drift out of the target area; may cause unintended pollution in wetlands or other sensitive biological systems; but is easily and economically employed over large areas. Spot treatment, if the herbicide administrator is properly trained to identify the target species for selective control, has a much smaller ecological footprint but is more time- and labor-intensive (Hoshovsky, 2000). The preferred treatment method for all but the most extensive infestations is spot treatment of cut stems after above-ground biomass removal (Soll, 2004). Herbicide application is most effective in late summer to autumn when leaves are fully developed and plants are not moisture stressed, when translocation of the herbicide from foliage to root tissues may be highest (Oregon State University Extension Service, 2008; Soll, 2004). Older plants appear to be more resistant to herbicides than younger, and may require more repeated applications (Amor, 1974b). Use of chemical herbicides in wetlands areas must be done with great care and only where appropriate and allowed by regulations, but the “Rodeo” formulation of glyphosate is considered the most safe and effective in these areas (Bennett, 2004, 2006). Annual hand-pulling or spot herbicide applications are then necessary for several years to ensure native plants can become established and eradication is complete.

2.4 The Challenge of Managing *Rubus armeniacus* in Wetlands

Rubus armeniacus is listed as a noxious weed in both the states of Washington and Oregon. In Washington, it has been added to the state’s Noxious Weeds List as a Class C weed, a designation that recognizes its widespread distribution while allowing for control where local infestations are still small or are putting significant resources at risk (Washington State Weed List, 2009). As noted in an open letter to the Washington State Noxious Weed Control Board supporting its addition to the state list, King County’s specific reasons to support its listing were two-fold: allowing landowners to avoid permitting issues when controlling the plant, and allowing removal of the weed from biologically sensitive sites and wilderness areas with incipient infestations. Oregon lists it as a Class B weed, “a weed of economic importance which is regionally abundant, but which may have limited distribution in some counties” (Oregon Department of Agriculture, 2011). Its limited state-wide distribution can be accounted for due to the arid nature of the eastern half of the state where *R. armeniacus* is rarely found (Figure 1).

The impacts of *R. armeniacus* invasions have been studied in the US, and to a greater extent in Australia and New Zealand. There it has a major impact in both natural and agricultural areas and is the subject of various control and management efforts (Groves et al., 1998). In the US, particularly the Pacific Northwest, the plant is a common invader of disturbed sites with a tolerance for diverse topographic, physiographic, edaphic, and climatic conditions (Caplan & Yeakley, 2006; Gray, 2005). Some impacts are considered positive; it provides wildlife with edible fruits and foliage, nesting habitat, and vegetative cover after quickly colonizing disturbed sites (James & Lockwood, 1998). However, it harbors pest species (Dutson, 1974) and easily excludes other plants after quickly forming dense thickets, spreading vegetatively as it rapidly grows its arching canes (Ringold et al., 2008), or by seed in the guts of birds and other wildlife that consume the fruits and expel the seeds far from their parent plant, as previously discussed in detail. Several other factors affecting its invasive abilities have been shown to include a higher photosynthetic rate during longer periods of the year (McDowell & Turner, 2002) and high water use efficiency during periods of drought (Caplan & Yeakley, 2006). The plant also seems to invest greater resources to reproductive efforts, at lower relative resource costs, than native *Rubus* species (McDowell & Turner, 2002).

When considered in light of wetland and riparian areas specifically, *R. armeniacus* is a major invasive weed in wetlands and riparian areas of the Pacific Northwest (Fierke & Kauffman, 2006; Oregon State University Extension Service, 2008; Ringold et al., 2008), and can have persistent impacts on invaded sites once established. In early successional communities formed after disturbances in river corridors, it reaches peak infestation levels around years 12-16 post-disturbance, and can persist even in relatively closed canopy for much longer (Caplan & Yeakley, 2010; Fierke & Kauffman, 2006). Fierke and Kauffman (2006) also found that, while eventually able to develop within monocultures of *R.*

armeniacus infestations, deciduous tree species took significantly longer to recover in disturbed and infested river corridors. Instream biotic integrity also suffers significantly in *R. armeniacus*-invaded watersheds (Ringold et al., 2008), most likely due to changes in quality or quantity of leaf litter input for aquatic invertebrate use (Going & Dudley, 2007). These impacts, in concert with water quality degradation caused by other anthropogenic inputs, have had profound negative effects upon the ecological integrity of entire watersheds in the Pacific Northwest (National Research Council [US], 1996; Roni et al., 2002).

With *R. armeniacus* clearly more than a nuisance weed in wetland areas and continuing to have profound negative impacts on wetlands and watersheds over a large geographic region, it is a problem that cries out for a solution. Specifically, more needs to be understood about its tolerance to, adaptation for, or avoidance of wetland conditions in ways that illuminate how the plant is able to infest areas subject to flooding. Greater understanding of the plant's physiology may generate better techniques to protect or restore wetlands prone to *R. armeniacus* infestation.

3. Methods

To investigate the physiological response of *R. armeniacus* to flooding and submergence, I pursued a pair of greenhouse and laboratory experiments, eschewing the variability of a field experiment for these more controlled environments; subsequent field tests would not be without warrant, but are beyond the scope of this research. The first experiment was conducted in the Douglas Greenhouse at the University of Washington's Center for Urban Horticulture and was the larger, more ambitious, and more quantitative of the two experiments, the second narrower in focus, observational, and qualitative. The greenhouse experiment was designed to study the effects of partial submergence (10 cm above the soil line) while plants were under either sunny or shaded conditions, along with the effects of cutting most of the above-ground biomass away from some plants. The second laboratory experiment was designed to allow a single plant roughly 15cm tall to be completely submerged and observed for up to a month or longer. The specific materials and methods for these two experiments are detailed below.

3.1 The Greenhouse Experiment: Partial Flooding and Shade and/or Cutting Treatments

The greenhouse experiment was a 2x2x2 factorial design, involving flooding, shading, and cutting as the three possible treatments. This design created eight possible combinations of treatments to be applied to my test subjects, six-month-old *R. armeniacus* propagules. The flooding and cutting treatments were easily applied to individual plants, but practical considerations forced me to apply the shade treatment to groups of plants under large shade structures, as any attempt to shade individual plants would have inadvertently shaded those around them in the limited space within the greenhouse.

The three treatment types were selected to mimic either environmental conditions found in wetlands invaded by *R. armeniacus* or those potentially applied during weed control efforts or ecological restoration projects. The flooding treatment was designed to mimic the periodic partial flooding that *R. armeniacus* would experience in a typical riparian or upland wetland setting with occasional spring flooding or a perched water table. Experimental flooding was constant (water was never drained away from plants) to assess the worst-case environmental condition the plant might encounter. The shading treatment was chosen as a proxy for increased canopy cover in wooded wetlands, where *R. armeniacus* often persists after first establishing in early riparian succession (Fierke & Kauffman, 2006; Ringold et al., 2008). Lastly, mowing or other means of above-ground biomass removal is usually the first step in managing infestations of *R. armeniacus*. Thus, the third treatment, cutting the plant to 17 cm tall at the initiation of the experiment (a height that matched the top of the plant's enclosing bucket and easily measured), was a proxy for conditions created when plants are mowed and their photosynthetic capabilities are greatly reduced.

The experiment was conducted during the summer and fall of 2010 and consisted of several distinct phases: (1) propagation of *R. armeniacus* tip cuttings for use as experimental subjects, (2) assembly and installation of the experimental apparatus, (3) performance of the experiment and gathering data from live plants between August 23rd and November 17th of 2010, and (4) a post-experiment dry biomass assay.

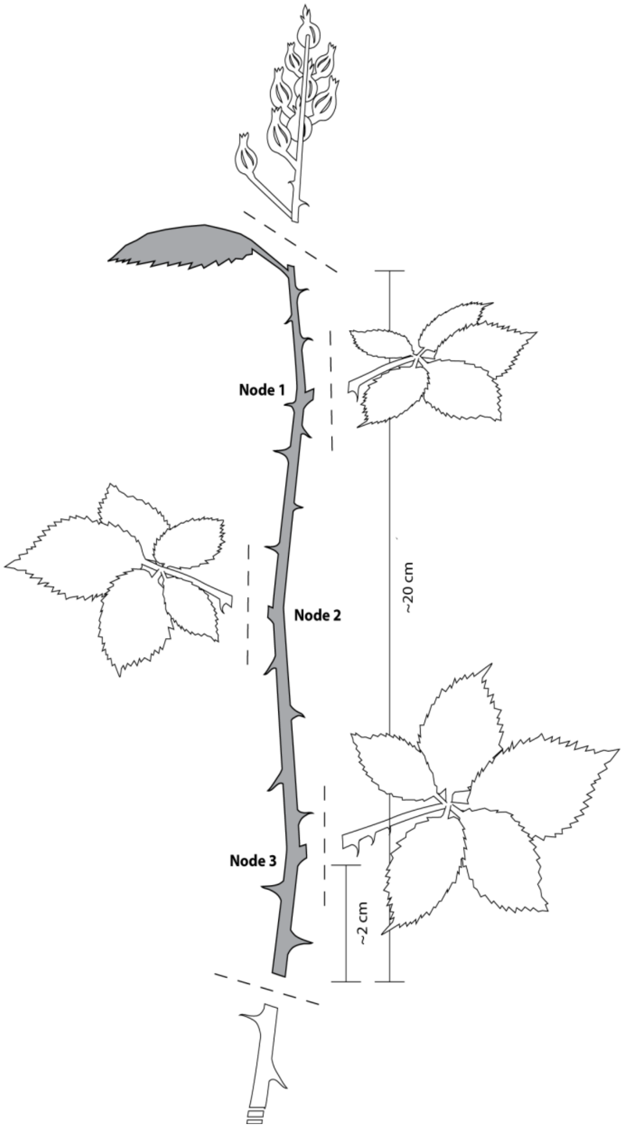


Figure 2: Diagram of cane tip cutting preparation for propagation.

3.1.1. Propagation of test subjects

Because of its strong tendency to reproduce asexually (Alice & Campbell, 1999; L, Sampaio, Foerster, & Bertol, 2003), the collection of *R. armeniacus* specimens for this experiment occurred in a small area near the Douglass Greenhouse with the assumption that they were genetically representative of a significant portion of the species at-large. The 200 floricane tip cuttings measuring 15-19 cm in length were collected in April of 2010, their flower or apical leaf buds were removed, and all but the newest full leaves near the tip were removed down to the third node (Figure 2). Cuttings were then inserted 3-4 cm deep into a 1:4 peat moss to Perlite potting mix and placed in a mist hood for ten (10) weeks. Roots successfully developed in 147 propagules (Figure 3a) and these were transplanted into 1-gallon pots filled with 0.75 liters of 1:6 mix of

Perlite and standard Lakeland Natural & Organic brand potting soil. To ensure uniformity of soil density and height, a simple jig was created to tamp the soil around the rooted cutting to a uniform height of 18 cm above the bottom of the 1-gallon pot (Figure 3b). 1-1.5 cm of paver sand was then poured on top of the soil surface to keep buoyant soil components from floating away from pots placed in flooded conditions. Potted plants were watered as needed and twice fed 150ml of 50% Hoagland solution prepared by Lloyd Nackley, Center for Urban Horticulture. Of the 147 cuttings that developed roots, 128 survived potting-up and grew for another 2 months before the healthiest and most uniform 120 plants were selected and assembled to begin the experiment.

Figure 3a: A rooted cutting.

Figure 3b: A potted cutting with the soil-tamping jig.

Figure 3c: Three potted cuttings placed within a labeled bucket showing it assigned to Block A, and treatments "not shaded" (0), "not flooded" (0), and "cut" (1):

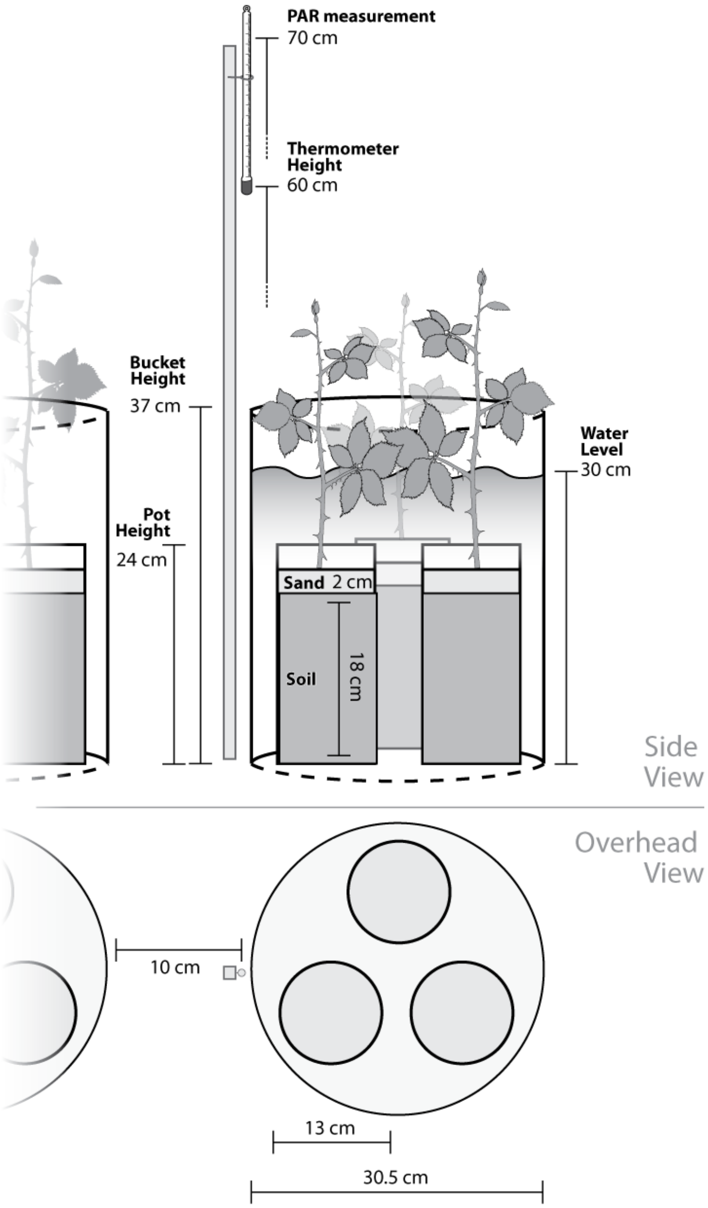


Figure 4. Detailed illustration of the buckets of plants and other experimental apparatus.

3.1.2. Experimental Design

The 120 propagules in their 1-gallon pots were placed into larger 5-gallon buckets so that three plants shared a single bucket (Figures 4 and 5). The buckets were filled with randomly selected plants, and the 1-gallon pots in each bucket were oriented in a triangular configuration with one long side, defined by any two pots as corners, perpendicular to the north and south walls of the greenhouse. Buckets on the east side of a greenhouse table had this long side facing south (towards the ventilation fans) and buckets on the west side of the tables had this pair of plants perpendicular to the north wall (away from the fans). This allowed for a consistent orientation of the plants as viewed by data collectors when standing on either side of a table, always orienting two plants to

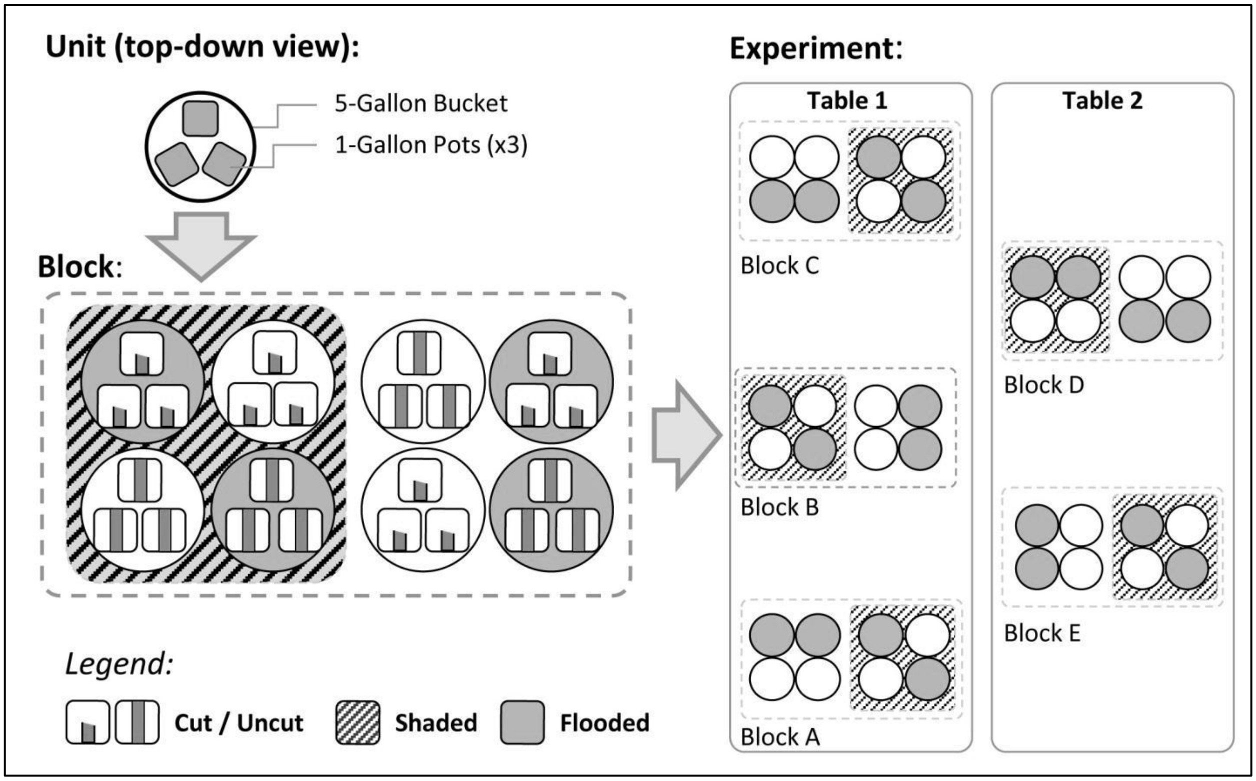


Figure 5. Experimental design, diagramed from experimental unit (a single bucket) to a replicated block, to the full layout of the experiment on two tables of the Douglass Greenhouse at the University of Washington's Center for Urban Horticulture.

the left and one to the right in each bucket. In this orientation, plants were numbered 1 to 3 in a counter-clockwise manner, starting with the plant in the far left part of the bucket.

The forty (40) buckets were placed in five replicate blocks (df=4) of eight (8) buckets each (Figure 5), and labeled to indicate which of the three treatments were to be applied to it (Figure 6). From this a bucket's unique identification code was derived with "1"s indicating a treatment was applied in this order: shaded/not shaded, flooded/not flooded, and

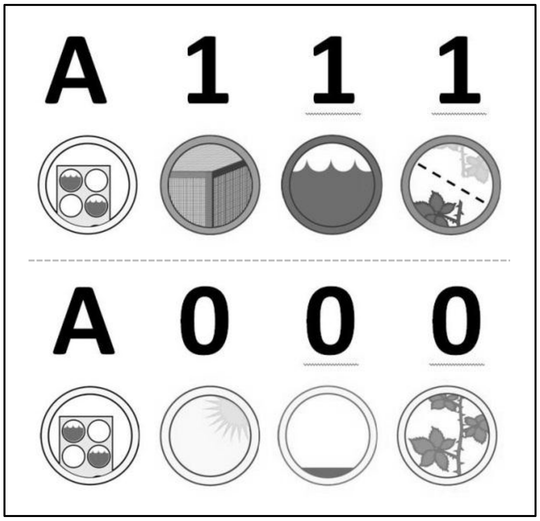


Figure 6. Bucket label symbology.

Figure 7. A panoramic view of the experimental setup.

cut/not cut. For example, a bucket in block B that was shaded, not flooded, and cut was labeled B101, and a bucket in block E that was not shaded, flooded, and not cut would be labeled E010, and so-forth; any bucket with the label “000” contained three control plants in full light and was neither flooded nor cut.

Shading treatment – Five large wooden shade structures were constructed from 1x2 untreated pine, each with a 82 cm^2 (32 $inch^2$) footprint and a height of 1.22 m (4’) tall (Figure 7). Tan 50% shade cloth was applied 91 cm (3 feet) down from the top of the structure (allowing a 1’ gap around their base for air flow, but lower than the top of the buckets) on three sides. The top of the shade structure had a second layer of shade cloth added to further reduce light penetration. This section of cloth was also used to form an access flap over the fourth side, was not stapled to the frame as the cloth on the other three sides was, and could be lifted away for access to the plants within. This flap was weighted securely at the bottom by 91 cm (3’) of 1.9cm x 3.8cm (3/4” x 1.5”) pine to prevent inadvertent opening from air currents in the greenhouse. It matched the other sides in height from the top and completed the fabric enclosure of the shade structure. Each of the five (5) replicated blocks of the eight (8) buckets were divided in half with the four (4) buckets designated for the shade treatment and representing all possible combinations of the other treatments (shaded only, cut, flooded, or cut and flooded) placed within a shade structure. Buckets both within the shaded and un-shaded halves of a block were placed 10 cm apart and a 1-meter dowel held fly paper and a thermometer 60 cm above the table top, placed at their center (Figure 4). The shade structure was placed randomly on either the east or west side of each block with the access flap facing towards the table edge.

Flooding treatment – Buckets designated to be used in the flooding treatment had 5 mm (¼-inch) holes drilled around their edges 30 cm from the bottom of the bucket to allow excess water to drain out and the exact depth of flooding to remain constant. Consequently these 20 flooded buckets had standing water to 10 cm above the soil level of the smaller 1-gallon pots within. Several 5 mm (¼-inch) holes were drilled into the bottom of the buckets that were not going to be flooded to allow them to drain quickly and completely after plants were watered. Within each replicated block, two flooded buckets were outside shade structures and two were within, placed randomly among the four buckets in these halves of the replicate. Flooded buckets were filled with Seattle municipal tap water the day before data collection began, and remained flooded for the duration of the experiment, with water added to replace loss to evaporation and/or transpiration. All other plants were watered whenever necessary to maintain moderate soil moisture.

Cutting treatment – Half of the plants were also cut to a height of 17cm, exactly even with the top edge of the 5-gallon bucket, on the day before the experiment and data collection began. These plants then had emergent stems 7cm above the water surface in the flooded buckets. The remaining uncut plants were staked and loosely tied to 1-meter bamboo rods with soft cotton garden twine.

Unless flooded, all plants were watered until water flowed freely from the base of their pots whenever their soil became perceptibly dry. The experiment was initially planned to continue until data indicated that an overwhelming majority of plants in the combined shaded, flooded, and cut treatment had died, on the assumption that was the harshest combination of treatments and would lead to the highest mortality rate among the possible treatment combinations. However, the duration of the experiment was later modified because mortality rates were far slower than anticipated. Instead, the experiment was ended after 3 months when the risk of losing biomass to decay was too great and “greenhouse effects” such as recurrent aphid and scale infestation were putting the plants in unintended jeopardy.

3.1.3. Data Collection

Environmental data were collected for each half-block (shaded and unshaded parts of a replicate) and experimental data were collected for each individual plant.

Environmental data included the ambient air temperature 60cm above the center of a half-block, and Photosynthetically Active Radiation (PAR) was averaged for each half-block from four observations taken 60 cm above each bucket. Experimental data gathered included: stem length of every cane on a plant that rose above the edge of the bucket (longer than 17 cm), a subjective plant health score, a SPAD Chlorophyll Index score from three (3) leaves per plant, whether adventitious roots were seen, and whether insects were observed infesting the plant. Specific data collection protocols were as follows:

Photosynthetically Active Radiation (PAR), µMol m^{-2} *second*$^{-2}$ –A LI-COR Biosciences light meter was used to measure PAR in Wm^{-2}. Measurements were taken above each bucket at a height of 60 cm above the greenhouse table, on all forty buckets either inside or outside shade structures. Light measurements were recorded only on days when light levels remained constant - near noon and when skies were clear of intermittent cloud coverage. Measurements were recorded per-bucket, but the measurements for the four sunny and four shaded buckets from each replicate were later averaged since individual observations could be greatly influenced by shadows from greenhouse superstructure, a larger plant's leaves, etc. The intent was to quantify the difference in light levels between the shaded and unshaded treatments, and to determine if there was a significant difference in light levels between experimental replicates.

Temperature, °C – Two thermometers were installed in each replicate block, one inside the shade structure and one outside, on a dowel 60 cm above table top (23 cm above the top of buckets) in the center of the four buckets. Ambient air temperature was recorded every time data was collected. The intent was to quantify differences in ambient temperatures that the experimental replicates might experience in different parts of the greenhouse.

Insects – Presence of more than three aphids, whiteflies, or other similar parasitic or herbivorous insects per leaf was recorded if detected anywhere on the plant, as observed casually while collecting other data. Spiders, hornets, or other non-plant damaging organisms were not to be recorded. Significant infestations were controlled as-needed with a sprayed solution of emulsified neem oil and 2% pyrethrum.

Stem Length, cm – All canes per plant were measured from the top of the pot edge to its apical bud tip, even if leaves extended further; thus all plants subjected to the cutting treatment started with a zero (0) height for analysis purposes, though their canes extended another 17cm from the bucket's top edge down to the soil line. Long canes were extended and fully measured beyond the shade structure if necessary, then returned to their previous position. Over time, these measurements provided growth rate data per stem, per plant, and also provided data on the number of stems per plant.

Normalized Daily Growth Rate[8]: I calculated a plant's growth rate per day, relative to the growth of all plants for that period, to adjust for changes in ambient light and other fluctuating environmental conditions related to weather events that affected growth rates by using the following equation:

---

[8] This should not be confused with the RGR, or Relative Growth Rate, commonly used in plant physiology studies and determined by multiplying a plant's measured Leaf Area Ratio (LAR) and its Net Assimilation Rate (NAR).

$$\frac{\left(\frac{Growth\ of\ an\ individual\ plant\ from\ total\ stem\ length\ changes}{Days\ between\ observations}\right)}{Per - day\ growth\ of\ all\ plants\ during\ the\ same\ period}$$

This measurement was then averaged per-bucket for analysis.

Plant Health Ranking, scale of 4-0 – After plants had been initially cleaned of any dead, yellowed, or dying leaves before the experiment began, plant health was evaluated subjectively using the following rubric:

> **4** - Plant was in full and vigorous health. No leaves were dead, and no more than two leaves were, yellowing, chlorotic, or withering; new growth was clearly evident.
>
> **3** - Plant was in relatively good health but up to 33% of leaves and/or stems were yellowing, chlorotic, withering, or decaying
>
> **2** - Plant showed yellowing, chlorotic, withering, or decay in 33% to %66 of its leaves and/or stems.
>
> **1** – The plant is more than %66 yellow, withering, or dead, but still has some living, green portions; little or no new growth is seen since last observation.
>
> **0** – The plant appears entirely dead, with no green and/or healthy leaves or stems.

SPAD Chlorophyll Index – A Konica-Minolta SPAD-502 Chlorophyll Meter was used to measure chlorophyll density of the 1st, middle-most, and youngest leaves of the largest cane of every plant, three times during the experiment. The last set of measurements was selected for analysis as they were better at showing differences in rates of leaf senescence among the treatment groups. Baseline measurements of a single large leaf per plant were also taken at the start of the experiment in the greenhouse. SPAD chlorophyll density was measured on plants outdoors on August 12th 2010 on the campus of the University of Washington's Center for Urban Horticulture, in both exposed and shaded sites, to use as a reference when comparing wild plant chlorophyll density levels to greenhouse plants.

Biomass weight (g): A final destructive biomass assay was performed after the experiment had run for 90 days. Plants were removed from their pots, soil was washed from their roots, their leaves, stems, soil roots, and adventitious roots were cut, separated, and placed in a drying oven for 72 hours. Samples were then and weighed, and a root:shoot ratio was calculated as follows:

$$\frac{(Soil\ root\ +\ Adventitious\ root)}{(Leaves\ +\ Stems)}$$

These ratios, and the original biomass weights by plant part, were recorded for analysis.

Other response variables: Anatomical changes such as number of stems (canes) and the appearance of adventitious roots and/or hypertrophic stem swelling was also recorded. Furthermore, plants infested with aphids and the numbers of dead plants per bucket were also recorded.

Data were initially collected every day, then over longer periods once a baseline of the rates of change of different data types were established and understood. Data collection forms were created, one for all data except SPAD Chlorophyll Index data, and another form for the latter. Data collection forms were electronically available for data collectors and immediately entered into a Microsoft Excel database upon completion. Paper copies were archived and electronic data was regularly backed up to removable media.

3.2 The Laboratory Experiment: Full Submergence and Time-Lapse Observation

The second experiment involved completely submerging small *R. armeniacus* plants to augment the first experiment with additional detailed observations of anatomical

Figure 8. Partially-filled submergence tank and time-lapse camera at the start of a submergence trial. The tank was completely filled during the experiment.

changes in fully submerged plants. The difference between partial and full submersion is important, as partially submerged plants (or those growing in anoxic soils) can invoke anatomical changes like aerenchyma development to facilitate gas exchange between emergent and submerged anaerobic tissues (Larcher, 2003). If the entire plant is submerged, these adaptive strategies alone cannot be successful unless the plant can somehow breach the surface of the floodwaters through adaptive changes in structural morphology.

Over the course of the summers of 2009 and 2010, four (4) *R. armeniacus* seedlings 10-15 cm tall were transplanted into 4" containers and grown in a greenhouse for one month before each was used as a test subject in this experiment. The experimental apparatus consisted of a submergence tank, lighting, and digital time-lapse camera (Figure 8). A 5-gallon glass-walled fish tank illuminated by two broad-spectrum 18-watt fluorescent light bulbs was used as the submersion tank. It was filled with laboratory tapwater (pH 6.9) and placed on a lab countertop within the Center for Urban Horticulture ecological restoration labs. A single test plant in its 4" pot, with an extra 1 cm of white horticultural sand added to prevent the soil or plant from becoming buoyant, was placed in the submergence tank 10 cm away from the side farthest from the time-lapse camera for optimal image capture.

The time-lapse camera consisted of a Canon A540 running alternative firmware found in the free Canon Hacker Development Kit (http://chdk.wikia.com/wiki/CHDK). This software enabled the use of a custom programming script written to be executed by the camera, allowing it to be programed to select a fixed 0.6m focal length, power on at 7 AM, and take a 5-megapixel image every hour until 7 PM. A timer synchronized the lights on the submergence tank to turn on and off just before and after this daily period of image capture. The camera was affixed to a small tripod, itself mounted to the laboratory countertop; the area was marked to prevent disturbance.

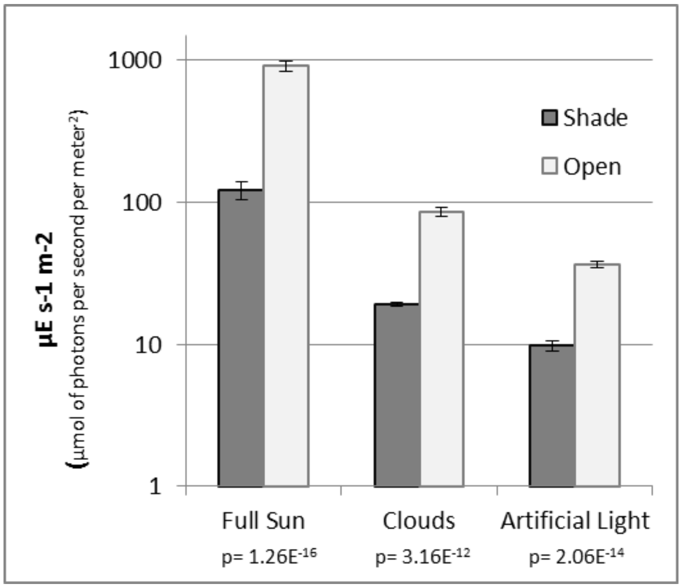
The initial experiment was intended to run for just two weeks, but lack of significant change in the first submerged plant's appearance after that period lead me to extent the experiment for 30 days. The subsequent plants were submerged for 35, 38, and 42 days. Once the submergence was ended and the tank was drained, the plant remained in the empty submergence tank and was observed for another two weeks while being regularly watered to determine if the plant was dead, survived the shock of re-exposure to open air, and was able to continue growing.

Data collected consisted of thousands of still images that were stitched together into 15-frame second^{-1} time-lapse videos using Quicktime Pro software. Videos were reviewed for changes in plant vigor, morphology, and number of days until new growth was observed. Post-submergence images were used to determine if the plants recovered from submergence and continued to grow.

4. Results

Both experiments were implemented successfully and yielded a wealth of data. In this section, analytical techniques and results are reported in order by experiment, general conditions encountered, hypothesis, analytical technique or observation employed, and the ensuing results.

4.1 The Greenhouse Experiment: Partial Flooding With Shade and/or Cutting Treatments



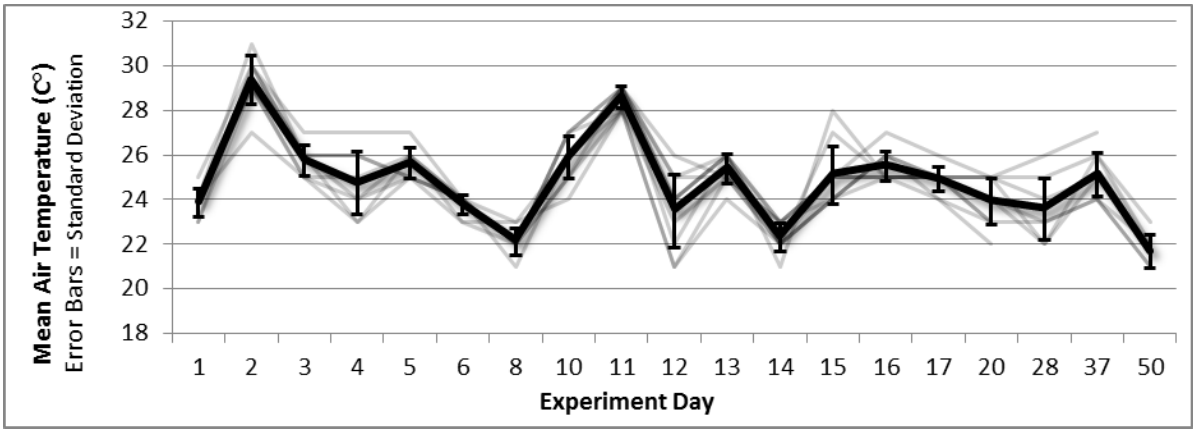
Mean $\mu E\ s^{-1}\ m^{-2}$	*Full Sun*	*Clouds*	*Artificial Light*
Shade	122.05	19.15	9.8
Sun	911.8	85.73	36.56

Figure 9. Light levels within shade structures and in open, unshaded blocks, during sunny, cloudy, and artificially illuminated night conditions.

Greenhouse environment: Light levels[9] and temperature measured within the greenhouse fluctuated due to short-term weather events and longer-term seasonal trends. Light levels were measured within and outside shade structures to quantify their effects during sunny, cloudy, and artificially-

---

[9] When recording light levels, μE = μEinstein, or μmol of photons, or $6.022x10^{17}$ photons. Light meter measurements were therefore the number of photons, in μE, detected per second, per square meter.



Day	*1*	*2*	*3*	*4*	*5*	*6*	*8*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*20*	*28*	*37*	*50*
Stdev	0.64	1.11	0.66	1.39	0.70	0.43	0.60	0.94	0.49	1.63	0.66	0.64	1.30	0.67	0.54	1.04	1.40	0.99	0.75

Figure 10. Mean temperature trend among all treatment groups, with error bars and table indicating daily standard deviations of temperatures. Pale grey lines are per-treatment means to illustrate the extent of variability.

lit evening conditions (Figure 9). Differences were significant between open and shaded blocks in all conditions, with p=1.26E-16, p= 3.16E-12, and p= 2.06E-14 for paired T-tests of mean sunny, cloudy, and artificially lit conditions, respectively, within and outside of shade structures at an alpha of 0.05. The greatest mean difference was under sunny conditions, where unshaded areas received 911.8 µE s^{-1} m^{-2} while shaded blocks averaged 122.5 µE s^{-1} m^{-2}. Temperatures were similar between block and within or outside shade structures, reflecting generally shared ambient conditions and constant airflow within the greenhouse (Figure 10). Single-day standard deviation from the daily mean ambient air temperature within the greenhouse never exceeded 1.39° C, with a mean standard deviation of 0.89° C a day. The daily mean temperature remained within a range between 21° and 29° C throughout the experiment.

Observations: The experiment was initiated on August 23 and concluded on November 17, 2010, a span of 87 days. This duration was longer than initially anticipated, as

I incorrectly assumed flooded plants would exhibit greater stress and have more rapid mortality rates than I actually observed. The experiment was extended until it became clear that confounding greenhouse influences such as aphid infestations (Figure 11) and loss of measurable biomass (rotting leaves or roots left for long periods in flood waters) might endanger the integrity of experimental data. Data on aphid infestation was collected, but when analyzed, appeared to be completely stochastic (T-test of infested and not infested plants, using their mean health scores, p = 25.61 with alpha = 0.05) and was not useful as an unplanned but potentially serendipitous additional measure of plant stress levels.

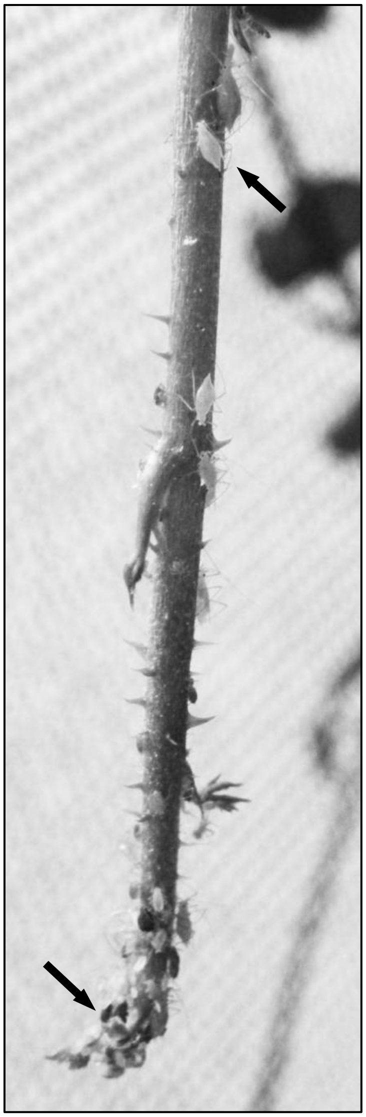


Figure 11. Aphids infesting the growing tip of a shaded plant's cane. Arrows denote younger aphids at tip and adults, with longer probosci, feeding farther up the stem.

Plant growth rate and size were also greater than anticipated, partly due to the experiment running longer than planned. By the 87th day, some plants had canes nearly 3 meters long, often despite being in the shaded treatments. This was mitigated by curving canes within shade structures into loops and using their recurved spines to attach them to the shade cloth, thus preventing them from spilling out of the shade structure and circumventing the light deprivation intended by the shade treatment.

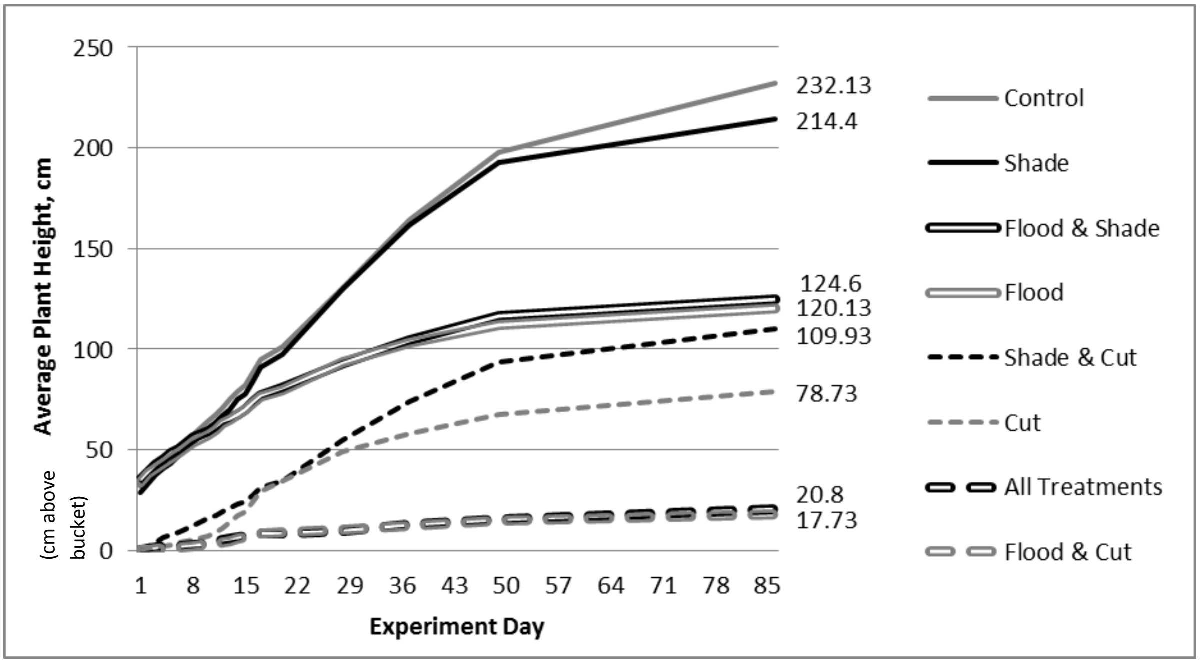


Figure 12. Average cane length of plants in the eight treatment groups, with final average height at the end of experiment shown in cm; n=15 for each treatment group.

4.1.1 Hypothesis 1: Plants subjected to multiple stresses will exhibit the greatest degree of physiological stress, as expressed by changes in (1) growth rate; (2) chlorophyll density in foliage; and (3) ranked vigor. Flooding is expected to be the most important stressor, but cutting and shading will also be significant.

The data show that the largest plants were in the control group, with a mean of 232.13 cm of canes by the last day of the experiment, while the smallest plants were in the flooded and cut treatment group, which averaged 17.73 cm in total cane length per plant (Figure 12). To calculate growth rates from the total cane lengths recorded from all plants in a treatment group, the previous like measurement was subtracted from the current one to calculate a gross difference, then divided by the number of days between measurements to determine the average daily growth rate for that period. This rate was then adjusted by dividing the treatment's growth rate by the average growth rate of all plants for that period, resulting in an "Normalized Daily Growth Rate," as discussed in the Methods section. Growth rates of the control and shaded groups were the highest, averaging 1.86 and 1.76 cm day^{-1} respectively, though the shaded group recorded a slower rate on the final day of the experiment. The groups that were flooded and cut, regardless of being shaded or not, shared the lowest average rate of 0.22 cm day^{-1}. Similarly, the flooded treatments, shaded or not, shared the same average adjusted growth rate of 1.09 cm day^{-1} (Figure 13).

Chlorophyll density in each plant's bottom, middle, and top leaves were measured. Results show that most plants allowed their older leaves (at the bottom of their canes) to senesce as they reallocated nutrients to their extending cane and younger leaves near the cane top (Figure 14 A-H). An ANOVA of all the top leaves' SPAD scores proved their

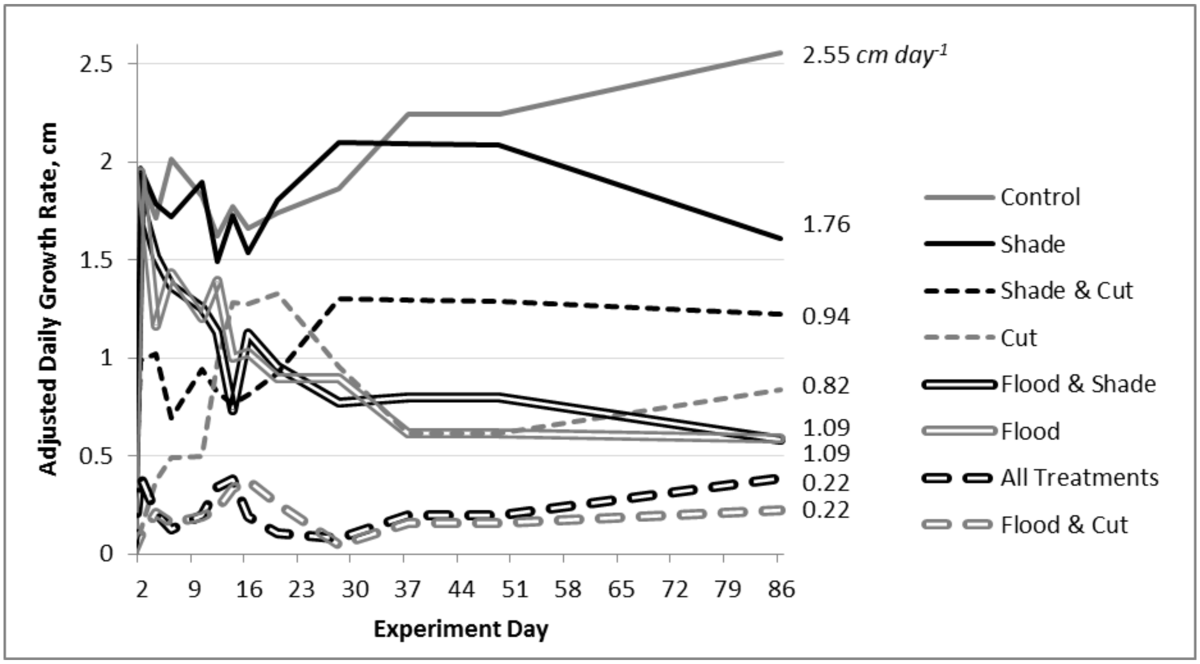


Figure 13. Normalized Daily Growth Rates for the eight treatment groups, and mean growth rates per treatment shown in cm^{-1} day^{-1}; n=15 for each treatment group.

differences to be non-significant (p=0.4268, with alpha = 0.05). With the flooded plants included in the ANOVA of all middle leaf SPAD scores, the ANOVA is significant (p=3.47E-5 with alpha =0.05). But remove that one flood-only treatment mean, and the differences among the chlorophyll levels of middle leaves is also non-significant (p=0.2006 with alpha=0.05). Only the bottom leaf chlorophyll levels are significantly different (p=2.66E-28

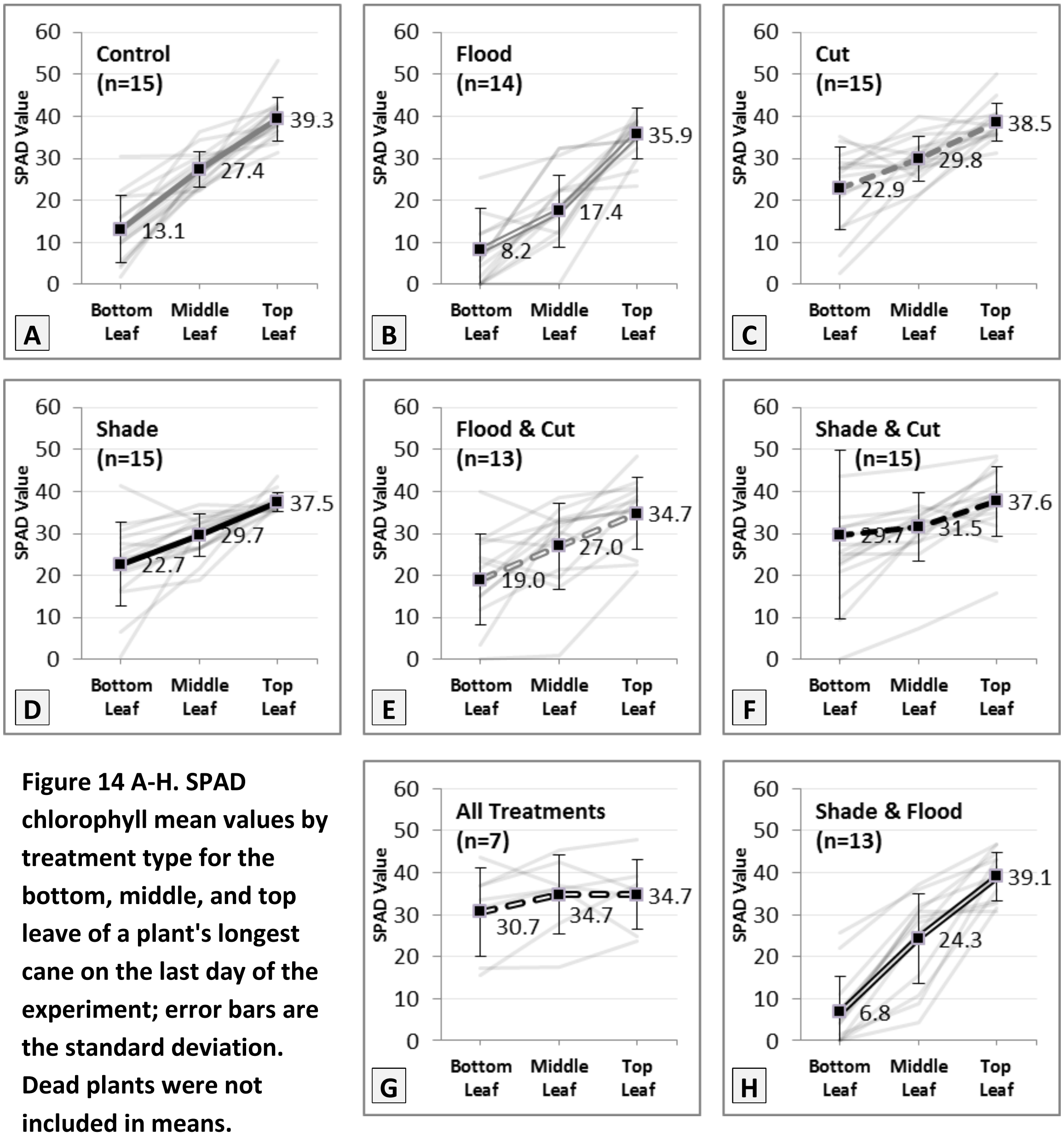


Figure 14 A-H. SPAD chlorophyll mean values by treatment type for the bottom, middle, and top leave of a plant's longest cane on the last day of the experiment; error bars are the standard deviation. Dead plants were not included in means.

with alpha=0.05) with the control, flooded, and flood/shade treatments lowest (Figure 14 A, B, and H).

To further analyze the SPAD leaf chlorophyll density data I plotted the mean of the three SPAD leaf scores per-plant, per-treatment, against the same treatment's mean plant size, and calculated the slope of the resulting linear models through the three leaf mean points (Table 2 and Figure 15, below). Larger plants generally had shallower slopes, indicating a slower pace of senescence along the leaves of their canes from bottom to top. The flood-and-cut treatment had the steepest linear model slope of 63.5° on fairly small plants, indicating a very fast change from senescing to new leaves along their short canes. It was also notable that the flooded and flood-and-shade treatments both had comparatively steep slopes of 13.29° and 15.01°, respectively, on relatively large plants. They correspondingly showed the greatest difference in total SPAD scores from bottom to top, 27.6 for flooded and 32.3 for flood-and-shade plants. Interestingly, the control plants were very large and had nearly the shallowest slope of 6.47° yet had almost as great a difference in SPAD scores from bottom to top leaves of 26.2 as the flooded and flood-and-shade treatments.

Table 2. Results of linear models (LMs) of mean SPAD values, as shown in Figure 15, with corresponding slopes and differences in SPAD values from bottom to top leaves, per treatment. Results are listed from steepest to shallowest angle of the LM slope.

Treatment	*Mean SPAD LM Equation*	*Angle of LM Slope*	*Difference, Bottom to Top Leaf SPAD*
Flood & Cut	y = 0.8949x + 19.055	63.50°	15.9
Flood & Shade	y = 0.259x + 7.2256	15.01°	32.3
Flood	y = 0.2298x + 6.6964	13.29°	27.6
Cut	y = 0.1984x + 22.594	11.44°	15.6
All treatments	y = 0.1923x + 31.367	11.09°	4.0
Shade & Cut	y = 0.1226x + 24.196	7.04°	13.5
Control	y = 0.1127x + 13.538	6.47°	26.2
Shade	y = 0.0689x + 22.578	3.95°	14.8

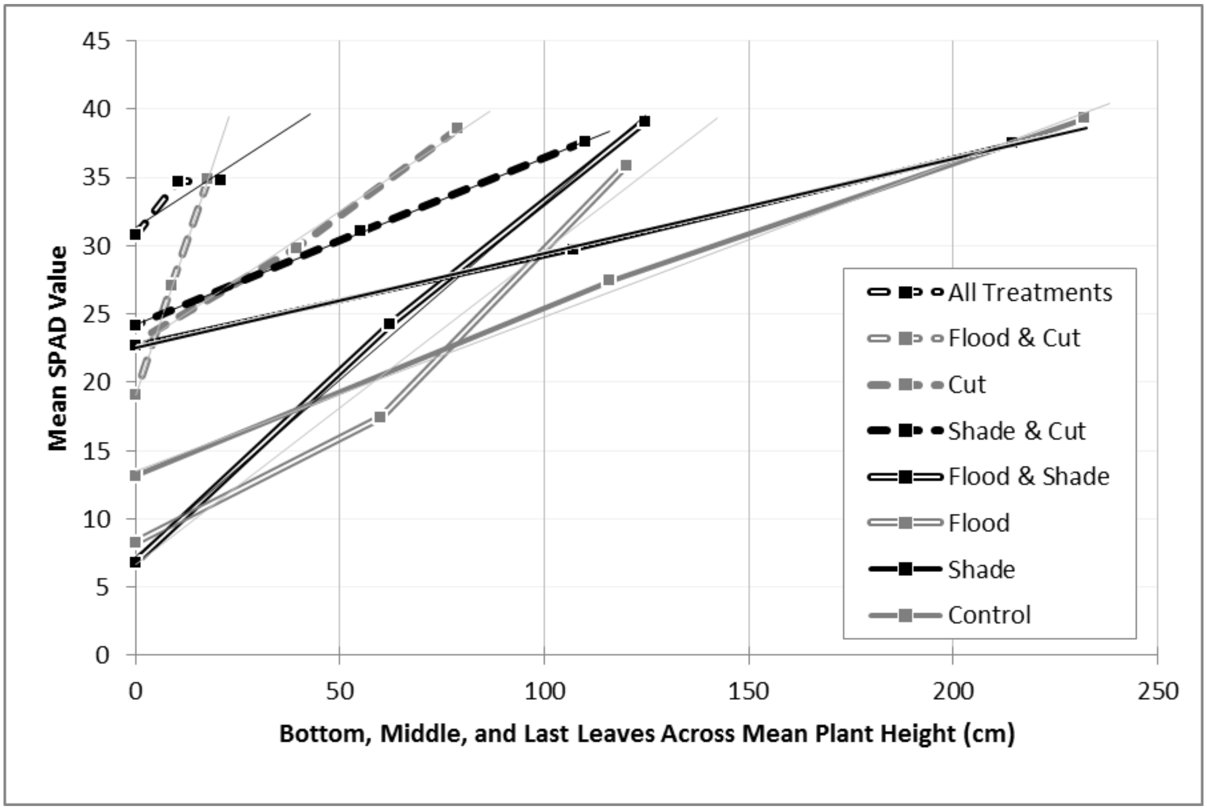


Figure 15. Mean SPAD values for the bottom, middle, and top leaves of each treatment, plotted against the mean length of all plants in those treatments on the day SPAD scores were recorded.

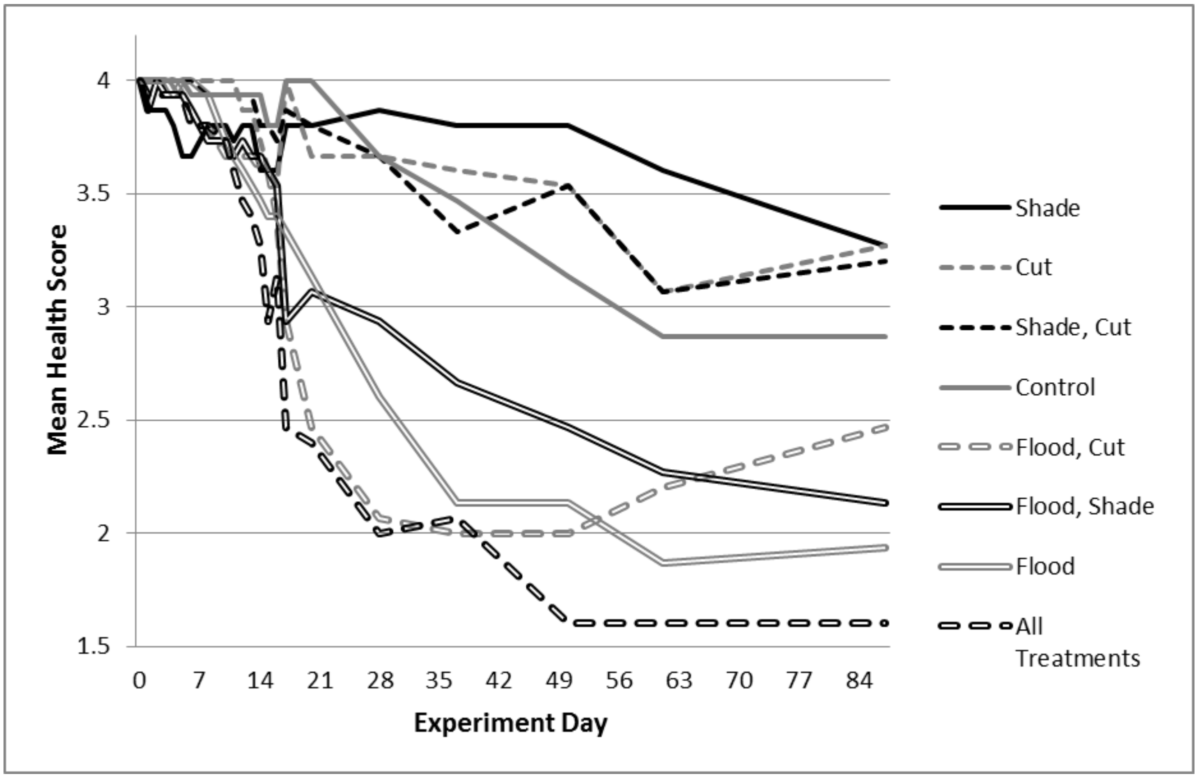


Figure 16. Mean health scores per treatment over the duration of the experiment.

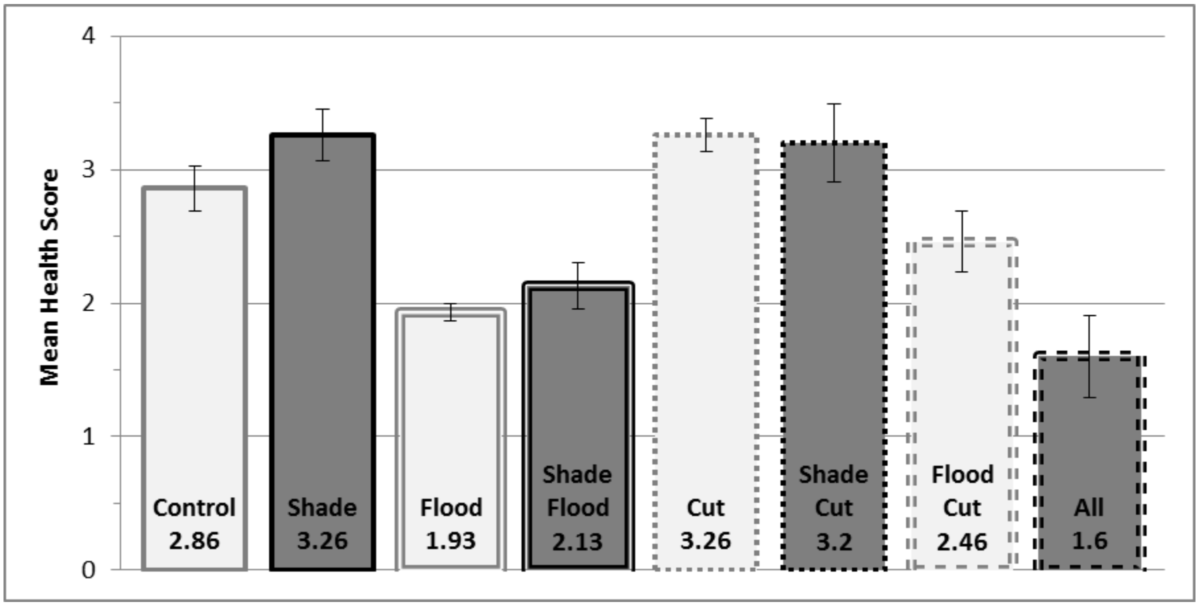


Figure 17. Mean health scores for all eight treatment groups. An ANOVA of these means proved significant (p = 1.5e-06 at alpha = 0.05).

To augment the previous quantifiable and objective observations of plant health and vigor, subjective health rankings were also recorded for each plant. Differences between treatment groups were analyzed with an ANOVA that indicated a significant difference between the mean health scores (Figure 17) of at least two of the treatment groups ($p = 1.5e^{-06}$, alpha = 0.05). A Tukey's HSD test found the following pairings caused the significant ANOVA results (Table 3). It appears that all treatments involving flooding, as well as the "Shade & Cut" treatment, had the most significant differences that contributed to the significant ANOVA results.

Table 3. Paired treatments that prove significant when analyzed by a Tukey's Honestly Significant Difference (HSD) test

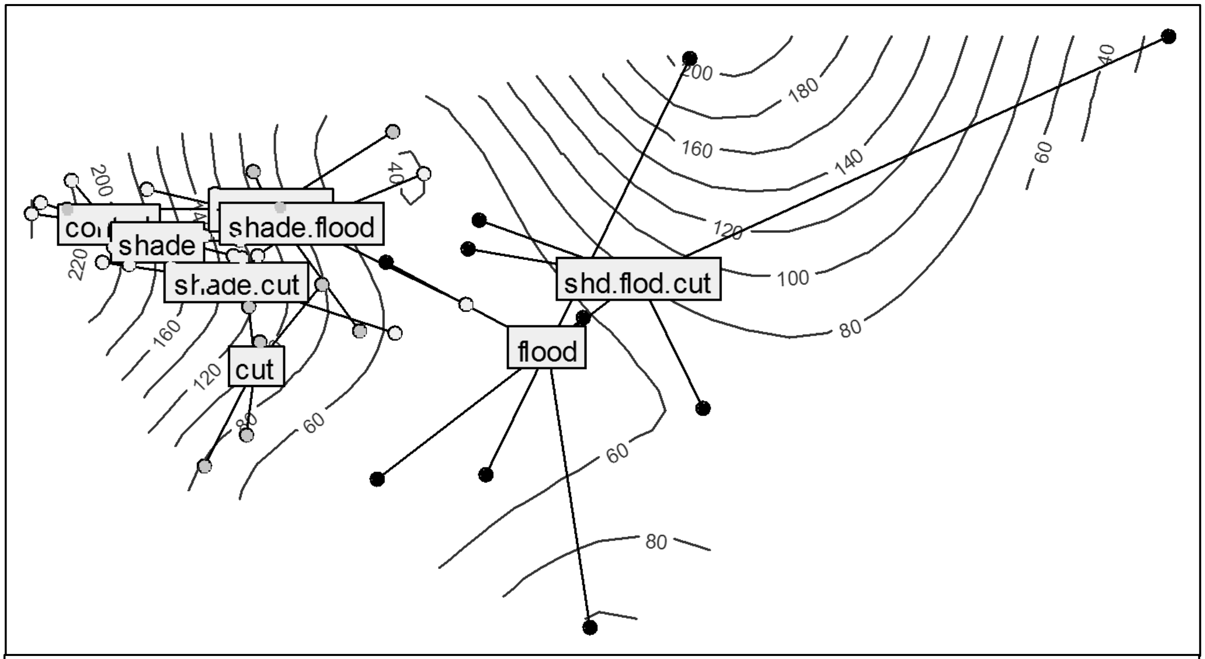
Treatment Pairs	*Difference*	*p (Adjusted) < 0.05*
All Treatments - Cut	-1.2	0.0470
All Treatments - Flood	-1.2	0.0470
All Treatments - Flood & Cut	-1.6	0.0029
All Treatments - Shade & Flood	-1.4	0.0123
Shade & Flood - Shade	1.2	0.0470
Shade & Flood - Shade & Cut	1.2	0.0470
Flood & Cut - Shade & Cut	-1.4	0.0123
Flood & Cut - Shade	-1.4	0.0123

Mean health scores for each treatment group were plotted against the duration of the experiment (Figure 16). After the first few days of the experiment the treatments began to show diverging levels of health, with the shade treatment consistently better than all others until the final days of the experiment, when the cut and cut-and-shade treatments recovered to nearly match it. All of the four treatments involving flooding, either alone or in combination with shade and cutting, were the least healthy and their scores are potted as a fairly discrete group by the end of the experiment. Interestingly, the flood-and-cut treatment recovered some health after the 50th day of the experiment.

Three multivariate datasets of response variables were produced during the course of the experiment. Two were homogeneous tables of either periodic observations of Plant Health Scores or Normalized Daily Growth Rates, and the third was a heterogeneous set of multiple metrics recorded at the end of the experiment. They were complemented by a single table of explanatory variables consisting of the three shade, flooding, and cutting treatments as well as block/replicate name, all controlled or manipulated as part of the experimental design. All datasets were cleaned of outliers and inspected for data errors (one error was found and corrected). Analysis of the homogeneous Plant Health Scores and Normalized Daily Growth Rates datasets were performed identically. “R” statistical analysis software was used to execute a PERMANOVA to identify significant treatments or treatment interactions evident in the data, followed by the creation of a 2-dimensional ordination using Non-metric Multidimensional Scaling (NMDS) to visualize relationships between treatment types. Each PERMANOVA was performed multiple times to detect problems with the order of arguments in the Type I model, but results were similar. A final model was selected for both the health scores or growth rates datasets with y as their respective multivariate table of data, and x as:

$$\textit{Treatment block} + \textit{shade} * \textit{cut} * \textit{flooding}$$

This order of arguments was chosen after inspecting initial trial PERMANOVAs to first detect block effect, and then allow what were observed to be weaker influences (shade, then cutting) to explain as much variation in the data before the overwhelmingly significant flooding treatment was added to the model. Normalized Daily Growth Rate and Plant

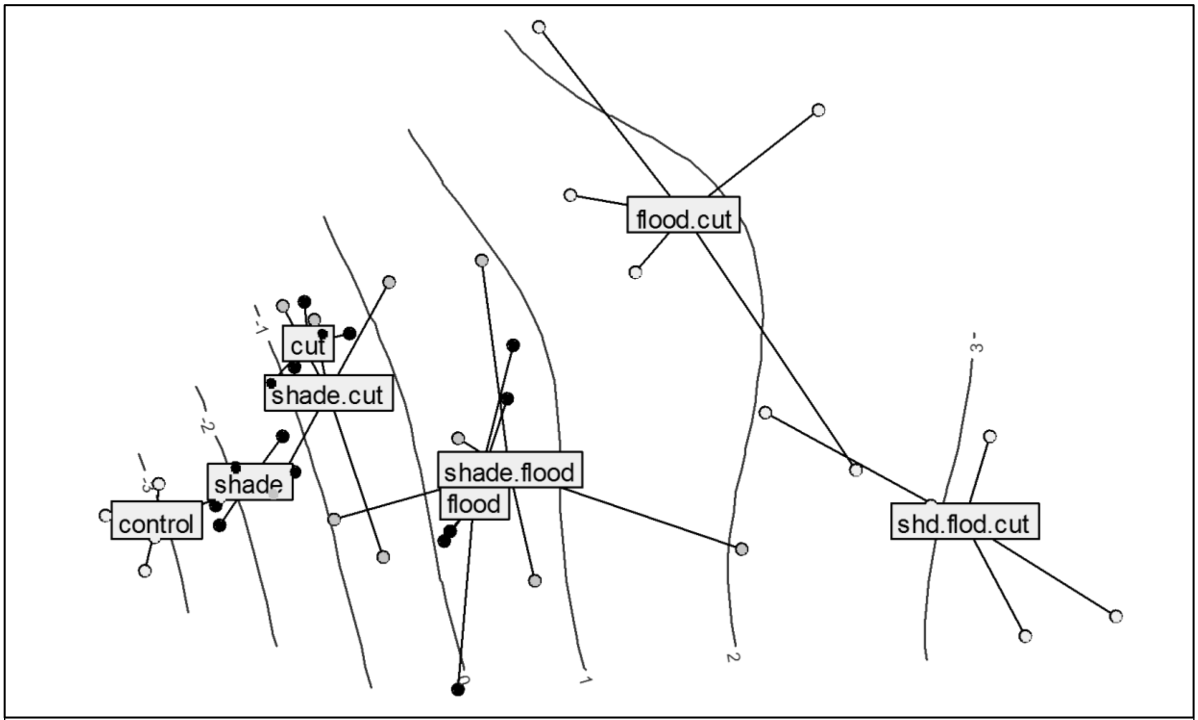


```
Call:
adonis(formula = grow.data[, 4:22] ~ grow.data$block + shade * cut *
flood, data = h.treat, permutations = 999, method = "euc")

                Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)    
grow.data$block  4     54.22  13.554  1.8046 0.10118  0.087 .  
shade            1      7.98   7.980  1.0625 0.01489  0.311    
cut              1     30.98  30.983  4.1251 0.05782  0.027 *  
flood            1    107.42 107.422 14.3023 0.20047  0.001 ***
shade:cut        1      9.39   9.387  1.2498 0.01752  0.243    
shade:flood      1      3.42   3.420  0.4553 0.00638  0.716    
cut:flood        1     37.73  37.731  5.0236 0.07041  0.011 *  
shade:cut:flood  1     74.40  74.403  9.9061 0.13885  0.001 ***
Residuals       28    210.30   7.511         0.39247           
Total           39    535.85                 1.00000           
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 18. Normalized Daily Growth Rates of eight treatment groups as an NMDS ordination with PERMANOVA results from "R" statistical analysis software. Stress was a low 9.65.

Health Score data were both ordinated using Non-metric Multi-Dimensional Scaling (NMDS) in two dimensions to visually represent relationships between treatment groups (Figures 18 & 19). The ordination of Normalized Daily Growth Rates appears to separate the plants in the flood and all-shade-flood-cut treatments from a closer grouping of the other treatment



```
Call:
adonis(formula = bio.max ~ grow.data$block + shade * cut * flood,
data = h.treat, permutations = 999, method = "euc")

                 Df SumsOfSqs MeanSqs F.Model      R2 Pr(>F)
grow.data$block   4    0.6236  0.1559   1.257 0.03991  0.249
shade             1    0.7680  0.7680   6.192 0.04916  0.004 **
cut               1    3.1780  3.1780  25.622 0.20342  0.001 ***
flood             1    6.0069  6.0069  48.429 0.38451  0.001 ***
shade:cut         1    0.4379  0.4379   3.530 0.02803  0.032 *
shade:flood       1    0.6245  0.6245   5.035 0.03997  0.011 *
cut:flood         1    0.2076  0.2076   1.674 0.01329  0.163
shade:cut:flood   1    0.3030  0.3030   2.443 0.01940  0.070 .
Residuals        28    3.4730  0.1240         0.22231
Total            39   15.6225                 1.00000
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Figure 19. Health Scores of eight treatment groups as an NMDS ordination with PERMANOVA results from "R" statistical analysis software. Stress was low at 9.64.

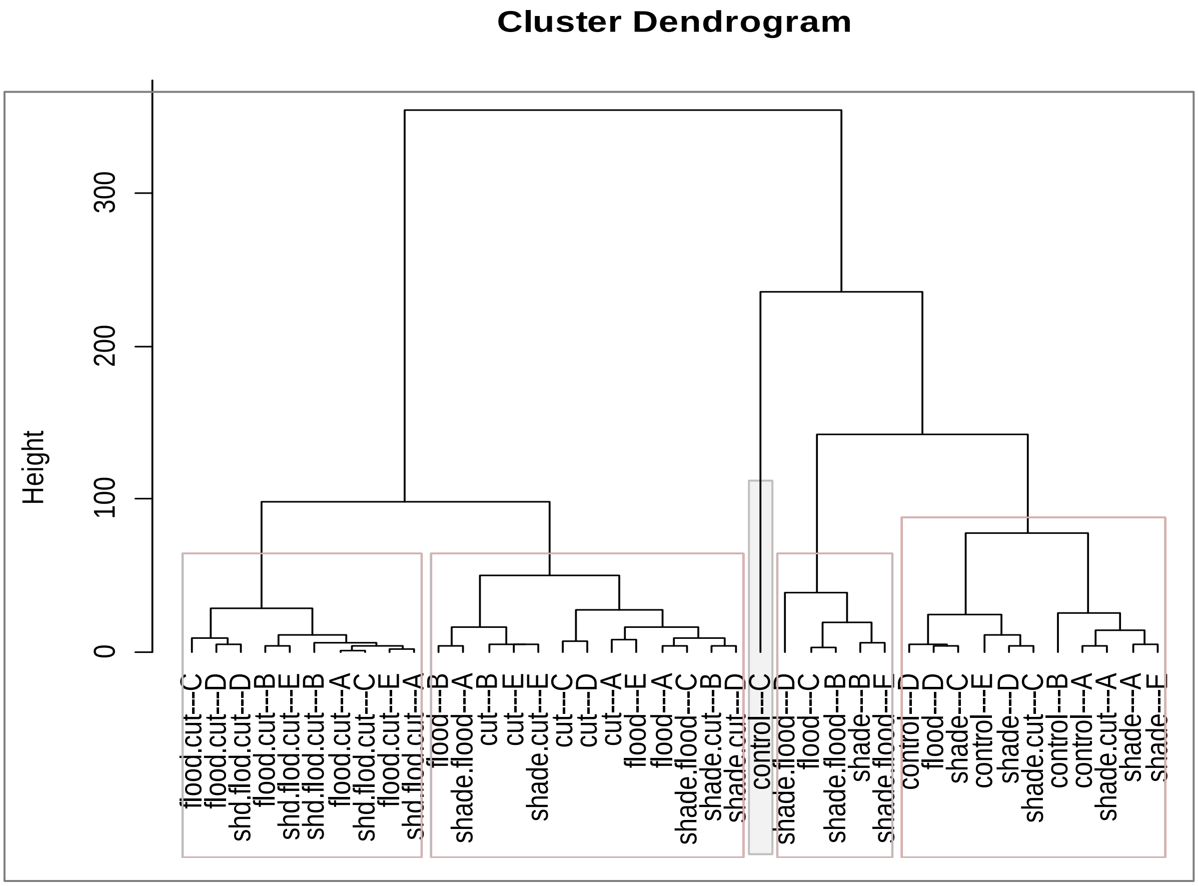


Figure 20. Dendrogram of the Hierarchal Cluster Analysis of the biomass and mixed dataset. Proposed groups are highlighted in grey boxes. An anomalous control bucket is highlighted.

types. The ordination of the Mean Health Scores provides a more easily interpreted ordination of all four treatments that involved flooding plotting to the right of positive ordination contours and all other non-flooded treatments grouped in the positive regions of the ordination.

A third heterogeneous multivariate dataset was comprised of response variables including dry biomass (g) of both root types (soil roots and adventitious “water roots”), leaves, and stems, final plant height, final heath score, and number of dead plants per bucket. This dataset was also subjected to a PERMANOVA after adjusting the data by column maxima to compensate for differing data types, but not by row, as it was not desirable to analyze the relative portions of these data within rows because of the heterogeneous nature of the data (McCune & Grace, 2002). In this case it was also appropriate to apply Principal Components Analysis (PCA), and perform a PERMANOVA on the Principal Components to calculate their significance. The PCA showed that the first three Principal Components (PCs) explained 0.86% of variance of the dataset, after which the screeplot shows a drop in PC importance. The absolute values of PC component levels showed a rough correlation of PC1 with plant above-ground biomass and health, and the remaining two PCs reflecting changes in water roots and health, and general root size and health, respectively.

A Hierarchal Cluster Analysis using Ward's method was built to examine relationships between the buckets of plants in differing treatment groups (Figure 20). Five groups within the Hierarchal Cluster Analysis were chosen (grey boxes in Figure 20) because a *k-means* cluster analysis performed with 2 to 6 groups found no change or improvement in within-group sums of squares or between-group sums of squares after five groups were used. One bucket of control plants was shown to be somewhat unique, and the remaining groups of buckets were a pair of similar groups including the harshest treatment (left-most pair of groups, Figure 20), and two

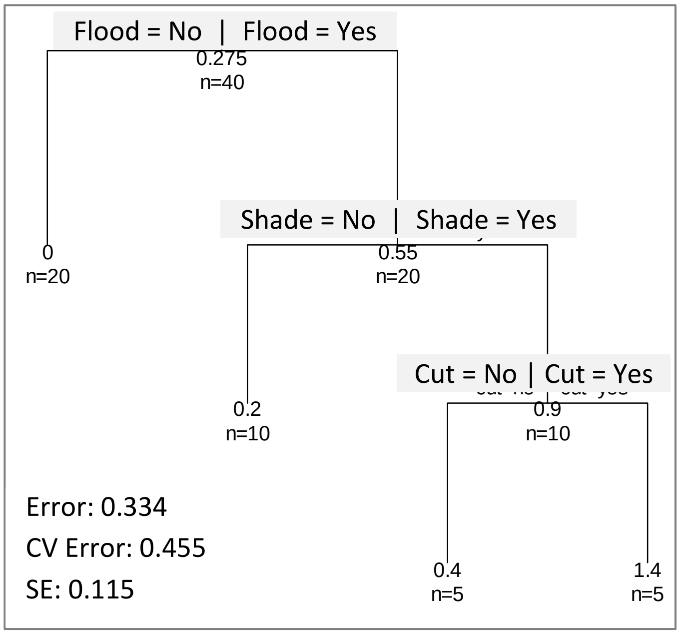


Figure 21. Univariate regression tree showing the effect of the treatments on plant mortality.

somewhat more dissimilar groups of buckets mostly containing flooded buckets with or without shade, and a group of single-factor or control plants.

A univariate regression tree (Figure 21) was created to determine what variables were most influential in causing plant mortality, and flooding proved to be the strongest factor, with shade and then cutting less important, respectively.

4.1.2 Hypothesis 2: Mortality rates of flooded plants under the various experimental treatments can be established to model the tolerance R. armeniacus has to partial inundation.

Mortality rates were forecast using linear models of Health Score data over the course of the experiment for each treatment type (Figures 22 and 23). Two-factor polynomial models provided the best fit for all data except the shade-only treatment, which had a higher R^2 with a logarithmic model. The polynomial model's equation and R^2 values for both the mean of the treatment data and the raw data points are provided in each chart. With one exception, all models using mean data had a >0.75 R^2 value at a significant p-value of $4.45E^{-18}$ or smaller (at alpha=0.05). The exception was the shade only treatment, which was nearly horizontal and non-significant (p= 0.2435 at alpha=0.05) and had a poor R^2 value with any regression model. Treatments groups involving flooding in any manner were also analyzed with a linear model (Figure 23) in order to force the model to intersect the X-axis (days) and forecast a mean time until all plants in a treatment group might be dead (a health score of "0"). These models showed that the shaded group had the longest forecast time to total mortality at 163 days, and the all-treatments group had the shortest at 105 days.

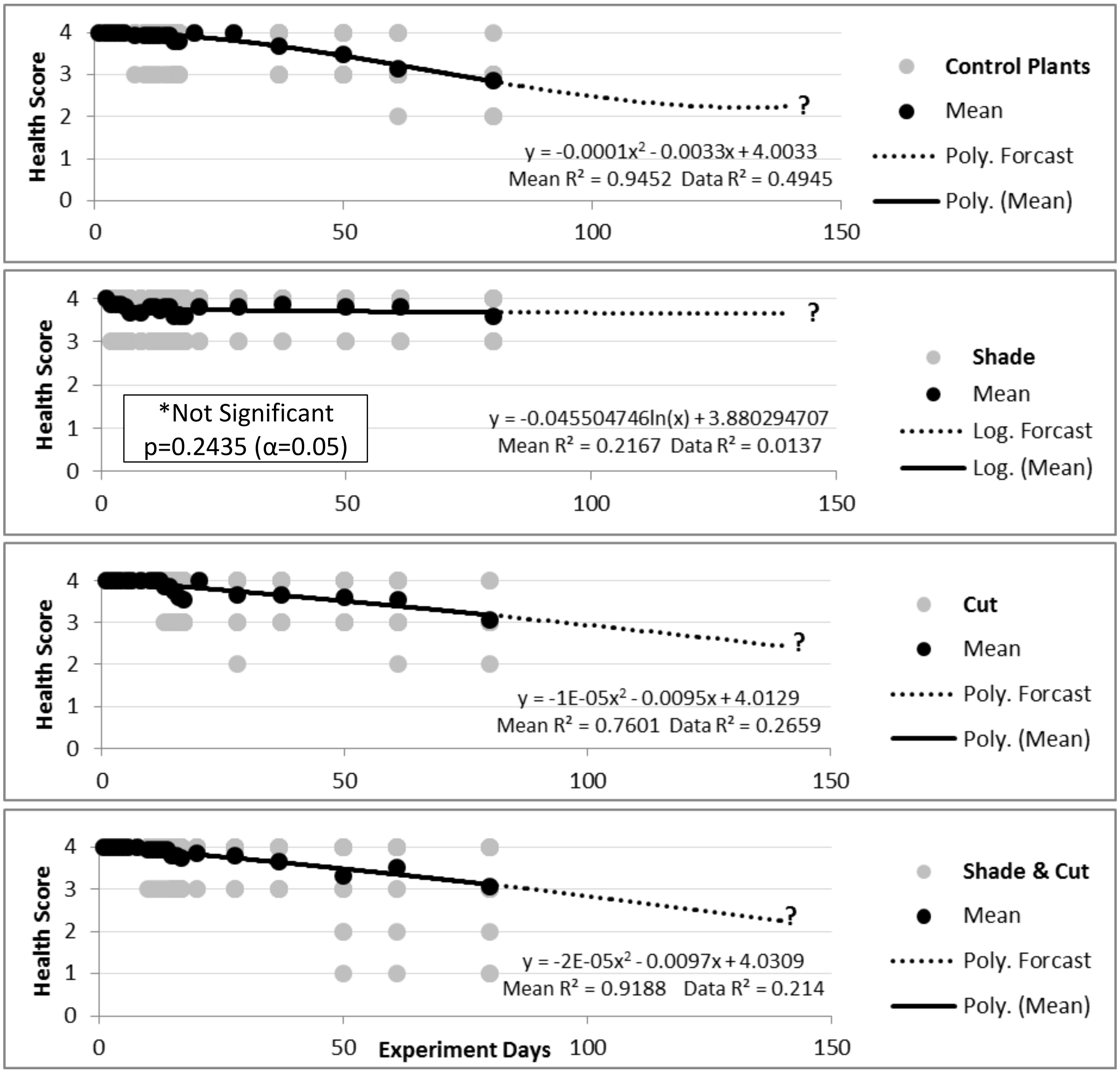


Figure 22. Linear models of health scores for non-flooded treatment groups. Data for each plant is in grey and means are shown in black. Dotted lines represent 60 day forecast using the chosen model. No plants died in these treatments (a Health Score of 0). Note that the experiment was not pursued longer than 87 days because the death and impending decay of flooded plants would have negatively impacted the data gathered from the final biomass assay.

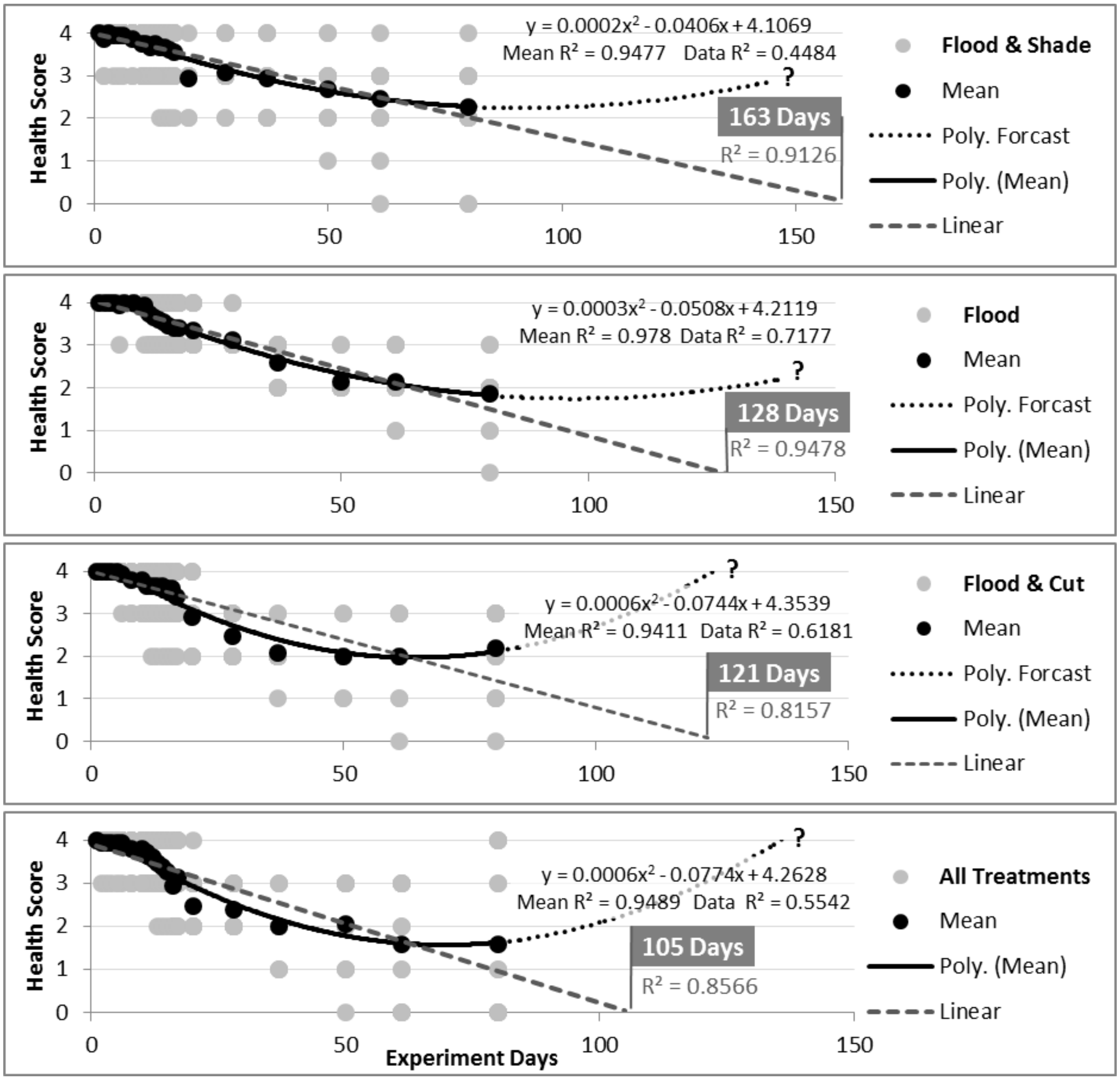


Figure 23. Linear models of health scores for flooded treatment groups. Data for each plant is in grey and means are shown in black. Dotted lines represent 60 day forecast using the chosen model, and dashed grey line shows a linear model projected to forecasted total mortality. Note that the experiment was not pursued longer than 87 days because the death and impending decay of flooded plants would have negatively impacted the data gathered from the final biomass assay.

4.1.3 *Hypothesis 3*: *Flooded plants that exhibit lower stress levels will be more likely to exhibit anatomical or morphological adaptations.*

Two anatomical changes, hypertrophic stems and adventitious rooting, were invoked by some flooded plants. 43% of flooded plants (26 of 60 plants in flooded treatments) grew adventitious roots into the water of their flooded buckets (Figure 24). Of these, 86% developed enough roots to also show hypertrophic stem expansion. Growth of adventitious roots was significant and positively correlated with health score (Figure 25) when comparing dry biomass weight of adventitious "water roots" and final plant heath score (R^2=0.1423, p= 0.0029, α=0.05).

Furthermore, I analyzed whether the appearance of adventitious roots could be

Figure 24. Adventitious roots (left) exposed after a flooded plant was removed from water, and detail (right) of hypertrophic stem swollen to accommodate aerenchyma; also note thick aerenchyma-filled roots.

significantly correlated with plant health using a chi-squared test. I prepared a 2x2 contingency table comparing counts of plants categorized either as healthy or unhealthy/dead (health scores of 1 or 0 for the latter) and plants with or without adventitious roots grown into flood waters. Results showed the relationship between plant health and root growth to be highly significant whether using an uncorrected chi-squared test, adding Yates' correction, or a Fisher's exact test, (p=0.0007, p=0018, and p=0012, respectively, α=0.05). Therefore it is significant that among flooded plants only 12% of unhealthy or dead plants had grown adventitious roots, but 60% of healthy plants did.

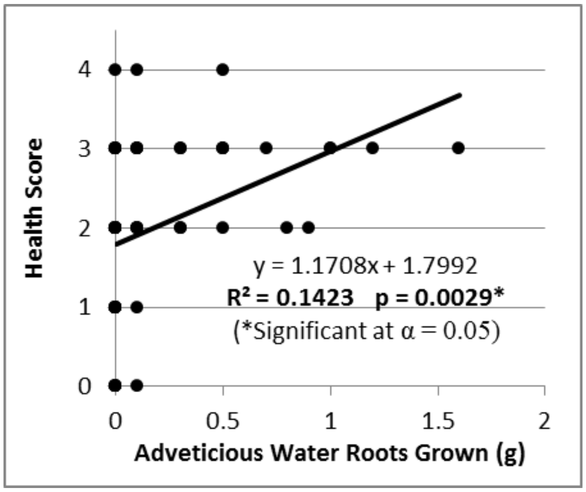


Figure 25. Regression of plant Heath Scores and adventitious roots grown into water by the end of the experiment.

4.2 The Laboratory Experiment: Full Submersion & Time Lapse Observation

The laboratory experiment resulted in four test plants being submerged and three were successfully time-lapse filmed; the fourth was partially filmed but an equipment failure resulted in a loss of images captured after the first few days. Laboratory conditions and experimental observations are summarized below.

Laboratory environment: Water temperature fluctuated slightly, but remained at a mean of 25° C. Laboratory water from the tap was measured with an electronic probe at a pH of 7.29, and tank water with a plant submerged was slightly more alkaline at pH 7.43. Tank water pH and temperature fluctuated within a small range but showed no significant trend and environmental conditions within the laboratory building remained fairly stable. PAR meter readings showed ambient light to be a neglidgeable influence within the submersion tank, with more than 90% of PAR originating from the two tank hood lights.

Observations: Videos if the first three plants are available online (See Table 3).

Table 4: Links to time-lapse videos of submerged *Rubus armeniacus* plants.

Plant 1 30 Days	http://www.youtube.com/watch?v=y-1yRmUbeus
Plant 2 35 Days	http://www.youtube.com/watch?v=epbuAQo5G4M (Submerged) http://www.youtube.com/watch?v=YX0HVZFXWd8 (After water removed)
Plant 3 40 Days	http://www.youtube.com/watch?v=cQdMV5eONcg
Plant 4 42 Days	Equipment failure – power outage reset camera script after 6 days.

1.3.1 Hypothesis 1: R. armeniacus will not evoke any morphological or anatomical changes while submerged.

Plants 1 and 4 exhibited little growth while submerged, but plants 2 and 3, the largest of the test plants, grew new shoots while submerged. Plant 2 began growing a new apical cane after 15 days underwater, while robust Plant 3 began growing several canes almost immediately after submersion and continued to grow vigorously for 40 days underwater. Petiole extension / leave elevation was observed in Plant 1, though this may have only been the result of leaf tissue degradation and not due to new tissue growth and true anatomical adaptation or submersion response.

1.3.2 Hypothesis 2: R. armeniacus plants will wither and appear visibly dead after 14 days of submergence.

Each of the four (4) plants survived total submersion in the water tank for 30, 35, 40, and 42 days, respectively. The largest leaves on all plants withered after removal from submersion, and Plant 1 lost all foliage, yet all resprouted new canes from leaf nodes within five (5) days of removal from submergence.

5. Discussion

The pair of greenhouse and laboratory experiments I performed were attempts to observe and quantify the effects of partial and full submersion, respectively, on this invasive weed. The plant's responses to these experimental treatments, and whether these results confirm my initial hypotheses, are discussed below.

5.1 The Greenhouse Experiment: Partial Flooding With Shade and/or Cutting Treatments

Ambient environmental conditions in the greenhouse were stable enough to ensure their effects were not significant; moderate differences in temperature and light levels (Figures 9 & 10) between blocks proved non-significant. However, unexpected effects of isolating the plants within a greenhouse did adversely affect the experiment, though not to such an extent as to require the experiments be abandoned. The principal issue was that the plant's isolation from the ecological community it usually thrives in meant disrupting the predator-prey relationship between its parasites, such as aphids and whiteflies, and the insects that control their populations. While a few hornets, wasps, and spiders could be found within the greenhouse, they were not enough to keep the aphid and whitefly population in check (Figure 11), and pesticides had to be used to control them. I assumed their impact on plant vitality retarded the growth of the fastest growing shoots, which seemed most often parasitized by aphids. However, when analyzed, aphid infestation appeared stochastic and could not be correlated with growth rate or health score data and was discarded as a factor when further analysis was performed. Regardless, it is recommended that aphids be actively controlled with regular applications of pesticide, should further experiments at this scale be performed in a greenhouse setting.

5.1.1 Hypothesis 1: Plants subjected to multiple stresses will exhibit the greatest degree of physiological stress, as expressed by changes in (1) growth rate; (2) chlorophyll density in foliage; and (3) ranked vigor. Flooding is expected to be the most important stressor, but cutting and shading will also be significant.

The mean plant sizes of each treatment type (Figure 12) were fairly indicative of all subsequent results and analysis in that they demonstrated that, of all the treatments, shading had the weakest influence and, in some cases, had a positive effect on plant size, while flooding had the greatest effect. Other results from the analysis of final mean plant sizes were less pronounced. Cut plants started out much smaller because of the treatment applied to them, but they grew relatively well when compared to plants both cut and flooded. All plants slowed their growth after the 50th day of the experiment, possibly because they had exhausted their supply of available nutrients accessible in either their potting medium or submersion water.

When growth rate was analyzed (Figure 13), the differences between treatments was slightly more pronounced and the positive influence of the shade treatment could be seen with cut plants and, to a lesser extent, for flooded-and-cut plants. Flooding had the overwhelmingly strongest influence on growth, with the cutting treatment further slowing growth, even when this data was normalized by total average plant growth for a recording period. The control treatment grew the fastest and the flood-and-cut treatment, exposed to full sun, grew the slowest. It is likely this difference was caused by higher transpiration rates and longer periods of stomata closure, leading to detrimental photoinhibition occurring in the photosynthetic tissues of the plants exposed to full sunlight; eventually they were unable to acquire enough nutrients, principally nitrogen, to build essential proteins to protect tissues from excess light (carotenoids and others) or enzymes like RuBisCO to enhance photosynthetic productivity. This may be more of an artifact of greenhouse conditions, and the one application of Hoagland solution fertilizer provided to the plants during the duration of the experiment, than an effect to be anticipated under field conditions. The shade-only treatment also had a slower growth rate by the end of the experiment, though for a short time these plants overtook the control plants in growth rate; evidently water loss / stomatal closure / photoinhibition was less of an issue for the control plants than for plants subjected to the cutting and/or flooding treatments. Cut plants, with their artificially unbalanced root:shoot ratio, were able to quickly respond with rapid shoot growth, though eventually the shaded cut plants grew faster than unshaded; a possible issue with stomatal closure and water loss as well.

The chlorophyll density data is more difficult to interpret, partly due to how the data collection was implemented and partly because of the variability in responses displayed by the plants (Figure 14), with the greatest variability shown in the most stressful treatments (See Figure 14 E, F, G and H). Differences in mean chlorophyll levels were non-significant across all treatments for the top leaves of all plants, showing that *R. armeniacus* leaves in young and growing parts of plants, even when the organism as a whole might be suffering from various levels of stress, are relatively similar in their chlorophyll density, though they may vary in leaf area. Without the flooded-only treatment's mean SPAD score, the difference between middle leaves was also non-significant, showing that the rate of senescence from middle leaves to top leaves was also fairly uniform except in the case of the flood-only treatment. However, the chlorophyll density of the bottom (oldest) leaves alone was very significant, though it was not a good proxy for plant health; the control plants were large and growing vigorously, yet their oldest leaves had senesced far more than the oldest leaves in many of the more stressful treatments. Furthermore, by the end of the experiment plant canes ranged from barely a centimeter beyond the edge of the plant's bucket to an experimentally unwieldy 3.4 meters in length. Therefore, choosing to measure the bottom (lowest and oldest), middle, and top (youngest) leaves on every plant's cane placed these measurements on leaves that were far from analogous. For example, some very small flooded plants had tiny, yet chlorophyll-rich leaves packed closely together on very short canes, while much larger plants had large, yet low-chlorophyll leaves along a majority of their cane. Therefore it seems that there are many influences on the rate of leaf senescence, as measured by chlorophyll density, and other variables must be controlled before this kind of data will provide meaningful results without more rigorous analysis; only reporting and comparing the means of SPAD levels between treatment groups is insufficient. This variability in growth habit, leaf size, and cane lengths (and number of canes) was a problem that had to be factored into further analysis of this SPAD chlorophyll density data.

A better analytical technique was to create linear models of the mean leaf SPAD scores plotted against mean plant size per treatment (Table 2 and Figure 15). Then the slopes of the resulting linear models could be used to quantify the rate of senescence along the canes of each plant in a manner that factored in the differences in plant sizes. Shallow slopes meant less change in chlorophyll density along the canes of the plants in a treatment group, and the results of this analysis are more interesting. Here the cut treatments all have relatively steep linear model slopes, and the cut-and-flooded treatments (shaded or not) are the steepest of all. Having been cut, these plants had less above-ground biomass from which to draw photosynthate or re-allocate nutrients, and therefore may have had to immediately begin scavenging nutrients from old leaves to grow new ones. Flood-and-cut plants were also disadvantaged by also having fewer root tissues to derive nutrients from because many of their roots may have greatly slowed their metabolisms or died in their anaerobic state. Overall, these results correspond well with other analyses with all treatments involving any flooding making up four of the top five linear models with the steepest slopes, again demonstrating the strong influence flooding had on plants.

Analysis of subjectively ranked Health Scores produced similar results (Figures 16 and 17). Differences between mean treatment scores were significant, with the all-treatments group in the poorest health by the end of the experiment. By the end of the experiment there is a distinct grouping of flooded and non-flooded treatments groups, with all flooded treatments showing the lowest health. Interestingly, the flood-and-cut treatment's mean Health Score improved over time. This may have been due to these plants' root-to-shoot ratio allowing sufficient gas exchange to occur in their abundant roots to support their smaller emergent stems, and thus hastening anatomical adaptations to flooding that allowed faster recovery from the initial onset of flood stress. The benefits of being shaded, as discussed previously in regard to my growth rate analysis and the possible effects of stomatal closure and photoinhibition on un-shaded plants, remain broadly true here as well; when paired with otherwise identical treatment groups, shade-only plants remained healthier than the control group, shade-and-flood plants fared better than flood-only, though cut and cut-and-shaded plants were similar and the all-treatments group fared the worst of all treatment types.

Multivariate analysis reinforced the theme of flooding being the overwhelmingly strongest factor influencing plant growth and health over the duration of the experiment. Both Non-metric Multi-Dimensional Scaling ordinations[10] (Figures 18 & 19) showed at least some flooded treatment types more closely associated with each other than the remaining treatment groups. The ordination of the growth rate data only had flooded or the all-treatments groups distinctly separated from other treatments (Figure 19), but the ordination of the Health Score data separated all flooded treatments from non-flooded, and also grouped the treatments that were flooded and cut along an outer ordination contour.

---

[10] Non-Metric Multidimensional Scaling (NMDS) are a way to visually represent relationships between entities (data points) that may reveal underlying processes though the perceived “nearness” of datasets. They are built by creating large matrixes of Euclidian distances between data points after assigning those points random locations in the scaling/ordination. This Dˆ matrix is then compared with an input D matrix of difference and/or similarity coefficients calculated using a PERMANOVA on a dataset. A stress test is employed to determine which direction each point should move within the ordination to best reduce its stress value, and this process repeats for multiple iterations until no point can move to lower its stress. Metric and non-metric scaling differ in their assumptions as to the relationships (linear or non-linear, respectively) between ordinated distances and distance coefficients; for my purposes, non-metric scaling was chosen as it can represent ecological data more accurately (Prentice, 1977).

Further multivariate analysis using Hierarchal Cluster Analysis to sort plants into a dendrogram (Figure 20) yielded only marginally useful results. Most interesting is a single bucket of control plants that was quite unique when compared to all others. Otherwise, groupings seem to loosely place most flood-and-cut and all-treatment buckets into one groups, but the others are more mixed.

A univariate regression tree (Figure 21) looking solely at influences on plant mortality (number of dead plants within a treatment group at the end of the experiment), predictably found that flooding was the strongest cause of plant death, but surprisingly showed that shading was more important than cutting. These results differ from the growth rate, SPAD scores, and Health Scores in that shading may have caused a bi-modal response; plants either stayed healthier and grew faster than their non-shaded counterparts suffering from frequent stomatal closure and photoinhibition, or they died due to photosynthate production rates too low to invoke adaptations to other stresses. This last result is an interesting one for the ecological restorers to consider – might canopy cover aid some plants while harming others, and what might be the cause of this bi-modal response?

Overall, the results of this experiment support the hypothesis that the plants subjected to flooding, shading, and cutting treatments exhibited stress levels that could be detected through analysis of growth rate, leaf chlorophyll density, and ranked Health Scores. However, while flooding was certainly the most important factor, the response of *R. armeniacus* to the shade and cutting treatments were more complex than anticipated.

5.1.2 Hypothesis 2: Mortality rates of flooded plants under the various experimental treatments can be established to model the tolerance R. armeniacus has to partial inundation.

Using mean Health Scores, mortality rates for each treatment type could only be loosely established as *R. armeniacus* proved far more resilient to flooding stress than anticipated. Among the non-flooded treatment types, most plants might have lived indefinitely or until overwhelmed by artificial environmental factors encountered in the greenhouse (Figure 22). Treatment groups that included flooding were analyzed with polynomial models that provided better fit (R^2 values) and linear models that were almost as good (R^2 values of 0.8157 or better). These linear models crossed the X axis and provided a forecast of when, generally, all plants in that treatment group might be expected to have died (Figure 23). However, both the previously discussed results from Health Score analysis and the polynomial models of these treatment groups point to the possibility that many plants could recover from flooded conditions and survive much longer than the mortality rate predicted by the linear models of Figure 24.

In light of all of these caveats, it does appear that the all-treatments group had the highest predicted mortality rate, the flooded-and-cut the next highest, and the flood-and-shade group the slowest. Therefore the implications for ecologists trying to restore infested sites are that mechanical treatment (cutting) before anticipated flooding events may help control *R. armeniacus*, and the presence of canopy cover will further impair the plant's recovery.

5.1.3 *Hypothesis 3*: *Flooded plants that exhibit lower stress levels will be more likely to exhibit anatomical or morphological adaptations.*

R. armeniacus certainly invoked anatomical changes as a response to flood stress, as evidenced by inspection of flooded plants removed from their pots for final biomass analysis (Figure 24). Furthermore, the positive correlation between Health Score and adventitious root biomass (Figure 25) supports the hypothesis that these acclimations do improve the plant's ability to tolerate anaerobic conditions. I consider these results conclusive evidence of *R. armeniacus*' adaptations to flood stress and the key to its ability to invade freshwater wetlands by invoking cell death to build aerenchyma in existing roots and shoots, or to grow new aerenchyma-filled adventitious roots into floodwaters or hydric soils.

5.2 The Laboratory Experiment: Full Submersion & Time Lapse Observation

This second, smaller experiment was more of a proof-of-concept than rigorous scientific inquiry, yet it did provide several interesting results: Small *R. armeniacus* plants can easily survive total submersion for more than 40 days, healthy plants can extend their shoots while underwater, and they can survive post-submersion desiccation. These results add to the understanding of *R. armeniacus*' impressive ability to tolerate anaerobic conditions.

With this basic experimental apparatus proven as effective, further experimentation should extend the length of submersion, and control plant size and age to determine the effects of those variables on plant survival rates. This apparatus could also be used to examine other *Rubi*, or perform a broader survey of morphological responses to submersion throughout a broader range of plant taxa.

6. Conclusion

Rubus armeniacus commonly invades seasonally flooded freshwater wetland areas of the Pacific Northwest (Bennett, 2004, 2006; Soll, 2004). My research has demonstrated the plant's innately substantial tolerance of, and rapid adaptation to, otherwise stressful and deleterious conditions caused by flooding. This information is valuable to ecological restorers planning to restore areas infested, or potentially infested, with this aggressive exotic species in a setting prone to seasonal flooding.

While there remains hope that either natural or introduced biological control agents may reduce the impact of exotic *Rubi* in the Pacific Northwest (Osterbauer et al., 2005), for the foreseeable future most management of *R. armeniacus* infestations is likely to remain dependent on the traditional combination of mechanical and chemical treatments. When the weed is found in areas that are seasonally flooded, or will be flooded after restoration efforts restore impaired hydrological regimes, its ability to tolerate these conditions is remarkable and must be taken into consideration if native plants are to be established in its place (Nash & Graves, 1993).

It is apparent that flooding alone is no substitute for other methods of controlling *R. armeniacus*. However, in combination with mechanical cutting, and in a shaded setting, the effects of flooding may be enough to obviate the need for chemical treatment of the plant. This saves restoration funds for other tasks and reduces the risk of ecological damage from herbicide use. However, a direct correlation between my greenhouse experiment and field conditions is tenuous, and field studies should be conducted before this method is employed on an active restoration site.

Further experimentation along similar themes is warranted, and a re-design and re-focusing of my efforts would help distinguish and illuminate some of my preliminary findings. The most important refinement would be to run a similar partial-submergence experiment without concern for preserving the test plant's tissues for a biomass assay, therefor allowing the experiment to run its course over six months or more. This would obviate the need to use linear models to forecast mortality rates of plants in various conditions, as I did with my analysis shown Figure 22 and Figure 23.

Finally, it must be said that *R. armeniacus* is a remarkable organism, and for many reasons: It has an impressively high photosynthetic capacity (Osaceae, 2002), reproduces prodigiously and efficiently (McDowell & Turner, 2002), and lives within a broad range of edaphic and climatic conditions (Caplan & Yeakley, 2006, 2010). What is most astounding is that this amazing phenotypic plasticity is available to the plant despite its acute lack of genetic diversity. Astounding as this is, it begs the question: Is this dichotomy common among invasive plants? Further investigation is warranted to establish a ratio of phenotypic plasticity to genetic diversity among a broader survey of invasive taxa (through various measurable traits or conditions that, in combination, can act as proxies for these larger concepts), to eventually determine if this radio is at all predictive of invasive behavior, risks of spread, ease of control, or other useful correlations.

List of References

Alice, L. A., & Campbell, C. S. (1999). Phylogeny of Rubus (Rosaceae) based on nuclear ribosomal DNA internal transcribed spacer region sequences. *American Journal of Botany*, *86*(1), 81. Botanical Soc America. Retrieved from http://www.amjbot.org/cgi/content/abstract/86/1/81

Alice, L. A., Riksson, T., Riksen, B., & Campbell, C. (2001). Hybridization and gene flow between distantly related species of Rubus (Rosaceae): evidence from nuclear ribosomal DNA internal transcribed spacer region. *Systematic botany*, *26*, 769-778. Retrieved from http://www.bioone.org/doi/abs/10.1043/0363-6445-26.4.769

Amor, R. L. (1973). Ecology and control of blackberry (Rubus fruticosus L. agg.). I. Rubus spp. as weeds in Victoria. *Weed Res*, *13*(4), 218–223. Wiley Online Library. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Ecology+and+control+of+blackberry+(Rubus+fruticosus+L.+agg.)+I.+Rubus+SPP.+as+weeds+in+Victoria#0

Amor, R. L. (1974a). Ecology and control of blackberry (Rubus fruticosus L. agg.). II. Reproduction. *Weed Research*, *14*(4), 239-243. doi:10.1111/j.1365-3180.1974.tb01048.x

Amor, R. L. (1974b). Ecology and control of blackberry (Rubus fruticosus L. agg.). III. Response of R. procerus to mechanical removal of topgrowth and to foliage applied herbicides. *Weed Research (UK)*, *14*(4), 239-243. doi:10.1111/j.1365-3180.1974.tb01048.x

Bailey, L. H. (1945). The genus Rubus in North America. *Gentes herbarum*, *5*(1), 851-854.

Bennett, M. (2004). Restoration of Himalayan blackberry-dominated riparian zones in interior southwest Oregon: A thorny issue. *16th International Conference, Society for Ecological Restoration* (Vol. 129, pp. 1-9). Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:No+Title#0

Bennett, M. (2006). *Managing Himalayan Blackberry in Western Oregon Riparian Areas*. *Reproduction* (p. 16). Oregon State University Extension Service. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Managing+Himalayan+blackberry#0

Blokhina, O. B., Chirkova, T. V., & Fagerstedt, K. V. (2001). Anoxic stress leads to hydrogen peroxide formation in plant cells. *Journal of Experimental Botany*, *52*(359), 1179. Soc Experiment Biol. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11432936

Blom, C. W., & Voesenek, L. a. (1996). Flooding: the survival strategies of plants. *Trends in ecology & evolution*, *11*(7), 290-5. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21237846

Boyd, R. (1999). *The Coming of the Spirit of Pestilence* (pp. 22-24). Vancouver, BC: UBC Press.

Brown, R. T., Agee, J. K., & Franklin, J. F. (2004). Forest restoration and fire: principles in the context of place. *Conservation Biology*, *18*(4), 903–912. Wiley Online Library. doi:10.1111/j.1523-1739.2004.521_1.x

Brunner, H., Harris, R., & Amor, R. (1976). A note on the dispersal of seeds of blackberry (Rubus procerus PJ Muell.) by foxes and emus. *Weed Research*, *16*(3), 171–173. Wiley Online Library.

Caplan, J. S., & Yeakley, J. A. (2006). Rubus armeniacus (Himalayan blackberry) occurrence and growth in relation to soil and light conditions in western Oregon. *Northwest Science*, *80*(1), 9. NORTHWEST SCIENTIFIC ASSOCIATION. Retrieved from http://web.pdx.edu/~yeakleya/Caplan and Yeakley 2006.pdf

Caplan, J. S., & Yeakley, J. A. (2010). Water relations advantages for invasive Rubus armeniacus over two native ruderal congeners. *Plant Ecology*, *210*(1), 169-179. doi:10.1007/s11258-010-9747-4

Chaloupka, M. Y., & Domm, S. B. (1986). Role of anthropochory in the invasion of coral cays by allen flora. *Ecology*, *67*, 1536-1547.

Daar, S. (1983). Using goats for brush control. *The IPM Practioner*, *4*(4), 4–6. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Using+goats+for+brush+control#0

Dutson, V. J. (1974). The association of the roof rat (Rattus rattus) with the Himalayan blackberry (Rubus discolor) and Algerian ivy (Hedera canariensis) in California. *Proceedings of the 6th Vertebrate Pest Conference (1974)* (p. 10). Retrieved from http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=1009&context=vpc6

Ferguson, R. B. (1981). Use of rosaceous shrubs for wildland plantings in the Intermountain West. *Monsen, SB; Shaw, N., comps. Managing Intermountain rangelands—improvement of range and wildlife habitats proceedings of symposia* (pp. 15–17). Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Use+of+rosaceous+shrubs+for+wildland+plantings+in+the+Intermountain+West#0

Fierke, M. K., & Kauffman, J. B. (2006). Riverscape-level patterns of riparian plant diversity along a successional gradient, Willamette river, Oregon. *Plant Ecology*, *185*(1), 85-95. doi:10.1007/s11258-005-9086-z

Going, B. M., & Dudley, T. L. (2007). Invasive riparian plant litter alters aquatic insect growth. *Biological Invasions*, *10*(7), 1041-1051. Springer. doi:10.1007/s10530-007-9182-1

Gray, A. N. (2005). Eight nonnative plants in western Oregon forests: associations with environment and management. *Environmental monitoring and assessment*, *100*(1), 109–127. Springer. Retrieved from http://www.springerlink.com/index/W632111146222570.pdf

Groves, R., Williams, J., & Corey, S. (1998). Towards an integrated management system for blackberry (Rubus fruticosus L. agg.). *Plant Protection Quarterly*, *13*(4), 148-199. RG and FJ Richardson.

Heslop-Harrison, Y. (1959). Natural and induced rooting of the stem apex in Rubus. *Annals of Botany*, *23*(2), 307. Annals Botany Co. Retrieved from http://aob.oxfordjournals.org/content/23/2/307.short

Hobbs, R. J., & Humphries, S. E. (1995). An Integrated Approach to the Ecology and Management of Plant Invasions. *Conservation Biology*, *9*(4), 761-770. doi:10.1046/j.1523-1739.1995.09040761.x

Hoshovsky, M. (2000). Rubus discolor Weihe & Nees. *Invasive plants of California's wildlands. Berkeley, Los*, *22209*(703). Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Rubus+discolor#1

Ingham, C. S. (2008). Himalayan blackberry (Rubus armeniacus) and English ivy (Hedera helix) response to high intensity-short duration goat browsing, 126. Oregon State University. Retrieved from http://scholarsarchive.library.oregonstate.edu/xmlui/handle/1957/10211

Jackson, M. B. (2005). Response and Adaptation by Plants to Flooding Stress. *Annals of Botany*, *96*(4), 501-505. doi:10.1093/aob/mci205

Jackson, M. B., Ishizawa, K., & Ito, O. (2008). Evolution and mechanisms of plant tolerance to flooding stress. *Annals of Botany*, *103*(2), 137-142. doi:10.1093/aob/mcn242

James, R., & Lockwood, M. (1998, July 29). Economics of blackberries: current data and rapid valuation techniques. *Plant Protection Quarterly*. Retrieved from http://ecite.utas.edu.au/34746

Jones, D. K. (2004). Factors affecting the regrowth of Himalya (sic) blackberry (Rubus armeniacus). *Defense*. Retrieved from http://scholarsarchive.library.oregonstate.edu/xmlui/handle/1957/10999

Kauffman, J. B., Beschta, R., Otting, N., & Lytjen, D. (1997). An Ecological Perspective of Riparian and Stream Restoration in the Western United States. *Fisheries*, *22*(5), 12-24. doi:10.1577/1548-8446(1997)022<0012:AEPORA>2.0.CO;2

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World Map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, *15*(3), 259-263. doi:10.1127/0941-2948/2006/0130

L, R., Sampaio, F., Foerster, F., & Bertol, R. (2003). Rubus discolor Weihe & Ness: Himalayan blackberry. *Agriculture*, 1996-1999.

Larcher, W. (2003). *Physiological Plant Ecology* (4th ed., p. 513). Springer.

Mallah, T. S. (1954). Natural hybridization between Rubus ursinus Cham. and Schlecht and other Rubi in California. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Natural+hybridization+between+Rubus+ursinus+Cham.+and+Schlecht+and+other+Rubi+in+California#1

Markarian, D., & Olmo, H. P. (1959). Cytogenetics of Rubus: I. Reproductive Behavior of R. procerus Muell. *Journal of Heredity*, *50*(3), 131-136. Retrieved from http://jhered.oxfordjournals.org/content/50/3/131.extract

McCune, B., & Grace, J. B. (2002). *Analysis of Ecological Communities*. Gleneden Beach, OR: MjM Software.

McDowell, S., & Turner, D. (2002). Reproductive effort in invasive and non-invasive Rubus. *Oecologia*, *133*(2), 102-111. doi:10.1007/s00442-002-1006-5

Miles, E. L., Snover, A. K., Hamlet, A. F., Callahan, B., & Fluharty, D. (2000). Pacific Northwest Regional Assessment: The Impacts Of Climate Variability And Climate Change On The Water Resources Of The Columbia River Basin. *Journal of the American Water Resources Association*, *36*(2), 399-420. doi:10.1111/j.1752-1688.2000.tb04277.x

Mommer, L., & Visser, E. (2005). Underwater photosynthesis in flooded terrestrial plants: a matter of leaf plasticity. *Annals of botany*, *96*(4), 581-9. doi:10.1093/aob/mci212

Moore, S. (2009). *Class B Noxious Weed List, designated in King County 2. Add Himalayan blackberry (Rubus* (pp. 2008-2010).

Munz, P. A., & Keck, D. D. (1973). *A California Flora and Suppliment* (Vol. 1, pp. 784-785). Univ of California Press. Retrieved from http://books.google.com/books?hl=en&lr=&id=mCm-jmU5E1AC&oi=fnd&pg=PA1&dq=A+California+flora&ots=5-oUPtiWuP&sig=hvNe_dNfR6lBM152u3SSeBt476I

Nash, L., & Graves, W. R. (1993). Drought and flood stress effects on plant development and leaf water relations of five taxa of trees native to bottomland habitats. *Journal of the American Society for Horticultural Science*, *118*(6), 845-850.

National Research Council [US]. (1996). *Upstream: salmon and society in the Pacific Northwest* (p. 452). Washington, DC: National Academies Press. Retrieved from http://books.google.com/books?hl=en&lr=&id=QOA1SYQXyMEC&oi=fnd&pg=PA1&dq=Upstream:+Salmon+and+society+in+the+Pacific+Northwest&ots=ZccXaR7nFM&sig=DQvXNeZvdes0YWzKp6yK8FHTMc4

Northcroft, E. G. (1927). The blackberry pest. I. Biology of the plant. *New Zealand Journal of Agriculture*, *3*, 376-388.

Nutt, R., Oleskevich, C., Shamoun, S. F., & Punja, Z. K. (1996). The biology of Canadian weeds. 105. Rubus strigosus Michx., Rubus parviflorus. *Plant Science*.

Oregon State University Extension Service. (2008). Invasive Weeds in Forest Land: Himalayan Blackberry & Evergreen Blackberry. *Biological Control*. Salem, OR: Oregon State University.

Osaceae, R. U. R. (2002). Photosynthetic characteristics of invasive and noninvasive species of Rubus (Rosaceae). *American Journal of Botany*, *89*(9), 1431-1438.

Osterbauer, N., Trippe, A., French, K., & Butler, T. (2005). First Report of Phragmidium violaceum Infecting Himalaya and Evergreen Blackberries in North America Plant Health Progress Plant Health Progress. *Plant Health Progress*, *2*(September), 2-4. doi:10.1094/PHP-2005-0923-01-BR.In

Prentice, I. (1977). Non-Metric Ordination Methods In Ecology. *The Journal of Ecology*, *65*(1), 85-94. Retrieved from http://www.jstor.org/stable/10.2307/2259064

Ringold, P. L., Magee, T. K., & Peck, D. V. (2008). Twelve invasive plant taxa in US western riparian ecosystems. *October*, *27*(October), 949-966. doi:10.1899/07

Robertson, K. R. (1974). The genera of Rosaceae of the southeastern United States [prim .]. *Journal of the Arnold Arboretum*, *55*, 303-332. Retrieved from http://biostor.org/reference/63663

Roni, P., Beechie, T., Bilby, R., Leonetti, F., Pollock, M., & Pess, G. (2002). A Review of Stream Restoration Techniques and a Hierarchical Strategy for Prioritizing Restoration in Pacific Northwest Watersheds. *North American Journal of Fisheries Management*, *22*(1), 1-20. doi:10.1577/1548-8675(2002)022<0001:AROSRT>2.0.CO;2

Soll, J. (2004). *Controlling Himalayan Blackberry (Rubus armeniacus [R. discolor, R. procerus]) in the Pacific Northwest*. *Retrieved February* (Vol. 8, p. 14). Retrieved from http://www.invasive.org/gist/moredocs/rubarm01.pdf

Stevens, M. (1993). Restoring wetlands in Washington. *Washington State Dept. of Ecology Publication*, (March). Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Restoring+Wetlands+in+Washington#5

Thompson, M. M. (1997). Survey of Chromosome Numbers in Rubus (Rosaceae: Rosoideae). *Annals of the Missouri Botanical Garden*, *84*(1), 128. doi:10.2307/2399958

Tirmenstein, D. (1989). Rubus discolor. *Fire Effects Information System*. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Retrieved from http://www.fs.fed.us/database/feis/

U.S. Department of Agriculture, B. of P. I. (1913). *The Himalaya Blackberry* (pp. 23-26).

USDA NRCS. (2011). The PLANTS database. Retrieved July 13, 2011, from http://plants.usda.gov

Visser, E. J. W. (2003). Flooding and Plant Growth. *Annals of Botany*, *91*(2), 107-109. doi:10.1093/aob/mcg014

Williams, H. S. (1915). *Luther Burbank: His life and work*. Hearst's International Library CO.