Phalaris arundinacea Control and Riparian Restoration within Agricultural Watercourses in King County, Washington

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CHAPTER 1. INTRODUCTION

The biological and economical threat of invasive non-indigenous species has been well established over the past two decades (Facon et al. 2006, Pysek and Richardson 2006, Richardson 2004, Reichard and White 2001, Sakai et al. 2001, D'Antonio and Vitousek 1992, Rejmànek and Richardson 1996, and many others). Indeed, almost one half (42%) of the threatened or endangered species listed under the US Endangered Species Act are in jeopardy due to competition or predation by non-indigenous species. This proportion balloons to as much as 80% in other regions of the world (Pimentel et al. 2000). Invasive organisms incur losses and damages, resulting in annual costs of $136,630 billion including control (Pimentel et al. 2000). Invasive non-indigenous plants are estimated to encroach upon roughly 700,000 hectares of native habitat per year. These invasives then threaten the native plants and wildlife on the site, biodiversity on a grand scale, as well as negatively impact entire ecosystems (Pimentel et al. 2000). In addition to diminishing biodiversity and disrupting ecosystem functions, invasive species seriously impact agricultural systems and can be hazardous to livestock and humans (Facon et al. 2006).

S.L Mitchill (1810), L.D. de Schwenitz (1832) and Asa Gray (1879), provided some of the earliest documentation of biological invasions. Within their essays, the authors describe the condition of urban, rural, agricultural and natural settings within the eastern United States with regard to “weedy” plants, and the aggressive nature of many of these species, most from Europe (Stuckey 1978). Charles Darwin also makes note of the “large proportional addition” of genera to the United States when commenting on the false assumption that successful introduced species would be of similar genera to those species found at the site of introduction (Darwin 1858). Charles Elton published the first book on invasive species in 1958, alerting the public to the serious topic of invasive species, predicting homogenization of flora and fauna by the breaking down the biogeographic barriers via human-mediated transport.

Even our National Parks, habitats which we feel are generally safe from harm, and well taken care of by stewards, are heavily impacted. Current estimates indicate that more than 2.6 million acres (3-5%) are dominated by invasive plants (Welch et al. 2007). Locally, the exotic plant management team for the North Cascades National Park reports that of the 13,587 acres inventoried, 13,228 acres have invasive species with 3,311 of those acres completely infested (Welch et al. 2007). Our national forest and grasslands are not faring any better, with approximately 420,000 acres in the Pacific Northwest Region being degraded by invasive plants (USDA Forest Service 2007).

Many of these invasive plants are capable of forming monocultures on a given site, completely displacing native plants as well as altering the structure, productivity, fire and flooding regimes and soil nutrient properties (Reichard and White 2001, D'Antonio and Vitousek 1992, Booth et al. 2003). The results from a survey of restoration ecologists within western Washington revealed that invasive non-indigenous plants were the leading cause of failure of restoration projects (Seebacher 1999, unpublished data).

In addition to direct competition, herbivory/predation, and parasitism, additional impacts of non-indigenous species include physical or chemical alteration of the habitat and soil, and introduction of pathogens. van der Velde et al. (2006) asserts that the impacts of the introduced species are especially problematic when the impacted species are keystone species, causing disturbance of the food web structure and biodiversity functions. Additionally, by removing the natural barriers between non-indigenous and native species as humans are doing at a phenomenal rate, we are altering the genetic diversity of the native species and native community. If the introduced invasive species hybridizes with a native species, these hybridizing events are potentially triggering outbreeding depression. Consequently, this introduction can also influence allopatric speciation and therefore, increase biodiversity within the bioregion (van der Velde et al. 2006). The potential escalation in biodiversity due to hybridization events may alter the genetic integrity and local adaptation of the native species involved.

Non-indigenous species have many labels; aliens, non-natives, exotics, introduced, immigrants, biological pollution and an additional term for plants, noxious weeds. Non-native species as defined by Boersma et al. (2006) refers to those that have been “moved to new places by humans.” From the same publication, an invasive species is determined to be a “non-native organism that causes harm to native habitats or species.” Official U.S. definitions for invasive species provided in Executive Order 13112 signed by President William Clinton in 1999 state that "Invasive species means an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health.” "Alien species means, with respect to a particular ecosystem, any species, including its seeds, eggs, spores, or other biological material capable of propagating that species, that is not native to that ecosystem” (Federal Register V 64 - 25 1999).

Pysek and Richardson (2006) go even further classifying invasive plants into three categories: “casual alien plants” as those that flourish and reproduce occasionally out of cultivation, but do not form self-replacing populations; “naturalized plants” as those with self-replacing populations and capable of independent growth; and “invasive plants” which are a subset of naturalized plants that reproduce in large numbers able to spread quickly over large areas. For the duration of this dissertation, “invasive plants” refer to those non-indigenous species that cause harm to native habitats or species and fall into the invasive category.

My dissertation focuses on a challenging invasive perennial, reed canarygrass (RCG), (*Phalaris arundinacea*) that is capable of forming monocultures within freshwater wetlands, riparian areas and agricultural fields. This invasive is responsible for generating substantial acreage (some infestations over 100 ha) of monocultures degrading biodiversity, displacing wildlife (Tu 2006) and altering invertebrate assemblages on the site (WSU Research Team 2006, unpublished data). Additional negative impacts of *Phalaris arundinacea* include changes in hydrology, which can increase the risk of flooding nearby agricultural fields and adjacent areas; and increasing the elevation of a site, eliminating ponds and watercourses utilized by waterfowl, amphibians and invertebrates.

Chapter One discusses the biology and ecology of invasive species, susceptibility of receiving sites, and the impacts of invasive plants on the community and ecosystems involved.

Chapter Two covers a field research project on *Phalaris arundincaea* control, and the restoration of the riparian zones within agricultural systems. This research project is associated with and a component of a comprehensive agricultural watercourse project allied with WSU researchers and King County. Three RCG control treatments were applied and replicated on three sites within eastern King County along agricultural watercourses. These treatments were followed for two consecutive seasons for successful RCG reduction and control. The data collected included the returning stem count of RCG as well as the native plant survival and density based on percent cover.

Chapter Three introduces the prospect that a native emergent sedge, *Scirpus microcarpus* (small fruited bulrush, (SFB)) could be able to effectively compete with the invasive RCG. A controlled greenhouse study was carried out to determine whether *Scirpus microcarpus* would reduce the above and/or below ground biomass of the reed canarygrass when grown together within one gallon pots for one growing season (inter-specific competition versus intra-specific competition).

Chapter Four covers the energy storage mechanism for reed canarygrass and how this strategy allows for increased aggressiveness and negatively impacts control methods for this species. Forty-five randomly chosen rhizome pieces were placed within one gallon pots which were buried in the field. These were then covered with an opaque fabric fixed in place. Fifteen were removed after three months, the next fifteen after six months and the last fifteen after nine months. The rhizomes were then stored within a freezer until being analyzed with a near-infrared spectrophotometer (NIRS) for fructosans. The time necessary to cover RCG rhizomes to achieve depletion of carbohydrates was then extrapolated from these data.

1.1 INVASIVE SPECIES BIOLOGY

1.1.1 Stages of Species Invasions

There are several stages involved in species invasions (Allendorf and Lundquist 2003). The first is the introduction of the species. The first records of species introductions in the United States dates back to 1628 when species such as *Isatis tinctoria* and *Cannabis sativa* were brought back to the US on the Endicott expedition. Many other common invasives were transported into the Plymouth colony and New England throughout the 1600's, such as *Rumex acetosella*, *Tanacetum vulgare*, *Foeniculum vulgare*, *Hypericum perforatum*, *Polygonum persicaria* and *Salvia sclarea*, all of which are still problematic throughout North America today (Mack 2003).

Generally, the pathways of these species to their new locations have been primarily horticultural, especially for woody species (Reichard 1997). Herbaceous invasive species have been introduced largely as crop seed contaminants, through ship ballast (Reichard and White 2001), as ornamentals, and for medicinal and/or for fodder purposes. These species are then further spread by: impure crop seeds, adhering to domestic and wild animals and birds; within the soil of ornamentals from nurseries; (Sakai et al. 2001) as seed traded and sold via arboreta, garden and horticultural clubs and the internet; (Reichard and White 2001), attached to vehicles along roadways (Von der Lippe and Kowarik 2007), as well as innocently attached to the clothing or boots of natural resource workers and hikers as shown by an anecdotal study by Reichard (1998).

After the introduction stage the species must become established and continue to survive and reproduce. Propagule pressure, the number of individuals introduced and/or the number of introduction events becomes significant in the establishment of the species. A larger number of introduced individuals would allow for a greater amount of genetic variation, which would in turn reduce any potential impacts of a population or founding bottleneck due to the original introduction. Multiple introductions may create the same results, especially when the plants are from different source populations. This may release genetically diverse individuals allowing for greater heterozygosity of the founding population and a higher chance of adaptation to the novel environment (Allendorf 2003). The exchange of genetic material between the introduced populations may also result in the distribution of an invasive genotype (Sakai et al. 2001) or the swamping of locally beneficial alleles (Allendorf 2003).

The second stage of invasiveness would be the dispersal and spread into new habitats and the replacement of native species by the introduced species. Range expansion is facilitated by high dispersal rates, which could potentially bring a high amount of gene flow and the probability of spreading into novel conditions within the new ranges. However, this gene flow from the central site of initial colonization to the periphery of the range may prevent local adaptation, impacting further range expansion (Sakai et al. 2001) causing the boundaries to this species to remain static. This same scenario can take place when this peripheral population is deficient in the phenotypic variation necessary for local adaptation (Lavergne and Molofsky 2007).

Beginning in the mid 1980s, Williamson and Brown (1986) employed a statistical approach to analyze the success of invaders, (that successful invasions are rare), which is referred to as the “tens rule.” This statistical rule states that one in ten species that are imported will become casual or “introduced”, one in ten of those casual species will become established, and one in ten of those established species will become a “pest.” (Williamson and Fitter 1996). The definitions for these terms are important for understanding the principles of the rules. Imported species are those “found in collections or accidentally brought into the country,” introduced (casual) species are found outside of cultivation in the wild, established species are those forming self sustaining populations, and pest species are those species that have a “negative economic impact. The transitions between these potential stages are identified as escaping, establishing and becoming a pest. Williamson and Fitter (1996) emphasize that an acceptable variation would mean between 5 and 20 percent for the tens rule.

There is often “lag time” or a delay between the initial introduction and colonization and the expansion of the species to surrounding habitats. This may occur because of the introduced species requires time to adatpt to the new environment and/or the evolution of invasive traits, or the “purging of a genetic load responsible for inbreeding depression” (Sakai et al. 2001). Founder effects, genetic drift and the rapid evolution generated by stressful conditions in the new environment may all initiate swift evolution of a non-indigenous species allowing for an increase in spread and invasiveness (Allendorf 2003).

1.1.2. Archetypical traits of invasive species

One of the most perplexing questions within the field of invasion biology is why some species become invasive while other introduced species remain benign? A list of the traits one might expect to find in the “ideal weed” that is commonly cited was generated in 1965 by Herbert Baker. This list (with minor modifications added by Baker a decade later) is as follows: 1) germination requirements fulfilled in many environments; 2) discontinuous germination (high seed longevity); 3) rapid growth through vegetative phase to flowering (short vegetative phase); 4) continuous seed production during adequate growing conditions; 5) ability to be self-compatible; 6) unspecialized or wind cross pollination; 7) high seed output; 8) able to produce at least some seed in range of environmental conditions (tolerant and plastic); 9) short and long dispersal adaptations; 10) vigorous vegetative reproduction and/or regeneration from fragments for perennials; 11) brittleness, not easily extracted; 12) ability to compete interspecifically via a rosette and/or allelochemicals. There is, of course, a wide spectrum within these traits and the invasiveness of a plant (Baker 1974). Two of these traits were also found to be correlated with invasiveness by Reichard and Hamilton (1997); absence of germination requirements and vegetative reproduction. Basu et al. (2004) added just a few additional traits: 1) deep root system, allowing the weed to thrive during a drought; 2) environmentally plastic, changing growth form in response to environmental factors; and as noted by Baker, Reichard and Hamilton, 3) the ability to reproduce both sexually and asexually.

Successful colonists typically employ *r*-selected life histories, such as short generation time, high fecundity and growth rates, as well as the ability to transfer between r-selected and *K*-selected strategies (Sakai et al. 2001). Grime (1977) expanded on this method of characterizing plant strategies by placing plants at any point within a triangle based on the morphology, life history and physiology of the plant. At the extreme of the triangular tips one would find those plants that are predominantly competitive, stress tolerant or ruderal. Weedy species are considered to be either (R) ruderals, those species that tolerate frequent disturbance by short reproductive times, or a (C) competitor, which can be found in undisturbed habitats with a reduced amount of resources being allocated to reproduction and more to vegetative biomass, or a mixture of the two (CR). Both the (C) strategist and the (R) strategist generally indicate productive habitats (Booth et al. 2003).

Baker (1953) introduced the idea of the formation of local races for introduced species. These ecological races or ecotypes would be selected for and would show genetically fixed characters based on the situation at the site. For example, continuing disturbance would select for a local adaptation by the species, perhaps existing as low growing rosettes during the season the site is usually mowed, if it is to survive and thrive at the site. Species capable of cross pollination would be able to adapt more quickly. However, multiple introductions and therefore, the availability of differing genotypes also allows for rapid race formation to develop. An example would be crop mimics that have adapted its growth and reproduction period, and even seed size for dispersal assistance, with the crop that they invade. Weedy species that have adapted to roads and railways are typically low growing with flat rosette leaves which are finely divided and pubescent. The stems are soft and flexible, not brittle, allowing for treading, and the fruits are dry and can be dispersed by adhesion to shoes or wheels. For invasives that tend to invade agricultural communities which are regularly mowed or grazed are selected for those species that can reproduce via rhizomes or stolons and eventually over many generations, rely on this means of reproduction and may even produce only sterile seed (Baker 1974).

As noted above in section 1.1.1, commercial horticulture is a primary pathway for invasive species. The traits which are selected by the breeders of floriculture have a propensity to also apply to invasive species. These include “disease and pest resistance, drought tolerance, high fertility, lack of seed dormancy, rapid germination, high yield potential, short generation, hybrid vigor and large plant size” (Anderson 2006).
When comparing six previous studies using numerous plant traits to predict invasiveness (Reichard 1997) found that those species that had “invaded elsewhere” were positively correlated to becoming an invasive species in a new range. Those species for which the native range would match the climatic conditions (precipitation and temperature regime) of the introduced region may also be a good indicator. A wide latitudinal range may also imply an ability to adapt to a wide range of environmental conditions. Other species attributes tested in the same studies that were found to be good predictors were: a short juvenile period (a positive trait in four out of the six studies); seed bank type or longevity (for *Pinus* sp. and annuals); and seed mass and size (smaller seeds allowing for greater dispersal).

Many invasive species have the ability to reproduce asexually via agamospermy or vegetatively and are able to avoid the complications of potential inbreeding depression associated with a small initial colonizing population (Allendorf 2003). Baker previously observed this connection, especially for annuals, stating that autogamy or agamospermy is a prominent feature for many weeds. This trait allows the plant to reproduce, creating a colony from a single immigrant or from the regeneration of a single plant left after weed removal operations. For perennial species, vegetative reproduction accomplishes the same goal, “rapid multiplication of individuals with appropriate genotypes”. This also allows for the rapid expansion of a population which would be as well adapted to the new environment as the founder individual (Baker 1974). Allendorf (2003) asserts that local adaptation of the native species may not be necessary except for during periodic episodes, such as long term extreme environmental situations such as serious flooding events or episodic fires or droughts. Additionally, numerous invasives are polyploids and therefore, this genetic variation is retained as fixed heterozygosity (Allendorf 2003).

About half of the studies Bossdorf et al. (2005) reviewed showed that the invasive species were more "plastic" than the native species. Phenotypic plasticity as defined by Pigliuccci (2002) can be defined as "the property of a genotype to produce different phenotypes when exposed to different environments. Plasticity is a property of the reaction norm of a genotype."

Some invasive species may be fundamentally better competitors as they have evolved in highly competitive environments (Allendorf 2003). One hypothesis concerning plant invasions is the "Evolution of Increased Competitive Ability (EICA)." This hypothesis states that the plant is released from pressure of certain pest in its native habitat that would keep that species "in check." In turn, the plant evolves the ability to reallocate the resources it used to draw on for defense into elevated reproduction rates and increased growth (Bossdorf et al. 2005). After reviewing field studies of various native and introduced plants and the impacts of herbivores, plant size and fecundity, Bossdorf et al. (2005) found that 56% and 55% of the studies found increased growth and decreased resistance for the introduced species, providing moderate support for EICA.

Invasive species may possess similar traits with the native species or conversely, possess different traits than the native species, thereby finding an empty niche (Sakai et al. 2001). Those introduced species with native congeners may share characters which allow for the plant to be more adapted to the site of introduction. In contrast, Darwin's theory on plant invasions was that the more successful invaders would have traits that were distinct from the native species (van der Velde et al. 2006). This theory plays well with the EICA theory noted above in which the non-native genera success can be at least in part due to the fact that many of the resident herbivores and pathogens would not be able to switch to species that are phylogenetically distant from the native host (Rejmànek 1996). One example reported by Rejmànek is a common aggressive and highly detrimental invasive species in the PNW as well as in California, *Cytisus scoparius* (scotch broom). There are no native phytophagous insects found on scotch broom in North America, yet there are at least thirty five phytophagous species in its native England.

Callaway and Aschehoug (2000) maintain some invasive species are successful due to "novel mechanisms" that they bring to the new environment and native plant community. They compared the impacts of *Centaurea diffusa* on the biomass and phosphorus uptake of North American grass species found in the native communities in which the *C. diffusa* invades, and its native Eurasian species. They found that the *C. diffusa* had decreased the biomass and P uptake of the North American species far more so than the Eurasian species. *Centaurea diffusa* produces allelochemicals which the Eurasian plant community neighbors had become somewhat adapted to but the new plant community in North America had not. A similar result was found by Prati and Bossdorf (2005), when looking at the interactions between native and introduced *Alliaria* and *Geum* sp., indicating that the origin of the plants within the impacted community can be significant when allelochemicals are at play (Bossdorf et al. 2005).

1.1.3 Community susceptibility

Elton introduced the theory that highly diverse ecosystems have been predicted to have greater resistance to invasion than those with lower diversity (Elton 1958). Intuitively, the greater the number of species within a community, the fewer resources and space available for newcomers, which coincides with the "empty niche" theory (van der Velde et al. 2006). Additionally, a higher number of species in a system increases the chance that a plant species would be available that could out-compete and exclude one which was recently introduced (Booth et al. 2003). Furthermore, a more diverse community would include a greater number of predators that could also prey on the new species (Levine and D'Antonio 1999). However, researchers have found differing results for different ecosystems and communities. For example, aquatic communities tend to be vulnerable if appropriate abiotic and dispersal conditions exist (van der Velde et al. 2006).

Conversely, van der Velde et al. (2006) suggests that a more diverse community may increase invasion susceptibility via the act of "facilitating" an invader, whether the facilitator is a native or non-native species. This may include measures such as a non-native species pollinating or dispersing seeds of an invasive species, or by amending the local biotic conditions (van der Velde et al. 2006) or more indirectly by competing with shared competitors (Levine and D’Antonio 1999). This new invasive may in turn allow additional non-indigenous species to invade, triggering an “invasional meltdown,” a term introduced by Simberloff and van Holle (1999).

Another explanation for the discovery that many highly diverse communities were actually more invasible could be that the site has biotic and abiotic conditions, such as suitable moisture, nutrients and “habitat heterogeneity” that make it advantageous (Levine and D’Antonio 1999). Stohlgren et al. (2003) use the term, “the rich get richer”, when refuting the long held belief that highly diverse plant communities are less likely to be invaded. After evaluating independent data sets, they found a positive relationship between native and non-native species richness, a trend which intensified as the spatial scale grew. The researchers make the conclusion that an elevated level of resources correlated with habitat heterogeneity may lead to high native species richness. Generally, high species richness is linked to high species turnover leading to amplified pulses of available nutrients, light and water and therefore more opportunities for the non-natives to edge in (Stohlgren et al. 2003).

Levine and D’Antonio (1999) state that “the factors controlling native diversity should similarly control invaders, indicating conditions favorable to invasion,” factors such as disturbance level and intervals, competition, and accessibility of resources. Furthermore, “the diversity of the native community is insignificant if the invader is satisfied with a different set of resources along the niche axis than the natives.” Most researchers agree that most systems are not found in the “stable state” that is necessary for the diversity hypothesis above to occur, and that frequent indirect abiotic and biotic interactions are more responsible for the invasive susceptibility of a community (Levine and D’Antonio 1999).

Levine (2000) determined that the native species diversity, the scale and the degree of disturbance can all make natural communities uniformly susceptible to invasions. The process of disturbance generally leads to an upsurge in the availability of light, nutrients and vacant areas for establishment (van der Velde et al. 2006) or may eradicate a potential successful competitor (Booth et al. 2003). When conditions exist for hybridization between the introduced species and a locally adapted native species, this allows for a potential increase in the fitness of the invasive, and the native community becomes even more susceptible (Sakai et al. 2001).

An alternative hypothesis for community vulnerability to invasive species is called the "fluctuating resource availability" theory which takes place when the community is exposed to increased resources either by reduced consumption or increased accessibility of unused resources. Contrary to the high diversity theory noted above, predictions of fluctuation resources indicate that high species diversity and risk of invasion are not correlated as both "species rich" and "species poor" communities are both capable of incomplete resource consumption (van der Velde et al. 2006).

Competition with the native species and the resource levels available for the introduced species can interact to affect the invasibility of a particular site. Low resource levels may prevent invasions, as the resource level may be below the threshold of the introduced species (Tilman 1999). Indeed, many researchers have found a connection between the levels of nitrogen and invasive species (Green and Galatowitsch 2002, Brooks 2003, Kercher and Zedler 2004).

Facon et al. (2006) discusses three scenarios from which invasion of a new species may arise. The first, "migration change" would occur when a match between the introduced species and the new environment exist, but the species does not reside in that region until introduced by human interference. In the second scenario, the species may have been introduced, yet has not "invaded" the site. The invasion takes place when the abiotic or biotic conditions at the site change to better suit the new species, thus allowing the proliferation even without adapting to the new conditions. Global warming is a potential perfect example of this scenario for many species. Under the third scenario, the invader has adapted to the new environment as a consequence of evolutionary forces, and is referred to as the “evolutionary change” scenario.

Zedler and Kercher (2004) maintain that wetlands provide opportunities for plant invasions by the numerous invasive opportunists that are available. Wetlands are particularly susceptible to invasion since they tend to accumulate materials from both terrestrial and upstream wetland disturbances. These substances would include excess water and debris, nutrients and sediments as well as pollutants such as heavy metals and contaminants. As a landscape sinks, invaded wetlands differ from invaded uplands in that they have to contend with flowing water, canopy gaps due to inflowing material, anoxic soils, and nutrient fluxes. Additionally, many wetland sites are continuously disturbed, especially riparian wetlands, by flood pulses creating bare areas by erosion and debris deposition, and are positioned within the dispersal routes of any invasive species upstream or upland from the site. Van der velde et al. (2006) agreed, stating that temperate freshwater, estuarine and coastal wetlands tend to be the most invaded systems due to the ample amount of introduction conduits and disturbance factors such as shipping, recreation and water diversions. Invasive wetland plant species tend to be water dispersed either via seed or plant fragments and most have copious aerenchyma cells. These species may also allow for the rapid uptake of the available nutrients during the high nutrient pulses permitting high growth rates (Zedler and Kercher 2004).

As noted above, many successful wetland invaders develop aerenchyma cells and wetland plants with a elevated amount of aerenchyma cells are able to attain “high plant volume per biomass investment,” and grow tall very quickly. Roots with aerenchyma are able to expand “further per unit biomass,” therefore allowing for greater nutrient uptake (Zedler and Kercher 2004).

1.1.4 Clonal Species Biology

There are benefits and costs for clonal organisms. A clonal, or asexual plant multiplies vegetatively generating a genetic duplicate of itself, or a ramet. Some of the advantages of this could be resource acquisition and storage and the ability to establish in a new site with a single individual. If a particular genotype is successful, vegetative reproduction allows this genotype to flourish in time and space as long as the habitat and environmental conditions remain analogous. If these conditions are not met, this genotype may not be successful (Sebens and Thorne 1985). Other disadvantages include a reduction in the available resources for sexual reproduction and therefore, potential valuable recombinations within a stochastic environment, the spread of any diseases for linked clonal plants (Klimes et al. 1997) and the increased potential for a pathogen to eliminate a genetically similar population.

A few of the traits of clonal organisms which contribute to the success of these species throughout many habitats include: the ability to seize and monopolize the available nitrogen with copious rooting systems and high growth rates, increasing the competitive impacts on neighboring species; and their capability to dominate a site by expanding relatively quickly laterally via stolons and rhizomes into a site, displacing other species (de Kroon and Bobbink 1997). This same ability to quickly expand into new areas forming a dense cover, allowing for dominance of the system, also gives clonal species an additional advantage from agricultural runoff within nitrogen rich habitats. Furthermore, by translocating the nitrogen withdrawn from senescing shoots to the rhizomes at the end of the season and reallocating the reserves to new growth the next season, the nitrogen attained each year is effectively exploited (de Kroon and van Groenendael 1997).

While researching clonal plant species and whether this trait permits a plant to become more invasive, Pysek (1997) identifies a variety of “pros and cons” for each phase of invasion for both clonal and non-clonal species. The favorable characters for clonal species are numerous. Firstly, the plants can be easily fragmented and dispersed and above ground removal procedures does not necessitate death of the individual. There are also no dormancy issues for reproduction. The plant can successfully and immediately reproduce even when only one individual is introduced. Therefore, due to this same characteristic, there is no need for specialized pollinators or dispersers for reproduction. If the clonal organism, especially one whose genotype may be somewhat adapted to the site, will benefit from a reduced lag phase and a rapid invasion and "occupation of the site." Additionally, once established, clonal species can persist and spread into conditions that are more stressful than those where it colonized (D'Antonio and Vitousek 1992).

Basically, clonal species can make up anywhere between 1% to 66.7% of the most aggressive invasive species worldwide. Pysek concluded that based on the available data, clonal and non-clonal species were equal with respect to invasiveness on a regional and global scale. One trend that did emerge was that clonal species, which were "less favoured by disturbance," were more likely to be found invading natural areas and in general, within wet and cooler habitats. Once established in these habitats, clonal species tend to be more competitive successfully occupying the site (Pysek 1997).

1.2 *PHALARIS ARUNDINACEA* BIOLOGY

Phalaris arundinacea (reed canarygrass), a C3 cool season grass, is a perennial with robust, hollow culms that reach up to 2 meters tall. These stems are ~1 cm. in diameter, with a reddish tinge at the top during the growing season. The leafblades are flat with prominent ligules. This species spreads predominantly by creeping rhizomes which can be stout with 6-10 nodes (Comes 1971). RCG is an obligate outcrosser exhibiting self-sterility (Lavergne and Molofsky 2004).

Reed canarygrass is one of 15-20 species of *Phalaris* distributed throughout the world within the northern temperate regions of five continents. It is reported to tolerate annual precipitation of 3-26 cm, annual temps of 5-23 C, and a soil pH of 4.5 to 8.2. RCG does not however, perform well in subtropical or tropical climates. Southern Virginia marks it southern boundary on east coast and across to southern California on the west coast (Lyons 1998).

This species is listed as a noxious weed by the US Federal government and is a class C noxious weed in Washington State. It is a notorious weed globally as well, cited as a serious or principle weed in numerous countries throughout the world.

Ecologically, RCG has the ability to exclude native species through competition. This species is extraordinarily successful at out-competing other vegetation due to several factors. There are no known dormancy requirements, and the seeds germinate immediately after ripening with a very high (97%) viability rate (Apfelbaum and Sams 1987). As noted above, the primary means of reproduction is by vegetative growth, i.e., spreading by aggressive rhizomes and stems (Naglich 1994). Each plant can produce a dense mat of rhizomes within one growing season (Apfelbaum and Sams 1987), and even seven to eight week old seedlings produce these rhizomes (Crockett 1996). RCG can reach heights of six feet or greater (Antieau 1998), easily shading out smaller, slower-growing shrubs and tree saplings.

Reed canarygrass also is well known for slowing water velocities, thereby inducing sediment deposition and resulting in a positive feedback loop of more flooding and increased sedimentation rates within affected channels. This is in large part due to the density of the shoots and is increased by a dense shallow root mass. A study of RCG growth characteristics found that at least 88 percent of the emergent shoots on established plants in the field originated from rhizome or tiller buds located in the top 5 cm of the soil (Apfelbaum and Sams 1987).

Within the introduced range of North America, RCG generally dominates anywhere from 50% to 100% of the site. Within just ten years, monitoring of restoration projects in the Midwest found that 66% of the sites had been invaded or re-invaded by RCG with almost 100% cover (Lavergne and Molofsky 2004). Additionally, as these stands of RCG dominate a site, not only does the biodiversity of the site decline, but the heterogeneity of microhabitats on the site is diminished as the lower voids are filled in with RCG biomass and sediment trapped by the RCG (Werner and Zedler 2002). Schooler et al. (2006) found that the native species in Pacific Northwest wetlands were impacted to a greater extent than other introduced species by RCG and purple loosestrife.

This species is particularly menacing in the Pacific Northwest (PNW), the northern Midwest and Northeastern states. The largest infestations within the PNW tend to be found within the wetlands, river floodplains, agricultural ditches, roadsides and pastures where it was planted for forage on the westside of the Cascades (Tu 2006).
An additional significant trait of RCG is the ability to take advantage of an extended growing season. It initiates growth very early in the spring, or late winter and continues growth late into the fall, usually October. Species that are capable of an extended growing season are typically able to out-compete surrounding species without having a higher photosynthetic rate. Not only is RCG able to capture maximum sunlight when it emerges in January, but it is able to successfully compete with its neighbors by photosynthesizing for a longer period of time each year (Zedler and Kercher 2004).
Many researchers consider RCG to be native to the inland Pacific Northwest, Europe and Asia, while others reason that it was introduced from Europe. A third view is that the aggressive North American genotypes are hybrids of native populations and the introduced European cultivars (Merigliano and Lesica 1998). Early collectors found RCG throughout the inland northwest between 1825 and 1911. Ten herbarium specimens predate Euro-American settlement in that region or were collected from remote, undeveloped areas. Of those specimens that were collected from riverine habitats, many indicated that RCG was abundant while several specimens from meadows and springs indicated that the plant was uncommon or rare (Merigliano and Lesica 1998).

RCG is known to have three cytotypes in Eurasia, mostly represented by an allotetraploid, with the subspecies name of *arundinacea*, along with a hexapoid form, subspecies, *oehleri*. There is also a diploid cytotype, *rotgesii*. Merigliano and Lesica, (1998) stated that the herbarium specimens from the inland northwest most closely resembled the diploid, but recent evidence by Lavergne and Molofsky (2004) shows that the invasive plants occurring in Vermont and North Carolina, are tetraploid and more similar to the cytotype in Eurasia. The cytotype of the aggressive RCG within the Pacific Northwest is unknown at this time.

Repeated introductions of RCG cultivars for a variety of purposes have been documented in the US. Cultivation for agronomic purposes began in Sweden in 1749. The first trials in the United States took place in the mid 1830s, using the *picta* form due to its higher palatibility. During the 1850s, RCG received a great deal of attention for reclamation projects and was recommended for reclaiming peatlands and marshes. Most of the stands on the Pacific coast are attributed to a cultivated stand in Coos County, Oregon established in 1885 (Comes 1971, Merigliano and Lesica 1998).

As noted, the aggressive RCG found in the Midwest and PNW may be a hybrid or hybrids of the native and introduced cultivars. Generally, hybridization events allow for the rapid reshuffling of varying adaptations. Elements of an entirely foreign genetic adaptive system can be carried over into a previously stabilized one. Each hybrid produced by these species may deliver different recombinations, each of which may be able to adjust to different niches. The ever increasing heterozygosity brought in by hybridization would be capable of generating increased variation generation after generation. This genotypic diversity would confer an advantage as the different genotypes could allow greater adaptive response to environmental influences and new niches which would allow a selective advantage for the hybrids (Anderson and Stebbins 1954). Hence, a hybridization event with RCG would allow a mixture of a native that has become very well adapted to the environmental conditions within the PNW with cultivars that have been bred to be vegetatively vigorous and drought tolerant. Ellstrand and Schierenbeck (2006) presented evidence of 28 examples of hybridization events which preceeded invasiveness, such as with *Spartina anglica* x *S. alterniflora* producing *S. foliosa* in CA, *Typha x glauca*, and some of the *Tamarix* spp.

Lavergne and Molofsky (2007) performed genetic testing on the invasive *Phalaris arundinacea* (RCG) from the eastern United States, (Vermont and North Carolina) verifying what many researchers have suspected. European strains of RCG were introduced into North America on many occasions as a large number of alleles unique to French and Czech populations were found within the populations from North America. Additionally, new genotypes have been created in North America through widespread recombination events with 85% of the total allelic diversity being shared between North American and European strains but only 1.5% of the NA genotypes occurring in the European populations. The researchers determined that evolution of many phenotypic traits may have been responsible for the observed invasiveness in the NA strains of RCG based on consistent differences between the European and NA genotypes.

Several processes can trigger the evolution of invasive traits. The first could be hybrid vigor where recombination would create a genotype more invasive than the parents. The second process might be the increase in genetic variation due to the large number of introductions and subsequent recombinations, followed by natural selection for those traits leading to invasiveness. A third process would bring about a great amount of phenotypic plasiticity, allowing the population to thrive within a wide range of environmental conditions (Lavergne and Molofsky 2007) as well as survive stochastic events. The researchers did not find evidence for hybrid vigor, but did find that the North American strains displayed superior heritability which would allow for a “greater response to natural selection for a number of phenotypic traits such as emergence time, tillering rate, and root biomass” (Lavergne and Molofsky 2007).

Reed canarygrass (RCG) is the classic opportunist of the many prospects for invasion that are presented within many wetland communities. A list provided by Zedler and Kercher (2004) illustrate that RCG is able to take advantage of almost every opportunity provided by a wetland to invade. Some of the more noteworthy would be the canopy gaps from flooding and debris. RCG with its rapid height growth via hollow stems would flourish. Another is the availability of fresh sediment from scouring or sediment deposition. RCG possesses the ability to rapidly anchor and has viable floating propagules. RCG acquires adventitious roots and allows for floating rhizome mats allowing this species to manage the increased water depth and moving water conditions found in wetlands. Another common situation, standing water does not seem to deter RCG. RCG is able to emerge above standing water with the use of adventitious roots and by producing copious amounts of above ground biomass (Zedler and Kercher 2004) and this species has a sizeable percentage of aerenchyma cells available for gas exchange (Miller and Zedler 2003).

Phenotypic plasticity seems to also play a role in the invasiveness of reed canarygrass in North America. Lavergne and Molofsky (2007) found that the invasive genotypes were more phenotypically plastic than the European strains for stem height, leaf number and for a variety of morphological traits examined. The observed aggressiveness of RCG may be due to fact that the RCG introduced into North America was bred for agronomic purposes, and therefore, with the traits discussed by Anderson (2006) such as drought tolerance, high fertility, rapid germination, high yield potential, hybrid vigor and large plant size. However, if this were the case, one would expect to see low genotypic diversity within the populations in the introduced range, yet, the opposite is true for the RCG strains examined in their study. The research by Lavergne and Molofsky (2007) was performed on RCG populations from the east coast of North America, Vermont and North Carolina only. There is no evidence that a native RCG strain existed on the east coast as there is here in the Pacific Northwest (Merigliano and Lesica 1998).

A study by Coops et al. (1996) also found evidence of both genetic diversity and phenotypic plasticity within RCG populations. The researchers examined biomass allocation patterns of RCG and how this allocation changed in response to vegetative cover. RCG allocated more resources to belowground biomass when grown within dense vegetative cover. This is probably giving the plant a competitive advantage in the next growing season, as it over-winters as root stock and is one of the first perennials to emerge in the spring. This morphological plasticity was also important for surviving within various water depths. Plants grown in deeper water allocated more biomass to elongating the stem, while plants grown in up to five cm of water allocated more biomass to the roots (Coops et al. 1996).

Maurer and Zedler (2002) also found morphologically plastic behavior by RCG when testing root:shoot ratios and the lateral expansion rates in different nutrient conditions. RCG spread nearly 50% farther and produced twice as many tillers under high nutrient conditions and produced fewer tillers closer to the parent clone under low nutrient conditions. This combination of the “guerilla” and “phalanx” strategies (consolidation strategy) allows RCG to dominate the vegetation year after year by maintaining its position in poor conditions and/or years and spreading into new areas during high nutrient conditions and favorable years. High levels of genetic diversity increase the likelihood that a particular genotype will flourish and spread into new areas. Thus, genetic diversity coupled with suitable environmental conditions frequently enable reed canary grass to aggressively take over entire plant communities.

The plant architecture of this species may also play a significant role in its competitive abilities. Grime and Hodgson (1987) listed characteristics of species with high competitive ability: “(1) a robust perennial life form with a strong capacity to ramify vegetatively; (2) the rapid commitment of captured resources to the construction of new leaves and roots; (3) high morphological plasticity during the differentiation of leaves and roots; and (4) short life spans of individual leaves and roots.” Gaudet and Keddy (1988) found that tall shoots, leaf shape (length:width ratio), and large canopy diameter were morphological characteristics that were significantly correlated with increased competitive ability in wetland plants.

The horizontally oriented leaves and tall culms of RCG improve the efficiency of light utilization. Wetzel and van der Valk (1998) found that *Carex stricta* and *Typha latifolia* were both heavily impacted when grown with RCG. In this study, RCG maximized the capture of light and nutrient resources by maximizing vegetative growth, even under low nutrient or soil moisture conditions. RCG is a superior competitor, producing exceptional biomass despite the environmental conditions, is consistent with theories of Grime (1979).

1.3 *PHALARIS ARUNDINACEA* CONTROL LITERATURE REVIEW

The majority of researchers confirm that invasive species threaten the continued survival of endangered species, and are one of the leading causes in the loss of biodiversity. Invasive species are also extremely costly, both monetarily through losses in agricultural and due to the cost of controlling the invasives. Prevention of the introduction of potentially invasive species is paramount and continued research is needed for the reliable and cost effective means of controlling our current invasive species (Allendorf 2003).

In spite of decades of study, there is currently no comprehensive strategy for the effective removal of existing RCG and establishment of alternative native vegetation (Perry and Galatowitsch 2004, Perry et al. 2004, and Forman et al. 2000). The management techniques utilized to date include chemical control (glyphosate), mowing and grazing, excavation of the substrate, water level manipulation, micronutrient management (boron), macronutrient management (nitrogen), burning, and shading (black plastic mulching and/or competitive exclusion).

Reed canarygrass responds quickly after mechanical removal by growing back from rhizomes and seeds remaining in the soil (Apfelbaum and Sams 1987). However, repeated shoot removal damages plants via stress when disturbance events are frequent. Available carbohydrate reserves are greatest during the winter months, declining to a low point in mid-summer. Depletion events happen as the growing point is elevated in spring and as the seed heads develop in early summer (Comes 1971).

Green and Galatowitsch (2002) found that agricultural runoff and the associated nitrogen addition contributes to the increasing colonization and dominance of reed canary grass. After testing three comparable levels of nitrogen on RCG and native species, they found that the total shoot and root biomass of the native community was suppressed by RCG, at all levels, and that shoot growth of the native community was reduced by nearly one-half at the highest N level. Kercher and Zedler (2004) had similar results with inorganic nitrogen additions. RCG reduced a native sedge biomass by 91% while the sedge did not impact the RCG. In contrast, in a carbon enriched soil, the competition by the sedge reduced the RCG biomass by 82% while RCG competition reduced the sedge biomass by only 32%.

Adding carbon sources has been proven to have a negative effect on the nitrogen availability within the soil where they have been applied in combination with shading undesirable species (Duryea, et al. 1999, Stout 2002). There have been many successful studies utilizing some form of carbon, such as wood chips and/or sawdust and sucrose, to reduce nitrogen, trying to give native species an competitive edge on exotics. However, these have generally been practiced in prairie and grassland systems (D’Antonio 2004, Blumenthal 2003, Reever-Morghan and Seastedt 1999). Davis (2000) found that carbon additions were effective in suppressing weed biomass and promoting native species within a wetland prairie system in Oregon. Generally, the optimum carbon to nitrogen ratios is approximately 10:1. Examples of a few amendments and their carbon to nitrogen ratios include corn stalks at 60:1, sawdust (weathered 2 months) at 625:1 and Douglas fir bark at 491:1.

Shade material has been frequently utilized to control weeds. There are several inadequacies when using shading fabrics with a species such as RCG. Most fabrics have the tendency to break down as a result of prolonged exposure to sunlight, allowing the re-growth of RCG from underground rhizomes and seed (Stannard and Crowder 2001). Additionally, typically the material is too light to remain in place, thus allowing RCG re-growth to literally push up the fabric from underneath due to the extensive amount of carbohydrate storage within the rhizomes (Naglich 1994, Wisconsin DNR 2002). Therefore, simply using a typical manufactured shade cloth, has not been shown to be effective over the long term.

Mixed canopy layers allow for a reduced transmittance of light with a lower red:far-red light ratio than direct sunlight. Lindig-Cisneros and Zedler (2001) exposed RCG seeds to low red:far-red ratios and found that germination decreased by nearly 30 percent. Canopy gaps were shown to increase invisibility in this study, as RCG did not germinate in no-gap treatments, regardless of species richness. RCG did germinate under a canopy with only one species, but was 43 percent lower in mixed canopy treatments.

However, during a greenhouse experiment with three-month-old reed canarygrass clones, Maurer and Zedler (2002) tested the effects of shading on the expansion of new tillers. They found that new growth was not significantly affected for those that remained attached to an un-shaded parental clone. Therefore, it is noteworthy, that after a clonal invader such as RCG establishes, the shade cast by neighboring plants may no longer inhibit growth or vegetative spread.

Based on consultations with King County DNR employees and landowners as well as the constraints of working on operating agricultural land, I established a set of objectives for this research project that complied with King County’s regulations for farm land. The control methods examined for *Phalaris arundinacea* control and riparian restoration were founded on the RCG literature review and supplementary perennial invasive species control literature.

The following chapters will focus on developing methods for controlling RCG and restoring infested riparian zones using carbon reduction, multiple canopy layers, competition from native species and techniques for depleting the RCG carbohydrate reserves.

CHAPTER 2. *PHALARIS ARUNDINACEA* CONTROL AND RIPARIAN RESTORATION

2.1 INTRODUCTION AND PROJECT BACKGROUND

The agricultural industry in western Washington can be traced back as early as the 1820s (Kantor 1998). A majority of the agricultural land within King County lies within river valleys and floodplains which are subject to frequent flooding and saturated soils. The watercourses utilized for drainage of the floodplains typically flood due to the accumulation of fine sediment, associated with the spread of the invasive grass species, *Phalaris arundinacea* (reed canarygrass (RCG)). The RCG biomass and dominance is increased due to nitrogen enriched agricultural runoff (Green and Galatowitsch 2002). This in turn, leads landowners to clear the channels by dredging, a practice which decreases the quality of habitat for native birds, wildlife, invertebrates and salmonids.

The re-establishment of vegetated riparian buffers along agricultural watercourses is a significant challenge, in large part due to competition by dense monocultures of RCG. In addition to clogging watercourses, RCG does not provide sufficient shade or instream habitat structure in the form of large woody debris (LWD) needed to constitute high quality riparian and in-stream habitat. Furthermore, it is believed that RCG may harbor a different and perhaps less desirable assemblage of invertebrates when compared to native woody streamside vegetation (WSU & UW Coop Research Team, unpublished data 2007). Thus, finding effective and economical control measures for reed canarygrass is imperative for these watercourses.

Reed canarygrass can alter the surrounding habitat by: 1) constricting flow in watercourses; 2) filling shallow lakes and ponds, degrading fish and wildlife habitat; 3) greatly increasing evapotranspiration, which can affect local shallow groundwater characteristics (Antieau 2002); 4) degrading water quality particularly by elevating biological oxygen demand (BOD) during the dieback of excessive biomass each year (WSU & UW Coop Research Team, unpublished data 2007); and 5) arresting natural plant succession on the site (Antieau 2002). These alterations can result in a complete passage blockage (physically and due to high temperature and/or low dissolved oxygen (DO)) for anadromous salmonids during a portion of the year, generally late-summer and early-autumn (WSU & UW Coop Research Team 2007).

2.2 *PHALARIS ARUNDINACEA* CONTROL RESEARCH PROJECT OBJECTIVES

The objectives of the riparian vegetation enhancement section of this project include:

a) finding a Best Management Practices (BMP) protocol for the effective control/eradication of reed canary grass, and;

b) determining a method for providing native ground cover and woody riparian vegetation that is vigorous, shade producing and provides habitat for insects that constitute prey for salmonids.

2.3 PILOT PROJECT DESCRIPTION

As a part of this research project, a successful method for reducing the vigor of RCG and eventually removing RCG was investigated. To test numerous treatments of interest on the potential suppression of RCG, a pilot project was implemented in the fall of 2002 and spring of 2003. Response data were collected throughout the spring, summer and early fall of 2003.

Study Questions and Hypotheses:

1. Will the application of steam provide a significant kill of the RCG?

2. Does the allelopathic plant, *Gaultheria shallon* effectively compete with reed canary grass?
3. Will the cover crop *Trifolium repens/pretense* effectively suppress RCG?
4. Will an allelopathic mulch placed on top of RCG successfully suppress RCG?
5. Will shading RCG with a heavy opaque material effectively suppress the RCG?

Ho: The treatments of steam, a cover crop, an allelopathic plant, an allelopathic mulch and shading do not successfully suppress reed canary grass as measured by stem density compared to untreated sample plots.

Ha: The treatments of steam, a cover crop, an allelopathic plant, an allelopathic mulch and shading do successfully suppress reed canary grass as measured by stem density compared to untreated sample plots.

Steam has been proven to be an effective treatment for numerous weedy species (Norberg et al. 1997, Quarles 2001). In most cases, the efficiency of steam has been equal to the use of herbicides. Most annual species are killed immediately, however, as with herbicide, perennial species typically require additional applications (Quarles 2001). The use of steam has not been attempted on RCG to date. Most studies have been completed with a system from the Waipuna Company (http://www.waipuna.com/), whose steamers reach a temperature of 98º C (~208º F). This study employed a steamer that is programmed to reach much higher temperatures. The steam was at 149º C (300º F) within the pressurized machine and exited the hose at 138º C (~280º F).

The native shrub, *Gaultheria shallon* (salal), is an allelopathic plant that releases an allelochemical called tannins from the flowers, leaves and roots (Preston 2002). Various studies have indicated that salal has a negative impact on the re-growth of conifer seedlings after logging (Preston 2002), sometimes called a “salal complex” (Boateng and Comeau 2002). Tannins are able to bind proteins in a manner that negatively impacts the availability of nitrogen (Cornell University 2001). Salal was used in this study to gauge the impact that tannins and nitrogen reduction may have on the re-growth of the RCG. Salal is also evergreen and could provide year round shade after establishment within the drier areas of the agricultural sites along the top of the bank where the soil is generally much drier, especially during the later part of the growing season. Additionally, a variety of wildlife species consume the leaves, flowers and berries of salal (Boateng and Comeau 2002).

Clover was tested in the pilot project due to a direct observation from a site visit during the summer of 2002 where it seemed to be surviving in the presence of surrounding RCG. Planting fast growing cover crops to compete with and suppress aggressive invasive species while the desired species become established has been utilized in some agriculture and prairie restorations (Perry and Galatowitsch 2003). Gunti et al. (1999) found that red clover (*Trifolium pratense*) reduced the biomass of the invasive hedge bindweed (*Calystegia sepium*) in a greenhouse experiment.

Adding carbon sources have been proven to reduce nitrogen availability within the soil, resulting in weed suppression where they have been applied in addition to shading weeds (Stout 2002; Duryea et al. 1999). At the time of this study, mulch has been tested (Reever-Morghan and Seastedt 1999, Davis 2000, Zink and Allen 1998, Davis 2000, Blumenthal et al. 2003, Corbin and D'Antonio 2004) however, the use of a potentially allelopathic mulch for weed suppression has not been reported from a scientific study. Hogfuel is the debris which falls off of the first saw in a sawmill (the hog). This usually includes strips of bark as well as an array of wood chip sizes. This allows for a more densely packed material. Red Cedar Hogfuel was also tested for allelopathic tendencies on lettuce seed germination, seedling growth and RCG rhizome regrowth.

Shade material has been frequently utilized to control weeds. The principle drawback with using most shading fabrics with a species such as RCG is the propensity for the material to either break down as a result of prolonged exposure to sunlight, allowing the re-growth of RCG from underground rhizomes and seed, or that the material is too light to remain in place, thus allowing RCG re-growth from underneath. RCG stores an extensive amount of carbohydrates within the rhizomes. Therefore, simply using a typical manufactured shade cloth has not been shown to be effective over the long term. Carpet was tested in this study due to the weight of the material and resistance to break down in ultraviolet light. A biodegradable material of equal weight and density will be generated if this material is successful.

2.3.1 Field Pilot Project Methods

The pilot project took place along a watercourse at an agriculture site within the Sammamish Valley Agriculture Production District in Woodinville, WA.

Each plot was placed linearly along a watercourse at the pilot study project site during the fall of 2002. Each plot is one treatment cell (subplot) wide and 10 cells long. There were three replicates of these 10 treatment cells. The cells are 1.5 meters by 1.5 meters. The RCG within all three plots and all treatment cells was mowed and tilled to remove the aboveground biomass and loosen the rhizomes in the top ~15 cm of soil. A trench of ~30 cm was placed around each cell and a rhizome barrier placed in each trench to remove any rhizomatous connection with the surrounding parental clones. The treatments were assigned randomly within each replicated plot. Examples of the treatments are listed below in Figure 2.1.

Steam

Red Cedar Hogfuel – 25 cm deep	**Densely planted salal -** Plugs / 90 per cell / 1 per 10 cm sq	**Cover Crop –** *Trifolium repens/pratense* seed mix, 0.5 pd per cell	**Shade**	**Control**

No Steam

Red Cedar Hogfuel – 25 cm deep	**Densely planted salal** – Plugs / 90 per cell / 1 per 10 cm sq	**Cover Crop -** *Trifolium repens/pratense* seed mix, 0.5 pd per cell	**Shade**	**Control**

Figure 2.1. Reed canary grass treatment design. Three replicates of each treatment were placed linearly along the watercourse.

The Steam Machine (Stinger 1) was mounted on the back of a pickup truck, along with a generator and a water tank. The water was heated within the steamer and steam was sprayed on the plots assigned to this treatment. After one week, the additional treatments noted were installed.

The cells assigned to the densely planted salal treatment were divided into 10 cm squares. One salal plug was planted within each square. Those cells designated for the cover crop treatment had *Trifolium repens / pratense* seeds spread on top, ~0.5 pounds per cell.

The allelopathic mulch composed of red cedar hog fuel was applied in an undecomposed condition in order to maximize the concentrations of allelochemicals within the wood and bark. A 25 cm layer of mulch was placed on top of the cells.

The shading material utilized for this pilot project was carpet cut in 2.25 meter squares to test whether carpet material provides both the strength and weight needed to suppress the re-growth of the RCG rhizomes. This fabric was used due to the low cost (free) and the density. With favorable results, a biodegradable fabric of similar density and weight could potentially be developed.

RCG stem density was measured bi-weekly to determine the success of each applied treatment. A one meter square was placed in the middle of each treatment. Dowels were permanently placed in each cell to verify that the measurements were taken in the same place. By counting the returning RCG stems within each plot, the stem density of the RCG was determined throughout the spring and summer of 2003.

2.3.2 Red Cedar Hogfuel Allelopathy Study Methods

To determine whether the red cedar hogfuel is allelopathic, lettuce seeds and seedlings were watered with either hogfuel tea for those in the hogfuel (HF) treatment or fresh tap water for the control treatment. Red cedar hogfuel was inundated with water for 72 hours to make the hogfuel tea. Ten replicates of five lettuce seeds were placed on filter paper and watered daily with either hogfuel tea or water for five weeks. The treatments were continued on the seedlings as the seedling radicle was measured from the point of germination until the end of the five week period.

Additionally, ten soil sample trays (~20 cm x ~20 cm) containing field soil and RCG rhizomes were collected from the experimental site for use in the green house at the Center for Urban Horticulture. The RCG rhizomes were equally divided and randomly placed within the trays with field soil. Five were chosen to be randomly watered with hogfuel tea and the other five were watered with fresh tap water.

The data were analyzed using a two sample paired t-Test for means. The seeds, seedlings and RCG rhizomes watered with hogfuel tea were compared with those watered with fresh water. The data included in the analysis were: 1) the number of lettuce seeds germinated; 2) the final lettuce seedling radicle length; and 3) the final number of RCG stems grown from the rhizomes which were present in that tray.

2.3.3 Soil Testing

Soil core samples were taken from the soil under the hogfuel treatments along with the control plots at the end of the summer, 2003. These samples were dried and sent to the University of Massachusetts soil laboratory for testing. The results indicated that the nitrogen was not significantly different for the soil under the treatments versus the control plots.

2.3.4 Pilot Project Results and Discussion

None of the treatments embedded within the steam treatment differed from the treatment without steam (cover crop - $p = 0.41$, salal - $p = 0.23$, mulch - $p = 0.26$, control - $p = 0.114$). All of the treatments, hogfuel, salal, cover crop, and shading resulted in reduced RCG growth ($p = 0.064$) (Table 2.1).

Table 2.1. ANOVA results for the final RCG returning stem counts for the pilot project.

	Df	Sum of Sq	Mean Sq	F Value	p
Treatment	4	58608.2	14652.05	2.643	.064
Steam	1	5880.00	5880.00	1.061	.315
Treatment:Steam	4	8027.00	2006.75	0.362	0.833
Residuals	20	110886.7	5544.33		

Figure 2.2 illustrates the reed canarygrass stem count for each treatment over the 2003 growing season. The RCG stem count within control plots which were exposed to the steam treatment increased over the season, even more so than the non-steamed control plots (Figure 2.2). The plots which were exposed to the steam treatment and were then planted densely with salal initially had fewer returning RCG stems at the beginning of the season. Nevertheless, the average of the three replicates indicate a higher number of RCG stems at the end of the season (141 for the steam/salal plots versus 77 stems for the no steam/salal plots). It should be noted that for all treatments, replicate three yielded a

higher number of RCG stems than the other two replicates, for almost every treatment. This higher value does not change the results of which treatments were more successful, however. As expected, those cells covered with the shade treatment resulted in no live RCG stems by the end of the season ($p = .03$)(Figure 2.3). The mulch/hogfuel treatment began the season completely covered with the hogfuel and with zero live RCG stems but producing a few RCG stems by the end of the season, an average of 23 for the steamed plots and 33 for the non-steamed plots ($p=.10$ compared to control plots).

The final stem count took place on September 13th giving the average results for each treatment used within the pilot project for the end of the 2003 season (Figure 2.3). Stem count data from the growing season of 2003 indicate that two treatments were particularly successful. With the high rate of variation (again, predominantly due to replicate #3), only two of the treatments were significantly different from the control cells. The hogfuel and shade material treatments (whether used with or without steam) suppressed the reed canarygrass significantly when compared to the control plots and the other treatments. These two treatments have been expanded upon and utilized within the principal project discussed in the next section, along with riparian vegetation restoration treatments. Again, the grass was mowed and tilled before each treatment, allowing for a reduction of live RCG after one season than what would be available if the site had not been tilled.

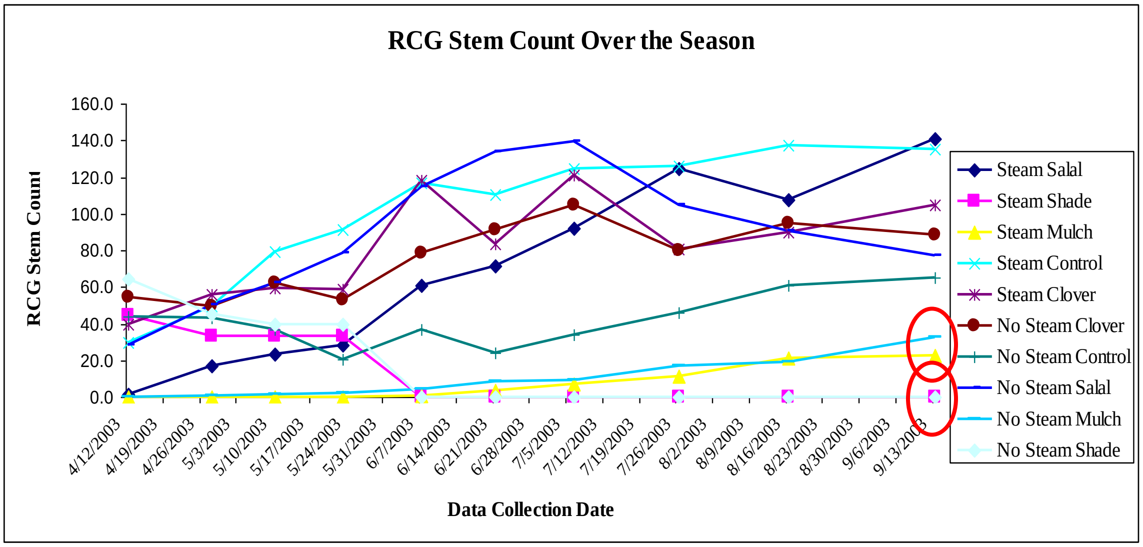


Figure 2.2. RCG Stem Count for each treatment over the course of the 2003 season.

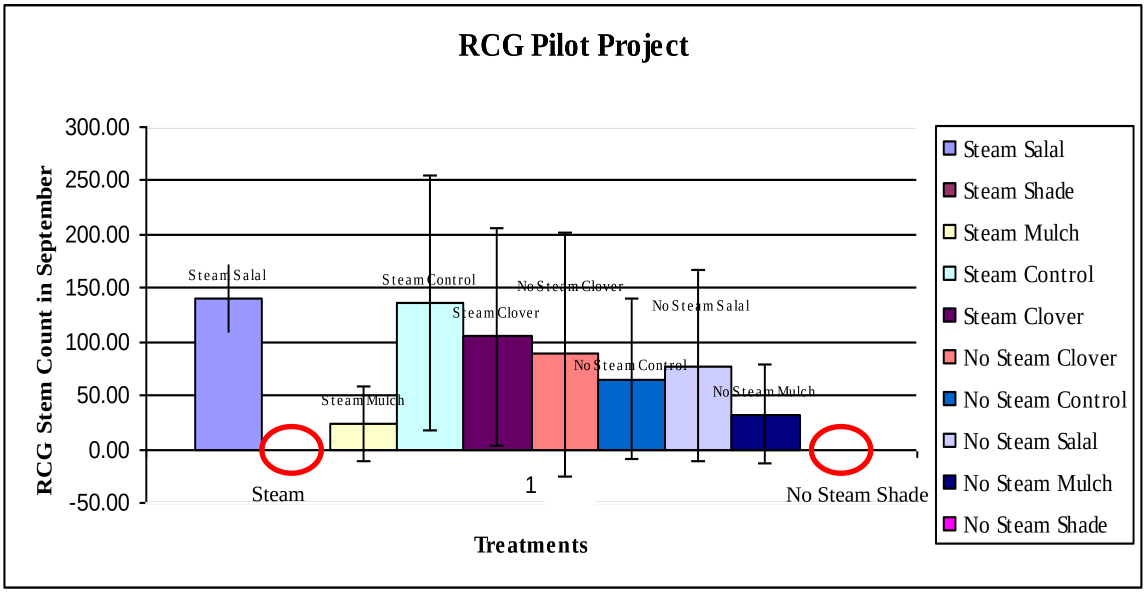


Figure 2.3. Pilot Project Results from 2003.

2.3.5 Hogfuel Allelopathy Test Results

The results indicate that the redcedar hogfuel is allelopathic for lettuce seed germination and growth by reducing the number of germinating seeds (T-test, df = 9, p = 0.005) and radicle length (T-test, df = 9, p = 0.000).

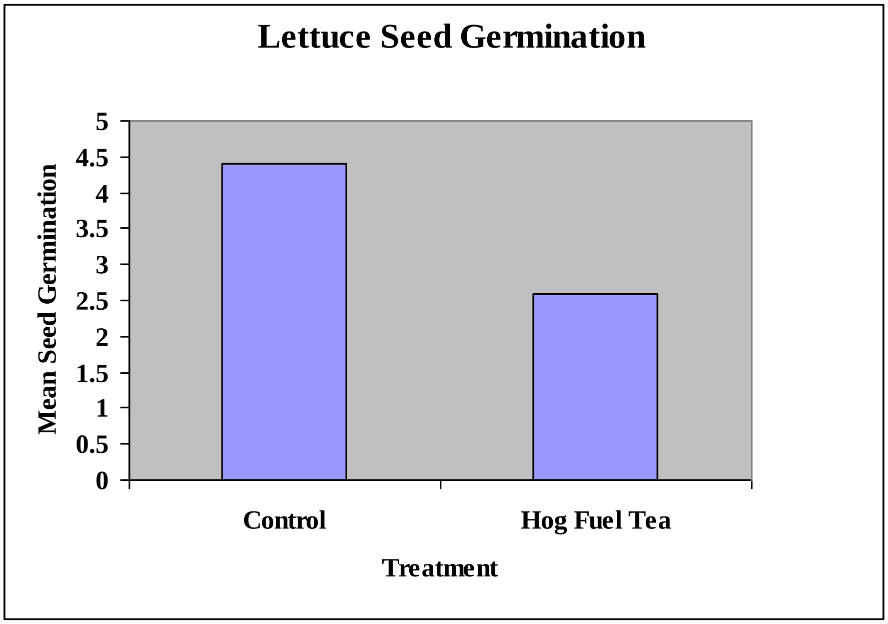


Figure 2.4. **Lettuce seed germination results for the hogfuel tea treatment.**

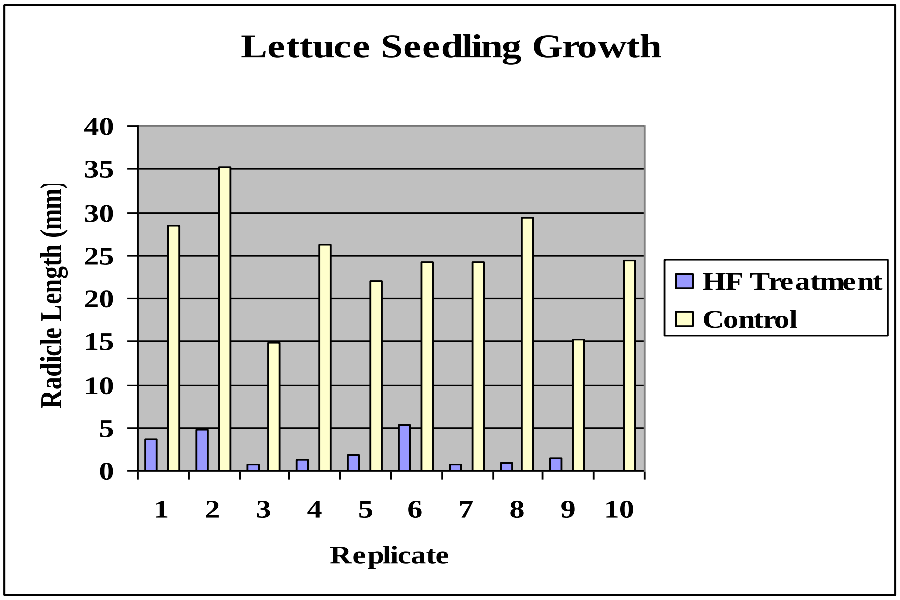


Figure 2.5. **Lettuce seedling radicle length for the hogfuel tea treatment.**

The red cedar hogfuel tea also reduced the RCG rhizome regrowth when comparing the stem count of the rhizomes grown with hogfuel tea versus those grown with water (T-test, df = 4, p = 0.05).

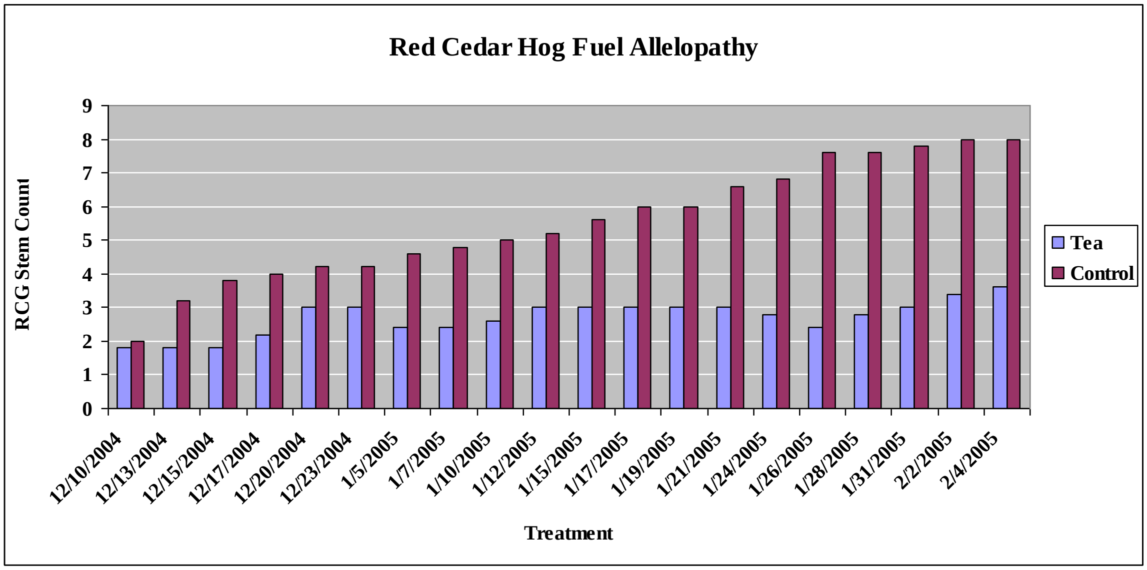


Figure 2.6. **RCG returning stem count within the hogfuel tea treatment.**

2.4 PRINCIPAL FIELD PROJECT DESCRIPTION

The treatments from the pilot project with significant RCG reduction were modified and re-developed for further study. These treatments included: 1) a red cedar hogfuel treatment and 2) a dense, heavy shade-cloth treatment, both of which were combined with the planting of native species for re-vegetating the watercourse as directed in the second objective (B) noted at the beginning of Chapter Two. Objective B is listed as: "determining a method for providing native ground cover and woody riparian vegetation that is vigorous, shade producing and provides habitat for insects that constitute prey for salmonids." These new treatments have been applied within the principal project implemented in the fall of 2003 and are described in further detail below.

The following research question was addressed in this field research section of my project:

1) Will the following treatments negatively affect the density of returning reed canarygrass?
    a) burlap/compost layers densely planted with native species providing multiple canopies (RCG barrier)
    b) red cedar hogfuel densely planted with willow species (*Salix sitchensis*)
    c) red cedar hogfuel placed on top of RCG with the RCG barrier on top of the hogfuel

The following two research questions were addressed in response to observations in the field from the first research season and are addressed in Chapters Three and Four:

2) Is *Scirpus microcarpus* an effective competitor with reed canarygrass?

3) How long do reed canarygrass rhizomes need to be covered with a weed block fabric to deplete the carbohydrate reserves?

2.4.1 Field Project Methods

2.4.1.1 Study Sites – Three sites were chosen within the Snoqualmie and Sammamish Agricultural Production Districts (APD) of King County (Figure 2.7). These are an agricultural farm (Woodinville, Sammamish APD) (Figure 2.8), a livestock site (Woodinville/Redmond, Sammamish APD) (Figure 2.9) and a "natural" site (Duvall, Snoqualmie APD) (Figure 2.10). These sites were chosen due to the occurrence of thick and continuous swards of RCG along the agricultural waterbody, ease of access, and the lack of planned dredging by the other researchers participating in this project.

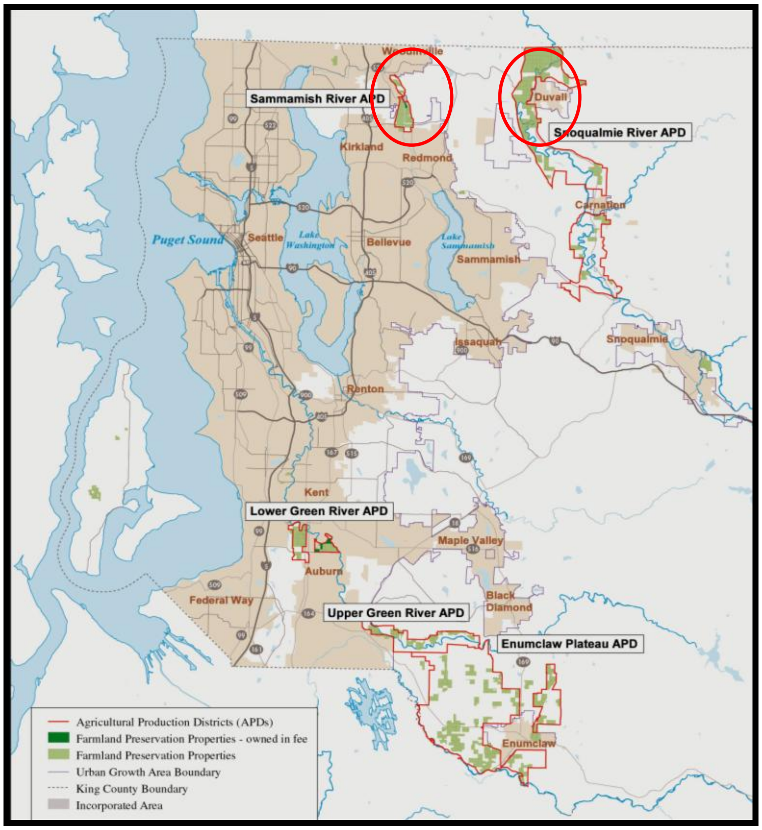


Figure 2.7. King County Agricultural Production Districts.

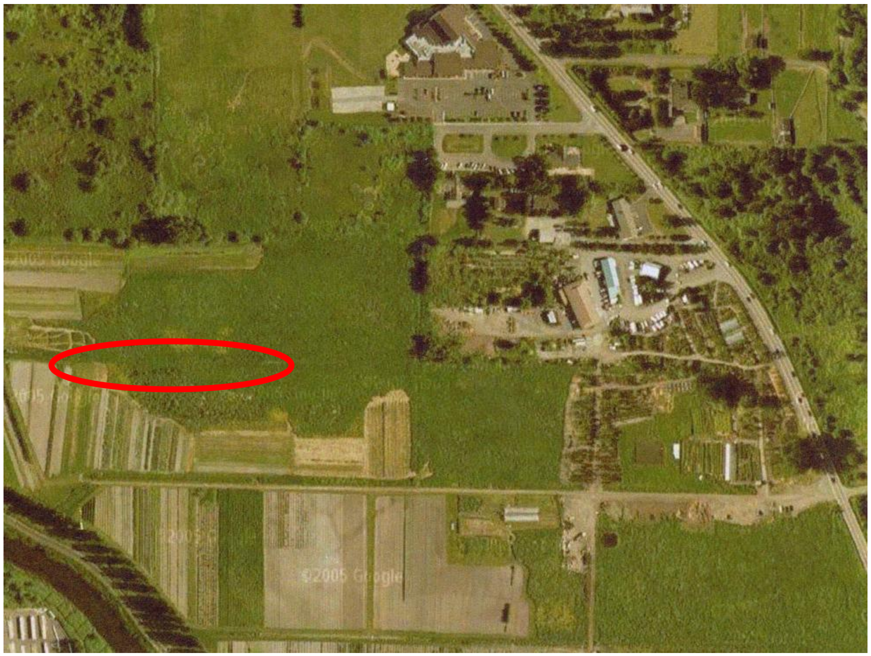


Figure 2.8. **Plot Location at the Agricultural Site. (Samammish APD)**

Figure 2.9. **Plot Location at the Livestock Site. (Samammish APD)**

Figure 2.10. Plot Location at the Natural Site. (Snoqualmie APD)

2.4.1.2 Treatment Design

Treatment #1 - Reed canarygrass barrier - treatments for the field project consisted of employing a RCG barrier that was developed specifically for this study. Burlap fabric was placed on top of mowed RCG and compost (~25 cm deep) was placed on top of the fabric within two by three meter plots. Burlap fabric was then placed on top of the compost and staked on each side. The burlap/compost "pillows" were used as a biodegradable alternate to carpet and due to the weight and shade that they provide (as shown by the carpet in the pilot project to suppress RCG) as well as the ability to plant within the compost allowing for re-vegetation of the watercourse and additional competition for RCG. Carpet is predominantly comprised of nylon, olefin (a polypropylene material), and/or polyester (Moxy Media 2007), most of which are not biodegradable, or certainly not within a sufficient period of time, for example, nylon takes 30-40 years to biodegrade.

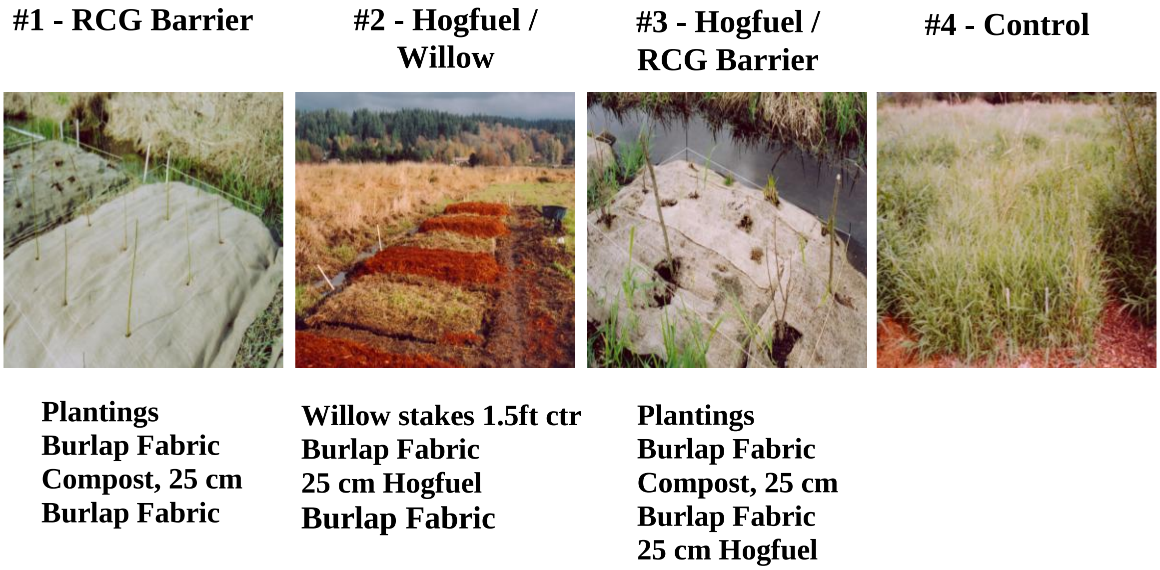
When plants or stakes are placed within the fabric, RCG competes with the plant/stake, and grows up through the hole and again on top of the fabric. With this design, the RCG underneath the mat will not be exposed for many years and is not capable of competing with the more desirable planted vegetation. Therefore, RCG seed and rhizome fragments from elsewhere are the chief concern for re-growth on top of the mat. In accordance with objective B, and in order to provide a habitat which will be competitive with the potential available RCG seeds and rhizome fragments, plants were chosen for re-vegetation that will provide the maximum shade possible and numerous canopy layers. As noted in the RCG control literature review, multiple canopy layers were proven to be successful in preventing RCG germination under mixed canopy layers (Lindig-Cisneros and Zedler 2001). Maurer and Zedler (2002) also found that RCG regeneration and growth from rhizome fragments was limited by 95 percent (25 percent reduction in survival) under heavy shading.

Based on the results from Dr. Zedler and her students and from the RCG control section in Chapter One, the plant species for the RCG barrier treatment were chosen due to their ability to: a) provide two or three canopy layers at any point within the plot; b) emerge early in the season, as does RCG; and c) tolerate wet to dry conditions depending on placement on the bank. Additionally, three species (*Rubus spectabilis, Vaccinium ovalifolium* and *Ribes bracteosum*) were chosen due to their ability to attract invertebrates to the site. Allan et al. (2003) noted that these understory species tended to harbor a high percentage of invertebrate biomass, many of the taxa were also found in the stomach samples of juvenile salmon. The species that will be used within the “RCG barrier” treatment of the principal project are listed below in Figure 2.12.

In addition to the RCG barrier treatment, two additional treatments were tested.
Treatment #1 – RCG Barrier treatment = Burlap fabric was placed on mowed RCG with 25 cm of compost and another layer of burlap placed on top with a designated planting design.

Treatment #2 – Hogfuel/Willow treatment = Burlap fabric was placed on top of mowed reed canarygrass with 25 cm of hogfuel and another layer of burlap on top with 90 cm willow stakes (three foot) placed within at a density of 12 stakes per plot. The willow stakes were planted 25-30 cm.

Treatment #3 – Hogfuel/RCG Barrier treatment - Twenty five centimeters of hogfuel was placed on top of the RCG with the same barrier used in Treatment #1 placed on top of the hogfuel.



#1 - RCG Barrier

Plantings
Burlap Fabric
Compost, 25 cm
Burlap Fabric

#2 - Hogfuel / Willow

Willow stakes 1.5ft ctr
Burlap Fabric
25 cm Hogfuel
Burlap Fabric

#3 - Hogfuel / RCG Barrier

Plantings
Burlap Fabric
Compost, 25 cm
Burlap Fabric
25 cm Hogfuel

#4 - Control

Figure 2.11. Treatment description and layout example.

The three treatments (plus one control) for the principal project are illustrated in Figure 2.11. Three replicates of the four plots were placed at each of three different sites in two Agricultural Product Districts.

Plots at the natural site and agricultural sites, were 2 x 3 meters and the plots at the livestock site were 1 x 2 meters. A randomized block experimental design was implemented with three treatments (plus control) within three blocks (or replicates) chosen randomly at each site, or nine replicates of each treatment in total. The reed canarygrass was mowed with string trimmers to height of ~5 cm and a trench of ~30 cm was placed around each cell and a rhizome barrier placed within the trench to remove any rhizomatous connection with the surrounding parental clones.

2.4.1.3 Data Collection

RCG Stem density was measured bi-weekly during the growing season (February through November) for two years, 2004 and 2005, to determine the success of each applied treatment within the principal project over two growing seasons. Appendix A displays the treatment arrangement at each site.

Percent cover of vegetation within each plot was recorded at the end of the second growing season to determine survival and competitive efficacy of the planted native species with the RCG.

Data were analyzed using a Two Way Analysis of Variance. The Tukey HSD procedure was utilized to guarantee the overall alpha level of 0.05.

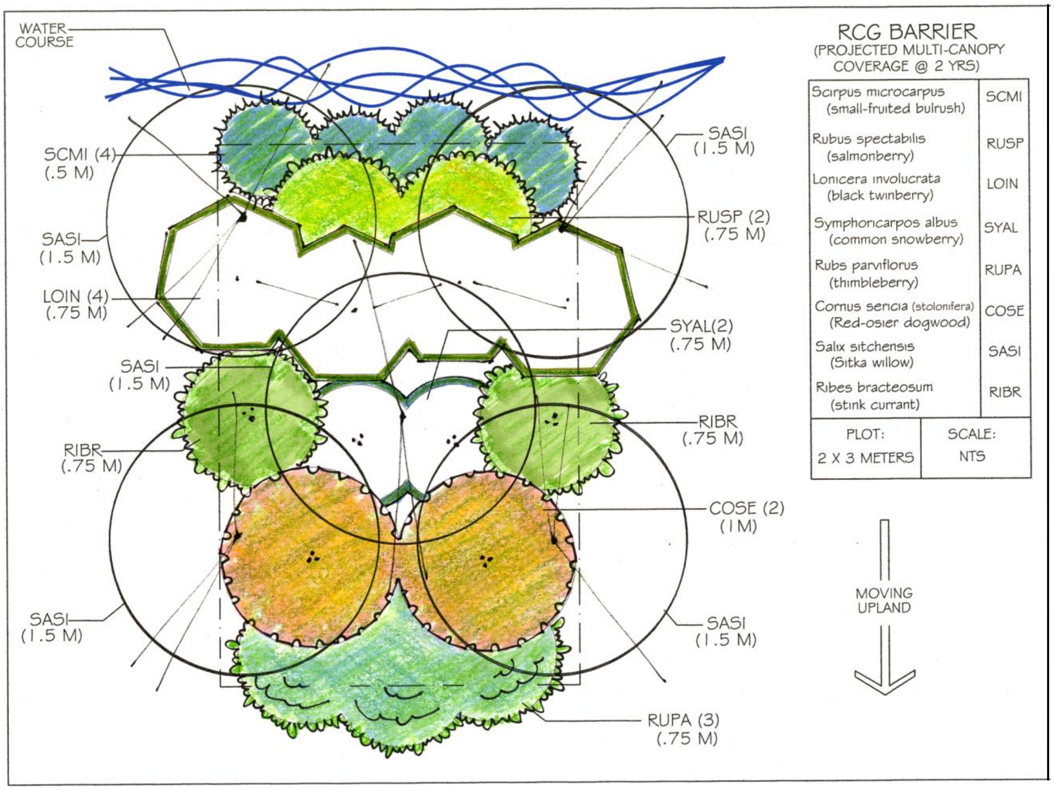


Figure 2.12. Spacing and design for the “RCG Barrier” treatment plots.

2.5 FIELD PROJECT RESULTS

A significant treatment and site interaction exists (p = 0.043) (Table 2.2). This interaction is due to the HF/RCG Barrier treatment obtaining a higher RCG stem count at the Agriculture site than at the other two sites (Figure 2.13). If the line were parallel there would not a site by treatment interaction. This interaction effect presents a situation in which one is unable to definitively state that any one treatment or independent variable significantly impacts the dependent variable (the returning RCG) in the ANOVA.

Table 2.2. Two-way ANOVA results for the final RCG returning stem counts.

	Df	Sum of Sq	Mean Sq	F Value	p
Treatment	3	3337438	1112479	101.74	0.000
Site	2	132895	66447	6.07	0.008
Treatment:Site	6	178229	29705	2.72	0.043
Residuals	20	218695	10935		

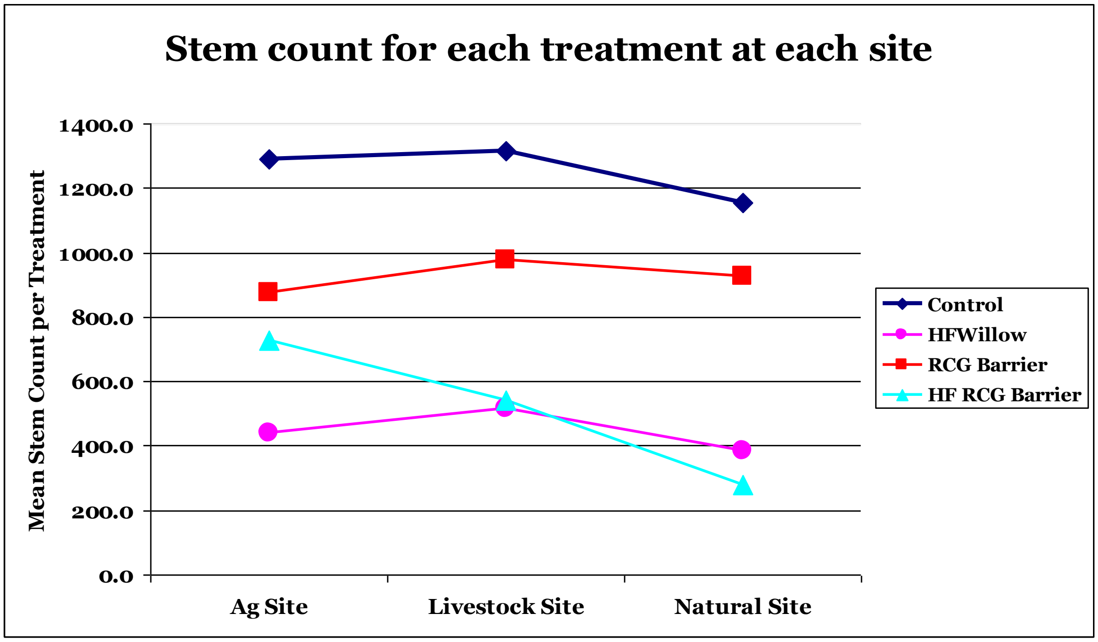


Figure 2.13. RCG returning stem count for each site and treatment.

With the interaction in mind, all three treatments resulted in fewer returning RCG stems than what established in the control plots as stem counts for the control plots are higher than for all three treatments at each site (Figure 2.13). The RCG Barrier alone treatment was the least successful at all three sites at reducing the returning RCG stem count and was not statistically significant (Figure 2.14 and Table 2.2).

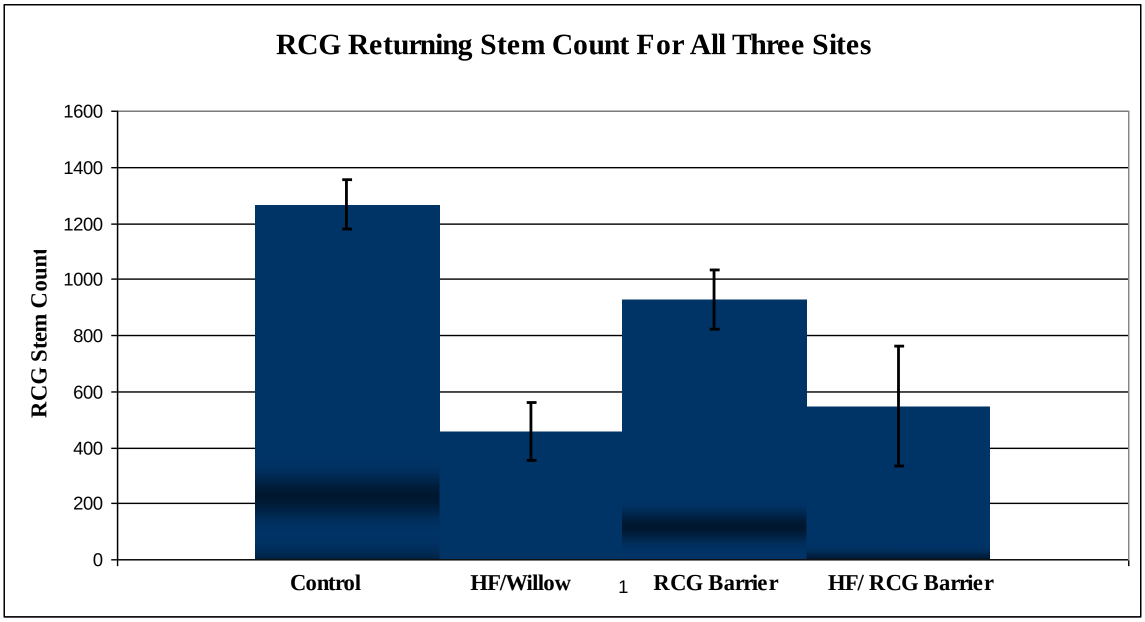


Figure 2.14. Average RCG returning stem count for all three treatment sites.

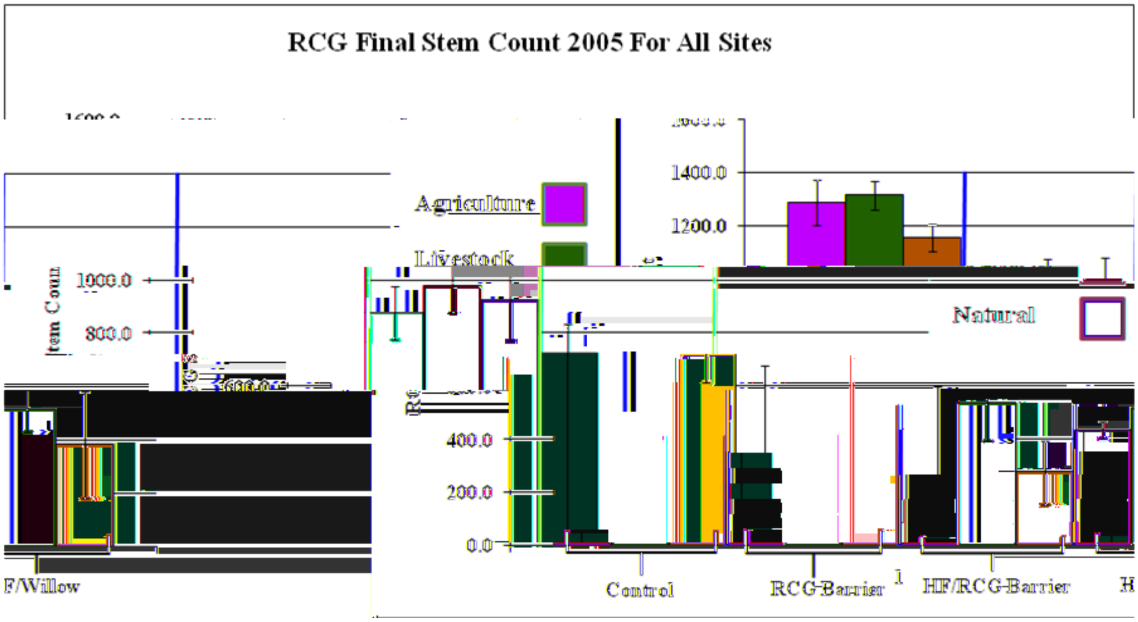


Figure 2.15. RCG Final Stem Count From The Three Sites Grouped by Treatment.

The treatments, Hogfuel/Willow (HF/Willow) and the HF/RCG Barrier were the most successful overall, but varied by site. The HF/Willow treatment was more successful at the Agriculture site (Figure 2.16) and the HF/RCG Barrier treatment was the most successful at the Natural site (Figure 2.18). The HF/Willow and HF/RCG Barrier treatments were not significantly different at the Livestock site (Figure 2.17). Additionally, except for the HF/RCG Barrier treatment at the Agriculture site, the Livestock site had higher stem counts than the other two sites for the control and the other two treatments.

The HF/Willow treatment reduced the returning RCG stem count when compared to each of the other two treatments at the Agriculture Site, but was not statistically significant due to the variation at the Livestock and Natural Sites (Figures 2.16, 2.17, 2.18). However, the HF/Willow treatment was the most successful treatment at two of the three sites, the Agriculture and Livestock sites.

The most successful treatment at the Natural site was the HF/RCG barrier treatment, although when looking at the high variation among the replicates, the two treatments, HF/Willow and HF/RCG Barrier, are not statistically significantly different from each other (Figure 2.18). The stem counts are from two replicates at the natural site, as replicate number one was completely flooded in late spring of 2004 after the watercourse channel moved during a major flood event. This site was also impacted by other flood events and beaver predation on all of the woody species.

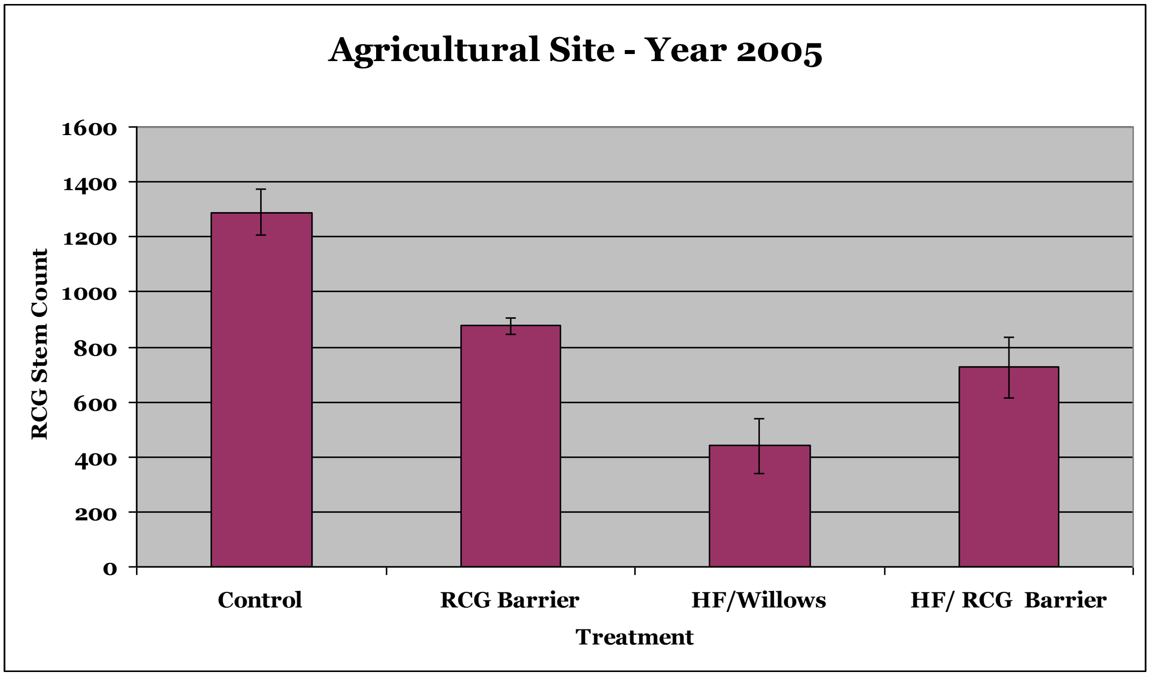


Figure 2.16. **Average RCG returning stem count for each treatment at the Agricultural Site for the ending period of 2005. Error bars indicate the standard deviation among the three replicates for each treatment.**

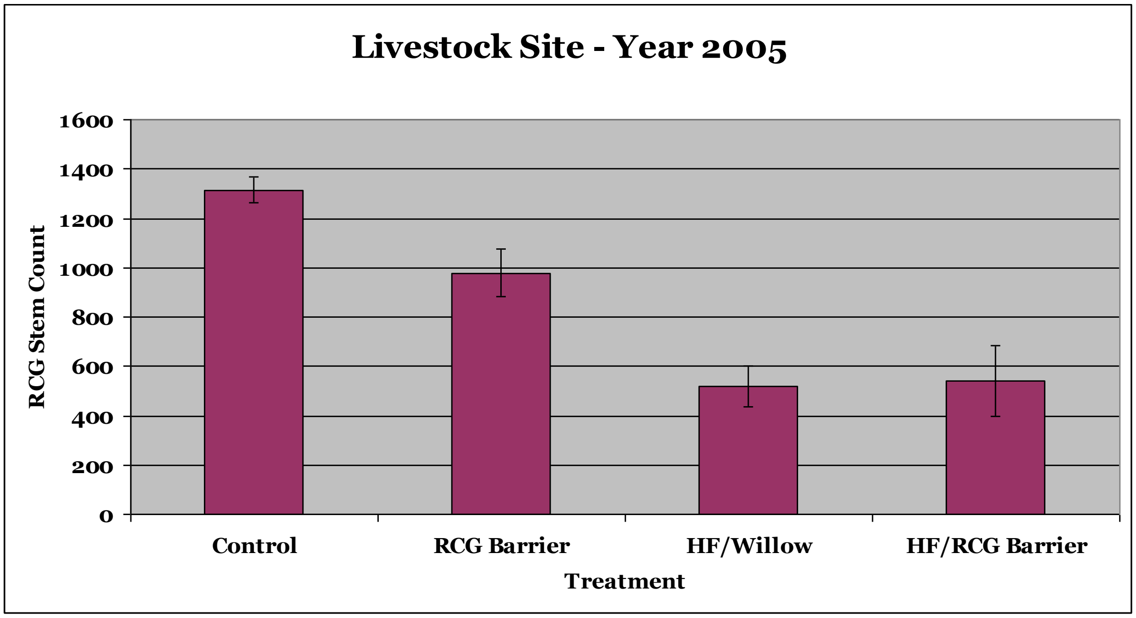


Figure 2.17. Average RCG returning stem count for each treatment at the Livestock Site for the ending period of 2005. Error bars indicate the standard deviation among the three replicates for each treatment.

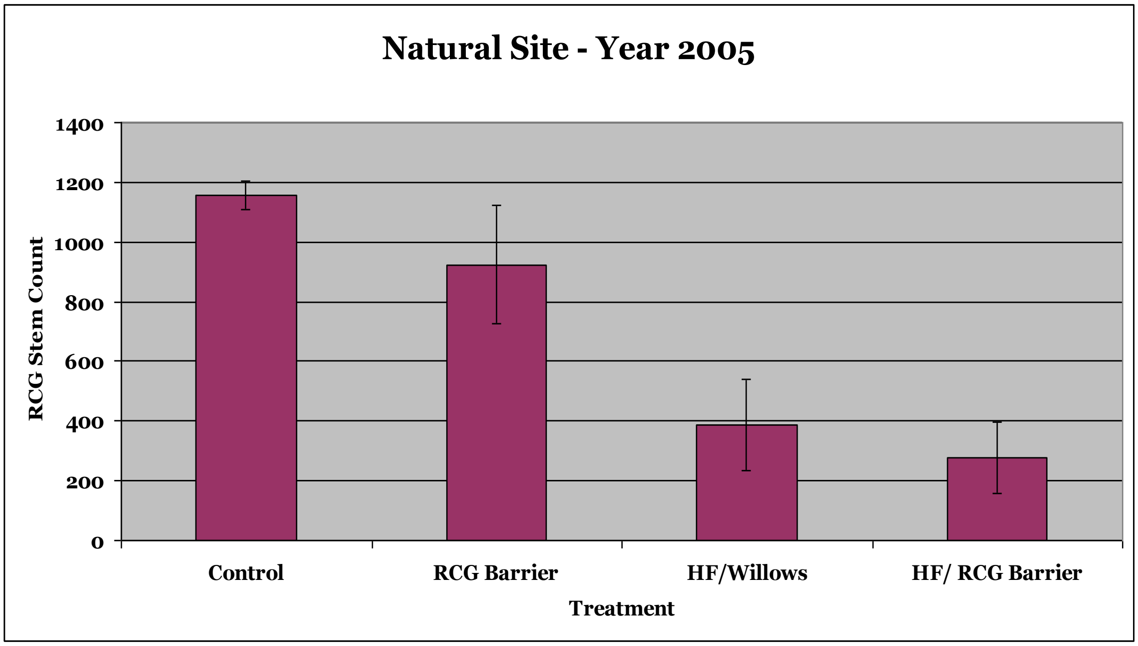


Figure 2.18. Average RCG returning stem count for each treatment at the Natural Site for the ending period of 2005. Error bars indicate the standard deviation among the three replicates for each treatment.

A Two-way Analysis of Variance (ANOVA) was used to examine the treatment main effect, site main effect and their interaction effect on the stem count. The ANOVA results are presented in Table 2.2 and the Tukey HSD pos hoc results are listed in Table 2.3.

Each of the treatments were statistically significant when compared to the control RCG stem count return and when compared to the other treatments overall (p-value = 0.000).

Both treatment and site have a statistically significant effect on the stem count (p=0.000 for treatment and p=0.008 for site). Post hoc comparisons shown below illustrate the pair wise difference among all of the combinations of treatment and site.

Table 2.3. **Post-hoc multiple comparison results for the final RCG returning stem counts.**

	Comparison	Estimate	Lower Bound	Upper Bound	Sig[1]
Agriculture Site	CON – HFWill	850	536	1160	*
	CON – HF/RCGbar	563	249	877	*
	CON – RCGbar	413	99.1	727	*
	HFWill – HF/RCGbar	-286	-600	27.5	
	HFWill – RCGbar	-437	-751	-123	*
	HF/RCGbar – RCGbar	-150	-464	164	
Livestock Site	CON – HFWill	798	484	1110	*
	CON – HF/RCGbar	775	461	1090	*
	CON – RCGbar	336	22.5	650	*
	HFWill – HF/RCGbar	-23.3	-337	291	
	HFWill – RCGbar	-462	-776	-148	*
	HF/RCGbar – RCGbar	-439	-753	-125	*
Natural Site	CON – HFWill	770	385	1150	*
	CON – HF/RCGbar	878	494	1260	*
	CON – RCGbar	231	-153	616	
	HFWill – HF/RCGbar	109	-275	493	
	HFWill – RCGbar	-538	-922	-154	*
	HF/RCGbar – RCGbar	-647	-1030	-263	*

Note[1] - * denotes significant at 0.05 level.

In addition to counting the returning RCG stems, the percentage of vegetative cover was also determined for each plot at each of the sites (Figures 2.19 through 2.24). Due to high variation within each plot, the six (out of 12) quadrates closest to the watercourse were utilized for the Agriculture and Livestock sites. These charts and affiliated pictures reveal which species used in this study not only survived the harsh conditions of competing with established RCG, high variations in water levels, flooding, beaver predation and the willow borer, but which species thrived.

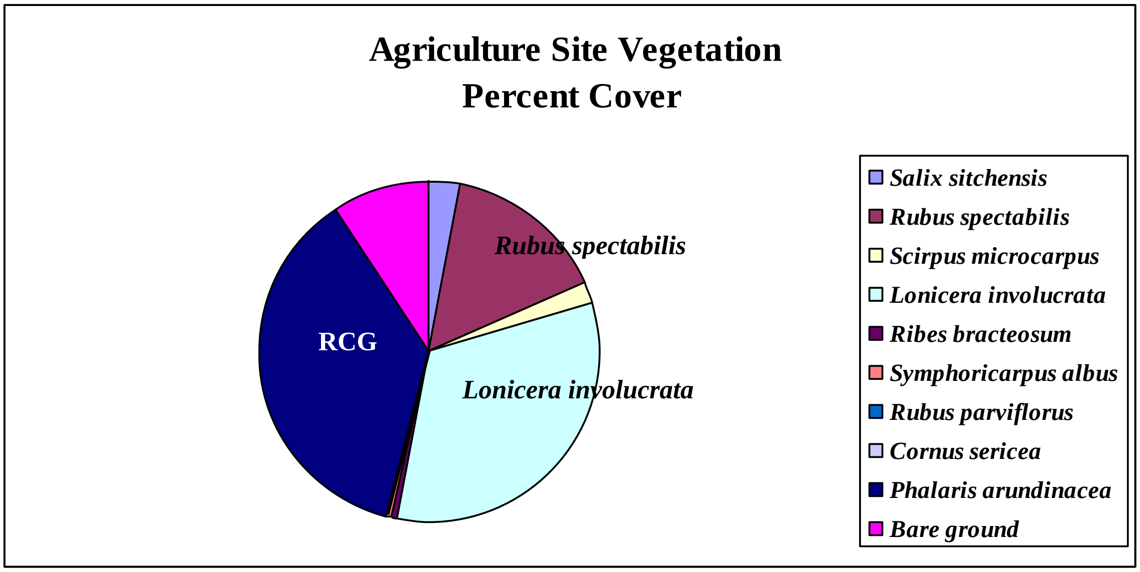


Figure 2.19. **Percent cover for the Hogfuel/RCG Barrier treatment. Average of the 3 replicates for the 6 quadrates closest to the watercourse.**

Figure 2.20. **Photos of the quadrats shown above in the pie chart. Photos from the end of the 2004 season.**

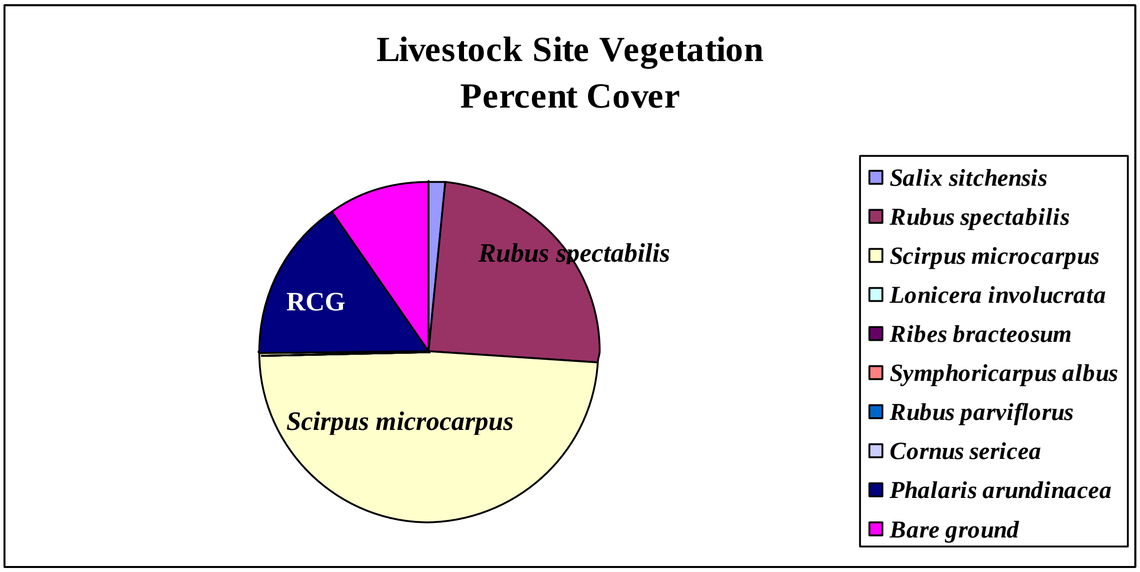


Figure 2.21. **Percent cover for the Hogfuel/RCG Barrier treatment. Average of the 3 replicates for the 6 quadrates closest to the watercourse.**

Figure 2.22. **Photos of the quadrats shown above in the pie chart. Photos from the end of the 2004 season.**

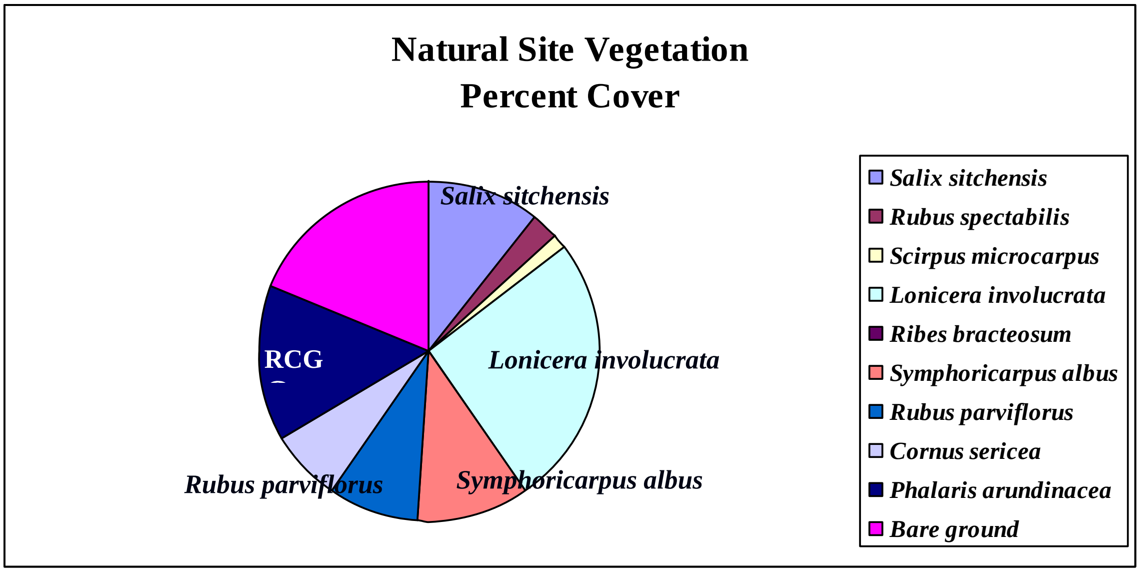


Figure 2.23. **Percent cover for the Hogfuel/RCG Barrier treatment. Average of the 3 replicates for the entire plot.**

Figure 2.24. **Photos of the quadrats shown above in the pie chart. Photos from the end of the 2004 season.**

2.6. PROJECT DISCUSSION AND CONCLUSIONS

Both the treatment and the site affected RCG stem count (RCG regrowth). The treatment effect varied by site for one treatment (the HF/RCG Barrier treatment at the Agriculture site). The post hoc comparisons quantified the differences among treatments at each site. These tests indicate that the control stem counts were significantly higher than all three treatments at all sites, except at the livestock site, where control count is not significantly different than the RCG Barrier alone treatment.

However, the HF/Willow treatment has lower stem count numbers overall (Figures 2.16-2.18). This may be due to the unexpected positive effects provided by the compost added to the RCG Barrier and HF/RCG Barrier treatment for planting within. The compost most likely provided additional moisture and possibly nitrogen for the RCG.

One of the objectives of this research project was to provide maximum shade and preferred invertebrates for salmonids, whether to benefit salmonids within the watercourse itself or downstream within the main channel into which the watercourse flows. The HF/Willow treatment alone would not be able to adequately meet this objective. Past research by Joy Zedler and many of her students have shown that RCG responds negatively to numerous canopy layers (Lindig-Cisneros and Zedler 2001, Maurer and Zedler 2002). Stem count results from my mixed canopy layer plots echo those results. Additionally, a more diverse herbaceous and woody plant species assemblage would allow for a more diverse invertebrate community (Allan et al. 2003).

My recommendation for controlling RCG and restoring agricultural watercourses would be the Hogfuel/RCG Barrier treatment with the multiple species plantings. Providing multiple canopy layers would allow for lower light levels and the additional species (*Rubus spectabilis, Vaccinium ovatum* and *Ribes bracteosum*) would assist in providing preferred prey for salmonids.

Salix sp. generally do not leaf out until mid to late spring (~mid April) while RCG generally begins growth in January or February in the PNW. If other species are utilized within the restoration/replanting, there is a greater chance for a few of the species to leaf out earlier in the spring (for example, the *Scirpus microcarpus, Rubus spectabilis and Lonicera involucrata*) providing some shade to the early returning RCG and supplementary shade for the water course. The use of conifers may also be desirable but were inappropriate for this study due to the height requirement of the Farm Preservation Program (FPP) program in King County under which this land operates.

With the Hogfuel/Willow treatment, the success of the restoration could be heavily impacted by the poplar and willow borer (*Cryptorhynchus lapathi*), an introduced species now ubiquitously established throughout western Washington. This weevil attacked the Agriculture and Livestock sites, at the end of the 2005 season and destroyed up to 80% of the willows at both sites (See Figure 2.25). The damaged willows were cut off, removed from the premises and destroyed so that the larvae inside would not mature. Many of the willows recovered from the rootstock, however, the shade supplied for RCG control was reduced dramatically and would not recover for at least 2-3 years.

Figure 2.25. An adult willow borer (*Cryptorhynchus lapathi*) on an impacted willow stem.

Placing woodchips / mulch within wetland and/or riparian settings, even for restoration projects, is considered the filling of a wetland and the project is therefore subject to inspection by the US Army Corps of Engineers. The Corp will evaluate the project, and unless the some aspect of the filling does not comply with the Endangered Species Act, the Corps will be able to allow the project under the Nation Wide Permit 27 (Bennett, pers. comm. 2007). Using red cedar hogfuel for RCG suppression was successful in this study and is recommended for use due to the weight, nitrogen reduction and its allelopathic tendencies. A study published after the red cedar hogfuel was tested in this research project also indicated inhibition of germination and suppression of hypocotyls and radicle growth of lettuce seeds and seedlings. Five species of trees from the southeast United States were tested. The southern red cedar (*Juniperus silicicola*) also significantly inhibited the growth of a common weed in the southeast, *Desmodium tortuosum* DC (florida beggarweed) when compared to a gravel mulch and control (Rathinasabapathi et al. 2005).

Finally, if financially feasible, a thicker fabric should be used in the future in place of the burlap fabric for either of the treatments described above. A thick, compact, heavy, and fully biodegradable fabric is in the process of being developed for weed control within restoration projects but is not publicly available at this time.

It was obvious that some species were superior in their ability to survive, compete and thrive within the harsh conditions in which they were planted. A list of recommended species from the species that were employed in this project include: *Scirpus microcarpus, Rubus spectabilis, Lonicera involucrata, Salix sitchensis, Cornus sericea,* and *Rubus parviflorus* (See Figure 2.12 for full list of species used).

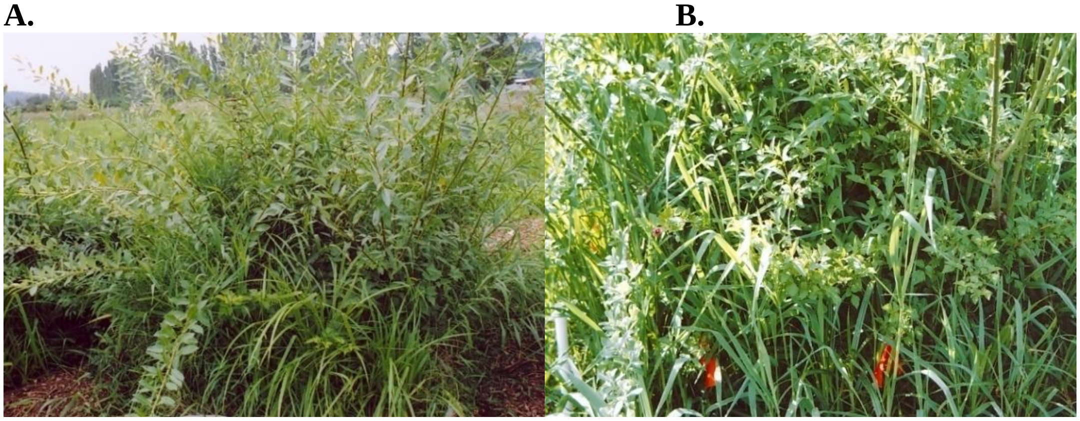


Figure 2.26. Photos from the Agriculture site (A) and Livestock site (B) from the end of the 2004 season.

CHAPTER 3. INTERSPECIFIC COMPETITION BETWEEN *PHALARIS ARUNDINACEA* AND *SCIRPUS MICROCARPUS*

3.1 INTRODUCTION

Field observations from both the experimental field sites and in some natural areas indicate that *Scirpus microcarpus* (small fruited bulrush (SFB)) is capable of thriving and competing effectively with *Phalaris arundinacea* (reed canarygrass) (Ewing and Seebacher, personal observation). A competition study between reed canarygrass (RCG) and small fruited bulrush was conducted within the greenhouse at the Center for Urban Horticulture (CUH), University of Washington to test the competitive abilities of these two species in a controlled setting.

3.2 PLANT COMPETITION ECOLOGY

Plant competition, specifically inter-specific competition is defined by Tilman (1997) as "an interaction in which an increase in the population density or biomass of one species leads to the decrease in the population growth rate and the population density or biomass of another species." The extent of growth inhibition by the adjacent species determines the degree of competition (Crawley 1997). The biodiversity of a plant community and site is largely determined by inter and intra-specific competition among the plant species involved. The effects of this competition on the plant community and biodiversity are modified however, by herbivores, pathogens and parasites (Crawley 1997).

Species such as RCG are typically found in monocultures and are faced with intra-specific competition. Bonsall (2007) partitions intra-specific competition into two categories; scramble, which takes place when resources are allocated in a uniform manner, and contest, when the resources are unequally divided. Intra-specific competition takes place when two or more plants or clones in the case of RCG, of the same species vie for the same resource (Bonsall 2007). Many clonal species have been found to dominate a site with one or two of the most fit clones covering literally hundreds of kilometers or hectares (Sebens and Thorne 1985). There are several consequences of intra-specific competition. The first could be a diminished size of the plant as the number of individuals mount, referred to as the density effect (Park et al. 2003). Another could be a shift in the gradient of sizes of individuals within the population, and thirdly, the potential loss of individuals as population density in the population increases, referred to as self-thinning.

Plants compete for one main above-ground resource, light, and numerous below ground resources from within the soil. These below ground resources include water, mineral nutrients, valence and oxidation state and space (Casper and Jackson 1997). There are several conflicting theories in resource competition. One such theory states that competition intensifies in habitats with higher productivity due to the high growth rates and subsequent biomass. This in turn increases the competition for space and light. Less productive habitats would tend to be less competitive, attracting those species that have a lower competitive ability (Wilson and Tilman 1991). Another theory advocates that more intense competition may take place in less productive habitats, primarily for soil resources as they would tend to be limited (Wilson and Tilman 1991).

If one species is to prohibit the survival of another or exclude another species, there are several prerequisites. First, the essential resources must be restricted. Second, interaction between the two species must overlap for space and resources, and third, one of the species would need to be better at capturing the shared resources (Aarssen 1983).

Connell (1983) proposed ranking species based on competitive ability. He defines competition as when a particular species has a harmful impact on another, but when only one species is impacted, the term “asymmetrical competition” should be used. When this asymmetry is strong, the species involved are able to be ranked based on superiority of how affected one was by the other in the experiment. Of those species in the research studies examined that indicated competition, asymmetry was robust with 61% demonstrating asymmetrical competition (Connell 1983). When comparing previous studies for intra and inter-specific competition differences, Connell found that in 75% of the experiments, intra-specific competition was equal to or stronger than inter-specific competition.

Scores of researchers have tried to connect species traits with their competitive potential in the field, both to predict distribution of species along a production gradient (Wilson and Tilman 1991, Gaudet and Keddy 1995, Keddy et al. 1998,) and to determine competitive abilities of a particular species with regard to controlling invasive species (Graustein 1995, Wetzel and van der Valk 1998, Green and Galatowitsch 2001, Perry et al. 2004).

Two traits noted by Keddy et al. (1998) that have been shown to be good predictors of a successful competitor are the relative growth rate and what Keddy refers to as competitive effect. Competitive effect takes place when a plant is able to inhibit other plants, and in addition to competitive response, being able to evade the suppression, encompasses the competitive ability of a species (Keddy 1998, Rosch et al. 1997).

Plants that are fast growing and are generally tall within their community have elevated competitive effect scores. RCG was determined to have a score of 89% out of 100 (Keddy et al. 1998). The only other species that had a higher competitive effect score out of the forty eight in this study were *Bidens cernua* (91%), a native species, yet considered weedy by several weed references, and *Lythrum salicaria* (96%), an extraordinarily aggressive invasive species. The study by Keddy et al. (1998) compared forty eight wetland species and the competitive response of those species with a sward of species (*Acorus calamus, Lythrum salicaria, Typha angustifolia, Carex crinita, Penthorum sedoides, Eleocharis smallii, and Scirpus acutus*). RCG responded negatively to the competition with a relatively low competitive response value.

If RCG has a high competitive effect score (the ability to suppress others), but a low competitive response score (the ability to avoid being suppressed), a symmetrical species which has a high score for both competitive effect and response may be able to out compete RCG in certain field conditions. *Scirpus microcarpus* was not one of the species included in the study and therefore, the competitive effect and response score for this species is unknown.

RCG and SFB are both long lived perennials which are highly rhizomtatous, similar in height, up to 2m for RCG, up to 1.5m for SFB, (Fern 2004) have similar rooting depth, 25 cm for RCG, 30 cm for SFB (Comes 1971, Azim et al. 2001) and similar specific leaf area ratios (SFB blades 23–60 cm × 5–15 mm (Flora of North America) and RCG leaf blades 10–35 cm × 10–18 mm and tapering (Flora of China)).

3.3 *SCIRPUS MICROCARPUS* BIOLOGY

Scirpus microcarpus is an obligate wetland herbaceous perennial sedge, predominately found in freshwater wetlands. This species has two synonyms, *S. sylvaticus* and *S. rubrotinctus*, (Guard 1995), and two varieties, *Scirpus microcarpus* var. *longispicatus* and var. *rubrotinctus*, and three common names, Small fruited bulrush (Guard 1995), Small flowered bulrush (Pojar and Mackinnon 1994) and Panicled bulrush (USDA, NRCS 2007). As with the reed canarygrass, I will use the acronym of the common name of the small fruited bulrush, SFB. SFB is found along the entire west coast throughout the upper Midwest and in the Northeast (USDA, NRCS 2007). The plants may grow as tall as 1.5 meters tall with plump, triangular stems arising from thick rhizomes (Pojar and Mackinnon 1994). Small mammals, such as the wandering shrew, use this species for cover (Ingles 1961), muskrat eat the seeds, while waterfowl, geese and swans consume the shoots and rhizomes (Guard 1995). Unlike RCG, which has been the subject of numerous research studies undertaken and articles written on biology and control, SFB is generally only referred to in vegetation surveys and in species lists within a few articles

(Mallik et al. 2001, Jurries 2003, Lee et al. 2001, La Force et al. 2002, Schuller 2006, Patterson and Cooper 2007, Treberg and Husband 1999, Schuyler 1976, Magee and Kentula 2005, Mockler et al. 1998, Stevens and Vanbianchi 1993).

3.4 COMPETITION PROJECT DESCRIPTION AND OBJECTIVES

The objective of the greenhouse competition experiment was to determine if SFB is an effective competitor with the aggressive, invasive RCG. An effective competitor was defined as a species that negatively impacts the performance of its neighbor by limiting growth. In this case, limiting growth is considered the reduction of above-ground and/or belowground biomass of RCG when grown with SFB (with inter-specific competition) versus with intra-specific competition. In this experiment, I looked at resource competition. The ecosystem for the field experiment is a high resource ecosystem (agricultural fields with drainage watercourses). This system experiences generally high levels of nitrogen from fertilizer and livestock and high soil moisture levels close to the watercourse and high levels of disturbance due to mowing and flooding. It is assumed that the species with high relative growth rates will be the most effective competitor as rapid growth would allow them to dominate the available space, produce more biomass and acquire the most resources while doing so.

Along with the observations that the planted SFB was thriving and competing with RCG, it was observed that SFB was most successful when placed closer to the watercourse and within a site with higher nitrogen due to the adjacent livestock farm (Seebacher, personal observation). The competitive ability of a species typically changes with differing nutrient and environmental conditions, the neighboring species and developmental stage. In addition to testing the competitive abilities of SFB with RCG, two soil moisture and nitrogen regime treatments were tested. This allowed me to determine if SFB is as competitive with RCG within a wide range of environmental and nutrient conditions or only within particular conditions and therefore, more site specific.

The goal of this restoration project is to control RCG along and within watercourses on agricultural land in King County. Within these systems, the RCG is mature, heavily clonal and in most every case, a monoculture on the site. Hence, testing the competitive abilities of the seedlings of these two species does not coincide with the current conditions of many impacted sites nor the conditions found for the majority of the restoration projects in the PNW. I therefore, used more mature bare-root plants for each species for this experiment.

3.4.1. Competition Experiment Methods

Sixty SFB plants were purchased from Storm Lake Growers and 140 RCG plants were collected from the field. The plants were chosen based on similarity in number of nodes and rhizome length. Ten plants were randomly chosen for an initial dry weight analysis. These data were compared with the mean dry weight analysis for each treatment at the end of the study. Of the remaining plants, the RCG and SFB were randomly chosen for each treatment (two soil moisture regimes plus two nitrogen regimes), the above-ground stems were clipped at 10 cm and the plants were weighed. Past experience with planting bare root RCG and SFB has shown that the above ground biomass (stem length) of the plants can be quite variable and generally dies immediately after planting. It was therefore, removed before weighing and planting. After weighing, the clipped plants were assigned a number for tracking purposes. This information was used to determine if there is a correlation between the initial fresh weight of each plant and the final biomass.

An additional tagging method was used within the control pots (RCG/RCG) where one of the RCG plants was randomly chosen to be the RCG plant weighed at the end of the experiment. Using bare-root plants that are of a similar size based on number of nodes, root length, and same length of above ground biomass reduced the variation at the beginning of the experiment. Randomly choosing which plants were placed in each treatment provided a Gaussian distribution among the treatments.

Each two gallon pot with either two RCG plants or one RCG plant and one SFB plant was placed independently within its own tray for watering and fertilizing. The trays were placed in a complete randomized block design on a table within zone 2 of the CUH greenhouse (~21◦C daytime, ~17◦C night, 14 hour photoperiod/1/8 full sunlight). There were 10 blocks with one of each of the eight treatments placed randomly in each block (Figure 3.1).

3.4.1.1. Treatments - Nutrient, Soil Moisture and Competition

The high nitrogen level for this experiment was 66.08 mg per kg (mean from three samples with the NH^4 and NO^3 added together from the site) based on soil samples from the livestock site in Woodinville. The low nitrogen level was 9.46 mg per kg based on soil samples from the “natural” site in Duvall.

For the high soil moisture regime, the water level within the tray was kept at a depth of ~2.5 cm. The low soil moisture regime was determined by averaging the precipitation data from the Monroe and Tolt South Fork Reserve weather stations (Monroe for its proximity to the Sammamish sites and Tolt for its proximity to the Snoqualmie site). I used the data for typical summer months, June through August, averaged over the last six years, from 2000 through 2005. The average rain per day for the two sites was 2.15mm. Adjusting this to the surface area of the pots used for this experiment allows for 800 mL of water per week for the low soil moisture treatment watering regime.

The plants were planted immediately after receiving. Two RCG plants were planted per pot (two gallon pots) for the control pots and one RCG plus one SFB plant were placed together for the competition treatment (two gallon pots). The soil moisture and nitrogen treatments were applied once each week. The plants were grown together for 22 weeks.

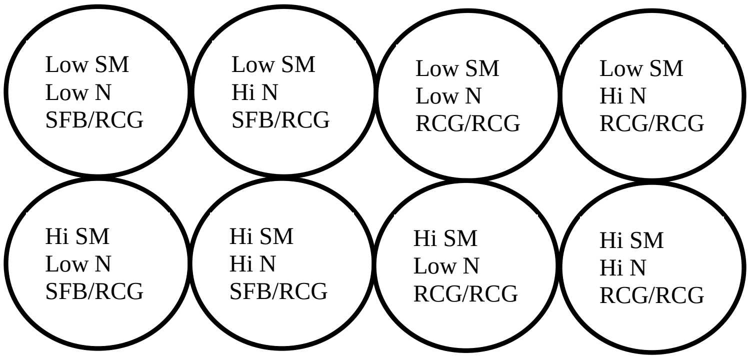


Figure 3.1. **Example of the eight treatments within one of the ten blocks.**

3.4.1.2. Data Collection and Statistical Analysis

Above and below-ground biomass was harvested for both species within each treatment. The above ground plant material was clipped 2 cm above the soil surface and dried and weighed. The below ground biomass was removed from the pots, washed, dried and weighed.

The mean biomass values of RCG grown alone and RCG grown with SFB was analyzed employing a Three Way Analysis of Variance. As noted above, soil moisture, nitrogen and competition are the three factors involved and there are two levels for each factor (Table 3.1). The above ground, below ground and total biomass means were analyzed separately to determine exact impacts of the SFB competition.

Table 3.1. **Factor and treatment layout.**

FACTORS →	**Nitrogen**	**Soil moisture**	**Competition**
Level 1	High	High	Intraspecific (RCG/RCG)
Level 2	Low	Low	Interspecific (RCG/SFB)

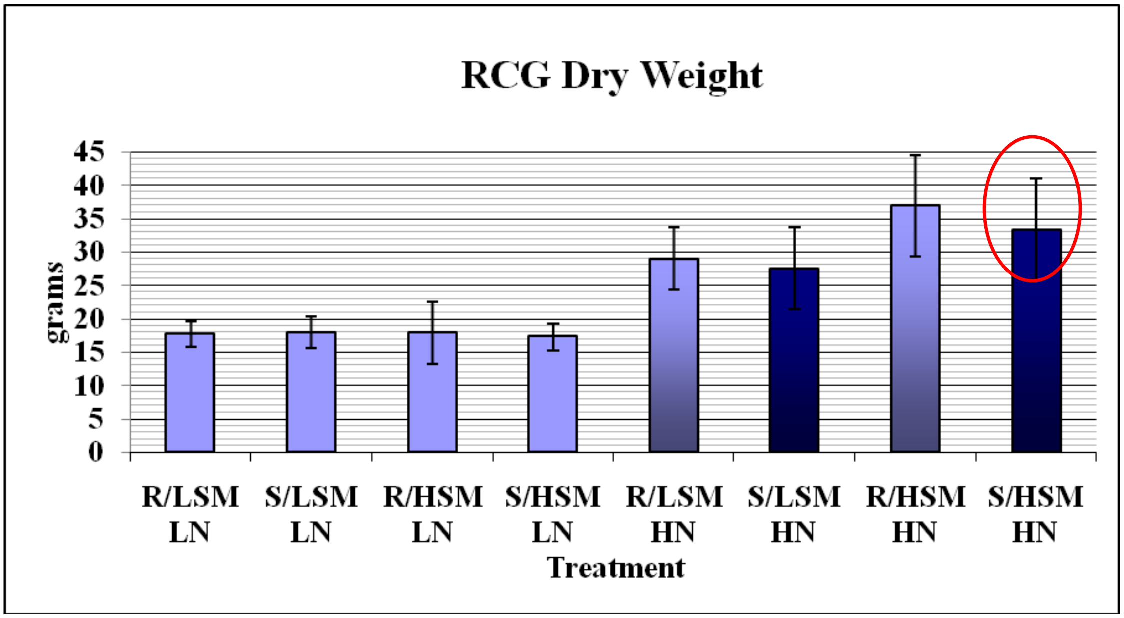
3.6 RESULTS

Reed canarygrass and small fruited bulrush responded favorably to the high nitrogen treatments. The RCG plants within the low nitrogen treatments did not respond differently to high or low soil moisture treatments or to inter versus intra specific competition. Conversely, those RCG plants exposed to the high nitrogen treatments did exhibit greater biomass in the high soil moisture treatments. Although not statistically significant, within the high nitrogen/high soil moisture treatments, the RCG grown with inter-specific competition (with SFB), showed evidence of lower total plant biomass (above and below ground) overall than when grown with intra-specific competition (with another RCG plant). (Table 3.2 and Figure 3.2)

Table 3.2. Three-way ANOVA results for the final RCG biomass.

Source	Df	Sum of Sq	Mean Sq	F Value	p
Moisture	1	220.91	220.91	9.241	0.003
Nitrogen	1	3939.06	3939.06	164.77	0.000
Competitor	1	36.100	36.100	1.51	0.223
Moisture*Nitrogen	1	255.68	255.68	10.695	0.002
Moisture*Competitor	1	11.220	11.22	.469	0.495
Nitrogen*Competitor	1	28.728	28.72	1.202	0.277
Moisture*Nitrogen*Competitor	1	1.80	1.80	.075	0.785
Residuals	72	1721.24	23.906		

Tests of Between-Subjects Effects



LSM = Low Soil Moisture
HSM = High Soil Moisture
LN = Low Nitrogen
HN = High Nitrogen

R = RCG vs. RCG
S = RCG vs. SFB

Figure 3.2. Total RCG dry weight. Circle indicates lower RCG total biomass when grown with SFB under high soil moisture and high nitrogen treatment.

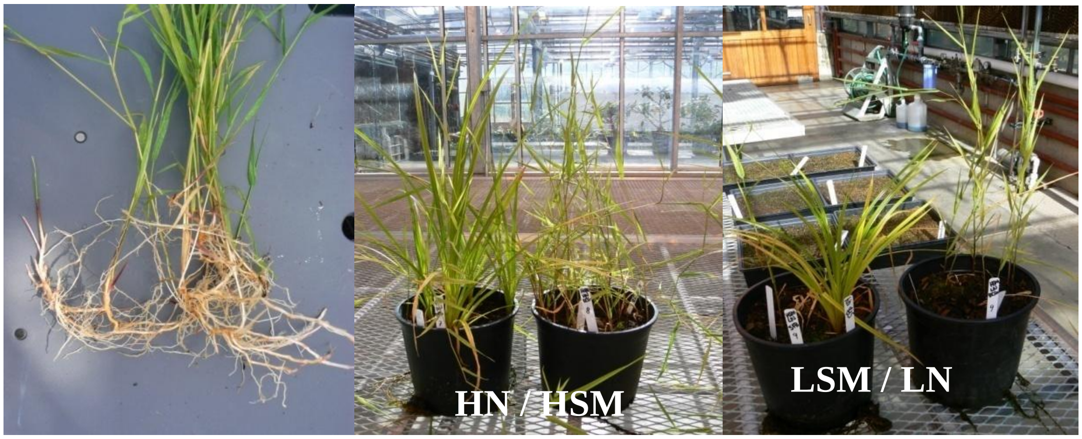
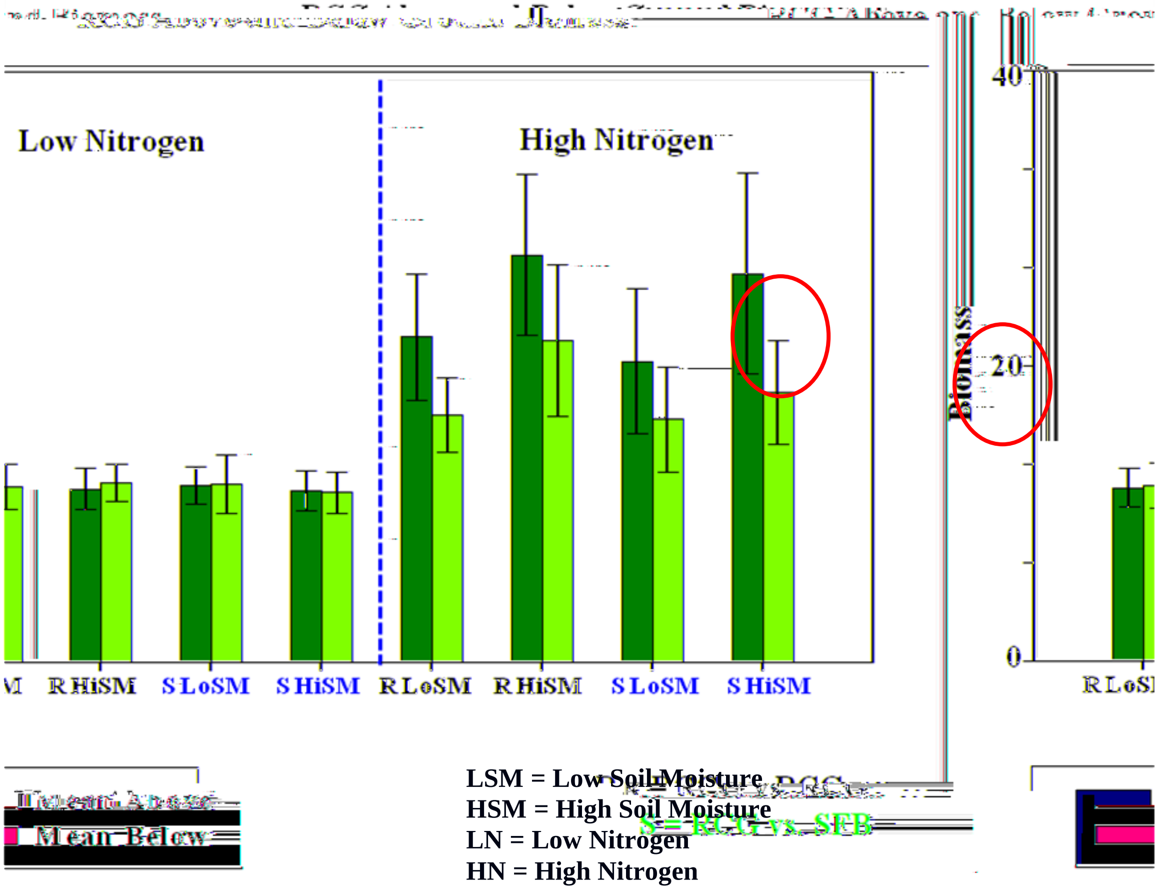


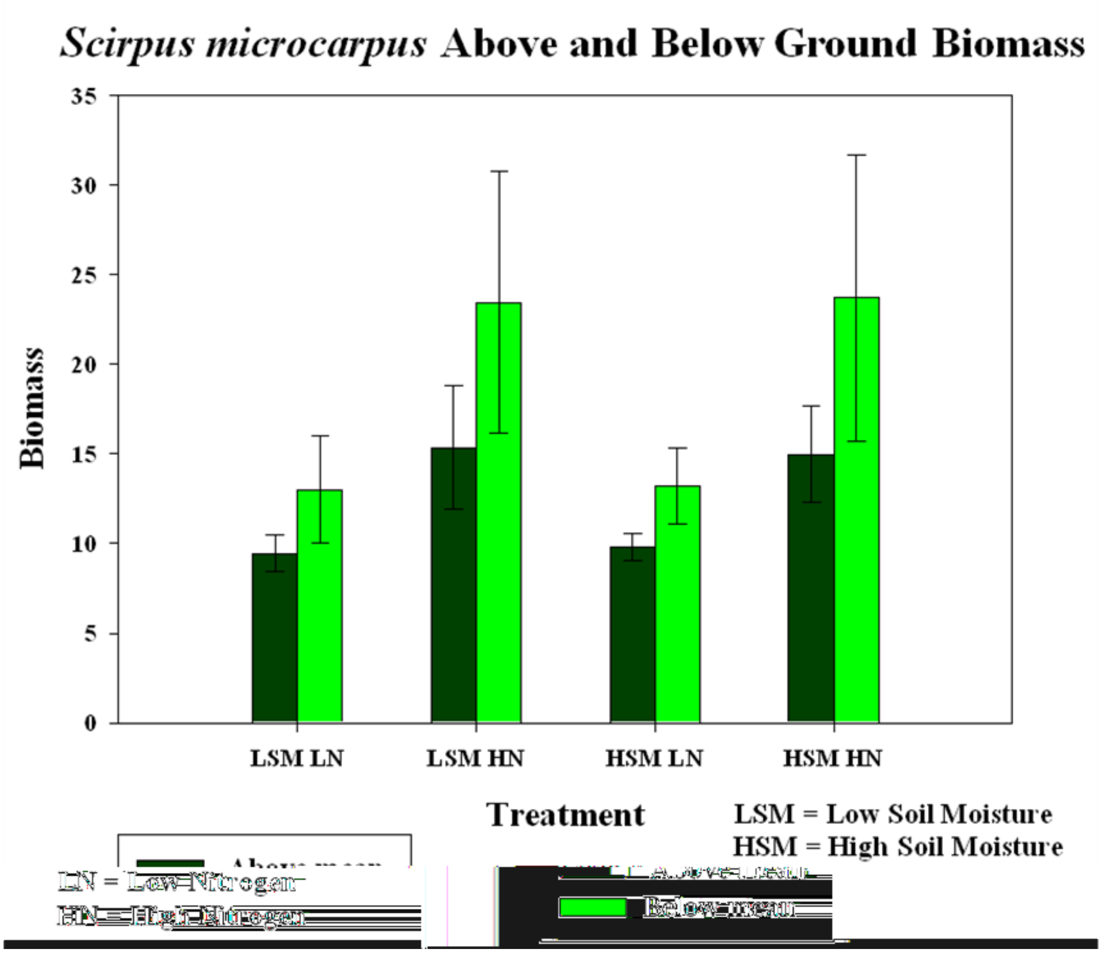
Figure 3.3. Photos of the plants for two of the treatments just before and after harvesting.

While examining the above and below-ground biomass, it was observed that *Phalaris arundinacea* (RCG) consistently allocated more resources to above ground biomass (18-20 grams for RCG versus 14-15 for SFB) (Figure 3.4) within the high nitrogen treatments while the *Scirpus microcarpus* (SFB) consistently allocated more resources to below ground biomass within all treatments (22-23 grams for high nitrogen treatments for SFB versus 12-13 grams for RCG) (Figure 3.5). In fact, both above and below ground RCG biomass was reduced when grown with SFB in high nitrogen environments, although this was not statistically significant (Figure 3.4). Within the HSM/HN treatment, SFB reduced the RCG below ground biomass more than above ground biomass (Figure 3.4). It seems plausible that the SFB is employing a disproportionate amount of below ground biomass when competing with other species (Figure 3.5).



LSM = Low Soil Moisture
HSM = High Soil Moisture
LN = Low Nitrogen
HN = High Nitrogen

Figure 3.4. **RCG above and below ground dry weight for each treatment. Red circles highlight a reduction in RCG below ground biomass when grown with SFB in the high nitrogen, high soil moisture treatments (S/HSM HN) compared to grown in the same treatments with another RCG plant (R/HSM HN).**



LSM = Low Soil Moisture
HSM = High Soil Moisture
LN = Low Nitrogen
HN = High Nitrogen

Figure 3.5. **SFB above and below ground dry weight for each treatment.**

3.6 DISCUSSION AND CONCLUSIONS

Both species in this study responded negatively to the low nitrogen treatments, whether grown with high or low soil moisture or with inter or intra specific competition. SFB did show slightly higher biomass levels under low nitrogen/low soil moisture treatments, but did not suppress RCG under these conditions any more than the RCG plant grown with intra-specific competition.

An interaction effect between the nitrogen and soil moisture treatments was due to the nitrogen level impacts on soil moisture, ie., the soil moisture level impacted the resulting plant biomass only within the high nitrogen treatments.

A similar study by Perry et al. (2004) provided a different result. In their study, they enriched the soil with carbon, reducing available nitrogen and then added four NH_4-N treatments up to 1.25 grams per week (the high nitrogen treatment for my study was 66 milligrams for comparison). They found that the native sedge they tested, *Carex hystericina* (bottlebrush sedge) was successful at competing with RCG when grown from seed in a carbon enriched soil, reducing the RCG biomass by 82%. When both species were grown together in soil that was not enriched with carbon and at the highest nitrogen treatment level noted above, RCG suppressed the bottlebrush sedge by 91% (Perry et al. 2004).

The difference in results could be due to the fact they there were comparing the two species grown from seed whereas in my study, I tested two species grown from bare root specimens, which is more indicative of the conditions one would find in restoration projects in the field. Additionally, SFB may not have the same nitrogen uptake efficiency under nitrogen poor conditions as does the sedge tested in the above mentioned study and therefore may be less able to provide the suppression of RCG. *Carex hystericina* tested had an uptake efficiency almost twenty times greater than RCG nitrogen uptake efficiency in the lower nitrogen level treatments (Perry et al. 2004).

While looking at competition intensity and asymmetry, Johansson and Keddy (1992) found that the intensity of competition increases with species that are morphologically more similar and similar individuals have more symmetrical interactions. However, the authors also state that they believe their results may support the theory of co-existence proposed by Aarssen (1983) and Keddy and Shipley (1989).

This theory is stated as: “similar species will coexist because inter-specific competition is approximately equal to intra-specific competition, thereby weakening differential inter-specific interactions, which lead to exclusion” (Johansson and Keddy 1992). Perhaps, the competitive interactions between SFB and RCG is overly symmetrical and therefore, the theory that this weakens inter-specific interactions is accurate in this situation. If this is the case, using SFB to outcompete RCG may only be successful in highly specific situations, such as in sites with very high nitrogen and direct sunlight, as seen in the conditions at the livestock site initially. I used 66.08 mg of nitrogen as my high nitrogen treatment based on the soil samples taken from the livestock site. However, this site may very well receive much higher pulses of nitrogen during rain events or possibly when the horse waste is not removed before a rain event. Higher nitrogen levels may have changed the results of the experiment and would be an interesting future study.

Gaudet and Keddy (1988) found that biomass, especially above-ground biomass, was determined to be the plant trait that was most strongly correlated ($r^2 = .75$) with competitive ability and the suppression of the phytometer used (*Lythrum salicaria*). Below-ground biomass, plant height and canopy area (cm^2) were also highly correlated, but not to the same extent (Gaudet and Keddy 1988). The authors theorize that when species with similar biomass levels are co-occurring, other factors such as height or other life history or morphological variables determine the outcome of the competitive interaction (Gaudet and Keddy 1988). Again, possibly the two species, SFB and RCG are too similar in height and life history traits, and therefore, may only co-exist in most conditions.

I found that SFB consistently produced more below-ground biomass over all treatments and less above ground biomass over all treatments than RCG. RCG did the opposite, at least in the high nitrogen treatments, producing more above-ground biomass than below-ground. Conceivably, the limitation of the pots in the greenhouse did not allow the SFB to produce sufficient below ground biomass to support additional growth above ground to effectively compete with RCG as this species did in the field for the first two seasons.

Small fruited bulrush competition impacted the RCG total plant biomass within the high nitrogen/high soil moisture treatment, although the difference was not statistically significant. These results also contradict a study by Green and Galatowitsch (2002) where total shoot and root biomass of the native community was suppressed by RCG, at all three levels of nitrogen used.

The SFB planted at the Agriculture site thrived and competed effectively with RCG, especially in the first season of this experiment, (Figure 3.6B) and even more so at the Livestock site during the 2005 season, (Figure 3.6A). These observations prompted the controlled competition experiment in the greenhouse. However, after several years, the shade from the surrounding vegetation and RCG on the other side of the bank seemed to negatively impact the SFB.

Grime (1977) would consider RCG to be a C-selected species or a competitive species. This study contradicts Grimes' hypothesis that regardless of environmental conditions, species that exhibit competitive characteristics produce a greater amount of biomass. SFB produced more below ground biomass under the low nitrogen, low soil moisture treatments, yet SFB may be considered a C-selected species as well.

RCG possesses other attributes that are typical for Grimes' competitive species. These attributes include: early growth in the late winter; along with rapid expansion of stems and leaf canopy due to the mobilization of carbohydrate reserves from the previous season. Coops et al. (1996) found that RCG allocates more resources to below ground biomass when grown in dense vegetative cover adding to the belowground biomass. These traits provide the plant a competitive advantage from the additional height and canopy produced early in the season (Grime 1979) and additional carbohydrate reserve to draw from (Coops et al. 1996). This could also explain the initial domination by the SFB at the livestock site but then the drop in SFB and increase of RCG three years later.

Even with the contradictory results, I recommend using SFB in certain re-vegetation situations. The livestock site received higher nitrogen levels than what was found at the other two sites and was the only site where the plots were facing south, getting direct, full sunlight throughout the day. Since SFB is a shade intolerant species, only using this species for restoring watercourses in situations where this emergent would be able to get full sunlight within high nitrogen environments is recommended.

Additionally, herbaceous wetlands, also referred to as Palustrine emergent wetlands by Cowardin et al. (1979) are in serious decline nationally and within the Pacific Northwest. Although protected federally by the Section 404 of the Clean Water Act and in Washington State by the Shoreline Management Act and State Water Pollution Control Act (Granger et al. 2005), emergent wetlands have been extensively filled and drained for development, farming and for livestock. Excessive loss of acreage by non-native species invasions adds to this loss of acreage. Restoring impacted wetlands with woody vegetation in order to control exotics only exacerbates the loss of herbaceous wetlands by creating forested wetlands in their place. Adding emergent species to the re-vegetation of these agricultural watercourses would allow for the intended diversity one could obtain from multiple canopy layer situations.

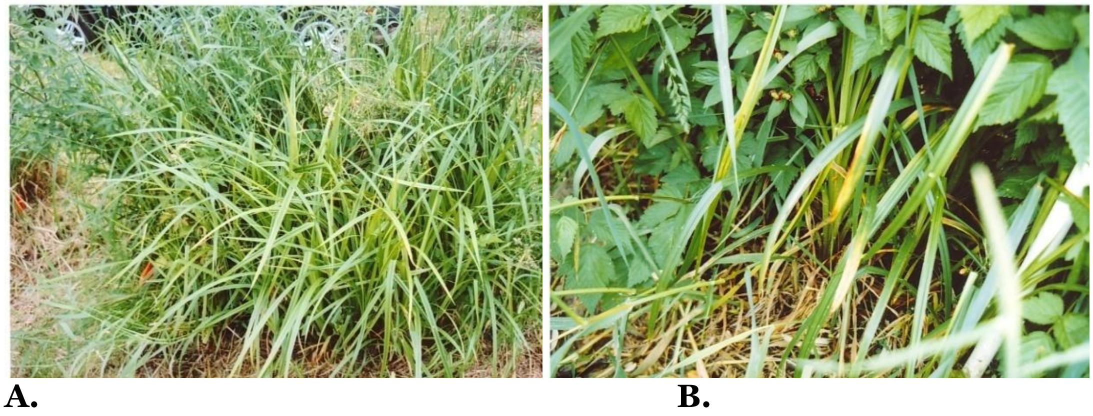


Figure 3.6. **A.** ***Scirpus microcarpus*** **growth at the Livestock site in 2005.**
B. ***Scirpus microcarpus*** **growth at the Agriculture site in 2004.**

CHAPTER 4. *PHALARIS ARUNDINACEA* CARBOHYDRATE RESERVES

4.1 INTRODUCTION

Perennial grasses use underground storage organs for absorption of water and nutrients, stabilization of the plant, storing nutrient reserves for winter survival, and for the initial growth in the spring and re-growth after grazing (Weinmann 1948, White 1972). This energy source is used for new growth until there is sufficient photosynthesis available for the respiration of the plant (White 1973). Many researchers agree that the total available carbohydrates (TAC) within the belowground biomass of a weedy perennial is the best way to gauge the fitness of the plant with regard to managing environmental conditions and control techniques by humans (Comes 1971).

Weinmann, 1947 clarifies the term total available carbohydrates to be those carbohydrates used by the plant "as a source of energy or as building material, either directly or indirectly after having been broken down by enzymes." Total Nonstructural Carbohydrates (TNC) is considered to be those carbohydrates that are immediately available to the plant, mobilized for metabolism or translocated throughout the plant (Smith 1969). Smith (1969) prefers the term total nonstructural carbohydrates (TNC) as it is applicable for both plant and animal researchers.

4.2 CARBOHYDRATE STORAGE AND UTILIZATION

Those carbohydrates that are considered to provide the majority of reserves, the nonstructural carbohydrates are the reducing sugars (glucose and fructose), a non-reducing sugar (sucrose) and the fructosans and starches (White 1973). Fructose and glucose are monosaccharides, while sucrose and maltose are disaccharides, all of which are the prevailing sugars found in grasses. The storage carbohydrates, starches and fructosans, are nonstructural polysaccharides (Smith 1969). The structural carbohydrates which do not supply significant reserves include hemicellulose, the pentosans and hexosans, and cellulose (White 1973).

In general, the prevailing reserve constituent for temperate region grasses are sucrose and fructosans and sucrose and starch for tropical grasses (White 1973). In addition to the Poaceae, fructans are also the main reserve constituent of the Compositae (Duffus and Duffus 1984).

In plants, carbohydrates are typically transported as sucrose while in animals carbohydrates are transported as glucose. It is believed that this is due to the fact that sucrose is “less reactive and less easily metabolized than glucose” and therefore, not as easily metabolized during herbivory by the herbivore or other stressful event by the plants enzymes (Duffus and Duffus 1984). The degradation of these polysaccharides for use by the plant takes place through various enzymes, particularly fructan and other polysaccharide hydrolases. Regulation of this degradation by the numerous enzymes is usually determined by either pH, phosphate concentration and/or light activation (Duffus and Duffus 1984).

The chief storage regions for these grasses are not necessarily only in the underground organs, but also in the stem bases which include the rhizomes, stolons and corms. Although the nonstructural carbohydrates can be stored in all plant parts provisionally (White 1973).

Several grass species demonstrate a noticeable diurnal variation of carbohydrate reserves, increasing during the day to a highest point just before sunset and are at a lowest point just before sunrise. Seasonal variation of these reserves vary by species. For instance, many species with a low reserve level a month or so after first emergence obtain the lowest reserve level during or after seed ripening. The seasonal variation as well as the reserve rate are dependent on environmental conditions such as water and nutrient availability and temperature in addition to the development stage of the given plant (White 1973).

Temperate grass species and tropical grass species have different optimum growth and photosynthesis temperature levels. Optimum temperate species temperatures are 20 – 25° C and for tropical species, temperatures of 30 to 35° C are the most favorable. This dissimilarity is based on the different temperature optima of the major CO2 fixing enzymes involved, given that tropical grasses contain both C3 and C4 pathways and the temperate grasses possess only C3 pathways (White 1973).

4.3 *PHALARIS ARUNDINACEA* RHIZOME CARBOHYDRATE STORAGE

Several researchers have determined that the predominate polysaccharide reserves for reed canarygrass (RCG) are fructosans (Smith 1968, Reinhardt 2004). Smith (1968) specified that longchain fructosans prevail in RCG, however, many species that accumulate fructosans in this manor tend to contain short chain molecules during the time in the storage cycle where the TNC's are at their lowest point. Fructosans are fructose polymers found in two forms, inulins and levans, containing a terminal glucose residue and are water soluble (Smith 1969).

Reed canarygrass rhizomes predominately originate from buds at the nodes of other rhizomes below the soil surface. In a study from northern Ohio, new shoots for this species develop early in the season, around April and May in the mid-west, probably earlier here in the PNW, and in August until the end of the season. The shoots which developed early in the season are from rhizomes which developed late the prior year. Rhizomes which developed in mid season, such as May and June terminate into shoots that same season. New rhizome growth reached a maximum, double of any other month, in June of each year during this study as seen also by Evans and Ely (1935).

The rhizomes of RCG originate at a bud and grow outward, then turning upward to the surface producing an aboveground shoot. At this point, a new rhizome develops from a bud near the tip of the original rhizome or from a leaf axil and tends to grow in the same direction as the older one and a third rhizome also follows this same pattern. When comparing rhizome growth of five different species, one of which was RCG, Evans and Ely (1935) found that quackgrass was superior in the final diameter of the plant and rhizome length, showing the relationship between rhizome length and the area occupied by the plant. However, reed canarygrass was found to make up for less length by developing seven orders (number of branching events from the original rhizome) of new rhizomes during which most of the other species only formed four orders of rhizomes (Evans and Ely 1935). This may also explain the substantial density of the rhizome mass of reed canarygrass.

Restoration of wetlands invaded by RCG is particularly problematic as this species is extremely difficult to eradicate or control. In addition to the highly competitive nature of RCG and other plant characteristics described in Chapter One, the predisposition of the rhizomes to persist with stored energy and produce new shoots after treatment makes control exceptionally arduous (Lyons 2002, Apfelbaum and Sams 1987, Lavergne and Molofsky 2004, Reinhardt 2004). The ability to store the nonstructural carbohydrates in the rhizomes also allows RCG to successfully overwinter and produce new tillers early the next season as well as continue productivity later in the season, which increases the competitiveness of this species (Lavergne and Molofsky 2004). This mat of rhizomes can also produce a sod layer up to ½ a meter thick, making the establishment of native species challenging (Tu 2004). Additionally, the energy stored allows for the new tillers to actually lift and break through standard weed fabrics permitting continued growth of the plant from underneath.

Depleting the carbohydrate storage capacity of the RCG rhizomes is vital. Reducing the capability of the rhizomes to produce new plants after mechanical or chemical treatment would be advantageous for wetland restoration projects throughout western Washington.

The objective of this project is to determine how long a land manager should cover RCG rhizomes with opaque material for complete depletion of the carbohydrate reserves before removing the material for planting or before planting within the material.

4.4 CARBOHYDRATE RESERVE PROJECT DESCRIPTION

4.4.1 Carbohydrate Reserve Project Methods

Reed canarygrass rhizomes were analyzed for total nonstructural carbohydrate (TNC) reserves, or fructosans. Samples were taken during mid June of 2005 during anthesis, when the reserves are at their lowest point (Comes 1971, Reinhardt and Galatowitsch 2004). Forty-five rhizome samples of similar length and weight were randomly chosen and removed from the agriculture site.

Five gallon containers were placed in holes that had been excavated at the agriculture site, with the top of the containers level with the surrounding soil. The forty five rhizomes were placed within sterile potting soil in two gallon containers set within the five gallon containers with a root barrier placed at the bottom of the larger container so that any roots or rhizomes from potential growth could not escape. A thick, dense fabric was placed on top to inhibit any photosynthesis and stakes were used around the edges of the fabric. Fifteen samples were removed after three months and frozen. Another fifteen samples were removed after six months and the last fifteen after nine months and was stored in a frozen condition below 0° C.

A Fructan Assay Kit was obtained from Megazyme International Ireland Ltd., Wicklow, Ireland. This kit includes sucrose, fructanase, fructan control flour, sucrose control flour, and fructose standard solution. Additional chemicals required were ingredients for two buffers, a sodium maleate buffer and a sodium acetate buffer and several reagents; a PAHBAH reducing sugar assay reagent and alkaline borohydride (Megazyme Intl. 2004).

The samples were removed from the freezer, rinsed and dried at 70° C in a forced air oven at the Center for Urban Horticulture to a constant weight. The rhizomes were ground in a Wiley mill with a 0.5 mm screen for analysis. The samples for each of the three time frames (3,6,9 months) were combined and 10 replicates were analyzed for each time frame. Randomly selected samples were used to calibrate the NIRS identifying fructosan specific wavelength spectra.

The Megazyme kit employs purified enzymes to hydrolyse sucrose, starch and fructans. The sucrase enzyme "hydrolyses sucrose but has negligible activity on I-kestose and other fructo-oligosaccharides." The final reading solution is measured with the near-infrared spectrophotometer (NIRS) at the absorbance level of λ= 409.64 nm (Megazyme International Ireland Ltd., Wicklow, Ireland). The data were then calculated with the following equation: $\triangle$ A x F x 5 x V s 1.1/0.2 x 100/W x 1/1000 x 162/180

Where: $\triangle$A = sample absorbance – sample blank absorbance (read against reagent blank)
F = factor to convert absorbance values to µg of D-fructose (54.5 µg D-fructose) / (absorbance for 54.5 µg D-fructose)
5 = factor to convert from 0.2 mL as assayed to 1.0 mL
V = volume (mL) of extractant used
1.1/0.2 = 0.2 mL was taken from 1.1 mL of enzyme digest for analysis
W = weight (mg) of sample extracted
100/W = factor to express fructan as a percentage of flour weight
1/1000 = factor to convert from µg to mg
162/180 = factor to convert from free

D-fructose, as determined, to anhydrofructose (and anhydroglucose), as occurs in fructan This analysis took place at the Center for Urban Horticulture, University of Washington.

4.5. CARBOHYDRATE RESERVE PROJECT RESULTS

The results indicate an extensive variation of the fructosan levels within the rhizomes which were covered for three months among ten replicates analyzed. Five replicates were able to be analyzed for the six month batch and three replicates for the nine month batch. The average percent of fructosans for those rhizomes covered for three months is 2.25%, for the six month rhizomes, 1.65% and for the nine month rhizomes, 0.773% (Figure 4.1).

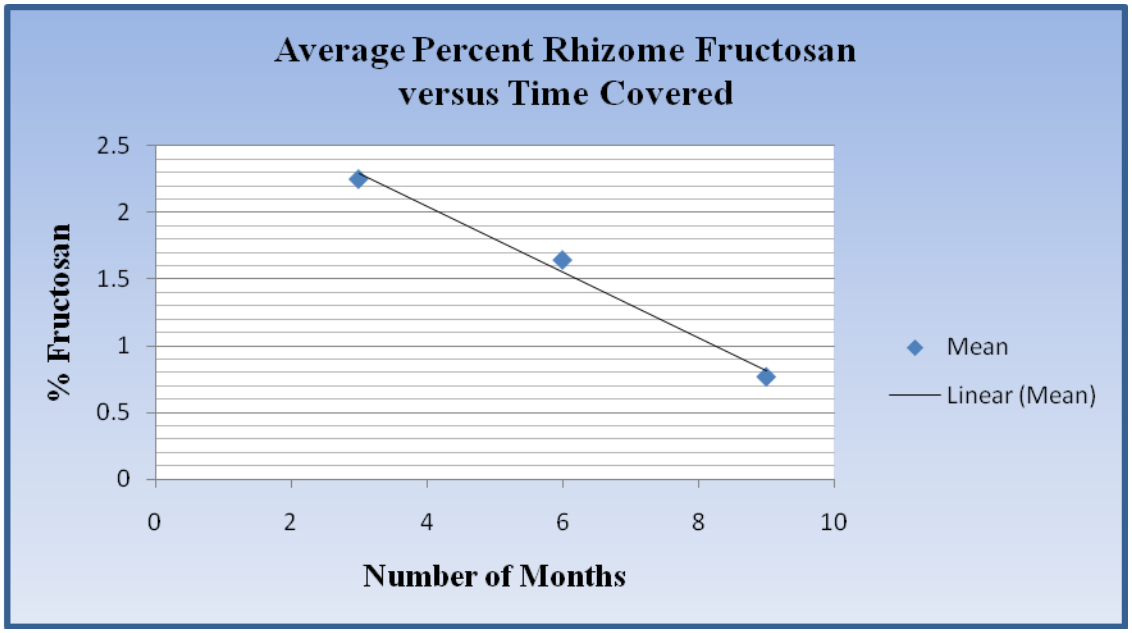


Figure 4.1. Average Percent Fructosan Level for three, six and nine months.

Due to the variability among individuals, the R^2 value of the regression of fructosan levels over time is very low (R^2 - 0.1262) (Figure 4.2).

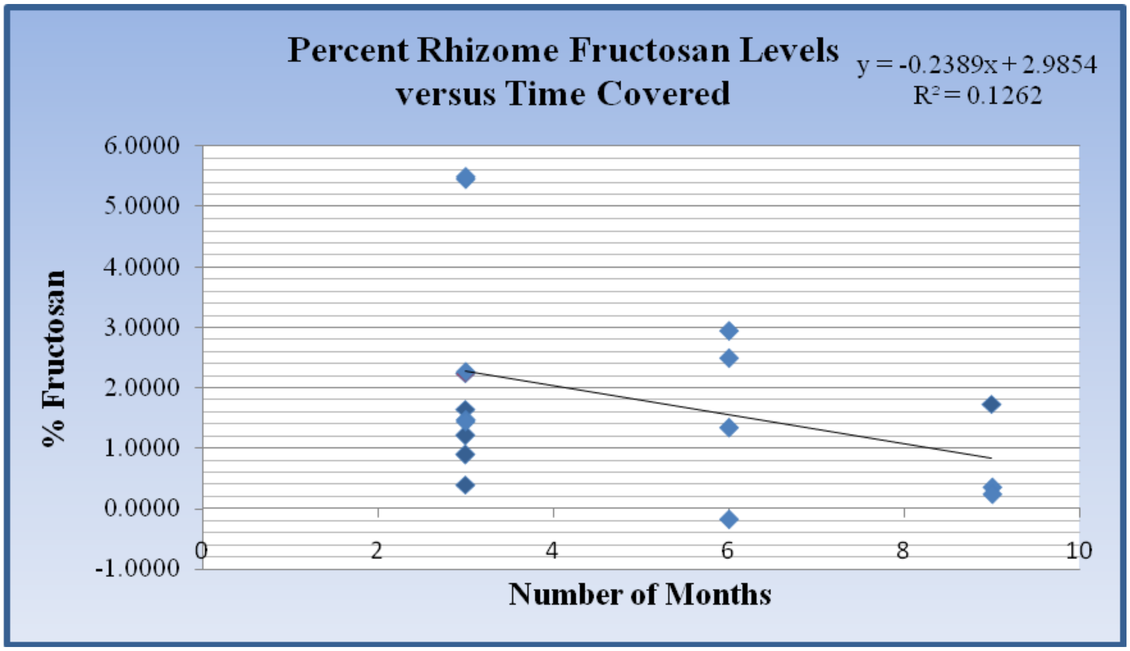


Figure 4.2. **Percent Rhizome Fructosan Levels for three, six and nine months.**

One rhizome sample within the six month batch was almost black after being removed from the field, while every other rhizome was tan or tanish pink. I read this sample out of curiosity. It did indeed give me a skewed fructosan result with a negative number of -0.18. After removing this sample as it was obviously a dead rhizome that had desiccated in the field, the R value decreasing further to a 0.11 (Figure 4.3). Also, after removing the dead rhizome, the means of the three and six month samples become equal at 2.25% compared to the 0.77% for nine month sample.

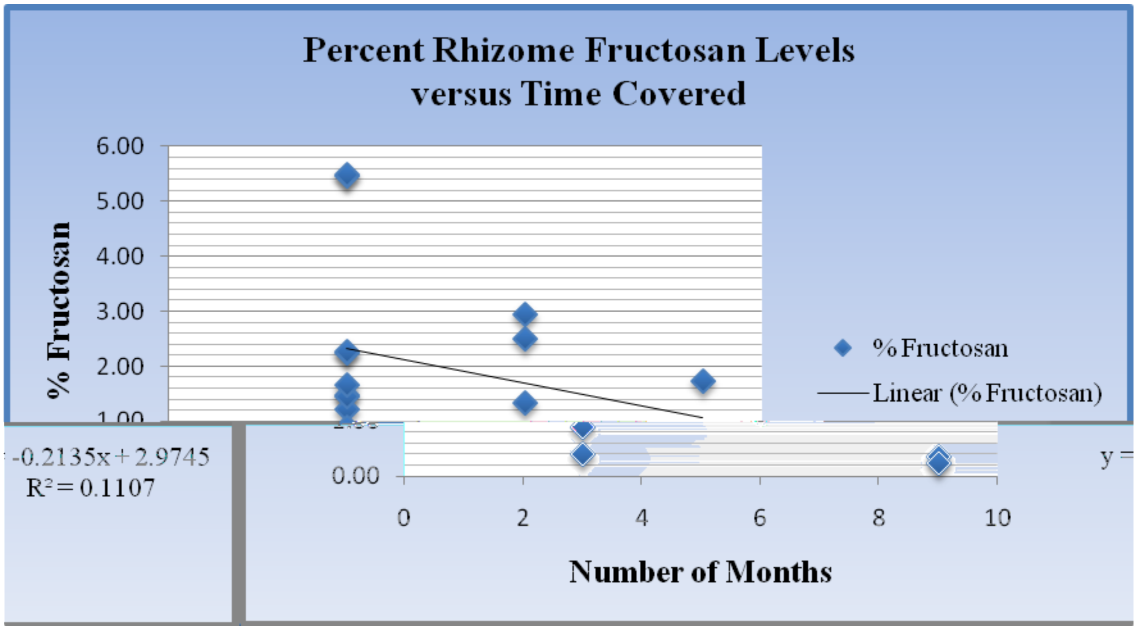


Figure 4.3. **Percent Rhizome Fructosan Levels for three, six and nine months after removing the dead rhizome (negative) data point.**

Log transforming the data does not improve the R value ($R^{2=}$.2) indicating that the variability is too high and there are not enough data to show a significant non-linear fit of the data to the line. However, log transforming the data for a Single Factor Analysis of Variance test (Single Factor ANOVA, df = 2, p = 0.08) indicated the lack of a difference between the three month mean and the six month mean. Yet, there is enough of a difference between the three and six month mean when compared to the nine month mean to provide a p-value that is marginally significant (Figure 4.4).

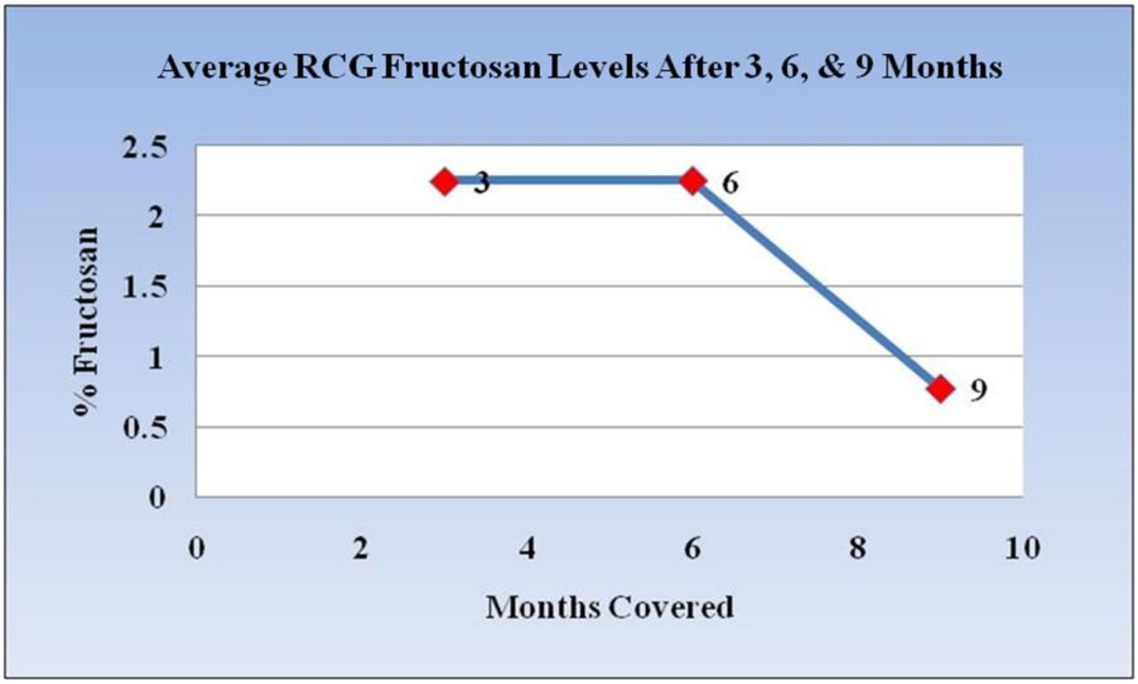


Figure 4.4. Mean RCG Fructosan Levels after removing negative data point.

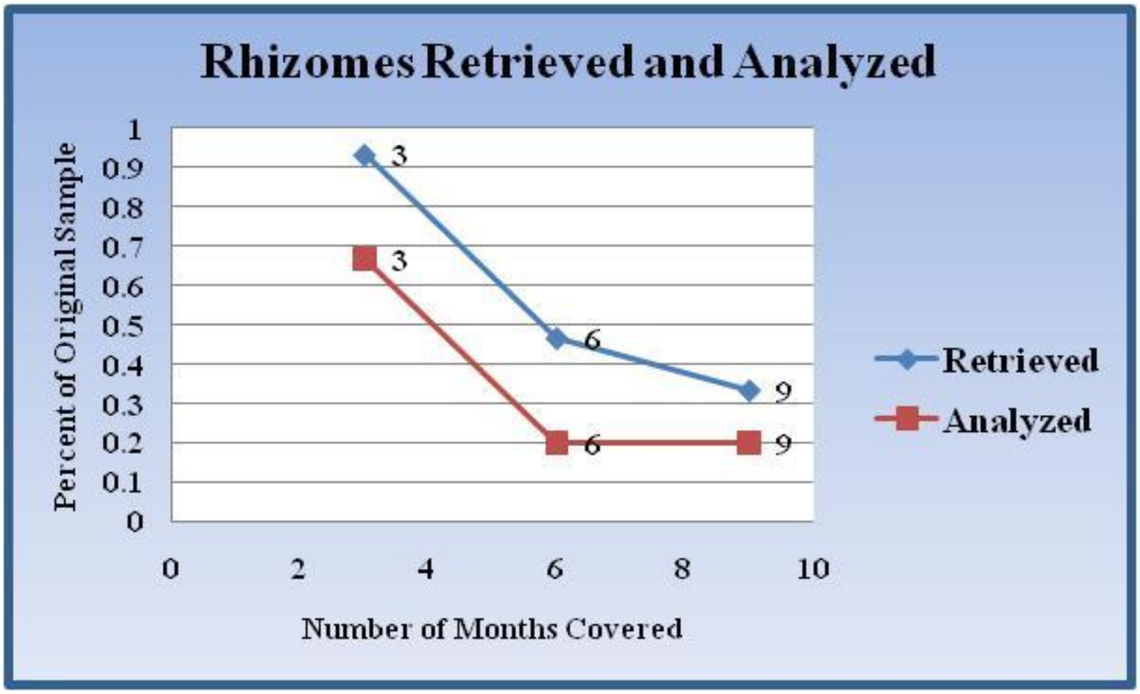


Figure 4.5. Percent Rhizomes Retrieved and Analyzed versus Original Sample.

4.6. CARBOHYDRATE RESERVE PROJECT DISCUSSION

One six month sample, replicate number two was considered an outlier with a value of 18.16% fructosans and was removed as it meets the standards of outliers for regressions, with eleven standard deviations above the mean. When examining the raw data for this sample, the sample absorbance was 1.5089% and 1.4635% which was much higher than every other sample absorbance in this experiment, ranging from 0.001 to 0.6 percent. All of the other rhizome samples that were analyzed in the same batch as this sample, with the same fructose and sucrose cellulose and 4D-Fructan standards, were normal.

Reinhardt (2004) analyzed RCG rhizomes every two weeks throughout the growing season in Minnesota to determine the seasonal variation and the most favorable time for herbicide application. As with many perennial species, the pattern of RCG reserves illustrated a decrease early in the season as the new shoots developed, followed by a leveling off in reserves as the plant matured and increased photosynthesis levels. Each year showed a marked decrease in reserves in early July at which point the plants were flowering and setting seed. At the end of July, the reserves began to increase until the end of the season. All three years strongly varied in the levels of carbohydrate content for Reinhardt's study, yet the carbohydrate levels followed a similar pattern. The rhizome content at flowering was estimated to be ~5% for the year 2000, ~ 33% for the year 2001, and ~17% for the year 2002 (Reinhardt 2004).

Comes (1971) also found the lowest seasonal carbohydrate level for RCG took place at the time of anthesis, increasing gradually in the fall. While looking at the TAC levels in the roots and rhizomes of RCG for plants treated with herbicide versus plants that were not treated, the untreated plants averaged between 26 and 33% of the total dry matter. It should be noted that this researcher used a different extraction method, sulfuric acid solution and it is therefore, difficult to compare the TAC levels with the levels that I obtained.

Although a decreasing trend in fructosan levels was observed over time, there was no significant difference between the three time periods that the rhizomes were covered. However, when averaging the data points for each of the three time frames, there is a significant drop in fructosan levels after the six month time frame from just over two percent to just under one percent for those rhizomes covered for nine months. The rhizome carbohydrate levels seem to persevere through the first six month period but after that time frame, when removed from the parent clone, the fructosan levels begin to drop consistently.

RCG rhizome health diminished as time separated from the parent clone passed from three months to nine months. The number of rhizomes found in the pots decreased over time, and the size of many of the rhizomes found dwindled from ~ten cm long with several nodes to some less than half of the original size (Figure 4.5).

The degradation of the rhizomes once separated from the clone indicates that tilling the rhizomes seems to weaken their ability to survive over time, especially when keeping the rhizomes from exposure to solar radiation. The reduction in rhizome numbers and size may indicate that the wounds caused by breaking apart the rhizome mat may permit entry of microorganisms which in turn could instigate decay of the rhizome. This type of infection is well known with wounds in the bark of trees potentially causing the death of the individual (Harris et al. 1999).

Additionally, the propensity of a rhizome fragment to degrade without the connection to a parental clone may signify that the connections are important during periods of stress. The connection would allow for the rhizome or ramet to draw from a larger resource supply permitting endurance rather than disintegration as with stressed isolated individuals (Tomasko and Dawes 1989). When examining the TNC levels in grazed and un-grazed *Carex lyngbyei* by Canadian geese, Crandell (2001) did not find a significant difference between the samples. She noted that overall belowground biomass may be a better indicator of fitness than TNC concentrations. In other words, the sum of carbohydrate reserves available to the RCG clone from the entire rhizome biomass may be more significant than the concentration of reserves. If this is accurate, removing the rhizomes from the parent clone and reducing the size of the rhizome material is paramount in winning the battle with RCG domination due to rhizome re-growth after typical control methods such as mowing or herbicide treatments.

The results from this project support that covering the tilled rhizomes for at least one year without disturbance or degradation of the shade material should significantly reduce the viability of the RCG rhizomes in the soil and most rhizomes should be completely depleted of fructosans. This should allow for the re-vegetation of the site with significantly reduced competition from reed canarygrass rhizome regeneration.

CHAPTER 5.0 CONCLUSIONS AND RECOMMENDATIONS

A flooding event took place in 1999 in which numerous salmon became trapped within agricultural and rural man made and/or maintained watercourses filled with the aggressive invasive plant, *Phalaris arundinacea* (reed canarygrass). This invasive also creates monocultures in riparian areas displacing native plants. Much of this agricultural and grazing land lies within river valleys and floodplains connected via these watercourses to major river systems within western Washington, most of which sustain salmon and trout (Washington Dept of Fisheries et al. 1993). Reed canarygrass (RCG) biomass and dominance in these watercourses is likely augmented by the nitrogen enriched agricultural runoff (Green and Galatowitsch 2002, Kercher and Zedler 2004). The accumulation of RCG biomass and subsequent sediment also cause flooding of the nearby agricultural fields increasing the practice of dredging. This escalation of disturbance by the landowner decreases habitat quality for native wildlife, invertebrates and fish as well as increasing the ability of RCG to flourish as shown by Kercher and Zedler (2004).

Two of the treatments tested in this research project were successful in reducing the RCG biomass on the site and re-establishing a vegetated riparian buffer dominated by native wetland species. As Blumenthal et al. (2003) found in prairie systems and Davis (2000) found in wetland systems, the application of mulch as a nitrogen reducer and invasive plant suppressor was significant. The hogfuel added, densely planted willow treatment and hogfuel added and the densely planted multi-canopy treatment (RCG barrier) reduced the returning RCG biomass by 64% and 56% respectively compared to the control RCG stem counts. The RCG barrier alone treatment was different than the controls and therefore, adding the hogfuel delivered the additional weight and solar radiation reduction necessary to suppress RCG re-growth.

The red cedar hogfuel did show allelopathic tendencies in the greenhouse, however whether allelopathy played a role in the reduction of the returning RCG count in the field was not tested.

Two species in particular were found to be reasonably competitive in the field with RCG, *Salix sitchensis* and *Scirpus microcarpus*. During the first two years of the research project, it was observed that *Scirpus microcarpus*, (small fruited bulrush (SFB)) covered almost the entire half of the plot next to watercourse at the livestock site and successfully straddled the watercourse, yet in a thinner band at the agriculture site. After testing for competitive interactions with RCG in a controlled setting, the results were too variable to obtain statistically significant results. However, positive trends were certainly observed within the high nitrogen treatments with a notable reduction of RCG total biomass. In situations with high nitrogen and sunlight, using SFB in emergent wetland restoration projects is suggested. As noted in Chapter Three, the competitive interactions between SFB and RCG may be overly symmetrical and SFB may only outcompete RCG in specific situations, but in the very least, SFB should be able to co-exist with RCG retaining space in the wetland for a native emergent.

Salix willow stakes (*Salix sitchensis*) planted densely at 0.5 meter centers within the hogfuel was the most successful treatment overall for this study for two of the three sites. Kim et al. (2006) also found significant results when testing *Salix lasiandra* and *Salix scouleriana* competitively against RCG at the 0.60 and 0.91 meter spacing. These treatments reduced the RCG biomass by 68% and 56% respectively.

Two of my field sites were impacted heavily by the willow borer (*Cryptorhynchus lapathi*). Ewing (personal communication, 2008) also affirmed that willows used in restoration projects at the University of Washington’s Union Bay Natural Area have been attacked by the willow borer. Newly emerged adults consume the bark, primarily of young stems. In the larvae stage, this weevil bores into the bark, wood and into the pith of the tree, for feeding excavating tunnels (Rosetta 2006). This causes many of the branches and in some cases, the entire main stem to break completely off of the tree. However, these willows are so robust that most grew back, with dense low branches. It took two years for the trees to get as tall as they were before the attack at the sites in this study, yet, it only took about a year for the trees at the UW UBNA site. The non-native willow borer is something to consider when using willows for RCG control and restoration projects. Furthermore, several of the landowners considered the willows to be quite "weedy" within the watercourses and difficult to eradicate (Galoach personal communication 2008, Quigley personal communication 2005, Calhoun personal communication 2005).

Carbohydrate reserves seem to vary for the first six months, yet after nine months of being covered by an opaque material, drop to less than one percent. These levels indicate the drop in fructosans when the rhizomes are removed from the parent plant, or tilled, at flowering, which is mid July here in the Pacific Northwest. If one is able to till the site, the results imply that the reserves should be depleted after one year of being covered. Tilling is also important as this management technique breaks the individual plants away from the connection with other clones. When looking at the effects of shading on RCG tiller expansion, Maurer and Zedler (2002) detected that those plants which were connected to parental clones were not impacted by the shading treatment.

The hogfuel added, RCG barrier treatment proved to be significant in this study. The multi-canopy layers suppressed the RCG re-growth while providing higher biodiversity and structural diversity for the site. Lindig-Cisneros and Zedler (2002) established that a treatment of 15 species reduced RCG survival and growth from seed by 48% when compared to a single species treatment, especially when canopy gaps exist. Maurer and Zedler (2002) verified similar results when looking at the re-growth of rhizome fragments under dense canopies versus a more open canopy. Other factors which favor a multi-canopy treatment are consideration for wildlife and invertebrate habitat quality.

Wipfli (1997) found that terrestrial invertebrates were as important for certain salmonid species as aquatic invertebrates during the spring, summer and fall and that the riparian vegetation influences the terrestrial invertebrates available for salmonids. A compact and diverse lower canopy layer with a broadleaf alder upper canopy provided a more diverse and productive terrestrial invertebrate community. Alan et al. (2003) went further in looking at specific over and understory species and their contribution of invertebrate mass. Salmonberry (*Rubus spectabilis*), currant (*Ribes sp*.) and blueberry (*Vaccinium ovalifolium* and *V. alaskaense*) and alder (*Alnus rubra*) were all noted as contributing a high level of invertebrate biomass and therefore should be considered when looking at species for re-vegetation projects in the PNW. Alder was not used in this project as it is a nitrogen fixer and RCG is understood to be a nitrophilic plant, yet alder could be used after the RCG is under control or in other re-vegetation situations.

Based on the conclusions from this research, other research projects, relevant literature and observations in the field, my recommendations for RCG control and riparian re-vegetation and restoration are as follows:

Recommendations:

1) If tilling is possible on the site, till the site and cover with a thick and dense opaque material for at least one year without disturbing. This fabric should not break down for several years and should be staked down securely with stakes or mulch/hogfuel. Tilling the site will also allow for increased microtopography which will also allow for increased species diversity (Maurer et al. 2003).

2) Using hogfuel is recommended. The thick, dense, potentially allelopathic material suppressed RCG re-growth in this study.

3) Plant a dense multi-canopy of native vegetation. Species should be chosen which will supply multiple canopies providing dense shade for additional invasive plant control, a preferred assembly of invertebrates and habitat diversity.

4) Plant species that have characteristics which allow the species to compete effectively with RCG. These characteristics include: fast growing, leafing out early in the season, high leaf area index for shading.

LITERATURE CITED

Aarssen, L.W. 1983. Ecological Combining Ability and Competitive Combining Ability in Plants: Toward a General Evolutionary Theory of Coexistence in Systems of Competition. The American Naturalist 122:6 707-731.

Allan, J.D., Wipfli, M.S., Caouette, J.P., Prussian, A. and J. Rodgers. 2003. Influence of streamside vegetation on inputs of terrestrial invertebrates to salmonid food webs. Canadian Journal of Fish and Aquatic Science 60: 309-320.

Allendorf, F and L.L. Lundquist. 2003. Introduction: Population Biology, Evolution, and Control of Invasive Species. Conservation Biology 17:1 24

Anderson, E. and G.L. Stebbins, Jr. 1954. Hybridization as an Evolutionary Stimulus. Evolution 8: 378-388.

Anderson, N.O. 2006. Prevention of Invasiveness in Floricultural Crops. In Flower Breeding and Genetics: Issues, Challenges and Opportunities for the 21st Century. Springer Publications, Netherlands 177-214.

Antieau, C.J. 1998. Biology and Management of Reed Canarygrass, and Implications for Ecological Restoration. Washington State Department of Transportation.

Apfelbaum, S.I. and C.E. Sams. 1987. Ecology and Control of Reed Canarygrass (*Phalaris arundinacea* L.). Natural Areas Journal 7:2 69-73.

Baker, H.G. 1953. Race formation and reproductive method in flowering plants. Syrup Soc. Exp. Biology 7: 114-145.

Baker, H.G. 1974. The Evolution of Weeds. Annual Reviews Ecol. Syst. 5: 1-24.

Basu, C., Halfhill, M.D., Mueller, T.C. and C.N Stewart Jr. 2004. Weed genomics: new tools to understand weed biology. Trends in Plant Science 9:8 391-398.

Blumenthal, D.M., Jordan, N.R. and M.P. Russelle. 2003. Soil Carbon Addition Controls Weeds and Facilitates Prairie Restoration. Ecological Applications 13:3 605-615.

Boateng, J.O. and P. Comeau. 2002. Operational Summary for Vegetation Complexes, Salal Complex
http://www.for.gov.bc.ca/hfp/publications/00052/salal.pdf

Boersma, P.D, Reichard, S.H. and A.N. Van Buren. 2006. Invasive Species in the Pacific Northwest. University of Washington Press. Seattle, Washington.

Bonsall, M, PhD. 2007. Dept of Biological Sciences, Imperial College of London. http://www.bio.ic.ac.uk/research/mbbon/Ecology.html

Booth, B.D., Murphy, S.D. and J. Swanton. 2003. Plant Invasions, In: Weed Ecology in Natural and Agricultural Systems. CABI Publishing

Bossdorf, O., Auge, H., Lafuma, L., Rogers, W.E., Siemann, E. and D. Prati. 2005. Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1 1-11.

Callaway, R.M. and Aschehoug, E.T. 2000. Invasive Plants Versus Their New and Old Neighbors: A Mechanism for Exotic Invasion. Science 290 521-523.

Casper, B.B. and R.B. Jackson. 1997. Plant Competition Underground. Annual Rev. Ecol. Syst. 28 545-570.

Comes, R.D. 1971. The Biology and Control of Reed Canarygrass (*Phalaris arundinacea* L.) on Irrigation Ditchbanks. Doctoral Dissertation. Oregon State University.

Connell, J.H. 1983. On the Prevalence and Relative Importance of Interspecific Competition: Evidence From Field Experiments. The American Naturalist. 122:5 661-696.

Coops, H., van der Brink, F.W.B., and van der Velde, G. 1996. Growth and morphological responses of four helophyte species in an experimental water depth gradient. Aquatic Botany 54: 11-24.

Corbin, J.D. and C.M. D'Antonio. 2004. Can Carbon Addition Increase Competitiveness of Native Grasses? A Case Study from California. Restoration Ecology 12:1 36-46.

Cowardin, L.M., Carter, V., Golet, F.C., and E.T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. U.S. Department of the Interior. Fish and Wildlife Service, Washington, D.C. Jamestown, ND: Northern Prairie Wildlife Research Center Home Page. http://www.npwrc.usgs.gov/resource/1998/classwet.htm (Version 04DEC98).

Crandell, C. 2001. Effect of Grazing by *Branta canadensis* (Canada Geese) on the Fitness of *Carex lyngbyei* (Lyngbye's sedge) at a Restored Wetland in the Duwamish River Estuary. M.S. Thesis. University of Washington.

Crawley, M.J. 1990. The Population Dynamics of Plants. Philosophical Transactions: Biological Sciences. 330: 125-140

Crockett, R.P. 1996. Biology and Control of Reed Canarygrass (*Phalaris arundinacea* L.) PNW EPPC Newsletter, Summer/Fall 1996.

D'Antonio, Carla M. and P. M. Vitousek. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. Annual Review of Ecology and Systematics 63-87.

Darwin, C. 1958. The Autobiography of Charles Darwin, with original omissions restored. Edited with Appendix and Notes by his grand-daughter, Nora Barlow. Collins Clear-Type Press, London and Glasgow.

Davis, K.J. The Effects of Nitrogen Manipulation and Hydrology on the Establishment and Competitive Abilities of Wetland Prairie Plant Species (Western Oregon). 2000. Doctor of Philosophy Dissertation. Oregon State University.

de Kroon, H. and Bobbink, R. 1997. Clonal Plant Dominance Under Elevated Nitrogen Deposition, With Special Reference to *Brachypodium pinnatum* in Chalk Grassland. In: The Ecology and Evolution of Clonal Plants. Edited by: de Kroon, H. and van Groenendael, J. Backhuys Publishers, Leiden.

Duffus, C.M. and J.H. Duffus. 1984. Carbohydrate metabolism in plants. Longman Press, London, New York.

Duryea, M.L., English, R.J. and L.A. Hermansen. 1999. A Comparison of Landscape Mulches: Chemical, Allelopathic, and Decomposition Properties. Journal of Arboriculture 25;2 88-96.

Ellstrand NC, and K.A. Schierenbeck. 2006. Hybridization as a stimulus for the evolution of invasiveness in plants. Euphytica 148:1-2 35-46.

Elton, C.S. 1958. The ecology of invasions by animals and plants. Methuen Press, London.

Evans, M.W. and Ely, J.E. 1935. The Rhizomes of Certain Species of Grasses. Journal of the American Society of Agronomy 791-797.

Facon, B., Genton, B.J., Shykoff, J., Jarne, P., Estoup, A., David, P. 2006. A general eco-evolutionary framework for understanding bioinvasions. Trends in Ecology and Evolution 21:3 130-135.

Federal Register V 64-No. 25, February 8, 1999 / Presidential Documents. Executive Order 13112 of February 3, 1999.
http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=1999_register&docid=99-3184-filed.pdf

Flora of North America Association. 2007. http://www.efloras.org/florataxon.aspx?flora_id=1&taxon_id=242357969

Forman, D.J., L.H. Hardesty and R.D. Sayler. 2000. Reed Canarygrass Working Group Conference. March 15, 2000.

Gaudet, C.L. and P.A. Keddy. 1988. A comparative approach to predicting competitive ability from plant traits. Nature 334: 242-243.

Gaudet, C.L. and P.A. Keddy. 1995. Competitive Performance and Species Distribution in Shoreline Plant Communities: A Comparative Approach. Ecology 76:1 280-291.

Granger, T., Hruby, T., McMillan, A., Peters, D., Rubey, J., Sheldon, D., Stanley, S., and E. Stockdale. 2005. Wetlands in Washington State – Volume 2: Guidance for Protecting and Managing Wetlands. Washington State Department of Ecology. Publication #05-06-008. Olympia, Washington.

Graustein, J.S. 1995. Competitive Effect of an Invasive Grass, *Phalaris arundinacea* L. on the Growth of a Rhizomatous Sedge, *Carex stricta* LAM. M.S. Thesis, Northern Illinois University.

Green, E.K. and Galatowitsch, S.M. 2001. Differences in wetland plant community establishment with additions of nitrate-N and invasive species (*Phalaris arundinacea* and *Typha xglauca*). Canadian Journal of Botany 79:2 170-178.

Green, E.K. and Galatowitsch, S.M. 2002. Effects of *Phalaris arundinacea* and nitrate-N addition on the establishment of wetland plant communities. Journal of Applied Ecology 39:1 134.

Grime, J.P. 1977. Evidence for the Existence of Three Primary Strategies in Plants and its Relevance to Ecological and Evolutionary Theory. The American Naturalist 111: 1169-1194.

Grime, J.P. 1979. Plant strategies and vegetation processes. John Wiley, New York, New York.

Grime, J.P. and J.G. Hodgson. 1987. Botanical Contributions to Contemporary Ecological Theory. New Phytologist 106:1 283-295.

Guard, B.J. 1996. Wetland Plants of Oregon and Washington. Lone Pine Publishing. Redmond, Washington.

Gunti, D., Burgos, S., Kump, I., Heeb, M., Pfirter, HA and Defago, G. 1999. Biological control of hedge bindweed (*Calystegia sepium*) with *Stagonospora convolvuli* strain LA39 in combination with competition from red clover (*Trifolium pretense*). Biological Control 15:3 252-258.

Harris, R.W., Clark, J.R. and N.P. Matheny. 1999. Aboriculture, Integrated Management of Landscape Trees, Shrubs, And Vines. Prentice-Hall, Inc., New Jersey.

Ingles, L.G. 1961. Home Range and Habitats of the Wandering Shrew. Journal of Mammalogy 42:4 455-462.

Johansson, M.E. and P.A. Keddy. 1991. Intensity and asymmetry of competition between plant pairs of different degrees of similarity: an experimental study on two guilds of wetland plants. Oikos 60: 27-34.

Jurries, D. 2003. State of Oregon, Dept of Environmental Quality, Biofilters – Bioswales, Vegetative Buffers, & Constructed Wetlands For Storm Water Discharge Pollution Removal.
http://www.deq.state.or.us/wq/stormwater/docs/nwr/biofilters.pdf

Kantor, Sylvia. 1998. Farming in King County: A Treasure in Peril. WSU Cooperative Extension, King County
http://king.wsu.edu/foodandfarms/documents/FarmInKC.pdf

Keddy, P. and B. Shipley. 1989. Competitive hierarchies in herbaceous plant communities. Oikos 54: 234-241.

Keddy, P., Fraser, L.H. and I.C. Wisheu. 1998. A comparative approach to examine competitive response of 48 wetland plant species. Journal of Vegetation Science 9: 777-786.

Kercher, S.M. and J.B. Zedler. 2004. Multiple disturbances accelerate invasion of reed cnaarygrass (*Phalaris arundinacea* L.) in a mesocosm study. Oecologia 138: 455-464.

Kim, K.D., Ewing, K. and D.E. Giblin. 2006. Controlling *Phalaris arundinacea* (reed canarygrass) with live willow stakes: A density-dependent response. Ecological Engineering 27:3 219-227.

Klimes, L, Klimesova, J. Hendriks, R. and J. van Groenendael. 1997. Clonal Plant Architecture: A Comparative Analysis of Form and Function. In: The Ecology and Evolution of Clonal Plants. Edited by: de Kroon, H. and van Groenendael, J. Backhuys Publishers, Leiden.

La Force, M.J., Hansel, C.M. and S. Fendorf. 2002. Seasonal Transformations of Manganese in a Palustrine Emergent Wetland. Soil Science Society of America Journal 66: 1377-1389.

Lavergne, S. and J. Molofsky. 2004. Reed Canary Grass (*Phalaris arundinacea*) as a Biological Model in the Study of Plant Invasions. Critical Reviews in Plant Sciences 23: 5 1-15.

Lavergne, S. and J. Molofsky. 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. PNAS 104:10 3883-3888.

Lee, B.D., Graham R.C., Laurent, T.E., Amrhein, C. and R. M. Creasy. 2001. Spatial Distributions of Soil Chemical Conditions in a Serpentinitic Wetland and Surrounding Landscape. Soil Science Society of America Journal 65: 1183-1196.

Levine, J.M. and D'Antonio, C.M. 1999. Elton revisited: a review of evidence linking diversity and invisibility. Oikos 87:1 15-26.

Lindig-Cisneros, R. and J.B. Zedler. 2001. *Phalaris arundinacea* seedling establishment: effects of canopy complexity in fen, mesocosm, and restoration experiments. Canadian Journal of Botany 80: 617-624

Lyons, K.E. 1998. Elemental Stewardship Abstracts for Reed Canarygrass. Edited by: J.M. Randall, M. Robison, T. Morisawa, B. Meyers-Rice. The Nature Conservancy, Wildland Invasive Species Program.
http://tncweeds.ucdavis.edu/esadocs/documnts/phalaru.pdf

Magee, T.K. and Kentula M.E. 2005. Response of wetland plant species to hydrologic conditions. Wetlands Ecology and Mangement 13: 163-181.

Mallik, A.U., Lamb, E.G. and H. Rasid. 2001. Vegetation zonation among the microhabitats in a lacustrine environment: analysis and application of belowground species trait patterns. Ecological Engineering 18:2 135-146

Maurer, D.A. 2001. Effects of light availability and resource subsidy on the vegetative spread of *Phalaris arundinacea* L. (Reed Canarygrass) in wetlands. M.S. University of Wisconsin-Madison.

Maurer, D.A. and J.B Zedler. 2002. Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. Oecologia 131: 279-288.

Maurer, D.A., Lindig-Cisneros, R., Werner, K.J., Kercher, S., Miller, R. and J.B. Zedler. 2003. The Replacement of Wetland Vegetation by Reed Canarygrass (*Phalaris arundinacea*) Ecological Restoration 21:2 116-119.

Megazyme International. 2004. Fructan Assay Procedure for the measurement of Fructo-oligosaccharides (FOS) and Fructan Polysaccharide. AOAC Method 999.03, AACC Method 32.32.

Merigliano, M.F. and P. Lesica. 1998. The Native Status of Reed Canarygrass (*Phalaris arundinacea* L.) in the Inland Northwest, USA. Natural Areas Journal 18:3 223-230.

Mockler, A. Casey, L. Bowles, M, Gillen, N., Hansen, J. 1998. Results of Monitoring King County Wetland and Stream Mititgations. King Conty Dept of Development and Environmental Services.

Moxy Media. 2007. The Carpet Site. An Online Consumers' Guide to Carpet and Other Flooring Products. http://www.thecarpetsite.com/about.aspx

Naglich, F.G. 1994. Reed Canarygrass (*Phalaris arundinacea* L.) in the Pacific Northwest: Growth Parameters, Economic Uses, and Control. Master's Thesis. The Evergreen State College.

Norberg, G. Jaderlund, A., Zackrisson, O., Nordfjeli, T., Wardle, D.A., Nilsson, M.C. and A. Dolling. 1997. Vegetation control by steam treatment in boreal forests: a comparison with burning and soil scarification. Canadian Journal Forest Res. 2026-2033.

Park, S.E., Benjamin, L.R. and Watkinson, A.R. 2003. The Theory and Application of Plant Competition Models: an Agronomic Perspective. Annals of Botany 92: 741-748.

Patterson, L. and D.J. Cooper. 2007. The use of hydrologic and ecological indicators for the restoration of drainage ditches and water diversions in a mountain fen, cascade range, California. Wetlands 27:2 290-304.

Perry, L.G. and S.M. Galatowitsch. 2003. A Test of Two Annual Cover Crops for Controlling *Phalaris arundinacea* Invasion in Restored Sedge Meadow Wetlands. Restoration Ecology 11:3 297-307.

Perry, L.G. and S.M. Galatowitsch. 2004. The influence of light availability on competition between *Phalaris arundinacea* and a native wetland sedge. Plant Ecology 170: 73-81.

Perry, L.G., Galatowitsch, S.M. and C.J. Rosen. 2004. Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. Journal of Applied Ecology 41: 151-162.

Pigliucci, M. 2002. Distributed by the Pigliucci Lab. University of Tennessee. http://fp.bio.utk.edu/pgl, http://www.bio.unc.edu/biol258/fall02/Plasticity_101.pdf.

Pimentel, D., Lach, L, Zuniga, R. and D. Morrison. 2000. Environmental and Economic Costs of Nonindigenous Species in the United States. Bioscience 50:1 53-61.

Plants for a Future. 2007.
http://www.ibiblio.org/pfaf/cgi-bin/arr_html?Scirpus+microcarpus

Pojar, J. and A MacKinnon. 1994. Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia & Alaska. Lone Pine Publishing, Redmond, Washington.

Pysek, P. 1997. Clonality and plant invasions: Can a trait make a difference?
The ecology and evolution of clonal plants 405-427.

Pysek, P. and D.M. Richardson 2006. The biogeography of naturalization in alien plants. Journal of Biogeography 33:12 2040.

Quarles, W. 2001. Improved Hot Water Weed Control System. The IPM Practitioner 23:1 1-4.

Rathinasabapathi, B. 2005. Evaluation of allelopathic potential of wood chips for weed suppression in horticultural production systems. HortScience 40:3 711-713.

Reever Morghan, K.J. and T.R. Seastedt. 1999. Effects of Soil Nitrogen Reduction on Nonnative Plants in Restored Grasslands. Restoration Ecology 7: 1 51-58.

Reichard, S.E. 1997. Prevention of Invasive Plant Introductions on National and Local Levels. In Assessment and Management of Plant Invasions. Edited By: J.O. Luken and J.W. Thieret. Springer Publications.

Reichard, S.H. and Hamilton, C.W. 1997. Predicting Invasions of Woody Plants Introduced into North America. Conservation Biology 11:1 193-203.

Reichard, S. 1998. Reducing the Spread of Invasive Weeds. Hortus West 9:1 pg 22.

Reichard, S.H. and P. White. 2001. Horticulture as a Pathway of Invasive Plant Introductions in the United States. Bioscience 51:2 103-113.

Reichard, S.H. and P. White. 2003. Invasion Biology: An Emerging Field of Study. Annals of Missouri Bot. Gard. 90: 64-66.

Reinhardt, C. and S.M. Galatowitsch. 2004. Best Management Practices for the Invasive *Phalaris arundinacea* L. (Reed canarygrass) in Wetland Restorations. Final Report for the Minnesota Department of Transportation, Office of Research Services.

Rejmànek, M. 1996. A Theory of Seed Plant Invasiveness: The First Sketch. Biological Conservation 78 171-181.

Rejmànek, M. and Richardson, D.M. 1996. What Attributes Make Some Plant Species More Invasive. Ecology 77:6 1655-1661.

Richardson, D.M. 2004. Plant invasion ecology – dispatches from the front line. Diversity and Distributions 10, 315-319.

Rosch, H., Van Rooyen, M.W. and G.K. Theron. 1997. Predicting competitive interactions between pioneer plants by using plant species traits. Journal of Vegetation Science 8: 489-494.

Rosetta, R. 2006. Oregon State University, Horticulture Dept., Pacific Northwest Nursery IPM. http://oregonstate.edu/dept/nurspest/poplar_and_willow_borer.htm

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. and S.G. Weller. 2001. The Population Biology of Invasive Species. Ecology and Systematics 32:1 305

Schuller, R. 2006. High Peak/Moon Creek Research Natural Area, Guidebook Supplement 30, USDA, FS PNW Research Station. General Tech Report PNW-GTR-673.

Schuylerae. 1976. Chromosome Numbers of Some Eastern North American Species of *Scirpus*. Bartonia 44: 27-31.

Sebens, K.P. and B.L. Thorne. 1985. Coexistence of Clones, Clonal Diversity, and the Effects of Disturbance. In: Population Biology and Evolution of Clonal Organisms. Edited by: J. Jackson, L.W. Buss, R.E. Cook. Yale University Press, New Haven and London.

Seebacher, L. 1999. Unpublished data. Survey of Restoration Ecologist in Western Washington.

Simberloff, D. and B. Von Holle. 1999. Positive Interactions of Nonindigenous Species: Invasional Meltdown? Biological Invasions 1:1 21-32.

Smith, D. 1968. Classification of Several Native North American Grasses as Starch or Fructosan Accumulators in Relation to Taxonomy. Journal of the British Grassland Society 23:4 306-309.

Smith, D. 1969. Removing and Analyzing Total Nonstructural Carbohydrates From Plant Tissue. Research Division, college of Agriculture and Life Sciences, Madison, Wisconsin. Research Report 41, 11p.

Stannard, M. and W. Crowder. 2001. Biology, History, and Suppression of Reed Canarygrass (*Phalaris arundinacea* L.). U.S. Department of Agriculture, Natural Resources Conservation Service, Plant Materials #43.

Stevens, M.L. and Vanbianchi, R. 1993. Restoring Wetlands in Washington. A Guidebook for Wetland Restoration Planning and Implementation. Publication #93-17. Washington Dept of Ecology.

Stohlgren, T.J., Barnett, D.T. and J.T. Kartesz. 2003. The rich get richer: patterns of plant invasions in the United States. Front Ecol Environ 1:1 11-14.

Stout, T. 2002. Potential Problems Associated with Mulches. Center for Urban Horticulture, University of Washington. http://students.washington.edu/tls2/Potential%20Problems%20Associated%20with%20Mulch.

Stuckey, R.L. 1978. Essays on North American Plant Geography from the Nineteenth Century. New York Arno Press New York, New York.

Tilman, D. 1997. Mechanisms of Plant Competition. In: Plant Ecology, Edited by: M.J. Crawley p 239. Humana Press.

Tilman, D. 1999. The Ecological Consequences of Changes in Biodiversity: A Search for General Principles. Ecology 80:5 1455-1474.

Tomasko, D.A. and C.J. Dawes. 1989. Evidence for physiological integration between shaded and unshaded short shoots of *Thalassia testudinum*. Marine Ecology Progress Series, 54: 299-305.

Treberg, M.A. and B.C. Husband. 1999. Relationship between the abundance of *Lythrum salicaria* (purple loosestrife) and plant species richness along the Bar River, Canada. Wetlands 19:1 118-125.

Tu, M. 2004. Reed Canarygrass (Phalaris arundinacea L.) Control & Management in the Pacific Northwest. The Nature Conservancy's Wildland Invasive Species Team. The Nature Conservancy, Oregon Field Office. http://tncweeds.ucdavis.edu/moredocs/phaaru01.pdf

Tu, M. 2006. *Phalaris arundinacea* L. In: Invasive Species in the Pacific Northwest. Edited by: Boersma, D., Reichard, S.H. and An Van Buren.

Van der Velde, G., Rajagopal, S., Kuyper-Kollenaar, M., bij de Vaate, A., Thieltges, D.W. and MacIsaac, H.J. 2006. Ecological Studies 191, In: Wetlands: Functioning, Biodiversity Conservation, and Restoration. Edited by: Bobbink, B., Beltman, B., Verhoeven, J.T.A., Whigham, D.F. Springer Publishing – Verlag Berlin Heidelbirg, New York.

Washington Department of Fisheries, Washington Department of Wildlife and the Western Washington Treaty of Indian Tribes. 1993.

Weinmann, H. 1947. Determination of total available carbohydrates in plants. Plant Physiolgy 22: 279-280.

Weinmann, H. 1948. Underground development and reserves of grasses. A review. Journal of British Grassland Society. 3: 115-140.

Welch, B., Geissler, P., Latham, P., Beard, K., Brooks, M., Cutler, R., Edwards, T., Klinger, R., Loope, L., O'Neil, S., Sarr, D., Schrader, S. and K. Young. 2007. Early Detection of Invasives Handbook. USGS – NPS Inventory and Monitoring.

Wetzel, P.R. and A.G. van der Valk. 1998. Effects of nutrient and soil moisture on competition between *Carex stricta, Phalaris arundinacea,* and *Typha latif*olia. 1998. Plant Ecology 138: 179-190.

White, L.M. 1972. Carbohydrate Reserves of Grasses: A Review. Journal of Range Management 26:1 13-18.

Williamson, M. H., and K. C. Brown. 1986. The analysis and modelling of British invasions. Philosophical Transactions of the Royal Society 314: 505-522.

Williamson, M.H. and A. Fitter. 1996. The Characters of Successful Invaders. Biological Conservation 78 163-170.

Williamson, M.H. and A. Fitter. 1996. The Varying Success of Invaders. Ecology 77:6 1661-1666.

Wilson, S.D. and D. Tilman. 1991. Components of Plant Competition Along an Experimental Gradient of Nitrogen Availability. Ecology 72:3 1050-1065.

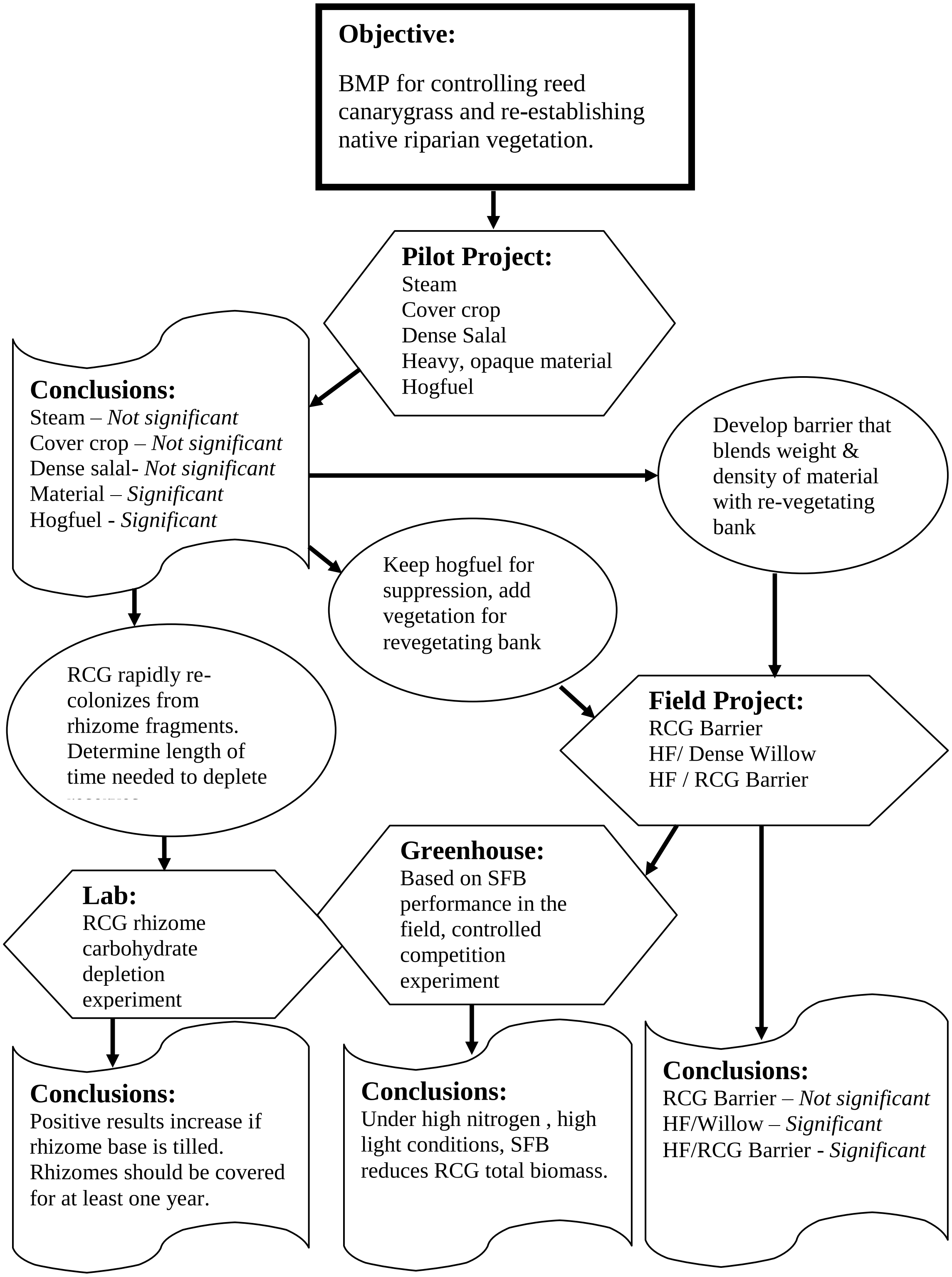
Wipfli, M.S. 1997. Terrestrial invertebrates as salmonid prey and nitrogen sources in streams: contrasting old-growth and young-growth riparian forests in southeastern Alaska, U.S.A. Canadian Journal of Fish. Aquatic Science 54: 1259-1269.

Zedler, J.B. and S. Kercher. 2004. Causes and Consequences of Invasive Plants in Wetlands: Opportunities, Opportunists, and Outcomes. Critical Reviews in Plant Sciences 23:5 431-452.

Zink, T. A. and M. F. Allen. 1998. The effects of organic amendment on the restoration of a disturbed coast sage scrub habitat. Restoration Ecology 6: 52– 58.

APPENDIX A:

Schematic flow of the research project development and process.


Objective:
BMP for controlling reed canarygrass and re-establishing native riparian vegetation.
Pilot Project:
Steam
Cover crop
Dense Salal
Heavy, opaque material
Hogfuel
Conclusions:
Steam – Not significant
Cover crop – Not significant
Dense salal- Not significant
Material – Significant
Hogfuel - Significant
Develop barrier that blends weight & density of material with re-vegetating bank
Keep hogfuel for suppression, add vegetation for revegetating bank
RCG rapidly re-colonizes from rhizome fragments. Determine length of time needed to deplete
Field Project:
RCG Barrier
HF/ Dense Willow
HF / RCG Barrier
Greenhouse:
Based on SFB performance in the field, controlled competition experiment
Lab:
RCG rhizome carbohydrate depletion experiment
Conclusions:
Positive results increase if rhizome base is tilled. Rhizomes should be covered for at least one year.
Conclusions:
Under high nitrogen , high light conditions, SFB reduces RCG total biomass.
Conclusions:
RCG Barrier – Not significant
HF/Willow – Significant
HF/RCG Barrier - Significant

Appendix B

½ meter between plots and one meter between replicates

Principal Project Layout, Agriculture Site

Each plot is two meters wide

Each plot three meters in length

Replicate 1

Control	RCG Barrier	Red Cedar Hogfuel / Willows	Red Cedar Hogfuel / RCG Barrier

Replicate 2

Control	Red Cedar Hogfuel / RCG Barrier	RCG Barrier	Red Cedar Hogfuel / Willows

Replicate 3

RCG Barrier	Red Cedar Hogfuel / Willows	Red Cedar Hogfuel / RCG Barrier	Control

Pilot Project Layout

Each plot is 1.5 meters by 1.5 meters

Replicate 1

Steam Salal	No Steam Shade	Steam Mulch	Steam Control	No Steam Clover	No Steam Control	No Steam Salal	No Steam Mulch	Steam Shade	Steam Clover

Replicate 2

No Steam Clover	Steam Control	No Steam Mulch	Steam Mulch	No Steam Salal	No Steam Shade	Steam Clover	Steam Salal	No Steam Control	Steam Shade

Replicate 3

No Steam Salal	Steam Shade	No Steam Mulch	No Steam Shade	Steam Salal	Steam Control	Steam Mulch	No Steam Clover	Steam Clover	No Steam Control