LARGE-SCALE COMPOSTING AS A MEANS OF MANAGING INVASIVE PLANTS IN THE RIO GRANDE RIVER VALLEY BASIN

THESIS

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Issues addressed in this proposal are consistent with the goals of a Transboundary Diagnostic Analysis of the Rio Grande Basin, in that the study will analyze the physical and biological environment of the Rio Grande Basin, as well as issues and challenges related to sustainable use of its water resources.

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ABSTRACT

LARGE-SCALE COMPOSTING AS A MEANS OF MANAGING INVASIVE PLANTS IN THE RIO GRANDE RIVER VALLEY BASIN

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The ecological impacts of invasive species are primarily due to their rapid growth, clogging waterways as well as outcompeting, even completely displacing native species. As a waste management system within agriculture, the composting process kills plant pathogens and weed seeds if high enough temperatures are obtained for long enough periods of time. Compost is used in the horticulture industry to decrease plant disease(s), increase the accessibility of nutrients by plants, and as an effective weed control agent. The purpose of this study was to investigate the effectiveness of a large-scale composting operation to manage invasive plants, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*), by rendering the seeds and other propagules non-viable while producing a valuable compost product for the agricultural and horticultural industries. Samples of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) plants were obtained during the mid to late summer

months (when flowering and fruiting). Oven mortality tests determined at which temperature seeds and other propagules were rendered nonviable. After being subjected to the oven mortality tests, analysis using tetrazolium tests (viability tests) revealed one water hyacinth (*Eichhornia crassipes*) seed to be viable from the 120.0 degrees Fahrenheit oven. However, all other seeds and propagules were rendered non-viable at all other temperatures. Therefore, in the field, achieving temperatures of at least 135.0 degrees Fahrenheit were necessary within the compost piles in order to effectively manage the invasive species.

In the field, windrow compost piles were constructed using the recipe: 50% woodchips, 25% cafeteria food waste, and 25% invasive species. A total of approximately 45,000 pounds of food waste, 52,200 pounds of woodchips, 7,680 pounds of water hyacinth (*Eichhornia crassipes*), 8,000 pounds of water lettuce (*Pistia stratiotes*), and 4,628 pounds of hydrilla (*Hydrilla verticillata*) – a total of 20,308 pounds of invasive plant species – were collected and utilized in this study. Composite samples of compost were collected from each compost pile, where they were either screened for seeds and other propagules and then analyzed by the researcher, or sent for analysis to the certified Compost Tests for U.S. Compost Council's Seal of Testing Assurance Program at Pennsylvania State University's Agricultural Analytical Services Laboratory. All seeds and propagules found through hand screening by the researcher, when analyzed using the tetrazolium test, were found rendered non-viable through the high temperatures achieved in the composting process. All compost samples analyzed by the certified Compost Tests for U.S. Compost Council's Seal of Testing Assurance Program, were determined as either within satisfactory to ideal levels for favorable compost and

therefore, a valuable compost product. Results demonstrate that the invasive species water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) can be used to produce a nutrient rich resource for various applications within the agriculture and horticulture industries, while also effectively managing invasive species.

CHAPTER I

INTRODUCTION

Compost is used in many states as both a waste management alternative, and a valuable horticultural and agricultural-based resource and is escalating as an environmental commodity for various types of crop applications (Glenn and Goldstein 1999; Faucette 2003). As a waste-management system within agriculture, composting is known to kill weed seeds if temperatures are high enough and maintained for long periods of time (Wiese, Bean, Salisbury, and Chenault 1998). Therefore, composting can potentially serve as a means to manage invasive aquatic species while producing a valuable product to the agricultural and horticultural industries.

The Rio Grande River Valley region is not only the home to approximately 13 million people but is one of the largest producers of vegetables in the nation, the center of citrus fruit (*Citrus* spp.) production, as well as a mass producer of cotton (*Gossypium herbaceum*), maize (*Zea mays*), melons (*Cucurbita* spp.), sugar cane (*Saccharum officinarum*), and various sorghums (*Sorghum* spp.) (Vigness and Odintz 2009). Given all of the agricultural production, the "Valley" is also a major producer of agricultural waste, and these 'biomass wastes' include cotton seed hulls (*Gossyspium thurberi*) and waste from the citrus juice plants (Vigness and Odintz 2009). Also, initially planted for erosion control, Georgia cane (*Arundo donax*) is another species found in riparian areas, but due to its invasiveness, has inundated areas along the river as well as agricultural

irrigation canals (Hodges 2010). Agricultural waste products and crop residues often go unused, and could be incorporated into a compost operation (Walker, Williams, and Waliczek 2006).

Due to the extensive length of the Rio Grande River, it is home to a variety of native and non-native aquatic plants in and along the river (Texas Parks and Wildlife Department [TPWD] 2009). Clearing of the native woodlands adjacent to the Rio Grande River for agriculture use resulted in much of the land bordering the river becoming susceptible to invasion of non-native species of plants which, in turn, reduced the biodiversity around and within the river (Vigness and Odintz 2009). Additionally, bog and invasive aquatic plant species blocked waterways and lowered the waters' dissolved oxygen content, resulting in a reduction of available oxygen for other plant and animal species (TPWD 2009). Non-native species found in the Rio Grande River that are considered more problematic due to their invasiveness include alligatorweed (*Alternanthera philoxeroides*), Eurasian watermilfoil (*Myriophyllum spicatum*), giant salvinia (*Salvinia molesta*), Georgia cane (*Arundo donax*), hydrilla (*Hydrilla verticillata*), water hyacinth (*Eichhornia crassipes*), and water lettuce (*Pistia stratiotes*) (Hodge 2004; Masser 2007; Hodge 2010). However, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) are considered by the United States Congress (2006) as the most problematic invasive aquatic species in the United States and are listed as federal noxious weeds. In addition, Georgia cane (*Arundo donax*) is an invasive riparian species listed as a noxious weed in Texas, an exotic plant pest in California, an invasive weed in Hawaii, and as an invasive, exotic pest in Tennessee (McWilliams 2004).

Currently, water hyacinth (*Eichhornia crassipes*) occupies approximately 30 major Texas reservoirs and all of the major rivers in eastern and southern Texas (Moran 2010); and according to the Texas Parks and Wildlife Department, water lettuce (*Pistia stratiotes*) and hydrilla (*Hydrilla verticillata*) have taken over stretches of the lower Rio Grande, "forcing the Rio Grande Watermaster to release up to 30% more water from [reservoirs] to push water through to irrigators" (Hodge 2004, p. 3; Hodge 2010). This is extremely detrimental, when long-term droughts dramatically reduce river and lake levels, where any loss of water is potentially damaging to fisheries, farms, and even cities that are dependent on the water (Hodge 2004; Hodge 2010). Additionally, dense populations of Georgia cane (*Arundo donax*) stands are found in abundance in riparian areas along the river as well as irrigation canals, which absorb large amounts of water daily altering water level and chemistry (TPWD 2009; Hodge 2010).

Problem Statement

The intent of this study is to investigate the effectiveness of a large-scale composting operation to manage various invasive aquatic plants found in the Rio Grande River, by rendering the seeds and other propagules non-viable, while producing a valuable product for the agricultural and horticultural industries. This study will investigate the utilization of invasive aquatic plants water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) in a large-scale composting operation, to determine the effectiveness of managing the invasive species.

Purpose

The purpose of this study is to investigate and begin the implementation of a multi-phase project in which a valuable agricultural product is obtained from the establishment of a large-scale composting operation to manage various invasive aquatic plants found in the Rio Grande River.

Objectives

1. To determine the aquatic plants in the Rio Grande River that are considered the most threatening invasive species.
2. To determine nitrogen and carbon sources available in regions of the Rio Grande River Valley Basin that would be consistently available composting feedstock sources.
3. To evaluate if the high temperature(s) achieved in the composting process has the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks.
4. To determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Hypotheses

The following hypotheses will be tested:

- Heat created by large-scale compost piles will render water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) seeds non-viable as well as decompose other propagules that would asexually reproduce the plant.

- Water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) will be an effective feedstock for use in a large-scale composting operation.
- Large-scale composting will be a safe and effective disposal system for water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*).
- The compost produced will be valuable and of acceptable quality determined by the Compost Tests for the United States Compost Council's Seal of Testing Approval Program for compost quality, analyzed by Pennsylvania State University Analytical Services Laboratory.

Definition of Terms

Adventitious Roots: root-like structures that arise from the stem (usually at a node), and not from root system (Urdang and Flexner 1968).

Axillary bud: "an embryonic shoot which lies at the joint of the stem and leaf petiole of a plant; may form a leaf, branch, or flower cluster" (Urdang and Flexner 1968, p. 95).

Biomass: "natural collection of a mass of living biological organisms and dead organic matter in a given area or ecosystem at a given time; can refer to species biomass, which is a mass of one or more species; or can refer to community biomass, which is a mass of all species in that community" (Urdang and Flexner 1968, p. 136).

Compost: "a mixture of various organic substances [materials] in which the natural decay of organic matter occurs, creating a nutrient-rich resource" (Urdang and Flexner 1968, p. 276).

Composting: a biological process in which microorganisms convert organic materials into a valuable soil-like material called compost (Dougherty 1999).

Culm: "a stem or stalk, especially the jointed and usually hollow stem of grasses" (Urdang and Flexner 1968, p. 325); "the stem of a grass" (Gould 2008, p. 258).

Curing: final stage of composting in which stabilization of the compost continues, but the rate of decomposition has slowed to a point where turning or forced aeration is no longer necessary – curing generally occurs at lower, mesophilic temperatures (Rynk *et al.* 1992).

Dioecious: Greek term meaning "two houses"; plant species population where the plant(s) bear one sex, either all male (staminate) flowers or all female (pistillate/carpellate) flowers on one individual plant, and therefore, can not produce seeds with only one plant (Harris and Harris 1994; Washington State Department of Ecology [WSDE] 2010a).

Ecosystem: "system formed by the interaction of a community of organisms with their environment" (Urdang and Flexner 1968, p. 419).

Evapotranspiration: "term used to describe the sum of evaporation and plant transpiration from the Earth's surface to the atmosphere; the subsequent loss of water as vapor through plants" (Urdang and Flexner 1968, p. 457).

Floret: "one of the closely clustered small flowers that make up the flower head of a composite flower" (Urdang and Flexner 1968, p. 506); as applied to grasses, the lemma (lower bract) and palea (upper bract) that encloses the flower (Gould 2008). "The floret may be perfect, pistillate, staminate, or neuter" (Gould 2008, p. 259).

Germinate: "to develop into a plant or individual, as a seed, spore, bulb, or the like" (Urdang and Flexner 1968, p. 553).

Germination: process by which a once dormant seed begins to sprout and grow into a seedling under the right conditions (Dougherty 1999).

Loam: "a rich, friable soil containing a relatively equal mixture of sand and silt and a somewhat smaller proportion of clay" (Urdang and Flexner 1968, p. 785).

Macrophyte: an aquatic plant that grows in or near water and is either emergent, submergent, or floating. In lakes, macrophytes provide cover for fish and substrate for aquatic invertebrates, produce oxygen, and usually act as food for some fish and other wildlife (Texas Parks and Wildlife Department [TPWD] 2009).

Mesophyles: organisms including actinomycetes, bacteria, fungi, and invertebrates present in compost at temperatures between 50.0 degrees Fahrenheit (10.0 degrees Celsius) and 105.0 degrees Fahrenheit (40.6 degrees Celsius). These organisms begin the process of composting and re-colonize the compost pile during the curing phase (Rynk *et al.* 1992).

Monoecious: Greek term meaning "one house"; plant species population that bear male (staminate) flowers and female (pistillate/carpellate) flowers on one individual plant, and therefore, individual plants can bear fruit (Harris and Harris 1994; WSDE 2010a).

Non-viable: "incapable of living, growing, and developing, as an infant, seed, plant, etc.; undevelopable" (Urdang and Flexner 1968, p. 1465).

Noxious weed: invasive; "any living stage (including seeds and reproductive parts) of a parasitic or other plant of a kind which is of foreign origin, is new to or not widely prevalent in the U.S., and can directly or indirectly injure crops, other useful plants, livestock, poultry, or other interests of agriculture, including irrigation, navigation, fish, and wildlife resources, or the public health" (United States Congress 2006, p. 2801).

Photoperiods: "the normal duration of natural daylight that an organism experiences"; refers to the physiological, biochemical, and behavioral processes of organisms' reaction(s) from exposure to the length of day and/or night periods (Urdang and Flexner 1968, p. 1000).

Phytochromone: a photoreceptor; a pigment that plants use to detect light (Urdang and Flexner 1968).

Phyotohormone(s): natural plant hormone(s) that regulate plant growth; phyotohormones can be modified by increase in artificial lighting and the use of plant growth regulators (PGRs) to accelerate and enhance plant growth (Urdang and Flexner 1968).

Pistil: "the female ovule-bearing reproductive part of a flower composed of at least one ovary, style, and stigma" (Urdang and Flexner 1968, p. 1011); the female part of the flower where pollination occurs (Harris and Harris 1994).

Pistillate/Carpellate: female flowers (Harris and Harris 1994); flowers which "have a functional pistil, capable of producing seeds – but either have no stamen(s) at all, or have stamens with anthers that are incapable of producing pollen" (Urdang and Flexner 1968, p. 1011).

Plankton/Planktonic: "any drifting or free-floating organisms (plants, animals, archeae, bacteria, detritus, minerals, etc.) that inhabit the pelagic (middle) zone of oceans, seas, rivers, lakes, and other bodies of water" (Urdang and Flexner 1968, p. 1015).

Plant Growth Regulators (PGRs): chemicals that target plant hormones (phyotohormones) which modify, accelerate, or enhance the natural phyotohormonal regulation of plant growth (Urdang and Flexner 1968).

Plume: refers to "anything that resembles a feather in shape or lightness" (Urdang and Flexner 1968, p. 1021).

Propagules: "any plant material (leaf section, stem, auxiliary buds, tubers, turions, or any number of other plant parts) that can be used for asexual reproduction of a plant; plant propagation" (Urdang and Flexner 1968, p. 1060).

Rhizome: "a characteristically horizontal stem of a plant that is usually found underground, often sending out roots and shoots from its nodes; serves as a vegetative reproductive structure" (Urdang and Flexner 1968, p. 1133).

Rosette: "any arrangement, part, object, or formation more or less, a cluster of leaves or other organs resembling a rose" (Urdang and Flexner 1968, p. 1147).

Scarification: refers to breaking a seed coat either through a mechanical process in which the outer seed coat is scratched, etched, cut, or through a chemical process in which the seed coat is broken down through the use of chemicals – breaking the seed coat exposes and allows water and oxygen into the seed, thereby helping to increase germination in some species (Urdang and Flexner 1968).

Spikelet: "a small or secondary spike in grasses; one of the flower clusters, the unit of inflorescence, consisting of two or more flowers and subtended by one or more glumes variously disposed around a common axis" (Urdang and Flexner 1968, p. 1266); "the basic unit of grass inflorescence, usually consisting of a short axis, the rachilla, bearing two 'empty' bracts, the glumes, at the basal nodes and one or more florets above, and each floret consists usually of two bracts, the lemma (lower) and the palea (upper), and a flower" (Gould 2008, p. 262).

Stamen: "pollen-bearing male reproductive part of a flower," comprised of at least one anther and a filament (Urdang and Flexner 1968, p. 1279; Harris and Harris 1994).

Staminate: male flowers; flowers which "have functional stamens, capable of producing pollen – but either have no ovary at all or an ovary which is not fertile" (Urdang and Flexner 1968, p. 1279; Harris and Harris 1994).

Stolon: "a prostrate stem (rootlike extension), at or just below the surface, that produces new plants from buds at its tips or nodes" (Urdang and Flexner 1968, p. 1293).

Substrate: "layers of strata underneath," – usually refers to the subsurface layers of strata underneath a body of water; is the composition of sediments and other materials that rests at and make up the surface bottom layer(s) in a body of water, in which organisms can grow in and/or be attached to; can consist of fine clay and silt, sand, and other granular sized particles up to pebbles, cobbles, and bolder sized rocks (Urdang and Flexner 1968, p. 1311; TPWD 2009).

Thermophiles: organisms including actinomycetes, bacteria, and fungi present in compost at temperatures between 105.0 degrees Fahrenheit (40.6 degrees Celsius) and 170.0 degrees Fahrenheit (76.7 degrees Celsius) (Rynk *et al.* 1992).

Tuber(s): various underground modified plant structures, which are like enlarged shoots or roots which store nutrients to provide energy and nutrients for growth or regrowth during the growing season; tubers bear buds in which new plant shoots can arise from and, therefore, are means of asexual reproduction (Urdang and Flexner 1968).

Turion(s): Latin "turio" meaning shoot; is a specialized bud produced over winter, by aquatic plants, and is a compacted vegetative bud (like an axillary bud) produced along

the stem, that detaches, and then forms a new plant (or plant colony) (Urdang and Flexner 1968).

Limitations

The limitations of the study may include the following:

- Any research conducted in a natural environment is subject to extraneous factors that can influence the results of the study.
- The study is limited to the invasive species water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*).
- Other compost feedstocks included in the study will be limited to the agricultural waste(s) found readily available in the Rio Grande Valley.

Basic Assumptions

- The water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) harvested will behave consistently, no matter if they are gathered from the San Marcos River and adjoining areas or the Rio Grande River.
- Ovens will be accurate and maintain three constant temperatures of: 120.0 degrees Fahrenheit (48.9 degrees Celsius), 135.0 degrees Fahrenheit (57.2 degrees Celsius), and 150.0 degrees Fahrenheit (65.6 degrees Celsius).
- Moisture meter, thermometer, and other measurement devices used in the study will produce accurate readings.
- Random samples taken from curing compost will be screened to capture any water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*),

hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) seeds and other propagules, if they are present after the composting process.

- Germination and growth tests will lead to precise and consistent results.
- Oven mortality tests will lead to precise and consistent results.
- The other carbon and nitrogen feedstocks found utilizable in the Rio Grande Valley, will be readily available and of a consistent quality.

CHAPTER II

REVIEW OF LITERATURE

Rio Grande River

The Rio Grande River is the twenty-fourth largest river in the world, the fifth largest in the United States, as well as the United States' second longest river (Owens, Grodowitz, and Nibling 2005; Storm Center Communications, Inc. [SCC, Inc.] 2009). The waters of the Rio Grande descend from snow-capped Sangre de Cristo and San Juan mountains in Colorado and New Mexico and drain 185,000 square miles to the Gulf of Mexico (SCC, Inc. 2009). This large portion of the river, stretching almost 2,000 miles, forms an international boundary between the United States and Mexico (Owens *et al.* 2005; SCC, Inc. 2009). The Rio Grande River is home to a variety of wildlife and is the base support of riparian ecosystems, as well as a water source for approximately 13 million people living throughout the basin valley (International Boundary and Water Commission [IBWC] 2008; SSC, Inc. 2009). The Rio Grande River sustains local agriculture, import-export trade, service and tourism, and a growing manufacturing sector, on both sides of the United State-Mexico border (IBWC 2008).

History and Utilization of the Rio Grande River Valley Basin

The Rio Grande Valley, is not actually a valley, but a delta or floodplain (Vigness and Odintz 2009). Since the region is a true delta with alluvial soils that vary from

sandy, to silty loam, to loam, to clay, the region is superior for agricultural use (Vigness and Odintz 2009). The development and population of the region occurred around the turn of the 19th century when Midwestern farmers and immigrants from Mexico migrated to the area (Vigness and Odintz 2009). This occurred at the same time as that of the construction of the railroad and the introduction of large-scale irrigation, making the Rio Grande Valley into a major agricultural center (Vigness and Odintz 2009). Today, the Rio Grande Valley is a center for agribusiness and tourism, being one of the nation's largest producers of vegetables such as beets (*Beta vulgaris*), cabbage (*Brassica oleracea var. capitata*), carrots (*Daucus carota*), green beans (*Phaseolus vulgaris*), onions (*Allium cepa*), tomatoes (*Lycopersicon esculentum*), and other minor crops (Vigness and Odintz 2009). Cotton (*Gossyspium* spp.), maize (*Zea mays*), sugar cane (*Saccharum officinarum*), and various sorghums (*Sorghum* spp.) are the leading crops in the valley; however, the principal crop of the region is citrus fruits (*Citrus* spp.) (Vigness and Odintz 2009).

Texas is the third largest producer of citrus fruit in the United States, and each year, grapefruit (*Citrus paradise*) makes up over 70% of the citrus crop in the valley (Vigness and Odintz 2009). Other citrus crops grown in Texas' Rio Grande Valley include oranges (*Citrus sinensis*), tangerines (*Citrus reticulate*), and tangelos (*Citrus tangelo*) (Vigness and Odintz 2009). According to Vigness and Odintz (2009), citrus fruit was introduced to the region around the same time as large-scale irrigation in 1904, and the "citrus fruit culture has survived severe freezes in 1949, 1951, 1961, 1983, and 1989" (p. 2).

The Rio Grande River is one of the only sources of fresh water in the semiarid, sun-scorched, region and is the lifeblood of the economic activity on both sides of the transboundary water source (Allen 2002; Patino-Gomez, McKinney, and Maidment 2007). However, as the river passes through the arid region, it tends to decrease in size as it flows downstream. Decreasing access of this limited resource has an impact on the health, wealth, agriculture, and industry of communities on both sides of the river (Allen 2002; Patino-Gomez *et al.* 2007).

Ecological Impacts of Invasive Aquatic Plants in the Rio Grande

The extensive length of the Rio Grande River allows for a diversity of native and non-native aquatic plant species. Aquatic vegetation is an extremely important component of freshwater ecosystems, providing habitat, refuge, and food for a diverse variety of organisms including fish, invertebrates, and waterfowl (TPWD 2009). However, a non-native or exotic species found along the Rio Grande River, Georgia cane (*Arundo donax*), in addition to a handful of non-native aquatic species found within the river, are considered extremely invasive and include alligatorweed (*Alternanthera philoxeroides*), Eurasian watermilfoil (*Myriophyllum spicatum*), giant salvinia (*Salvinia molesta*), hydrilla (*Hydrilla verticillata*), water hyacinth (*Eichhornia crassipes*), and water lettuce (*Pistia stratiotes*) (Hodge 2004; Masser 2007). Masser (2007) stated that, "Removed from their native environment, these [exotic] plants are freed from the presence of the indigenous herbivores, diseases, and pests that kept their populations under control or at least in ecological balance" (p. 1). These plants create dense mats on the surface and within the water column, that alter nutrient cycling, increase rates of siltation, displace native vegetation, alter the native ecology and water quality, choke

waterways, limit recreational uses, and decrease waterfront property values (Masser 2007).

Furthermore, in less than 200 years, the United States has been introduced to more than 800 non-native plant species, wreaking havoc on ecosystems throughout the United States (Hodge 2010). Hodge (2010) debated that since humans do not live for 200 years, it is difficult for them to see the long-term ecological impacts of non-native exotic species. When exotic, invasive plant species are introduced and become established, the native ecosystems die out, with areas of native birds, insects, fish, mammals, and plants disappearing (Hodge 2010).

The exotic species Georgia cane (*Arundo donax*) is a riparian species that chokes riversides and stream channels, crowds out native plant species, interferes with flood control, and reduces habitat for wildlife, including the Least Bell's vireo (*Vireo bellii pusillus*), a federally endangered bird (Texas Invasive Plant and Pest Council [TIPPC] 2010). The long, fibrous, interconnecting root mats of Georgia cane form a framework for debris dams around bridges, culverts, irrigation canals, and other structures that block water flow and can lead to damage of the structure (TIPPC 2010). Due to its rapid growth rate and ability to vegetatively reproduce, Georgia cane is able to quickly invade new areas and form pure stands at the expense of other species (TIPPC 2010). It can float miles downstream where root and stem fragments take root and initiate new infestations (TIPPC 2010). Once established, Georgia cane (*Arundo donax*) has the ability to outcompete and completely suppress native vegetation, altering the entire ecosystem (TIPPC 2010). In addition, Georgia cane is listed as a noxious weed in Texas,

an exotic plant pest in California, an invasive weed in Hawaii, and as an invasive, exotic pest in Tennessee (McWilliams 2004).

In addition to Georgia cane (*Arundo donax*), a submerged aquatic species found in the Rio Grande River is the exotic species hydrilla (*Hydrilla verticillata*) (Hodge 2010). Hydrilla has been dubbed by Hodge (2004) as well as many others as the "aquatic weed from hell" (p. 2). Additionaly, Moran (2009) states that, "Texas Parks and Wildlife released a grass carp species (*Ctenopharyngodon idella*) into the Rio Grande from 2003 to 2007, which reduced the extent of hydrilla problem in the Rio Grande" River, until recently; however, infestation of hydrilla on the Rio Grande mainly occurs around the entry and exit points of irrigation canals and other small, flowing bodies of water (Hodge 2004).

Along with hydrilla (*Hydrilla verticillata*), two other exotic plants found in the Rio Grande River, water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) are considered the most problematic, with water hyacinth being called the worst aquatic weed in the world (TPWD 2009; Washington State Department of Ecology [WSDE] 2010c). As well as clogging waterways, the ecological impacts of these invasive aquatic floating plants are primarily due to their rapid growth and formation of a dense floating canopy that outcompete and displace native submerged and planktonic vegetation for nutrients and light (Masser 2007). The lack of penetrating sunlight and nutrient depletion alter "biogeochemical cycles and water quality" (Masser 2007, p. 4). Dissolved carbon dioxide increases while dissolved oxygen drops, and particularly in small and/or limited circulated areas, oxygen can drop underneath the mats to critical levels, killing fish and invertebrates, such as crustaceans, insects, mollusks, and worms

(Masser 2007). Also, conductivity, pH, turbidity, temperature, and bicarbonate alkalinity values decrease, resulting in habitat alteration, where in some instances waterfowl do not utilize the aquatic habitat (Masser 2007). The consequences of anoxia of the water due to water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and/or hydrilla (*Hydrilla verticillata*) populations include the reduction of native population densities and species diversity or richness, potentially of the aquatic and terrestrial mammals and even waterfowl, who use and rely on the water and the displaced vegetative species as daily food sources (Masser 2007). Additionally, Georgia cane (*Arundo donax*) has the ability to outcompete and fully suppress native plant species, completely altering entire ecosystems (TIPPC 2010).

Water Hyacinth (Eichhornia crassipes)

History/Origin of Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) is a subtropical-tropical aquatic plant that originated in South America and is a native of the Amazon River, most likely from Brazil (Penfound and Earle 1948; WSDE 2010c). Water hyacinth was first introduced into the United States at the World's Industrial and Cotton Centennial Exposition of 1884 in New Orleans, Louisiana (WSDE 2010c). At the exposition, water hyacinth was the featured South American horticultural display and attracted great positive attention (Monsod 1979).

From the exposition in New Orleans, water hyacinth (*Eichhornia crassipes*) was transported to Florida by an attendee (Monsod 1979; WSDE 2010c). At that time, the water hyacinth was shared with fellow gardeners and planted in garden ponds across Florida and was subsequently released into the St. Johns River (Monsod 1979; WSDE

2010c). When a flood hit Florida, the water hyacinth was spread to Texas, and soon it was located from Virginia to Northern California (Monsod 1979). Becoming a nation wide aquatic weed problem during the last century, water hyacinth is presently found in all major rivers world wide, surviving in freezing conditions and has naturalized in [the subtropical and tropical climates of] Central America, North America [California and southern states], Africa, India, Asia, Australia, and New Zealand (Penfound and Earle 1948; WSDE 2010c).

Description of Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) is a free-floating aquatic plant and a member of the Pickerelweed family (*Pontederiaceae*) (WSDE 2010c). The plants vary in size from an inch tall to over three feet in height and the glossy, green, leathery leaf blades are up to eight inches long and two to six inches wide (WSDE 2010d). The leaves are attached to petioles that are often spongy and slightly puffy and they have numerous dark, branched, fibrous roots that dangle in the water from the underside (WSDE 2010d). The inflorescence is a loose terminal spike with showy light-blue to violet flowers with six bluish-purple petals joined at the base to form a short tube (WSDE 2010d). The fruit of water hyacinth is a three-celled capsule containing many minute, ribbed seeds (WSDE 2010d).

Vegetative Propagation of Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) reproduces by seeds and vegetatively through daughter plants that form on rhizomes and produce dense plant beds (WSDE 2010c). Water hyacinth primarily reproduces by vegetative means, by the stolons that produce offshoots at their ends (Barrett 1980). It has been found that water hyacinth will

double at least every 18 days, and given favorable conditions will double to twice its population in twelve and half days (Penfound and Earle 1948). In ideal conditions, water hyacinth will double as often as every six days (Penfound and Earle 1948; TPWD 2009).

In one study, two water hyacinth plants multiplied to an estimated 1200 daughter plants in four months, or 120 days (WSDE 2010d). In Louisiana, water hyacinth (*Eichhornia crassipes*) has been found to reproduce vegetatively in a typical spring season, where one plant could produce to over 65,000 daughter plants (Penfound and Earle 1948; Barrett 1980). Through this reproductive process, water hyacinth can form impenetrable mats of floating vegetation, where sporadic breezes and/or increased water currents due to rain can loosen the mat(s), allowing individual plants to break off and disperse (WSDE 2010c).

Sexual Reproduction of Water Hyacinth

Observation by researchers of sexual reproduction in water hyacinth (*Eichhornia crassipes*) populations have been limited, and the factors controlling flowering, seed formation, and seed germination are still being evaluated in order to be understood and determined (Penfound and Earle 1948; Barrett 1980). The seeds of water hyacinth can germinate in a few days or remain dormant for 15 to 20 years (WSDE 2010d). Seeds are commonly found on mud banks exposed by low water levels; however, seed germination is rare (WSDE 2010c). Reports on water hyacinth concluded that sexual reproduction in natural field conditions was rare to nonexistent (Barrett 1980). Studies in California failed to find any evidence of sexual reproduction (Mulcahy 1975).

However, in contrast, seedlings as well as seed production of water hyacinth (*Eichhornia crassipes*) were reported in Texas and Louisiana (Barrett 1980). Researchers

have proposed that environmental factors such as insufficient pollinators, unfavorable climate conditions that inhibit seed formation, as well as a lack of sites suitable for seed germination and seedling formation are the major sources limiting sexual reproduction of the non-native species (Barrett 1980). On the other hand, in a recent study Montoya and colleagues (2012) found that of 100 water hyacinth seeds tested, 62 successfully germinated within fourteen days at a stable temperature of 80.0 Fahrenheith (26.7 degrees Celsius) within the laboratory.

Flower(s), Fruit, and Seed Development of Water Hyacinth

For decades the ornamental flowers of the water hyacinth (*Eichhornia crassipes*) have been of vast curiosity and interest to botanists (Penfound and Earle 1948). Each flower has six purplish petals and the flowers are borne on a terminal inflorescens which is an attractive lavender (light blue to violet) spike subtended by two bracts and surmounted on an elongated peduncle (Penfound and Earle 1948; Barrett 1980; WSDE 2010c; WSDE 2010d). Individual spikes have 4 to 25 flowers usually, though numbers as high as 35 have been recorded (Penfound and Earle 1948). After all the flowers on the inflorescence have opened, the bulky inflorescence axis bows downward, ultimately submerging the flowers (Penfound and Earle 1948; Barrett 1980). Fruit development universally occurs while the inflorescence is submerged in water, though the fruit have also been known to develop outside of water (Penfound and Earle 1948).

Paramount fruiting of water hyacinth (*Eichhornia crassipes*) occurs at relative high percentages of humidity (Barrett 1980). Germination of water hyacinth seeds requires a combination of water and environmental temperature totals that range between 72.5 degrees Fahrenheit (22.5 degrees Celsius) to 95.0 degrees Fahrenheit (35.0 degrees

Celsius) (Barrett 1980). The fruit and seed production of water hyacinth vary greatly between different population locations (Penfound and Earle 1948; Barrett 1980). According to Agharkar and Banerji (1930), around 35% of flowers are pollinated under natural conditions (Barrett 1980). The number of seeds per fruit varies from just a few to over 450 (Barrett 1980). In some instances, a single water hyacinth plant can produce as many as 5,000 seeds during one seasonal period (WSDE 2010d). Each seed is relatively small, with a maximum size of about one millimeter in length and half a millimeter in width (Penfound and Earle 1948; Barrett 1980). Though some research has claimed sexual reproduction to be rare, under ideal conditions Barrett (1980) found water hyacinth seeds to take as little as 14 up to 32 days to germinate within the laboratory (WSDE 2010c).

Seed Germination of Water Hyacinth

There are numerous incompatible reports on the environmental requirements for seed germination of water hyacinth (*Eichhornia crassipes*) (Barrett 1980; Gopal 1987). The first reported germination tests of water hyacinth seeds were recorded by Mueller in 1883 (Gopal 1987). In this study, germination occurred only after the seeds were dried (Gopal 1987). Since Mueller's study, there have been many reports supporting, as well as contradicting, the conditions necessary for germination of the seeds of water hycainth.

In contrast to Mueller's study, a later study found that a drying out stage was not required for germination of the water hyacinth (*Eichhornia crassipes*) seeds (Penfound and Earle 1948). Another study found that alternating the wetting and drying of the seeds was necessary for germination (Robertson and Thein 1932). Other studies found that a storage period of 10 weeks for wet stored seeds and 16 weeks for dry stored seeds was

necessary for germination to occur (Mulcanhy 1975). A similar study by Barton and Hotchkiss (1951) found that dry seeds that were stored required twice the amount of time to germinate when compared to wet seeds that were stored (Mulcanhy 1975). However, in other research studies, 100% germination of water hyacinth was reported to occur in dry and wet conditions, as well as germination of fresh, untreated seeds in controlled environments (Chadwick and Obeid 1966; Obeid and Tag El Seed 1975).

Problems Caused by Water Hyacinth

Water hyacinth (*Eichornia crassipes*) is one of the most productive plants on earth and is considered the world's worst aquatic plant in terms of invasiveness (TPWD 2009; WSDE 2010c). Water hyacinth forms dense mats that interfere with navigation, recreation, irrigation, and power-generated water resources (Aquatic Ecosystem Restoration Foundation [AERF] 2009; TPWD 2009; WSDE 2010c). Water hyacinth blocks waterways used by boats, it impedes drainage canals for irrigation on farm lands, and destroys wildlife habitat by displacing native submerged and floating aquatic plants (AERF 2009; TPWD 2009).

Water hyacinth (*Eichhornia crassipes*) has been found to multiply and spread, clogging hundreds of feet of open river in just a matter of a few weeks (Allen 2002). It has been known to inhibit river water flow by 40 to 95% (Gopal 1987; AERF 2009). In five years, water hyacinth can render a drainage canal 5 feet in depth and 20 feet wide to be barely functional at all (Penfound and Earle 1948). Just as the plant can clog up a waterway, it can also clog up water distribution pipelines and aqueducts (Allen 2002).

Furthermore, water loss due to evapotranspiration is a problem caused by water hyacinth (*Eichhornia crassipes*) (AERF 2009). Water loss from evapotranspiration of

water hyacinth in open conditions, increases three to six times the amount of water lost compared to the normal evaporation rate of water lost from rivers and reservoirs (Penfound and Earle 1948; Masser 2007). According to the research entomologist for the United States Department of Agriculture – Agricultural Research Service [USDA-ARS], Dr. Moran (2009), *Eichhornia crassipes*, "water hyacinth is the most troublesome aquatic weed in both the Rio Grande Valley [RGV] and Texas statewide, as it occupies about 30 major reservoirs plus all of the major rivers in eastern and southern Texas." In Texas, water loss from reservoirs and rivers is found to be 88.3 billion cubic feet annually, and cost an estimated $83 million dollars (Benton, James, and Rouse 1978). Additionally, one hectare of water hyacinth is estimated to have an oxygen depleting load equal to that of the sewage produced by 80 people (Raynes 1964; Gopal 1987).

With this in mind, water hyacinth (*Eichhornia crassipes*) mats lower the pH and temperature of the water, altering the critical and stable habitats of native species (Reddy, Sutton, and Bowes 1983). Also, low oxygen conditions develop beneath the dense water hyacinth mats, preventing all fish but top minnows from utilizing the habitat due to oxygen depletion (Penfound and Earle 1948; WSDE 2010d). These dense mats of water hyacinth create excellent breeding grounds for mosquitoes; therefore, the mosquito populations increase in those areas, which lead to rise of disease spread in developing nations (AERF 2009; WSDE 2010c). Mosquito bites transmit some of the most devastating diseases in the world, such as dengue "breakbone fever," heartworms, encephalitis (ie. West Nile virus), malaria, and yellow fever (AERF 2009, p. 31-32). Therefore, public health is a concern in areas around large populations of water hyacinth (*Eichhornia crassipes*), as well as, water lettuce (*Pistia stratiotes*) (AERF 2009).

Furthermore, water hyacinth (*Eichhornia crassipes*) takes over areas of native submerged and riparian species, which are an essential part of daily nutrition of not only aquatic species, but waterfowl as well (Penfound and Earle 1948; AERF 2009). Aquatic native species are a critical part of a variety of riparian species' diet (AERF 2009); especially that of waterfowl, where the displacement of the native species by water hyacinth can cause waterfowl to abandon the area in search of food (AERF 2009).

Utilization of Water Hyacinth

Water hyacinth (*Eichhornia crassipes*) has a variety of possible utilizations and has been used as animal feed, for compost, to make paper, to make energy, and as a wastewater treatment (Gopal 1987). Water hyacinth has been used for fertilizer in some countries (Jianqing, Hill, Centre, and Julien 2001). Additional uses include "processing of the plant into...needed nutrients for humans, like protein, Vitamin A, Vitamin B-2 (Riboflavin), Vitamin E, Vitamin B-12, and Xanthophyll" (Monsod 1979, p. 31). In the Philippines, Indonesia, and Thailand, water hyacinth has been utilized to make rope, baskets, hats, handbags, stuffing for upholstery, and even shoe soles (Monsod 1979; Gopal 1987). In rural areas of China during the economic depression of the 1950s to the 1970s, water hyacinth was used as a food supply for animals when there were food shortages (Jianqing *et al.* 2001).

As early as 1925, water hyacinth (*Eichhornia crassipes*) was used as a feedstock for compost (Gopal 1987). It was made by layering a mixture of cow manure, wood ash, and soil in between layers of fresh water hyacinth that were then covered with soil (Gopal 1987). Water hyacinth has also been used in compost as dried material which was then mixed with farmyard manure, soil, wood ash, and vegetable refuse (Gopal 1987).

The utilization of the compost produced using water hyacinth (*Eichhornia crassipes*) has been viewed with caution because of and by raising the question: "What are the effects of heavy metals and toxic substances absorbed by water hyacinth on crops and soil characteristics when used as compost or mulch?" (Gopal 1987, p. 313). This "question" arose due to the decrease in some yields that were attributed to the relatively high potassium chloride (KCl) content of water hyacinth compost (Gopal 1987).

Managing/Controlling Water Hyacinth

The best and cheapest way to manage and control water hyacinth (*Eichhornia crassipes*) is to prevent it from ever becoming established (TPWD 2009). However, many methods such as harvesting, application of chemical agents like aquatic herbicides, and biological control agents, have been used to try to control the spread of water hyacinth (AERF 2009; TPWD 2009; WSDE 2010d). The use of herbicides for controlling riparian species is not the preferred use, since some aquatic habitats are ecologically sensitive or since, in some instances, communities utilize the same water being treated as drinking water (Jianqing *et al.* 2001; Allen 2002).

Numerous research studies have experimented with utilizing various biological agents for control and management of various plant species (WSDE 2010d). For water hyacinth (*Eichhornia crassipes*), the release of insects including two weevil species (*Neochetina eichhorniae* and *Neochetina bruchii*), a moth species (*Sameodes aliguttalis*), and a mirid or leafbug (*Eccritotarsus catarinensis*), have been studied and tested as biological agents to aid in controlling water hyacinth (Jianqing *et al.* 2001; WSDE 2010d). The weevil species (*Neochetina* spp.) decreased water hyacinth populations, but unfortunately did not result in large-scale reductions of the plant (Jianqing *et al.* 2001;

WSDE 2010d). One problem with using insects for biological control is being able to obtain a reliable supply (Hodge 2010). Due to undesirable results, the lengthy time required for biological methods to have a noticeable effect on large plant populations, and the risk of large insect populations toward public health, other means of controlling and managing invasive species are still being explored (WSDE 2010d).

Water Lettuce (Pistia stratiotes)

History/Origin of Water Lettuce

The exact origin of water lettuce (*Pistia stratiotes*) is unknown, but it is believed to have been brought to Florida by early Spanish monks of St. Augustine in the 1500s (Masser 2007). The confusion surrounding the origin of water lettuce is due to its extensive worldwide distribution (Chadwick and Obeid 1966). Fossils of water lettuce species (*Pistia stratiotes*) indicated that it is a descendant species that "originated in Eurasia some 65 million years ago and...has been widely dispersed for a long time" (Habeck and Thompson 1997, p. 2). It is commonly believed by some to be a native to subtropical South America, where many regionally native insects are associated with water lettuce (Habeck and Thompson 1997; Rivers 2005). Host-specific insect-plant associations are relationships that evolve and form over long periods of time, demonstrating that insects specific to a particular plant have been connected with the plant for a long time (Habeck and Thompson 1997). Habeck and Thompson (1997) believe that Africa is the "home of water lettuce [*Pistia stratiotes*], since African plants produced seeds while American plants rarely did, indicating an absence of pollinators" in America (p. 2).

Water lettuce (*Pistia stratiotes*) was first reported in the United States in 1765 in Florida and by 1946 had been discovered on the other side of the world in Australia (Habeck and Thompson 1997; Rivers 2005). Ships that traveled through the mats of water lettuce in South America, carried the fertile plant segments to new areas, where they were unknowingly distributed and established (Rivers 2005). Like water hyacinth (*Eichhornia crassipes*), the popularity of water lettuce (*Pistia stratiotes*) as an aquatic garden plant led to its spread even more (Rivers 2005).

Description of Water Lettuce

Water lettuce (*Pistia stratiotes*) is a free floating aquatic plant, and perennial monocot of the Arum family (*Araceae*) (Rivers 2005). Water lettuce is clearly identifiable because it looks like a floating head of lettuce (Fenner 2007). Water lettuce leaves are thick, soft, spongy, light green blades that are usually up to six inches in length and form a circular pattern or rosette that conceals a small female flower and the seed-bearing fruit of the plant (Rivers 2005). Water lettuce is supported by numerous feather-like roots that are submerged in the water beneath the leaves of the plant (Rivers 2005). The number and size of the primary roots correspond to the size of the plant that is exposed above the water's surface and can be up to three feet in length (Weldon and Blackburn 1967). The yellow flowers are nearly hidden on the short stalk in the center, where a single female flower is below a whorl of smaller male flowers above (Rivers 2005).

Vegetative Propagation of Water Lettuce

Water lettuce (*Pistia stratiotes*) reproduces similarly to that of water hyacinth (*Eichhornia crassipes*) using seeds as well as vegetative propagules (Chadwick and

Obeid 1966). Growth rates of water lettuce are similar to that of water hyacinth, but water lettuce are smaller plants and therefore, have a total biomass that is less (Masser 2007). Water lettuce reproduces vegetatively by daughter offshoots from mother plants on slightly thinner, brittle stolons when compared to that of water hyacinth, but can be connected to the mother plant by stolons up to two feet in length (Chadwick and Obeid 1966; Weldon and Blackburn 1967).

Increase in biomass of water lettuce (*Pistia stratiotes*) occurs principally through production of young plants (Hall and Okeli 1974). Rapid vegetative reproduction allows water lettuce to cover an entire lake with a dense mat of connected rosettes, from shore to shore, in a short period of time (Rivers 2005). It was found in Florida that water lettuce had reached densities of up to 35 rosettes per square foot in a four month period (Rivers 2005). Given ideal conditions, water lettuce (*Pistia stratiotes*) will double every seven days, compared to six days of that of water hyacinth (*Eichhornia crassipes*) (Penfounde and Earle 1948; Umali-Stuart and Stiuart-Santiago 2010; WSDE 2010c). "The maximum daily productivity of *Pistia stratiotes* [water lettuce] can thus be...comparable with, or perhaps slightly lower than, estimates published for its close associate of similar life-form, *Eichhornia crassipes*" (Hall and Okali 1974, p. 722).

Sexual Reproduction of Water Lettuce

Observation and reports by researchers of sexual reproduction in water lettuce (*Pistia stratiotes*) populations have also been scarce, and a key dilemma has been the "lack of complete developmental studies, including vegetative and flowering shoots" (Lemon and Posluszny 2000, p. 722). The flowering shoot of water lettuce consists of a linear series of articles; each article is made up of two leaves and a terminal inflorescence

(Lemon and Posluszny 2000). The flower opens by first exposing the pistil and then a few hours later the stamen is exposed (Lemon and Posluszny 2000). The stamen has between four and eight pollen chambers arranged in a circular pattern about one and a half millimeters in diameter (Weldon and Blackburn 1967). However, most water lettuce plants either abort the flower soon after opening, eliminating any time for seed germination, or do not produce an inflorescence at all (Weldon and Blackburn 1967; Lemon and Posluszny 2000).

Flower(s), Fruit, and Seed Development of Water Lettuce

Optimal growth of water lettuce (*Pistia stratiotes*) when compared to water hyacinth (*Eichhornia* crassipes) is essentially the same, requiring approximately the same high percentages of humidity and temperatures between 71.0 to 95.0 degrees Fahrenheit (21.7 to 35.0 degrees Celsius) (Hall and Okali 1974; Barrett 1980). Water lettuce is a subtropical-tropical plant that is susceptible to cold and does not thrive in northern states; however, recent studies have reported the first cold winter months of flourishing water lettuce (*Pistia stratiotes*) population in a temperate region of Central Europe (Sajna, Haler, Skornik, and Kaligaric 2007). This study revealed that only two years after its first appearance in that location, water lettuce (*Pistia stratiotes*) managed to cover most of the body of water where thermal springs caused an elevated water temperature of >77.0 degrees Fahrenheit (>25.0 degrees Celsius) year round and 88.0 degrees Fahrenheit (31.1 degrees Celsius) at the source of the stream (Sajna *et al.* 2007). New stolons and an increase in individual plant and biomass size were observed as well as seed production of water lettuce that, when taken back to the lab, were revealed to be viable (Sajna *et al.* 2007).

Moist and essentially the same temperature conditions that are required for flowering of water hyacinth (*Eichhornia crassipes*) are required for water lettuce (*Pistia stratiotes*), with maximum flowering of water hyacinth occurring at 70.0 to 95.0 degrees Fahrenheit (21.1 to 35.0 degrees Celsius), compared to that of water lettuce at 71.0 to 95.0 degrees Fahrenheit (21.7 to 35.0 degrees Celsius) (Chadwick and Obeid 1966; Weldon and Blackburn 1967; Barrett 1980). During the rainy season of early spring that occurs in many environments, water lettuce not only exhibits peak flowering, but maximum plant size as well (Hall and Okali 1974). Additionally, high levels of solar radiation contribute to enhanced growth rates of water lettuce during summer months due to the origin of its native region's proximity to the equator (Hall and Okali 1974).

For water lettuce (*Pistia stratiotes*), it typically takes seven to eight days from the time the flower buds are first noticeable to when the flower opens (Weldon and Blackburn 1967). Flowers will commonly fall from the plant within two weeks leaving little time for pollination to occur, and in most instances no viable seeds are produced (Weldon and Blackburn 1967).

Seed Germination of Water Lettuce

Reports and observations by researchers of seed germination in water lettuce (*Pistia stratiotes*) populations have also been limited, and when observed, despite the abundance of flowering, mature fruits were rare and no seedlings were found (Hall and Okali 1974). Lack of water lettuce seedling production has also been attributed to the same alteration of environmental factors that inhibit germination of seedlings of water hyacinth, such as climatic conditions that suppress flowering and pollination, and/or the absence of sites suitable for seed germination and seedling development (Hall and Okali

1974). Studies performed during the past century reported that water lettuce plants in the subtropics of Africa have been observed to produce seeds while those from North America rarely did; however, in the past decade or so, seeds and seedlings have been found in south Florida (Habeck and Thompson 1997).

Jing and Song (2008) administered a number of laboratory controlled studies on seed germination of water lettuce (*Pistia stratiotes*). The research they conducted reported the effects of alternative environmental factors used during germination, including dehydration, chilling, adjustment to phytochromes (sensory pigment) using various applications of white light, red light, far red light, and dark period(s), as well as the use of phytohormones or hormonal plant growth regulators (PGRs) on the seeds of water lettuce, concluding that germination is successful with the use of certain PGRs but is best achieved by control of phytochromes (sensory plant pigment) (Jing and Song 2008).

Jing and Song's study (2008) on water lettuce (*Pistia stratiotes*) included testing phytochromes (sensory pigment) in order to see if light affected germination. They documented that in dark and far red light, germination percent was zero (Jing and Song 2008). However, in the complete periods of white and red light as well as in the twelve hour alternating dark and day photoperiods of the white and red light, germination of the water lettuce seeds was promoted, with optimal germination of the seeds included in the complete white light experiment (Jing and Song 2008).

The study on phytohormones (plant hormones) included the use of hormonal plant growth regulators (PGRs) on seeds stored in complete darkness, where the application of indoleacetic acid, gibberellic acid, and benzyladenine did not increase seed germination

of water lettuce (*Pistia stratiotes*) (Jing and Song 2008). However, compared to the control group, there was increased germination of the water lettuce seeds attributable to affected phytohormones treated with the PGRs, ethrel (which mimics photoperiods) and sodium nitroprusside (which aids in generating shoots) (Jing and Song 2008).

Jing and Song's research (2008) concluded that germination of water lettuce (*Pistia stratiotes*) was successful with the use of the PGR ethrel mimicking photoperiods and was best achieved by influencing phytochromes with white light (bright light). This supports Hall and Okali's study (1974) that observed that the growth of water lettuce is optimal during spring months when solar radiation is highest due to proximity to the sun, which is conceivably due to the plant's native origin near the equator and its extended periods of high solar radiation levels.

Jing and Song (2008) also researched and studied seed dehydration, and found that the percent germinated water lettuce (*Pistia stratiotes*) seeds decreased with both slow and rapid dehydration; where the slow dehydration of the seeds caused greater damage and impairment in comparison to rapid dehydration of the seeds. Since it is a tropical plant, Jing and Song (2008) knew that water lettuce and their seeds cannot survive freezing temperatures but researched the minimum temperatures at which the seeds were still viable. These seed chilling experiments tested seed viability by putting the water lettuce seeds into dry, cool storage temperatures (Jing and Song 2008). The study found that water lettuce seeds can survive a minimum temperature of 65.0 degrees Fahrenheit (18.3 degrees Celsius), because at this temperature and above, the seeds were not susceptible to damage, indicating that the seeds have intermediate dormancy behavior (Jing and Song 2008).

Essentially, the same high percentage of moisture and temperature conditions are required for seed germination of water lettuce (*Pistia stratiotes*) when compared to water hyacinth (*Eichhornia crassipes*). Seed germination of water hyacinth occurred at 72.5 to 95.0 degrees Fahrenheit (22.5 to 35.0 degrees Celsius), compared to that of water lettuce which is 71.0 to 95.0 degrees Fahrenheit (21.7 to 35.0 degrees Celsius) (Chadwick and Obeid 1966; Weldon and Blackburn 1967; Barrett 1980).

Problems Caused by Water Lettuce

Water lettuce (*Pistia stratiotes*) causes all of the same severe ecological impacts on the environment and economy as that of water hyacinth (*Eichhornia crassipes*) (AERF 2009; TPWD 2009; WSDE 2010c). The problematic impact of water lettuce (*Pistia stratiotes*) includes creating dense mats on the surface that alter nutrient cycling, increasing rates of siltation, displacing native vegetation, altering the native ecology and water quality, clogging waterways, limiting recreational uses, and decreasing waterfront property values (Masser 2007; AERF 2009; TPWD 2009; WSDE 2010c). The evapotranspiration rates differ from that of water hyacinth which was three to six times normal evaporation, with water lettuce transpiring two to ten times more water when compared to normal water evaporation rates (Masser 2007). Water lettuce is now located all over the world and according to Chadwick and Obeid (1966) it "has become a nuisance so that it is ranked as one of the most troublesome of aquatic weeds" (p. 563).

Also, like water hyacinth (*Eichhornia crassipes*) "one of the most important problems caused by water lettuce [*Pistia stratiotes*] is the...nuisance...and disease association with mosquitoes" (Weldon and Blackburn 1967, p. 8). A sizeable percentage

of water lettuce is managed throughout the world, in order to try to gain biological control of mosquitoes (Weldon and Blackburn 1967).

Plant growth and productivity estimates depend more on the combined properties of site and species, than the effect of species alone (Hall and Okali 1974). Just as, "productivity is related to vigor of growth, the higher productivity of [water hyacinth], coupled with its taller growth-form and possession of petiolar bladders, may be part of the explanation of the observation that it frequently displaces [water lettuce] in sites of common occurrence of both species" (Hall and Okali 1974, p. 723). According to Moran (2009), water lettuce (*Pistia stratiotes*) is not as common in the Rio Grande Valley.

Utilization of Water Lettuce

Reported industrial applications and uses of water lettuce (*Pistia stratiotes*) are limited and rare; however, medicinal uses have been reported by Pliny in Egypt as early as 77 A.D. (Habeck and Thompson 1997). Folkloric medicinal uses of water lettuce include infusion of leaves to help treat for bladder and kidney afflictions, dropsy, and diabetes, the poultice of pounded leaves used to treat boils, hemorrhoids, ringworm, skin infections, and tumors, and powdered dry leaves mixed with honey have been used to treat syphilis (Umali-Stuart and Stiuart-Santiago 2010). Other folkloric uses of water lettuce (*Pistia stratiotes*) include using the juice of leaves mixed with coconut oil for a variety of chronic skin conditions (Umali-Stuart and Stiuart-Santiago 2010). Also, oil extracted from the leaves have been used for asthma, burns, dysentery, tuberculosis, worm infestations, and ulcers and in the Peruvian Amazon, the oil was said to have been used for arthritis (Umali-Stuart and Stiuart-Santiago 2010).

The chemical constituents and properties of water lettuce (*Pistia stratiotes*) include that the root is emollient, a laxative, and diuretic (Umali-Stuart and Stiuart-Santiago 2010). The leaves showed presence of sterols (Umali-Stuart and Stiuart-Santiago 2010). Studies have also reported that the leaves of water lettuce possess antifungal properties supporting some of its folkloric uses (Umali-Stuart and Stiuart-Santiago 2010). Also, studies of pharmacologic activities of water lettuce revealed hypotensive and bronchi dilating activity due to calcium channel and neuromuscular blocking action, from a methanol extract of the whole plant (Achola, Indalo, and Munenge 1997; Umali-Stuart and Stiuart-Santiago 2010). In these studies, systolic and diastolic blood pressures decreased in subjects that were given doses of the water lettuce (*Pistia stratiotes*) extract (Achola *et al.* 1997).

Research involving water lettuce (*Pistia stratiotes*) as a potential compost feedstock has been conducted, however, no credible evidence has reported water lettuce utilized as a beneficial compost source or feedstock, mainly because it is either too costly or dreadfully labor intensive to produce (Hodge 2010).

Managing/Controlling Water Lettuce

Prevention is the cheapest and best means of control and management of water lettuce (*Pistia stratiotes*) (Hodge 2010). Fifteen insects and larva (including six weevils) have been found to be herbivores of water lettuce (*Pistia stratiotes*) where some are not known to feed on any other plant species (Habeck and Thompson 1997). These insects have been and are still being observed for biological control of water lettuce (*Pistia stratiotes*) (Habeck and Thompson 1997). Released populations of water lettuce leaf weevil (*Neohydronomus affinis*) and water lettuce leaf moth (*Spodoptera pectinicornis*)

have been used for biological control of water lettuce (*Pistia stratiotes*) (Rivers 2005). However, due to the very short life cycle of both species (approximately 30 days), and the fact the moth only ate the water lettuce leaves and not the roots, as well as that the larvae only fed during the larval stage, and not during adulthood, plant populations were reduced by 40% for eighteen months after the release of the insects (Rivers 2005). After 18 months, water lettuce populations began to increase again (Rivers 2005). A problem with using insects for biological control of water lettuce is being able to attain a reliable supply (Hodge 2010).

Chemical agents such as kerosene oil have been used as a spray solution and have presented reasonable control of young water lettuce (*Pistia stratiotes*) (Weldon and Blackburn 1967). However, especially in mature water lettuce plants, re-growth following kerosene oil spray solution treatments was observed (Weldon and Blackburn 1967). Also, bromide and chloride formulations of the herbicide diquat have been used as chemical control agents for both water lettuce and water hyacinth, leading to only a slight burn of the leaf tissue (Weldon and Blackburn 1967). After five weeks following diquat treatment, the water lettuce population decreased by 85% and the water hyacinth was severely stunted, but only in individual size (Weldon and Blackburn 1967). Yet two months after treatment, the water lettuce population had increased by 35%, making up half of the original population size before treatment and the water hyacinth population had grown to fill in the remaining open water (Weldon and Blackburn 1967). While chemical treatment for managing water lettuce (*Pistia stratiotes*) has been effective, it is not only disfavored because of environmental effects, but it is also costly and must be

repeated relatively frequently for proper management to be successful (Habeck and Thompson 1997).

Hydrilla (Hydrilla verticillata)

History/Origin of Hydrilla

There has been controversy on the origin of hydrilla (*Hydrilla verticillata*). Due to its extensive, widespread habitat, it was originally thought to be native to parts of Asia, Africa, and Australia, but DNA analysis has recently discovered that two varieties or biotypes of hydrilla actually exist (Overholt *et al.* 2010; Union of Concerned Scientists [UCS] 2010; WSDE 2010a). The two biotypes are represented as either monoecious or dioecious; monoecious refers to plant species populations that have both male and female components on one individual plant and are able to produce seed(s), while dioecious refers to plant species populations that have plants with flowers of one sex, either male or female, and therefore, can not produce seed(s) with only one plant present (WSDE 2010a). As a result, the monoecious variety of hydrilla was discovered to be native to Korea, and the dioecious variety of hydrilla is recognized as native to India (Overholt *et al.* 2010; UCS 2010). Furthermore, these areas are also believed to be the original source of hydrilla because it was observed to not have invasive characteristics in these countries (Overholt *et al.* 2010).

Therefore, hydrilla (*Hydrilla verticillata*) is believed to be introduced into the United States at least twice (UCS 2010). The dioecious variety of hydrilla was introduced to Florida during the 1950s through the aquarium trade (UCS 2010). A fish and tropical plant farmer from St. Louis, Missouri imported and shared the hydrilla with a farmer in Tampa Bay, Florida. In Florida, the shared samples were stored in a wire cage

in a canal, where it flourished and quickly spread throughout the canal; by the 1970s it was well-established throughout Florida and had found its way into other southern states of the United States (UCS 2010).

The monoeious variety of hydrilla (*Hydrilla verticillata*) was introduced to the United States slightly later in separate introductions in the late 1970s the North Carolina and Washington D.C. areas, and then again in the early 1980s in California (UCS 2010). The monoeious variety of hydrilla was believed to be introduced when it "hitch-hiked" a ride with shipments of water lilies (UCS 2010; WSDE 2010b). The contaminated water lilies were then deliberately planted in a lake, commencing hydrilla's establishment (UCS 2010).

Hydrilla (*Hydrilla verticillata*) is now found in all states all along the southeast United States, and up along the eastern seaboard as far north as Cape Cod in Massachusetts, as well as all along the west coast of California and sporadic infestations in Washington (UCS 2010). In the United States, all of the dioecious variety of hydrilla are female (Overholt *et al.* 2010; WSDE 2010a).

Description of Hydrilla

Hydrilla (*Hydrilla verticillata*) is a submerged aquatic plant in the Frog's-bit family (*Hydrocharitaceae*) (Ramey 2001). *Hydr* is Latin meaning "water" and *verticillus* is Latin meaning "the whorl of a spindle," therefore the Latin names stands for "water plant with whorls of leaves" (Ramey 2001, p. 1). Hydrilla is a submerged aquatic plant (in either still or flowing water) that is rooted in the substrate and can grow up the surface and form dense mats (Ramey 2001). Hydrilla can grow virtually anywhere including freshwater springs, lakes, marshes, ditches, rivers, and even tidal zones, and can become

established in water depths ranging from a few inches to more than thirty feet of water (Ramey 2001).

Hydrilla (*Hydrilla verticillata*) stems are slender, light green, branched, and can grow up to 30 feet in length up to the surface of the water depending on the purity of water and soil substrate type (Ramey 2001; WSDE 2010b). The dark green leaves of hydrilla are small (five-eighths of inch or one to two millimeters), strap-like, pointed, and grow in whorls of four to eight around the stem, and the leaf margins are distinctly saw-toothed with one or more sharp teeth along the length of the underside of the midrib (Ramey 2001). Hydrilla produces miniature white flowers (one and a half millimeters in diameter) on long thread-like stalks, as well as rhizomes, stolons, adventitious white roots, turions at the leaf axils, and potato-like tubers attached to the roots in muddy substrates (Ramey 2001).

Hydrilla (*Hydrilla verticillata*) is winter-hardy to Zone 5, which means when submerged it can withstand temperatures as low as negative 20.2 degrees Fahrenheit (negative 29.0 degrees Celsius) (Ramey 2001; "Plants for a Future" 2008). According to Kasselmann (1995), 86.0 degrees Fahrenheit (30.0 degrees Celsius) is the maximum temperature at which hydrilla is able to flower (Ramey 2001). Research has concluded that optimum growth of hydrilla is at temperatures between 70.0 to 85.0 degrees Fahrenheit (21.1 to 29.4 degrees Celsius) (Netherland 1997); under these conditions it can grow as much as an inch a day (Netherland 1997; Ramey 2001).

Vegetative Propagation of Hydrilla

Hydrilla (*Hydrilla verticillata*) has numerous propagation methods, such as spreading through rhizomes, stolons, tubers, and turions, as well as through root and stem

fragments (WSDE 2010b). Therefore, it is extremely effective in development, distribution, and succession. However, hydrilla has been discovered to have two biotypes and is represented as either being monoecious or dioecious (WSDE 2010a). Monoecious refers to a plant that has both male and female components on one plant and is able to produce seed(s), while dioecious refers to a plant that has either all male or all female flowers and, therefore, cannot produce seed(s) (WSDE 2010a). In the United States, the dioecious variety of hydrilla (*Hydrilla verticillata*) reproduces strictly by asexual methods of vegetative reproduction including via rhizomes and stolons, as well as by forming vegetative propagules called tubers and turions (Robles n.d.). Research has found that both monoecious and dioecious varieties of hydrilla utilize more energy towards tuber and turion production, and is, therefore, reported to have a greater potential to spread by these means when compared to seed production (Spencer and Anderson 1986; WSDE 2010b).

A tuber is an enlarged part of the terminal node of a rhizome growing underground in the sediment substrate (WSDE 2010a). A hydrilla (*Hydrilla verticillata*) tuber looks like a tiny potato that is one-eighth to one-half an inch (four to ten millimeters) in length (Robles n.d.). Tubers can remain dormant in the sediment and maintain a viable state for years (WSDE 2010a). Tubers are produced in response to days with long photoperiods or days with long durations of sunlight, and the monoecious variety of hydrilla appears to produce tubers in a shorter period, four weeks, when compared to the dioecious variety which takes up to six to eight weeks to produce tubers (Spencer and Anderson 1986). Therefore, the monoecious hydrilla variety has an advantage in environments characterized by shorter growing seasons and it is speculated that this is

why the monoecious variety is found in the northeastern states and that the dioecious populations are associated with warmer geothermal-influenced waters in the southern states such as Florida and within Texas (Spencer and Anderson 1986; WSDE 2010a).

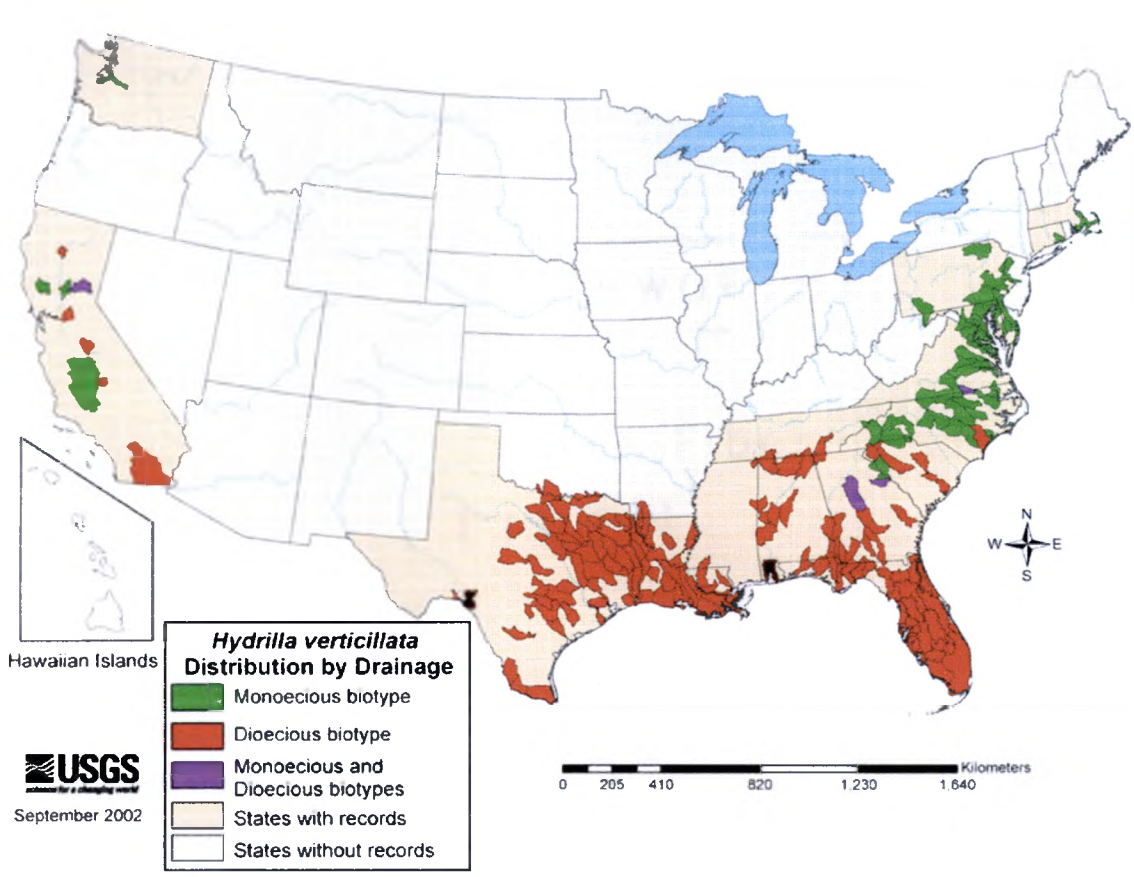

Figure 1. Map of the United States that illustrates the distribution by drainage of Hydrilla (*Hydrilla verticillata*). Map documents only the presences of the dioecious biotype of hydrilla within Texas as of Septmember 2002. Map provided by United States Geological Survey (USGS).

Hydrilla (*Hydrilla verticillata*) turions arise from a leaf axils and are tiny, spiny green buds one-eighteenth of an inch (two millimeters) in diameter and one-half of an inch (eight millimeters) in length (Robles n.d.). Turions are produced over winter months by many aquatic plants, and are like axillary buds produced along stems, that detach, and then have the potential to form a new plant (or plant colony) (Robles n.d.; Urdang and Flexner 1968). The tubers and turions of hydrilla (*Hydrilla verticillata*) can survive

drying, herbicides, ice cover, and ingestion and regurgitation by waterfowl (WSDE 2010a). This is a problem because one square meter of hydrilla (*Hydrilla verticillata*) can produce 5,000 tubers (WSDE 2010b). Furthermore, Netherland (1997) observed hydrilla tubers and turions and reported that within a period of two weeks, "sprouting of these propagules [was] often greater than 90%" (p. 8). Furthermore, optimal growth of hydrilla as well as optimal productivity of tubers and turions was found to occur in environments in temperatures between 70.0 to 85.0 degrees Fahrenheit (21.1 to 29.4 degrees Celsius) (Netherland 1997). Yet, hydrilla has been found to survive in temperatures as high as 95.0 degrees Fahrenheit (35.0 degrees Celsius) however, this is the maximum temperature hydrilla has been found tolerate and will not produce turions or tubers for further growth and distribution at this temperature (Netherland 1997).

In addition to reproducing vegetatively by rhizomes, stolons, tubers, and turions, hydrilla (*Hydrilla verticillata*) can also reproduce by means of root and stem fragments, which can develop into new plants (and plant colonies) (WSDE 2010a). In hydrilla, these stem fragments can be very small; it only requires as few as two nodes or whorls of leaves to generate a new plant (WSDE 2010b). Therefore, these small stem fragments can be easily spread from one body of water to another by not only recreational activities, but waterfowl and other riparian species as well (UCS 2010).

Sexual Reproduction of Hydrilla

Through research, hydrilla (*Hydrilla verticillata*) has been discovered to have two biotypes and are represented as either monoecious or dioecious plants (WSDE 2010a). Studies have shown that both biotypes of hydrilla (*Hydrilla verticillata*) flower similarly (WSDE 2010b). However, due to its efficiency and effectiveness of reproducing

vegetatively, as well as the lack of sexual reproductive organs on the dioecious variety, research on sexual reproduction and seed development is limited. Furthermore, research has found that both varieties of hydrilla utilize more of their energy towards tuber and turion production (Spencer and Anderson 1986; WSDE 2010b). Hydrilla tubers and turions also are reported to have a greater potential to spread when compared to their potential to spread by seed production (Spencer and Anderson 1986; WSDE 2010b).

Flower(s), Fruit, and Seed Development of Hydrilla

Through research, hydrilla (*Hydrilla verticillata*) has been discovered to have two biotypes and are represented as either monoecious or dioecious varieties (WSDE 2010a). However, studies have shown that both biotypes of hydrilla (*Hydrilla verticillata*), the monoecious and dioecious varieties, flower similarily (WSDE 2010b). The female flowers of hydrilla are minute (one sixteenth of an inch in diameter), white, and solitary (Ramey 2001). The isolated female flower is attached to a thread-like stalk, extending up to four inches in length, allowing the flower to float on the surface of water bodies (Ramey 2001). Once at the surface, the flower opens to form a wide funnel, where the petal rims hold the flower at the surface, as well as prevent water from getting into the flower (Ramey 2001). The minute hydrilla female flowers are composed of six petals, and three styles with stigmas (Ramey 2001).

The male flowers of hydrilla (*Hydrilla verticillata*) are the same size as the female flowers (Ramey 2001). The male flowers are comprised of six petals, three stamen, and are greenish, and the solitary male flower(s) are closely attached to the leaf axils near the stem tips (Ramey 2001). Also, the male flowers break loose from the plant and then rise to the surface; once at the surface, the free-floating male flower looks like an inverted

bell (Ramey 2001). Research has observed hydrilla flowering from May to October ("Plants for a Future" 2008); and, in areas of large populations, the free-floating male flower will randomly bump into the female flowers and fertilize them (Ramey 2001). Therefore, hydrilla (*Hydrilla verticillata*) seeds are pollinated by water ("Plants for a Future" 2008).

Hydrilla (*Hydrilla verticillata*) produces tiny brown seeds that have a smooth textural margin (Mulholland-Olson 2004). The hydrilla seeds are less than a millimeter in diameter and length, and can be found in groups of five or less in a single linear sequence within the ovary of the style (Mulholland-Olson 2004). Research on hydrilla has reported observation of the seeds germinating and developing in the summer and fall from June to October ("Plants for a Future" 2008).

Seed Germination of Hydrilla

Due to its efficiency and effectiveness of reproducing vegetatively, as well as the lack of the presence of necessary sexual reproduction organs of the dioecious variety in some environments, research on sexual reproduction and seed development of hydrilla in nature is limited. The lack of research on seed germination of hydrilla (*Hydrilla verticillata*) is also due to the fact that since the dioecious variety exists in nature, only the dioecious variety appeared to have been observed and studied within the United States, until recently; therefore, observation, research, and documentation of female hydrilla plants have just currently began to be differentiated between, observed, and studied (Spencer and Anderson 1986). Yet, since the discovery in Washington D.C. of the presence of the monoecious variety of hydrilla in the United States, "other monoecious populations have been identified in Virginia, North Carolina, Delaware, and

Maryland, [where] seed production has been reported" (Spencer and Anderson 1986, p. 551). The presence of "seedlings appears to be rare," so it is speculated that "seed production [is] a minor means of reproduction" (WSDE 2010b, p. 6). Furthermore, research has found that both varieties of hydrilla utilize more of its energy into tuber and turion production (Spencer and Anderson 1986; WSDE 2010b). Hydrilla tubers and turions are reported to have a superior potential to spread when compared to distribution through seed production (Spencer and Anderson 1986; WSDE 2010b).

Hydrilla (*Hydrilla verticillata*) seeds have been reported to germinate and develop between the months of June to October ("Plants for a Future" 2008). Furthermore, research by Lal and Gopal (1993) discovered that hydrilla seeds are light sensitive and will germinate within a week in the laboratory at temperatures between 74.0 to 83.0 degrees Fahrenheit (23.3 to 28.3 degrees Celsius). In the experiment, both dried and wet hydrilla seeds were stored in the dark for up to a year, and both germinated readily when exposed to light (Lal and Gopal 1993). Lal and Gopal (1993) concluded that the seeds portray a long term strategy for survival of hydrilla in regions of prolonged dry periods with monsoon conditions.

Problems Caused by Hydrilla

In the 1960's, hydrilla (*Hydrilla verticillata*) was imported into the United States for use in aquariums (Hodge 2004; Hodge 2010). However, it was stored and cultured in Florida canals, where it escaped, and is now established in virtually 700 bodies of water in twenty states, including at least 80 lakes in the state of Texas (Hodge 2004). Hydrilla continues to be sold by aquarium supply dealers and over the internet even though it is listed by the Texas Parks and Wildlife Department (2010) as an Invasive, Prohibited, and

Exotic Species, which are organisms legally classified as exotic, harmful, or potentially harmful, and include regulations that no persons may import, possess, sell, or place them into water of the states except as authorized by rule or permit issued by the department.

Hydrilla (*Hydrilla verticillata*) has been listed as a federal noxious weed since 1974 (when the *Federal Noxious Weed Act* was created) and is on the Washington State Department of Agriculture's Quarantine list. Hydrilla is not only exotic, but very invasive and problematic because it has several physiological and morphological adaptations that allow it to out-compete native aquatic vegetation (WSDE 2010b). Hydrilla can grow at lower light intensities when compared to many other aquatic plants, which makes it difficult to shade out and allows it to grow for longer periods during the day (WSDE 2010b). Hydrilla can absorb carbon from the water more efficiently than other aquatic plants; therefore, even though it is considered a winter-hardy plant, it can continue to flourish during warmer temperatures such as summer months when high temperatures and sunlight levels allow virtually all aquatic plant species to photosynthesize faster (WSDE 2010b). This causes the hydrilla to saturate the water column with oxygen and limits the availability of carbon (WSDE 2010b). Hydrilla can store extra phosphorus and use the stored phosphorus to continue to grow when phosphorous is limited in the environment (WSDE 2010b). Hydrilla (*Hydrilla verticillata*) can tolerate a wide range of water temperature and quality conditions, including sedimentary density and even salinity of up to nine to ten parts per thousand, and therefore can encroach upon the outer limits of estuaries (WSDE 2010b). Hydrilla can flourish in both flowing and still water (WSDE 2010b). Studies have shown that

hydrilla actually grows faster and more aggressively in areas of flowing water (WSDE 2010b).

Due to hydrilla's diverse physiological and morphological adaptations and the fact that it can grow up to an inch a day, hydrilla (*Hydrilla verticillata*) creates the same severe ecological impacts on the environment and economy as that of water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) (Ramey 2001; AERF 2009; TPWD 2009; WSDE 2010a; WSDE 2010c). Hydrilla is considered to be an invisible nuisance because it can go unnoticed below the water surface until it has completely filled in and taken over an aquatic area (Ramey 2001). Areas of hydrilla infestations "top out" at the surface, creating dense mats not only on the surface like water hyacinth and water lettuce, but throughout the water column as well (Ramey 2001). These dense mats alter nutrient cycling, displace native vegetation, alter the native ecology, water quality, and oxygen levels, decrease waterfront property values, and severely clog waterways and irrigation canals where mats or extensive fragments collect at culverts and clog essential water control pumping stations (Ramey 2001; Masser 2007; AERF 2009; TPWD 2009; WSDE 2010a; WSDE 2010c). Hydrilla (*Hydrilla verticillata*) significantly interferes with both recreational and commercial activities, including swimming, boating, and fishing (Ramey 2001).

In areas of infestations, hydrilla (*Hydrilla verticillata*) can critically alter the entire ecosystem; it can displace relied on populations of native plant species which influences native fish species who do not consume hydrilla as food (Robles n.d.; Ramey 2001; WSDE 2010b). Research reports that the grass carp species (*Ctenopharyngoden idella*) are the only known herbivorous fish species that prefer hydrilla (*Hydrilla*

verticillata) as a daily food source; therefore native fish species decrease in size and weight in hydrilla infested areas (Robles n.d.; Ramey 2001; WSDE 2010b). Alteration and even displacement of native plant and fish species also affect native waterfowl species, which rely on the native populations as daily food sources (Robles n.d.; Ramey 2001; WSDE 2010b).

Unlike water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*), there is no evidence that hydrilla (*Hydrilla verticillata*) is specifically linked to diseases associated with mosquitoes. However, hydrilla does absorb oxygen from the water, where it can deplete oxygen levels and create stagnant water, which are breeding grounds for mosquitoes (WSDE 2010b).

Utilization of Hydrilla

In the 1960's, hydrilla (*Hydrilla verticillata*) was imported into the United States for use in aquariums (Hodge 2004). Due to the diverse physiological and morphological adaptations of hydrilla, it is consider the perfect aquatic plant and for decades it was the number one plant to use as an aquarium plant (Hodge 2004; WSDE 2010b). However, due to its invasiveness, it is illegal to import, possess, sell, or acquire hydrilla unless authorized by rule or permit issued by the Texas Parks and Wildlife Department (2010); yet it controversially continues to be sold through aquarium supply dealers and over the internet (Ramey 2001).

Hydrilla (*Hydrilla verticillata*) has been found to be a fundamental source of food for only certain wildlife. Many fish species utilize dense areas of hydrilla as cover from predators; however, it was found that the herbivorous grass carp species (*Ctenopharyngodon idella*) is the only fish species known to prefer hydrilla over other

aquatic plants for food (Robles n.d.; McKnight and Hepp 1995; WSDE 2010). Other wildlife that utilize hydrilla as a daily food source include certain waterfowl species, such as ring-necked ducks (*Aythya collaris*), pied-billed grebes (*Podilymbus podiceps*), and American coots (*Fulica americana*) (Johnson and Montalbano 1987; Esler 1990; TPWD 2009).

Hydrilla (*Hydrilla verticillata*) has been utilized in the treatment of wounds (especially if there is debris in the wound), as well as boils and abscesses, where a dried detergent-like powder of the plant is applied to help accelerate healing ("Plants for a Future" 2008).

Raw hydrilla is not known to be edible to humans; however, in the past has been used in the sugar refinery industry, and today is made into a powder where it is utilized as an innovative nutritional supplement (BioSan Laboratories, Inc. 2003). Since hydrilla is a submerged aquatic plant, it is protected from airborne and floating contaminants and is therefore a cleaner aquatic plant than other well-known aquatic food supplements like spirulina (BioSan Laboratories, Inc. 2003). Furthermore, because it is a submerged aquatic plant, it not only absorbs and accumulates nutrients from the soil that it is rooted in, but the water as well, making it a nutrient dense plant (BioSan Laboratories, Inc. 2003). Therefore, an innovative use of hydrilla (*Hydrilla verticillata*) as a dietary supplement was investigated, and literature claims it to be rich in all the essential amino acids (including a high concentration of lysine), beta-carotene, calcium, copper, linolenic acid, magnesium, manganese, potassium, zinc, vitamin C, vitamin E, and vitamins B1, B2, B5, B6, and B12 (BioSan Laboratories, Inc. 2003). The making of this dietary supplement requires hydrilla to be dehydrated at low temperatures to remove the moisture

without damaging the living enzymes and nutrients, resulting in a green powder that is a "synergistic" complex of nutrients to the human body (BioSan Laboratories, Inc. 2003).

Controversially, even though hydrilla (*Hydrilla verticillata*) is considered a nutrient dense plant, some research results indicate that it may be limited as a potential feedstock and/or compost ingredient, where poor quality compost was produced (Parsons and Cuthbertson 2004). However, research has found that hydrilla is a nitrogen-rich species and therefore a potential feedstock (Little 2010). Hydrilla is a fibrous plant and discovered to require two weeks longer to cure, the extended curing time is thought to be why poor quality compost has been reported (Little 2010).

Managing/Controlling Hydrilla

Since hydrilla (*Hydrilla verticillata*) has been declared a federally listed noxious weed since 1974, scientific research and 35 years of practical experience have produced relatively successful management programs using biological agents, herbicides, mechanical removal, and physical habitat manipulation (WSDE 2010a). However, "in spite of long-term intensive management efforts, hydrilla is still a major weed problem" (WSDE 2010a, p. 5). Prevention is the cheapest and best means of control and management of hydrilla (Hodge 2010).

Currently, endothall and fluridone are two Environmental Protection Agency (EPA) registered herbicides that are effective against hydrilla (*Hydrilla verticillata*) (WSDE 2010b). Endothall is a contact herbicide that is fast-acting, but only used when necessary for immediate, short-term control (WSDE 2010b). Fluridone is a systemic herbicide that is an effective long-term control agent (WSDE 2010b). However, it is high in cost, slow acting, and non-selective toward other macrophyte species (WSDE 2010b).

In Florida and other southern states, populations of hydrilla have been observed, that have developed varying levels of resistance toward fluridone (Robles n.d.; Puri *et al.* 2007; WSDE 2010b). While chemical treatment for managing hydrilla (*Hydrilla verticillata*) has been effective, it is disfavored because it is costly and time-consuming as far as waiting for the agents to take effect and time spent for the necessary frequently repeated application for proper management to be successful (Robles n.d.) Furthermore, chemical treatment is disfavored due to associated environmental effects, especially in ecosystems that are declared "critical habitat" or areas where the local community would potentially use(s) the contaminated water sources as drinking water from the tap (TPWD 2009). Therefore, other methods of control have been and continue to be researched, in order to aid in managing hydrilla.

In 1981, a cooperative study between the United States Army Corps of Engineers, the United Stated Department of Agriculture, and the University of Florida was initiated in which numerous insects were identified, quarantined, tested, and eventually released in Florida and other states, to study biological control of hydrilla (*Hydrilla verticillata*) (WSDE 2010b). However, the majority of the insects were collected in the tropics and it was questionable whether these insect populations could survive and become established in northern states such as Washington (WSDE 2010b). It was observed that insects such as a weevil species (*Bagous hydrillae*), and two leaf-eating diptera fly species (*Hydrellia balciunas* and *Hydrellia pakistanae*) could be used as biological control agents to reduce photosynthetic capacity, biomass, and tuber number (Robles n.d.). Of these, the Asian hydrilla leaf-mining fly (*Hydrellia pakistanae*) is the most effective insect utilized as a biological control agent at this time (Robles n.d.). However, large populations and

frequently repeated releases of the insect(s) are necessary to impact the long-term growth of hydrilla (*Hydrilla verticillata*), where being able to obtain a reliable supply could be a problem (Robles n.d.; Hodge 2010).

Mycoleptodiscus terrestris is a plant pathogen under development with expectations of the pathogen having the ability to collapse and disrupt plant cells (Robles n.d.). Additionally, grass carp (*Ctenopharyngodon idella*) is a herbivorous grass carp species that is deemed the most effective biological control agent for hydrilla (*Hydrilla verticillata*) (Robles n.d.; WSDE 2010b). However, because it is an exotic species from Asia, the United States is taking precautions with tightly regulated release of only sterile triploid fish into waterways (Robles n.d.; McKnight and Hepp 1995; WSDE 2010b).

Mechanical methods such as mechanical harvesters and cutting/chopping machines have been used to help control hydrilla (*Hydrilla verticillata*) (Robles n.d.; Ramey 2001). However, mechanical methods do not provide long term control since hydrilla spreads efficiently from stem and root fragments (Robles n.d.; Ramey 2001). Therefore, these methods may actually increase the plant distribution rate and area (Robles n.d.; Ramey 2001). Cutting and harvesting methods should only be used when the infestation populates the entire body of water, because using mechanical methods while the hydrilla is still invading will tend to enhance the rate of distribution (Ramey 2001; WSDE 2010b). Furthermore, hydrilla produces a great biomass per square meter when compared to the majority of other aquatic plants (WSDE 2010b). Harvesting can be costly, $600 to $1,200 per acre, in terms of operation costs including the use and maintenance of the harvesting machine (i.e. gas) and certified personnel fees/wages (WSDE 2010b).

In some instances, reservoirs, irrigation canals, and lake systems have the ability to lower the water level by means of drawdowns, which exposes the submerged hydrilla plants causing them to dry out and die (Ramey 2001; WSDE 2010b). However, drawdowns do not provide long term control either, because they only lower the water level and do not affect the hydrilla tubers that are buried in the moist sediment below (Robles n.d.; WSDE 2010b).

Hand removal of roots and stems from the surface bottom of a body of water has been successful for scattered individual hydrilla plants or isolated areas; however, re-infestation is possible from buried tubers and turions (Robles n.d.).

Georgia Cane (Arundo donax)

History/Origin of Georgia Cane

Due to the widespread distribution and extensive utilization by man, Georgia cane (*Arundo donax*) has many common names: Georgia cane, giant cane, giant reed, donax cane, bamboo reed, cane, river cane, reedgrass, wild cane, and many others (Perdue 1958; Invasive Species Specialists Group [ISSG] 2006).

Georgia cane (*Arundo donax*) is an exotic species that was widely introduced into the southern United States and is an Old World species – an ancient, long-lived species existing and surviving historically throughout the continents of the eastern hemisphere before the discovery of the Americas (Perdue 1958; Gould 2008). Georgia cane is thought to originate in Egypt or Mesopotamia, where archaeological evidence indicates that reed woodwind musical instruments made from the stalk(s) of the plants were not only present but well-established in the life and culture of the people (Perdue 1958).

Also, Georgia cane has been used to make arrows used by the Egyptians and Greeks (Perdue 1958).

Primarily referred to as originating from Mediterranean Europe, Georgia cane (*Arundo donax*) was introduced in the Americas in the late 1700s and early 1800s (Decruyenaere and Holt 2001; Wijte *et al.* 2005). Since then and primarily through intentional human introductions, Georgia cane has become widely distributed in all of the subtropical and temperate areas worldwide including Australia and many islands of the Pacific and Atlantic oceans (Perdue 1958; TIPPC 2010). Georgia cane has primarily become established through plantings as an ornamental and through highway plantings along culverts and ditches for erosion and flood control (Gould 2008; TIPPC 2010). However, due to its broad environmental tolerance, by the 1990s, Georgia cane had infested tens of thousands of acres in riparian ecosystems worldwide (Decruyenaere and Holt 2001; Wijte *et al.* 2005). Consequently, in 1994, the California Invasive Plant Council declared it one of the 20 most critically widespread nuisance plants (Wijte *et al.* 2005). Georgia cane is found throughout the state of Texas, except for the High Plains region and found in 25 other states within the United States (Gould 2008; TIPPC 2010). In addition, Georgia cane (*Arundo donax*) is an invasive species listed as a noxious weed in Texas, an exotic plant pest in California, an invasive weed in Hawaii, and as an invasive, exotic pest in Tennessee (McWilliams 2004). According to Perdue (1958), "there are abundant wild growths [of Georgia cane] along the Rio Grande River" and the "most abundant growths occur on fertile soils well above the river bed that become flooded only during rare flash floods" (p. 370-371).

Description of Georgia Cane

Georgia cane (*Arundo donax*) is a hydrophyte, known to grow where water tables are near or at the soil surface, such as riparian areas along riversides, streams, ditches, and channels (ISSG 2006). Georgia cane is in the *Poaceae*, or Grass Family, and is a very tall and robust bamboo-like, perennial grass with stocky, spreading clusters of thick culms that grow to 20 feet (6.1 meters) tall (ISSG 2006; TIPPC 2010). The bamboo-like leaves of Georgia cane are hairless, numerous, approximately one and a half inches (five centimeters) wide and a foot (30.5 centimeters) long, and arranged oppositely in two rows along the culms (ISSG 2006; Gould 2008; TIPPC 2010). Georgia cane is the largest and tallest ornamental grass other than bamboo, and is the tallest grass that can be grown outside of the tropics (ISSG 2006). The leaf blades are thick, flat, elongate, and margins are sharp to the human touch and can cut if handled carelessly (ISSG 2006; Gould 2008). In the winter, the foliage of Georgia cane dries from a light green to a light brown, tan color and rattles in the wind (ISSG 2006). Georgia cane (*Arundo donax*) spreads through thick, knobby rhizomes, which form a framework of interconnecting root mats (ISSG 2006).

Georgia cane can tolerate a wide variety of conditions, including high salinity, and can thrive in many types of soil including everything from loose sands to heavy clays (ISSG 2006). However, Georgia cane grows best in riparian areas of well-drained soils where abundant moisture and sunlight is available (ISSG 2006). Georgia cane is well-adapted to the elevated disturbance dynamics of riparian systems and has also been demonstrated to prefer areas with enriched nitrogen levels (ISSG 2006).

Vegetative Propagation of Georgia Cane

Similarly to the other invasive species of interest in this study, Georgia cane (*Arundo donax*) reproduces primarily through vegetative reproduction of rhizomes and stem fragments, which root and sprout readily, as well as penetrate deep into the soil substrate (Wijte *et al.* 2005; ISSG 2006; TIPPC 2010). The growing season for Georgia cane usually begins in early March and continuous until the end of October, when there is the emergence of new shoots from the rhizomes (Wijte *et al.* 2005). Georgia cane stems are perennial and new stems of Georgia cane grow from nodes near the ends of the rhizomes (Wijte *et al.* 2005). Georgia cane's rhizomes are between the nodes and the stems that they grow from, which is approximately seven centimeters in length (Wijte *et al.* 2005).

Once established, Georgia cane tends to form immense, continuous, clonal root masses that can be more than a meter thick, sometimes covering several acres (ISSG 2006). Therefore, Georgia cane can form dense clumps in which one square meter may contain up to 80 stems (Wijte *et al.* 2005). Georgia cane's vegetative reproduction and spread by rhizome and stem fragments is allegedly the exclusive reason for its invasion and establishment in California (Wijte *et al.* 2005). Furthermore, intense floods can significantly increase the dispersal of Georgia cane, where the rhizomes of a stand can be undercut by the eroding action of fast-moving floodwaters (Wijte *et al.* 2005). This can break off rhizomes, stems, and even small stands that are still attached to the rhizome fragments, where they can be carried downstream and establish a whole new stand or buildup around drainage pipes and other flood control structures (Khudamrongsawat *et al.* 2004; Wijte *et al.* 2005).

Surface layering, in which stems touching the ground sprout roots, of Georgia cane has been reported to disperse as much as seven and a half times faster the spread of Georgia cane when compared to spread by rhizomes, but is thought only to occur within flood zones (ISSG 2006). Furthermore, the longevity of Georgia cane propagules has been studied, and if not waterlogged, the rhizomes can remain viable for six months after isolation from the parent plant (Decruyenaere and Holt 2001). Regeneration from stem fragments was observed to only occur with the presence of an axillary bud, where a new shoot sprouted from the axillary bud and was followed by root emergence from the base of the new shoot (Wijte *et al.* 2005). However, regeneration of Georgia cane from stem fragments with the presence of an axillary bud was observed to occur only 30% of the time, where only one out of every three regenerated with the presence of an axillary bud (Wijte *et al.* 2005). Furthermore, Georgia cane "branch during the second year of growth," therefore, axillary buds only form on second year growth culms (Perdue 1958, p. 369). At temperatures below 50.0 degrees Fahrenheit (10.0 degrees Celsius), it was observed that no rooting occurred (Wijte *et al.* 2005). Georgia cane's growth ability is due to the thick and deeply penetrating root mat development that reach deeply situated sources of moisture (Perdue 1958). New plantings of Georgia cane are exclusively started through vegetative propagation of rootstocks or roots attached to rhizome cuttings (Perdue 1958).

Sexual Reproduction of Georgia Cane

Georgia cane (*Arundo donax*) primarily spreads asexually by dispersal of stem and rhizome fragments (Khudamrongsawt *et al.* 2004). There have been limited reports regarding seed production; therefore, little is known about Georgia cane's sexual

reproduction and dispersal by seeds (ISSG 2006). Georgia cane flowers but has been found to generate seeds that are non-viable (Wijte *et al.* 2005); and according to Khudamrongsawat and colleagues (2004), "viable seeds have not been found in the United States" (p. 395). Furthermore, according to Gould (2008), Georgia cane "apparently does not produce fertile seeds" (p. 33). Likewise, the Texas Invasive Plant and Pest Council (2010) assert that little is known, but research on sexual reproduction of Georgia cane has the potential to yield improvements in the management of Georgia cane.

Flower(s), Fruits, and Seed Development of Georgia Cane

Georgia cane (*Arundo donax*) "flowers but does not produce viable seeds" (Wijte *et al.* 2005, p. 507). The inflorescences of Georgia cane form a feather-like plume panicle, which start appearing in the late summer and are initially purplish, aging to silver in the fall (ISSG 2006; TIPPC 2010). The inflorescence plumes are dense, one to two feet (0.3 to 0.6 of a meter) in length, and the plume stands above the foliage (ISSG 2006; TIPPC 2010). The dense plume is made up of hundreds of hairy, light-weight spikelets, in which one to two individual florets with the enclosed grain occurs (ISSG 2006; Gould 2008; TIPPC 2010). Typically, each floret consists of two bracts, the lemma (lower) and the palea (upper) bracts, and two to four enclosed flowers, and "the floret may be perfect, pistillate, staminate, or neuter" (Gould 2008, p. 259; TIPPC 2010).

Seed Germination of Georgia Cane

Since Georgia cane (*Arundo donax*) reproduces primarily through vegetative reproduction of rhizomes, few studies have been performed regarding seed production. Therefore, little is known about Georgia cane's sexual reproduction and dispersal by

seeds. Georgia cane produces seeds from September to November (Gould 2008); however, studies have reported that the seeds of Georgia cane are non-viable (Wijte *et al.* 2005). Additionally, according to Khudamrongsawat and colleagues (2004), "viable seeds have not been found in the United States" (p. 395). Controversially, according to Perdue (1958), "plants have been grown from seed collected in Afghanistan, Baluchistan, and Iran" (p. 371).

In spite of insufficient knowledge on sexual reproduction, seed viability, dormancy, germination, and seedling establishment of Georgia cane (*Arundo donax*), the Texas Invasive Plant and Pest Council (2010) emphasizes that research on these topics has the potential to yield improvements in management and control of Georgia cane.

Problems Caused by Georgia Cane

Populations of Georgia cane (*Arundo donax*) primarily affect riversides and stream channels, where it has been known to reduce and displace native vegetation (ISSG 2006). The reduction and displacement of native vegetation has resulted in habitat loss for birds, including the federally endangered Least Bell's Vireo (*Vireo bellii pusillus*) and the federally threatened willow flycatcher (*Empidonax traillii extimus*) (Wijte *et al.* 2005). Georgia cane is well adapted to the high disturbance dynamics of riparian systems and once established, it has the ability to outcompete and completely suppress native plant species, alter nutrient cycling, reduce habitat for wildlife, and cause severe ecological changes (ISSG 2006). The extensive, fibrous, interconnecting root mats of Georgia cane form a framework that becomes intertwined with debris usually around bridges, culverts, and other flood control structures, thus affecting the structures function and altering ecosystems (Wijte *et al.* 2005; ISSG 2006).

The rapid vegetative growth rate of Georgia cane (*Arundo donax*) is estimated at two to five times faster than native competitors, and is capable of invading new areas where it instantaneously can form pure stands (ISSG 2006). Georgia cane is extremely flammable, which increases the likelihood and intensity of fires (ISSG 2006). Furthermore, after fire situations, Georgia cane recovers three to four times faster than native species and outcompetes the re-growth of native vegetation (ISSG 2006). Georgia cane has a high rate of evapotranspiration, and therefore, lowers water levels, which has been found to be detrimental to ecosystems in arid Southern California (Wijte *et al.* 2005). Georgia cane is reported to use "three times the volume of water per unit biomass [than] used by native vegetation" (Khudamrongsawat *et al.* 2004, p. 395).

Georgia cane (*Arundo donax*) is listed as a noxious weed in Texas, an exotic plant pest in California, an invasive weed in Hawaii, and as an invasive, exotic pest in Tennessee (McWilliams 2004).

Utilization of Georgia Cane

The stalks or culms of Georgia cane (*Arundo donax*) have historically and continue to be used to make reeds for woodwind instruments and were once used to make organ pipes (Perdue 1958). Georgia cane has played a significant role in the culture of the western world through its influence on the development of music and its utilization in the creation of music can be traced back 5,000 years (Perdue 1958). Also, historically, Georgia cane has been found to be used to make arrows utilized by the Egyptians, Greeks, and other early peoples (Perdue 1958).

Georgia cane (*Arundo donax*) has been used as a source of cellulose for rayon and considered as a source of paper, however, requires twice as much chemical for bleaching

when compared to other plants used for paper (Perdue 1958). In Mexico, Georgia cane is soaked in water and then crushed and woven into mats (Perdue 1958). The culms of Georgia cane are widely used for fishing rods and walking sticks (Perdue 1958).

Georgia cane (*Arundo donax*) has been grown as an ornamental for its attractive appearance of towering, huge feather-like plumes of purplish flowers (ISSG 2006). The massive, robust, and fluffy purplish plumes have been used in floral arrangements (ISSG 2006). In folk medicine, the rhizomes and rootstock of Georgia cane was used for dropsy or edema (ISSG 2006). The rhizomes and rootstocks of Georgia cane have also been reported to be boiled in wine with honey and used to fight cancer (ISSG 2006). Georgia cane is a poor forage grass for livestock as well as for wildlife, and is only eaten by browsing or grazing animals when no other food is available (Gould 2008). However, Georgia cane provides cover for wild birds and small animals, especially when they come in for a drink (Gould 2008).

Georgia cane (*Arundo donax*) is frequently planted in wet soils and along stream banks and ditches and valued for its erosion and flood control properties (ISSG 2006; Gould 2008). In Texas and Australia, Georgia cane is utilized for stabilizing drifting sands (Perdue 1958); and in Argentina, it is recommended as reinforcement for the edges of mountain terraces (Perdue 1958).

Managing/Controlling Georgia Cane

Since isolated stems and rhizomes are capable of producing new plants, there has been some controversy over how to properly dispose of Georgia cane (*Arundo donax*) once it has been removed from an infested area (Decruyenaere and Holt 2001). According to Decruyenaere and Holt (2001), "chemical control with phloem-mobile

herbicides [are] most effective" in controlling Georgia cane (p. 760). Also, the use of other chemical herbicides such as glyphosate and fluazipop, applied as a cut stump treatment or foliage spray after flowering, have been found to control Georgia cane (ISSG 2006). However, while chemical treatment for managing Georgia cane has been effective, it is disfavored because it is costly, time-consuming, and requires frequently repeated application for proper management to be successful (TPWD 2009). Furthermore, chemical treatment is disfavored due to associated environmental effects, especially in ecosystems that are declared "critical habitat" or areas where the local community potentially use(s) the contaminated water sources as drinking water from the tap (TPWD 2009). Also, caution should be taken when using herbicides around water or in wetlands; most wetlands in the United States are federally protected (ISSG 2006). Therefore, other methods of control have been and continue to be researched, in order to aid in managing Georgia cane.

A popular method for treating Georgia cane (*Arundo donax*) is integrated management, where the stalks are hand-cut and treated with an herbicide, then the substrate biomass is removed by hand, and then repeated three to six weeks later when plants grow to approximately a foot tall (ISSG 2006). The primary advantages to this method of treatment for controlling Georgia cane is less chemical herbicide is needed to treat fresh growth compared to tall, established plants, and application and coverage is usually easier and improved due to the shorter and uniform-height of the plants (ISSG 2006). However, "cutting the stems may result in plants returning to growth-phase, drawing nutrients from the root mass, [and] as a result there is less translocation of herbicide to the roots" therefore, less roots and rhizomes are killed (ISSG 2006, p. 2).

Hand pulling Georgia cane (*Arundo donax*) has been found to be effective at removing small infestations, however, care must be taken to dig up and remove all rhizomes to prevent re-establishment (ISSG 2006). Cutting removes the above ground biomass but not the rooted rhizomes, and this method is not completely effective where in many instances the whole colony can re-sprout from crowns that were cut (Decruyenaere and Holt 2001; ISSG 2006). Burning is not recommended as a control agent, since Georgia cane has been demonstrated to re-grow three to four times faster after fire than native vegetation (ISSG 2006).

Mechanical methods, such as mowing, has been found to be relatively effective in controlling Georgia cane (*Arundo donax*), however, roots and rhizomes are left in the substrate and quickly re-grow (TIPPC 2010). Also, mechanical cutting of Georiga cane can leave small stem fragments that may root and re-grow (TIPPC 2010). Depending on the size of the body of water, mechanical methods have the potential to increase the spread of Georgia cane in that location (TIPPC 2010).

Native flora and fauna have not been found to be effective as potential biological control agents of Georgia cane (*Arundo donax*) (ISSG 2006). However, in Barbados, the grub form of a sugar cane moth-borer (*Diatraea saccharalis*) was reported to attack Georgia cane, but is also the dominant pest of sugar cane (*Saccharum officinarum*) devastating enormous sugar cane crop fields that are grown for commercial use (ISSG 2006). Also, in Pakistan, a leafhopper has been found to utilize Georgia cane as an alternate host; however, it primarily attacks corn and wheat crops (ISSG 2006). Recently, Flores (2011) reported that a study conducted by Dr. Goolsby found promising biological control agents such as the Arundo fly (*Cryptonerra* spp.), which eats the inside

of new shoots, and a leaf sheath miner (*Lasioptera donacis*), which destroys the plant's leaves. Dr. Goolsby also released a population of *Tetramesa romana* wasp in 2009, which has been found to attack the weed's main stem, weakening the plant, and reducing its overall height (Flores 2011). The most promising of the biological control candidates studied by Dr. Goolsby is a scale insect (*Rhizaspidiotus donacis*), which has an outstanding reproductive capacity and attacks the reed's roots and rhizomes (where most of the plant's biomass occurs), thereby debilitating the rhizome, which could potentially have a positive impact on deterring the plants growth and spread (Flores 2011). Georgia cane is not very palatable to cattle and they will only consume it when other food is limited, starting on the younger, greener shoots (ISSG 2006). Then again, it has been found that in areas of California the use of Angora and Spanish goats have the potential for controlling Georgia cane (ISSG 2006).

Compost

Composting Process and Uses of Compost in Horticulture

Combining the literary works Field Guide to On-Farm Composting (Dougherty 1999) and the Random House College Dictionary (Urdang and Flexner 1984), compost is best defined as a mixture of various organic substances (materials), in which the natural decaying of organic matter occurs through the biological process wherein micro-organisms convert organic material into a nutrient-rich resource. During the composting process, microorganisms consume oxygen from the organic matter and release carbon dioxide (Dougherty 1999). Active, functional composting produces a large amount of heat, releasing water vapor into the air (Dougherty 1999).

Today, composting is a technique utilized to accelerate the natural decay process, where it can take as long as a year or as little as 14 days, depending upon the amount of human control (Pennsylvania Department of Environmental Protection [PDEP] 2010). The primary ingredients of compost are carbon and nitrogen, and an ideal carbon to nitrogen ratio (C:N) of 30:1 is needed for fastest decomposition (Dougherty 1999). The volume of finished compost is 50% or less of the original volume of raw materials (Dougherty 1999). This makes composting an effective means of waste management and control (Dougherty 1999; Stoffella and Kahn 2001).

As a waste management system within agriculture, the composting process has been known to kill plant pathogens and kill weed seeds if high enough temperatures are obtained for long enough periods of time (Wiese *et al.* 1998; Dougherty 1999). For weed seeds of bindweed (*Convolvulus arvensis)*, johnsongrass (*Sorghum halepense*), kochia (*Erigeron kachinensis*), pigweed (*Amaranthus palmeri*), and sorghum (*Sorghum* spp.), temperatures of 120.0 to 180.0 degrees Fahrenheit (48.9 to 82.2 degrees Celsius), obtained and maintained for three to seven days are necessary for seed mortality to occur (Wiese *et al.* 1998). Also, it has been found that "several days of pile temperatures above 130.0 degrees Fahrenheit (54.4 degrees Celsius) are recommended to destroy pathogens and weed seeds" (Dougherty 1999, p. 47).

Compost is used in many states as both a waste management alternative, as well as a horticultural and agricultural based resource and is poised to become an essential environmental commodity for various types of soil enhancement applications (Glenn and Goldstein 1999; Scheurell and Mahaffee 2002; Faucette 2003). Additionally, preventing the loss or erosion of top soil by directly adding compost to the soil surface, makes

compost an effective means of erosion control (Stoffella and Kahn 2001). Also, compost has been used after mine reclamation and along highways for revegetation after construction, as a topsoil and/or soil amendment for disturbed and/or damaged landscapes (Dougherty 1999).

The main value of compost is in replenishing organic matter content in soil, a much needed part of a productive soil complex, which is critical for optimal plant growth (Composting Council 1996; Glenn and Goldstein 1999). In current studies, primary reasons cited for using compost were associated with building humus content of the soil, increasing soil tilth, and increasing potential plant growth, with secondary reasons including the replacement of chemical fertilizers as well as decreasing diseases and weeds in yard and garden plants (Walker, Williams, and Waliczek 2006). The composting industry has expanded significantly over a short period of time; however, a major hindrance in the agricultural and horticultural industries adopting composting practices, is due to lack of information and knowledge of the art and science of composting, as well as, the awareness of the economic advantages (Walker *et al.* 2006).

The horticultural industry currently has the highest demand for compost (Stoffella and Kahn 2001). Compost is utilized by horticulturalists in vegetable, fruit, ornamental, nursery, and turf crop production systems (Stoffella and Kahn 2001). Land developers and landscapers use compost as a fertilizer supplement, mulch, and as an alternative in the landscape or field for topsoil (Dougherty 1999). Compost has also been found to decrease plant disease(s), to increase the accessibility of nutrients by plants, and to be an effective weed control agent (Stoffella and Kahn 2001).

Importance of the Removal of Water Hyacinth, Water Lettuce, Hydrilla, and Georgia Cane from the Rio Grande

The Rio Grande River is a transboundary water source and the base support of diverse ecosystems, as well as home to approximately 13 million people living throughout the basin valley (IBWC 2008; SCC, Inc. 2009). The Rio Grande River sustains local agriculture, import-export trade, service and tourism, and a growing manufacturing sector on both sides of the river (IBWC 2008; SCC, Inc. 2009).

Found along the Rio Grande River and irrigation canals, Georgia cane (*Arundo donax*) has the ability to outcompete and completely suppress native plant species (TIPPC 2010). Additionally, found in the Rio Grande River, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) are considered as the leading problematic aquatic weeds with hydrilla and water hyacinth being called the worst aquatic weed in the world (TPWD 2009; WSDE 2010a; WSDE 2010c). The ecological impacts of these invasive aquatic plants are primarily due to their rapid growth and formation of dense canopy that outcompetes and displaces native submerged and planktonic vegetation for nutrients and light, thereby clogging waterways (Masser 2007). The lack of penetrating sunlight and nutrient depletion alter "biogeochemical cycles and water quality" (Masser 2007, p. 4). Dissolved carbon dioxide increases while dissolved oxygen drops (Masser 2007). In small and/or areas with limited circulation, oxygen can drop underneath the mats to critical levels, killing fish and invertebrates, such as crustaceans, insects, mollusks, and worms (Masser 2007). Also, conductivity, pH, turbidity, temperature, and bicarbonate alkalinity values decrease, resulting in habitat alteration, wherein some instances waterfowl do not utilize the aquatic

habitat (Masser 2007). The anoxia of the water due to infestations of water hyacinth, water lettuce, and/or hydrilla populations can result in the reduction of native population densities and species diversity and richness, affecting not only aquatic and riparian species but waterfowl as well, who use and rely on the water and the displaced fish and vegetative species as daily food sources (Masser 2007; WSDE 2010a; WSDE 2010b).

The aquatic invasiveness of these exotic species, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) is an increasingly serious problem. These species are considered by the United States Congress (2006) as noxious weeds, defined by the *Federal Noxious Weed Act of 1974* as "any living stage (including seeds and reproductive parts) of a parasitic or other plant of a kind which is of foreign origin, is new to or not widely prevalent in the U.S., and can directly or indirectly injure crops, other useful plants, livestock, poultry, or other interests of agriculture, including irrigation, navigation, fish, and wildlife resources, or the public health" (p. 2801). In addition to Georgia cane (*Arundo donax*) listed as a noxious weed in Texas, it is listed as an exotic plant pest in California, an invasive weed in Hawaii, and as an invasive, exotic pest in Tennessee (McWilliams 2004). Furthermore, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla vertillilata*) are listed by the Texas Parks and Wildlife Department (2010) as Invasive, Prohibited, and Exotic Species, which are organisms legally classified as exotic, harmful, or potentially harmful, and therefore, no persons may import, take, sell, trade, or place them into waters of the states except as authorized by rule or permit issued only by the department.

The purpose of this study is to investigate the effectiveness of a large-scale composting operation to manage invasive aquatic plants, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*), by rendering the seeds and other propagules non-viable. The overall goal of this study is to effectively manage water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) in an established large-scale composting operation, producing not only a nutrient rich resource for various applications within the agriculture and horticulture industries but determining if a large-scale composting operation effectively controls invasive species.

CHAPTER III

METHODOLOGY

Purpose

The purpose of this study was to investigate the effectiveness of a large-scale composting operation to manage various invasive plants found in the Rio Grande River, by rendering the seeds and other propagules non-viable, while producing a valuable agricultural and horticultural-based resource.

Objectives

The objectives of this study were:

1. To determine the aquatic plants in the Rio Grande River that are considered the most threatening invasive species.
2. To determine other nitrogen and carbon sources available in regions of the Rio Grande River Valley Basin that would be consistently available composting feedstock sources.
3. To evaluate if the high temperature(s) achieved in the composting process has the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks.
4. To determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Objective 1

The first objective of this study was to determine the aquatic plant(s) in the Rio Grande River that are considered the most threatening invasive species. An extensive literature research review was conducted on recommended species, and their interactions with and effects on each other and the ecosystems as a whole.

In addition to conducting the literature review, additional information was obtained personally, either in person, over the phone, or via email from professors, experts, professionals, and specialists in the field. During the Spring semester of 2010, researchers visited the Delta Lake Irrigation District, as well as the United States Department of Agriculture (USDA) – Beneficial Insects Research Unit in the Rio Grande River Valley. While visiting the Rio Grande River Valley, observations and meetings with Delta Lake Irrigation District manager(s), in addition to USDA Research Entomologist, Dr. Patrick Moran, verified literature review findings, and also allowed for verbal and visual confirmation of the species that pose the biggest threats to the river and the irrigation canals of the Rio Grande River.

Objective 2

The second objective of this study was to determine other nitrogen and carbon sources available in regions of the Rio Grande Basin Valley, which would be consistently available composting feedstock sources. An extensive literature research review was conducted in order to determine nitrogen and carbon sources that would be readily available sources in the Rio Grande Valley.

In addition to conducting the literature review, additional information was obtained personally, either in person, over the phone, or via email from professors,

experts, professionals, and specialists in the field, determining available nitrogen and carbon sources in the Rio Grande Valley. During the Spring semester of 2010, researchers visited the Delta Lake Irrigation District, as well as the United States Department of Agriculture (USDA) – Beneficial Insects Research Unit in the Rio Grande River Valley. While visiting the Rio Grande River Valley, observations and meetings with Delta Lake Irrigation District manager(s), in addition to USDA Research Entomologist, Dr. Patrick Moran, verified literature review findings, as well as allowed verbal and visual confirmation of nitrogen and carbon sources that would be readily available within the river as well as the irrigation canals of the Rio Grande Valley.

Objective 3

The third objective of this study was to evaluate if the high temperature(s) achieved in the composting process had the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks. The invasive plant species of potential interest included water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*). As a waste management system, the composting process kills plant pathogens and seeds if high enough temperatures were obtained for long enough periods of time (Wiese *et al.* 1998; Dougherty 1999). For seeds of bindweed (*Convolvulus arvensis)*, johnsongrass (*Sorghum halepense*), kochia (*Erigeron kachinensis*), pigweed (*Amaranthus palmeri*), and sorghum (*Sorghum* spp.), temperatures of 120.0 to 180.0 degrees Fahrenheit (48.9 to 82.2 degrees Celsius) obtained and maintained for three to seven days were necessary for seed mortality to occur (Wiese *et al.* 1998). Research also found that "several days of pile temperatures above 130.0

degrees Fahrenheit [54.4 degrees Celsius] are recommended to destroy pathogens and weed seeds" (Dougherty 1999, p. 47).

For the laboratory tests, samples of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) plants were obtained by the researcher during the mid to late summer months (when the plants are flowering and fruiting) from areas of the Aquatic Biology ponds and the banks of the San Marcos River and areas of Spring Lake – under the guidance and supervision of the Aquatic Maintenance Manager of the Rivers Systems Institute (RSI) when it was necessary. Plants bearing flowers and seeds were harvested, but otherwise plants and seeds were randomly selected. In the United States, hydrilla are dioecious and female (Overholt *et al.* 2010; WSDE 2010a); nevertheless, hydrilla (*Hydrilla verticillata*) seeds were sought, even though there was no expectation of actually obtaining hydrilla seeds. Therefore, other propagules such as tubers and turions were collected from the hydrilla plants, and treated in a similar manner to the seeds for the laboratory tests.

For the laboratory tests, the seeds, tubers, and turions were collected and sorted by hand by the researcher. Once seeds were dried and processed, they were stored in sealed plastic bags that were coded with reference numbers in a cool, dry place. Collected tubers and turions were kept moist, and stored in sealed plastic bags that were coded with reference numbers in cool, dark space. Any seeds, tubers, and turions that were not immediately processed into the experiments were stored in a refrigerator located in the greenhouses in the Agriculture Building at Texas State University-San Marcos.

All data was logged in field notebooks and was the responsibility of the researcher. The data collected during plant collection included:

- Name and location of collection site.
- Name of study manager.
- Date and time of data collection.
- Environmental conditions during data collection (climate conditions, field conditions, etc.).
- Documentation of external equipment used for sample treatment (dryers, ovens, etc.).
- Field assigned identification number, if necessary.
- Species collected.

Germination and Growth Tests

Seeds and other propagules of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) were tested for germination rate(s) and optimal growing conditions through germination and growth tests. The tests were conducted using an incubation chamber (Labnet Mini Incubator Model I 5110, Woodbridge, New Jersey), stored in research labroom 103 of the Agriculture Building at Texas State University-San Marcos. The germination and growth experiments tested ideal conditions at which germination and growth of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) seeds and other propagules occur. One hundred seeds and/or propagules from each species were tested separately. Half of the seeds (50 seeds) were subjected to chemical scarification.

In some instances, even though environmental conditions (temperature, moisture, oxygen availability, etc.) are favorable for germination, healthy seeds may fail to germinate and this condition is called dormancy (Patil and Dadlani 2009). One common cause of dormancy in many species is the impermeability of the seed coat, where

scarification is a method used to overcome seed dormancy and can be achieved using a mechanical or chemical process (Patil and Dadlani 2009). Scarification of seeds occurs naturally through freezing temperatures, microbial activity, water saturation during rainy seasons, as well as passing through the digestive tract of various animals (Evans and Blazich 1999). Exceptionally hard seed coats are usually broken by mechanical scarification by physically nicking, cutting, or cracking the seed coat to allow water to reach the embryo (Evans and Blazich 1999). Vinegar is an effective chemical scarification treatment used for species that do not have extremely hard seed coats (Evans and Blazich 1999).

In this study, the scarification process was chemical, in which the outer seed coat was broken down and softened which allowed water and oxygen into the seed, thereby helping to increase germination (Urdang and Flexner 1968). Seeds were chemically scarified using a 20% vinegar solution; the use of a 20% vinegar solution was recommended to break or soften the seed coat without damaging the seed embryo, while still encouraging germination (Patil and Dadlani 2009). The use of a vinegar solution as a chemical scarification treatment has been found to increase germination in seeds of species of catnip/catmint (*Nepeta* spp.), pumpkin (*Cucurbita pepo*), and redbud (*Cercis canadensis*), as well as other legume species (*Fabaceae* spp. or *Leguminoceae* spp.) (Evans and Blazich 1999).

Germination tests were conducted by placing the seeds in sterilized petri dishes containing filter paper moistened with distilled water as a growing media and placing those petri dishes in an incubator chamber held at 80.0 degrees Fahrenheit (26.7 degrees Celsius). Fifty scarified and 50 unscarified seeds (100 seeds) of each species per petri

dish were placed on filter paper moistened with distilled water. Growth tests for other propagules such as the hydrilla (*Hydrilla verticillata*) tubers and turions were setup in beakers containing filter paper moistened with distilled water, and had a higher water level (enough to submerge the propagule) replicating submerged aquatic conditions occurring in natural habitats. For hydrilla (*Hydrilla verticillata*),100 propagules of various developed sizes (maturity stages) were tested for growth; 50 tubers were placed on filter paper within three 600 milliliter beakers (15 in two and 20 in one) and 50 turions were placed on filter paper within two 600 milliliter beakers (25 in each one) and propagules were moistened and submerged using distilled water. Moisture levels were monitored and maintained, where the filter paper remained moistened in the petri dishes and propagules were maintained in submerged conditions to completely cover all propagules.

Research indicated that germination should occur within 14 days for water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) seeds (Barrett 1980; Gopal 1987). Since literature and research on seed germination of Georgia cane (*Arundo donax*) was insufficient, the Georgia cane seeds were subjected to the same germination conditions and were held for 14 days and then analyzed for radicle emergence. Research indicated that if hydrilla (*Hydrilla verticillata*) seeds were, in fact, produced, germination would likely occur within seven days (Lal and Gopal 1993). Spencer and Anderson (1986) reported the apparent growth of hydrilla (*Hydrilla verticillata*) tubers to have occurred within one week within laboratory conditions; and another study by Netherland (1997) found hydrilla turions producing roots and becoming fully developed into an individual plant within 33 days under laboratory conditions. However, Netherland

(1997) also observed both the hydrilla tubers and turions, and reported that "sprouting of these propagules [was] often greater than 90% within a two week period" (p. 8). Therefore, after 14 days any viable turions should have emerging roots, as well as stem growth even though they may not be fully developed plants within that time (Netherland 1997).

Therefore, for the germination and growth tests, seeds of the three species water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*), as well as, the tuber and turion propagules of hydrilla (*Hydrilla verticillata*) were allowed up to 14 days for radicle emergence and/or propagule growth. While the germination and growth tests were in process, the incubator chamber temperature was monitored and checked daily using a Thermo Scientific ERTCO® ASTM thermometer (Lafayette, New Jersey) and the moisture level was monitored and checked daily by sight, where the filter paper was kept moist and beakers were kept at a high water level in order to keep propagules completely submerged. Distilled water was used when necessary to maintain moisture and water levels.

After 14 days, germination and growth in the incubator chambers was analyzed, determining the average germination/growth rate amongst the scarified and unscarified treatments (highest number of seeds germinated of those seeds tested) of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*), as well as growth rate of hydrilla (*Hydrilla verticillata*) tuber and turion propagules.

All data was logged and was the responsibility of the researcher. The data collected from the germination tests included:

- Name and location of germination and growth test (building and room number).
- Name of study manager.
- Date and time of data collection.
- Environmental conditions during data collection (germination chamber temperature and humidity levels).
- Documentation of external equipment used for sample treatment (petri dishes, filter paper, etc.).
- Field assigned identification number.
- Number of seeds or propagules used per petri dish.
- Number of germinated or growth of plants growing per petri dish.
- Germination or growth rate per petri dish.

Oven Mortality Tests

Seeds and other propagules of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) were tested for mortality rate(s) through oven mortality tests. Testing determined temperatures at which water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*) seeds, as well as hydrilla (*Hydrilla verticillata*) tubers and turions are rendered non-viable. For the oven mortaliy tests, plants bearing seeds, flowers, and/or other propagules were collected and taken to the Agriculture Greenhouses, where the seeds and other propagules were separated from the plants by hand.

For the oven mortality tests, samples of already manufactured compost (certified by the Agricultural Analytical Services Compost Laboratory – Pennsylvania State University, University Park, Pennsylvania) were held in 24 soil sample containers three inches in diameter and each weighed approximately 140.0 grams. Each soil sample

container contained separately – hydrilla (*Hydrilla verticillata*) tubers and turions, and seeds of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*).

Prior to each of the oven mortality tests, the soil sample containers were prepped the day before. Prepping included sterilizing all equipment, adding the compost samples moistened with distilled water to each soil sample container, weighing each container, pre-labeling each container, placing containers in the appropriate oven, and then turning on the oven. Each oven was turned on 24 hours prior to experiments start, so that appropriate temperatures were obtained and maintained. Therefore, the compost samples acclimated to the given temperature of the oven once the study was initiated. The seeds and other propagules were then added to appropriate pre-labeled soil sample container within the corresponding oven and moisture levels were examined and adjusted accordingly to water loss from evaporation during initial heating.

Research observed seed mortality to occur at temperatures between 120.0 to 180.0 degrees Fahrenheit (48.9 to 82.2 degrees Celsius) maintained for three to seven days (Wiese *et al.* 1998). Furthermore, Wiese and colleagues (1998) found that all the seeds of barnyard grass (*Echinochola crus-gallia*), kochia (*Kochia scoparia*), johnsongrass (*Sorghum halepense*), sorghum (*Sorghum bicolor*), and pigweed (*Amaranthus* spp.) species "were killed within three days…exposed at 120.0 degrees Fahrenheit (48.9 degrees Celsius) (p. 377). Therefore, the seeds and other propagules of interest were subjected to the oven mortality tests for three days in stabilized temperatures of either 120.0 degrees Fahrenheit (48.9 degrees Celsius), 135.0 degrees Fahrenheit (57.2 degrees Celsius), or 150.0 degrees Fahrenheit (65.6 degrees Celsius). The oven mortality tests

used three different Model 20AF Quincy Lab ovens (Chicago, Illinois) located in lab room 103 in the Agriculture Building at Texas State University-San Marcos.

Temperatures, moisture, and humidity levels were checked, monitored, and recorded daily. Oven and sample temperatures were checked daily with thermometers; built-in oven thermometers traceable to National Institute of Standards and Technology (NIST) monitored temperature(s) within the ovens, and sample temperatures within containers were monitored with a TruTemp Thermometer Model 3505 (Commerce Township, Michigan) traceable to National Science Foundation (NSF®) which checked the actual temperature of the compost samples, seeds, and other propagules within the containers. Samples were also monitored daily using a Rapitest® moisture meter (Frederick, Maryland) and maintained at a 50 to 70% moisture level, which was representative of an active compost pile (Rynk *et al.* 1992). Distilled water was used when necessary to maintain moisture levels.

Each oven mortality tests included 20 seeds or propagules from each species and were replicated five times to ensure quality, consistency, and reliability. Also, to avoid potential overcrowding and to minimize contamination, eight soil sample containers were used to hold ten seeds or propagules. The oven mortality tests consisted of the following:

- Eight soil sample containers held in Oven 1 at 120.0 degrees Fahrenheit (48.9 degrees Celsius), with each separately containing:
    - Ten unscarifed water hyacinth seeds.
    - Ten scarified water hyacinth seeds.
    - Ten unscarified water lettuce seeds.
    - Ten scarified water lettuce seeds.
    - Ten unscarified Georgia cane seeds.
    - Ten scarified Georgia cane seeds.

  - Ten hydrilla tuber propagules.
  - Ten hydrilla turion propagules.

- Eight soil sample containers held in Oven 2 at 135.0 degrees Fahrenheit (57.2 degrees Celsius), with each separately containing:
  - Ten unscarified water hyacinth seeds.
  - Ten scarified water hyacinth seeds.
  - Ten unscarified water lettuce seeds.
  - Ten scarified water lettuce seeds.
  - Ten unscarified Georgia cane seeds.
  - Ten scarified Georgia cane seeds.
  - Ten hydrilla tuber propagules.
  - Ten hydrilla turion propagules.
- Eight soil sample containers held in Oven 3 at 150.0 degrees Fahrenheit (65.6 degrees Celsius), with each separately containing:
  - Ten unscarified water hyacinth seeds.
  - Ten scarified water hyacinth seeds.
  - Ten unscarified water lettuce seeds.
  - Ten scarified water lettuce seeds.
  - Ten unscarified Georgia cane seeds.
  - Ten scarified Georgia cane seeds.
  - Ten hydrilla tuber propagules.
  - Ten hydrilla turion propagules.

Once the compost samples had been held at each temperature for three days, the seeds and other propagules were analyzed. Seeds and other propagules were separated and screened from the compost sample using a quarter of a millimeter screener, and were then tested using the optimal germination/growing procedures identified in the

germination and growth tests in previous section, as well as the tetrazolium tests described in section belowdetermining the viability.

Tetrazolium Tests

All seeds and other propagules were subjected to tetrazolium tests using the biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC), which tested for respiration of living tissue and viability (Patil and Dadlani 2009). It usually takes an average of 30 to 45 minutes to perform (Patil and Dadlani 2009). Seed embryos and other tissues are tested by cutting or piercing the seed coat to expose the embryo (Patil and Dadlani 2009). Seeds are then imbibed by soaking them in water, followed by the biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC) (Patil and Dadlani 2009). While TTC is initially colorless, it is converted to formazan red when living tissue is present (Patil and Dadlani 2009). Therefore, seed embryos and other propagules that were respiring or living appeared stained when soaked in TTC. Dead embryos and other propagule tissue did not turn red (Patil and Dadlani 2009).

All data was logged and was the responsibility of the researcher. The data collected from the oven mortality tests included:

- Name and location of oven mortality test (oven and sample temperature).
- Source of sample compost.
- Source of sample seeds or other propagules.
- Name of study manager.
- Date and time of data collection.
- Environmental conditions during data collection (laboratory conditions, oven conditions), if necessary.

- Documentation of external equipment used for sample treatment (tools and instruments used to turn samples; thermometers for double-checking temperatures, spray bottles, etc.), if necessary.
- Field assigned identification number.
- Number of seeds or other propagules per temperature.
- Number of seeds or other propagules killed (rendered non-viable).

Objective 4

The fourth objective of this study was to determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Compost was produced and stored at the Bobcat Blend composting site at the Muller Farm owned by Texas State University-San Marcos. The Muller Farm was previously utilized as an alternative grazing source for the livestock kept at Texas State University Freeman Ranch and is approximately 125 acres. Of the five acres allocated for the compost site, 2.285 acres was transformed into a catchment pond that could withstand a twenty-five year 24 hour flood event. The remaining 2.715 acres was cleared and graded so that any water run-off from the compost piles would be captured by the catchment pond. Fences and gates were also installed to keep out any livestock and to contain the feedstocks used for composting.

At the compost site, a total of twelve compost piles were created using plants collected from the San Marcos River and surrounding area(s). Three piles contained 25% of each invasive species including water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*), independently; these three piles were replicated three times for a total of nine piles. Additionally, a compost pile was built containing 25% of all three species (water hyacinth, water lettuce, and hydrilla)

simultaneously and was replicated three times. In addition to these twelve piles, three more compost piles were created using plants collected from area(s) along the Rio Grande River within the Valley region. Of these three compost piles, two piles contained 25% of each invasive species (water hyacinth and water lettuce) independently, and the third pile built contained 25% of all three species (water hyacinth, water lettuce, and hydrilla simetaneously and porportionately).

All compost piles also incorporated other compost feedstocks, which included food waste and woodchips. The compost operation used food waste from the cafeterias on Texas State University-San Marcos campus in the compost feedstock blends. Some of the cafeteria food waste was processed through a grinder which makes all of the food of an even "cole slaw" consistency, while some of the cafeteria waste consisted of whole food parts. All food waste collected and utilized as a compost feedstock for this study was weighed in containers using a scale and quantities were recorded and documented appropriately. Tree and shrub branches that were pruned by the Bartlett Tree Company were used as a carbon source for the composting project. Therefore, size of chips were relatively consistent, but species of tree waste did vary, where in the Rio Grande Valley woodchips could potentially be substituted withGeorgia cane (*Arundo donax*) and/or sugar cane (*Saccharum officinarum*) if a composting operation was implemented in the region. The quatity of woodchips utilized as compost feedstocks in this study was recorded and documented appropriately. Various percentages of these feedstocks were tested and piles were modified as required to create ideal conditions for composting.

Piles were turned every five days and composting was completed within 30 days (Rynk *et al.* 1992). The temperature, pH, moisture, and maturity of each pile were

monitored and recorded, and the blend of the feedstocks modified as necessary maintaining desired conditions (as determined by the previously described laboratory tests and ideal composting situations). A moisture level of 50 to 70% is ideal (Rynk *et al.* 1992). Moisture levels were measured with a 60-inch Compost Moisture Meter (ReoTemp Instrument Corporations, San Diego, California) and with a "feel" test. A feel test involved taking a handful of compost, squeezing it, and determining whether it felt like a moist sponge; if the sample did not feel wet to the touch, then it was too dry and if water could squeeze out, then it was too wet (Rynk *et al.* 1992). Acidity and alkalinity (pH) were measured and recorded with a handheld Kelway® Soil pH sensor (Wyckoff, New Jersey), with acceptable ranges from 5.0 to 8.5 (Rynk *et al.* 1992). Carbon to nitrogen (C:N) ratios were measured by calculating the feedstock mixture percentages and observing the rate of decomposition. Since a C:N ratio of 30:1 is desirable (Stoffella and Kahn 2001), compost piles were constructed attempting to attain this ratio and using recommendation from previous successful research by Montoya and colleagues (2010).

The composting process has been known to kill plant pathogens and kill weed seeds if high enough temperatures are obtained for long enough periods of time, recommending "several days of pile temperatures above 130.0 degrees Fahrenheit (54.4 degrees Celsius) to destroy pathogens and weed seeds" (Wiese *et al.* 1998; Dougherty 1999, p. 47). Therefore, compost pile temperatures above 135.0 degrees Fahrenheit (57.2 degrees Celsius) are desired. However, if the oven mortailty test results indicate that greater temperatures are necessary to kill the seeds and propagules of any and/or all of the species of interest, appropriate alterations of pile temperature will be implemented. Piles were maintained at that temperature and monitored with a 60-inch Fast Response

Compost Thermometer (ReoTemp Instrument Corporations, San Diego, California). Oxygen levels were monitored and measured with an oxygen monitor (Model No. 0-21, Demista Instruments, Arlington Heights, Illinois). Temperature, oxygen, pH, and moisture levels of the piles were monitored, recorded, and maintained through scheduled turning of the piles. The goal was that the piles would remain aerobic for best microbial activity for decomposition, as well as the least odor emittance.

The ideal size for the piles was a height of five to six feet, with a width of ten to twelve feet (Rynk *et al.* 1992). This height and width allowed the piles to be insulated and generate enough heat to kill pathogens (including the invasive seeds, pathogens, and plant propagules of interest), but did not allow too much heat to be generated in the piles which would have resulted in spontaneous combustion (Rynk *et al.* 1992). According to Rynk and colleagues (1992), when turned every five days, composting of materials took approximately 30 days. Turning was conducted to ensure that formerly outer exposed surfaces were buried within the pile each time the pile was turned.

Compost was examined by sight and touch after 30 days (one month) ensuring that compost was decomposed and suitable for the curing stage. The curing stage occurs when there has been a sustained reduction in pile temperatures (50.0 to 105.0 degrees Fahrenheit/ 10.0 to 40.6 degrees Celsius) (Rynk *et al.* 1992). The compost was cured in the same piles where they were built. The curing stage generally takes approximately four to eight weeks (Rynk *et al.* 1992). Compost that is not allowed time for the curing process to occur can limit the availability of nitrogen to plants and introduce phytoxins that are harmful to plants (Rynk *et al.* 1992). After curing, compost was screened using a quarter-inch plate screener. Screening removed larger particles and foreign materials.

Compost Quality Tests

Once compost had cured, samples of compost were collected to observe whether the composting process destroyed seeds and other propagules of each of the invasive species. Since piles of compost often vary from location to location within the pile, the samples collected for analysis must be representative of all material being analyzed (Pennsylvania State University 2002). Therefore, samples that were either analyzed by the researcher or sent to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program for testing assurance were composite sample of materials collected from several locations and depths within the pile that was sampled. Sampling began as soon as compost batches had been determined to be in a cured state. At this time, 25 one-gallon-sized samples were taken from each pile of cured compost of each of the four compost invasive species' recipe, for a total of 100 samples. Each compost sample was drawn by collecting one subsample from the peak of the compost pile, in addition to subsamples from eight locations and at three different depths from the overall curing compost pile. These subsamples were then combined to create composite five-gallon-sized sample. When the five-gallon-sized samples were drawn, they were labeled appropriately to identify the corresponding curing compost pile from which they were gathered. Additionally, from the compost piles made from the plants collected from the Rio Grande River Valley region, a total of 45 samples were also collected and analyzed to observe whether the composting process destroyed seeds and other propagules of each of the invasive species. From each of these piles one-gallon-sized samples were collected from five locations and at three different depths within the piles for the total 15 samples collected from each of the Valley piles to create composite sample.

Determining the effectiveness of composting at rendering seeds and other propagules non-viable, any water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) seeds and other propagules were extracted from the composite samples of finished cured compost collected using a consecutive series of screens. Since the seeds and other propagules are very small (water hyacinth – 1.0 x 0.5 millimeters, water lettuce – 1.0 x 0.5 inches, Georgia cane – 0.1 x 0.1 millimeters and hydrilla – 0.1 x 0.1 millimeters as well as 4.0 x 10.0 millimeter tubers and 3.0 x 8.0 millimeter turions), it is likely that the plant material may be completely decomposed during the composting process (Mulholland-Olson 2004; WSDE 2010b; WSDE 2010d). Any water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) seeds and hydrilla (*Hydrilla verticillata*) propagules screened from the compost samples were subjected to the tetrazolium test described previously – to determine is they were still viable or in fact rendered non-viable through the high temperatures achieved in the composting process.

To determine if the compost produced from invasive aquatic plant species is of high quality compost valuable to the agricultural and horticultural industries, in addition to the field sampling collections and analysis mentioned previously four samples of the compost (one composite sample from each of the four invasive species' recipe compost piles made with plants collected from areas of the San Marcos River), were collected and sent to the Agricultural Analytical Services Laboratory (Pennsylvania State University, University Park, Pennsylvania) for Certified Seal of Testing Assurance analysis. At the Agricultural Analytical Services Laboratory, the compost samples were subjected to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program, which

analyzed the compost on each of the following: pH, soluble salt content, and nutrient content [total carbon (C), total nitrogen (N), arsenic (As), cadmium (Cd), calcium (Ca), copper (Cu), lead (Pb), magnesium (Mg), mercury (Hg), molybdenum (Mo), nickel (Ni), phosphorous (P), potassium (K), selenium (Se), zinc (Zn), moisture content, organic matter content, bioassay (maturity)], as well as stability (respirometry), particle size, and weed seed(s) viability (Pennsylvania State University 2002; United State Composting Council 2010). These samples were collected using recommendations from the Pennyslvania State Univeristy Agricultural Analytical Laboratory (University Park, Pennsylvania), in which samples were collected from each compost pile at five locations and from three depths at each location to create a composite sample (Pennsylvania State University 2002). Composite samples were collected from each pile separately in order to analyze each compost pile feedstocks independently.

Compost pile construction and monitoring was either done by or supervised by the study manager. Field data was logged in field notebooks by the study manager and trained compost personnel. Field notebook(s) were the responsibility of the study manager. The data collected from compost pile construction and monitoring included:

- Name and location of compost pile (pile in block of grid).
- Name of study manager.
- Date and time of data collection.
- Type and quantity/percentage of feedstock ingredients.
- Source of feedstock ingredients.
- Age/maturity of pile.
- Treatment of pile.
- Average temperature of pile (measured at six random locations at varying depths within the pile).

- Condition of pile (moisture content, pH, C:N ratio).
- Environmental conditions during data collection (field conditions, ambient temperatures, precipitation, etc.).
- Documentation of external equipment used for/on sample treatment (compost thermometers, pH test, moisture meter).
- Field assigned identification number.

Data Analysis

Frequencies and descriptive data will be reported.

CHAPTER IV

RESULTS

Purpose

The purpose of this study was to investigate the effectiveness of a large-scale composting operation to manage various invasive plants found in the Rio Grande River, by rendering the seeds and other propagules non-viable, while producing a valuable agricultural and horticultural-based resource.

Objectives

The objectives of this study were:

1. To determine the aquatic plants in the Rio Grande River that are considered the most threatening invasive species.
2. To determine other nitrogen and carbon sources available in regions of the Rio Grande River Valley Basin that would be consistently available composting feedstock sources.
3. To evaluate if the high temperature(s) achieved in the composting process has the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks.
4. To determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Objective 1

The first objective of this study was to determine the aquatic plant(s) in the Rio Grande River that are considered the most threatening invasive species. Research found that worldwide, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) are of the leading problematic invasive weeds (Wijte *et al.* 2005; TPWD 2009; WSDE 2010a; WSDE 2010b). These aquatic and riparian species have also been found to be particularly invasive in the Rio Grande River (TPWD 2009; WSDE 2010a; WSDE 2010c).

The ecological impacts of these invasive aquatic and riparian plants are primarily due to their rapid growth and formation of dense canopy that competes with and displaces native submerged and planktonic vegetation for nutrients and light, thereby clogging waterways (Masser 2007). The anoxia of the water due to infestations of water hyacinth, water lettuce, and/or hydrilla populations can result in the reduction of native population densities and species diversity and richness (Masser 2007; WSDE 2010a; WSDE 2010b). Invasion of these species, as well as Georgia cane (*Arundo donax*), profoundly affect the natural functioning(s) of riparian and aquatic ecosystems (Wijte *et al.* 2005). Alteration to these ecosystems is not only detrimental to the native aquatic and riparian species, but to waterfowl as well, who use and rely on the water source, in addition to the displaced fish and vegetative species needed as daily food sources (Masser 2007; WSDE 2010a; WSDE 2010b).

Research found that the aquatic invasiveness of these exotic species, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*) and Georgia cane (*Arundo donax*), is an increasingly serious problem. These

species are considered by the United States Congress (2006) as noxious weeds, defined by the *Federal Noxious Weed Act of 1974* as "any living stage (including seeds and reproductive parts) of a parasitic or other plant of a kind which is of foreign origin, is new to or not widely prevalent in the U.S., and can directly or indirectly injure crops, other useful plants, livestock, poultry, or other interests of agriculture, including irrigation, navigation, fish, and wildlife resources, or the public health" (p. 2801). In addition to Georgia cane (*Arundo donax*) being listed as a noxious weed in Texas, it is also listed as an exotic plant pest in California, an invasive weed in Hawaii, and as an invasive, exotic pest in Tennessee (McWilliams 2004). Furthermore, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) are listed by the Texas Parks and Wildlife Department (2010) as Invasive, Prohibited, and Exotic Species, which are organisms legally classified as exotic, harmful, or potentially harmful, and, therefore, no persons may take, import, sell or trade, or place them into any waters of the state except as authorized by permit or rule(s) issued by the department.

In addition to conducting a literature review, the Delta Lake Irrigation District, as well as the United States Department of Agriculture (USDA) – Beneficial Insects Research Unit in the Rio Grande River Valley, were visited by researchers. Observations and meetings with Delta Lake Irrigation District supervisors and USDA Research Entomologist, Dr. Patrick Moran (2010), verified that the three invasive aquatic species, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*), were those that posed the biggest threats to the irrigation canals of the Rio Grande River. The occurrence of the invasive species Georgia cane (*Arundo*

donax) is found in abundance in riparian areas along the river, as well as irrigation canals (Hodges 2010) and was observed while visiting.

Objective 2

The second objective of this study was to determine other nitrogen and carbon sources available in regions of the Rio Grande Basin Valley, which would be consistently available composting feedstock sources. The Rio Grande Valley was found to be a major agricultural center for agribusiness and tourism, as well as one of the nation's largest producers of vegetables such as beets (*Beta vulgaris*), cabbage (*Brassica oleracea var. capitata*), carrots (*Daucus carota*), green beans (*Phaseolus vulgaris*), onions (*Allium cepa*), tomatoes (*Lycopersicon esculentum*), and other minor crops (Vigness and Odintz 2009). Cotton (*Gossyspium* spp.), maize (*Zea mays*), sugar cane (*Saccharum officinarum*), and various sorghums (*Sorghum* spp.) are leading crops in the valley, with citrus fruits (*Citrus* spp.) – grapefruit (*Citrus paradisi*), oranges (*Citrus sinensis*), tangerines (*Citrus reticulata*), and tangelos (*Citrus tangelo*) being the principle crops produced in the region (Vigness and Odintz 2009). Vegetables such as these or essentially any type of food waste, acts as a nitrogen, as well as a moisture source within compost piles.

It was found that Texas State University-San Marcos disposes of over 300 tons of food waste a year, which is accompanied by a substantial amount of trash disposal fees. Furthermore, continuous cafeteria operation on campus allows for a consistent, as well as readily available nitrogen-rich waste material. Therefore, food waste from Texas State University-San Marcos cafeterias was substituted as a convenient available nitrogen

compost feedstock that would act similarly to the agricultural waste available in the Rio Grande Valley.

In addition to conducting a literature review, a visit to Delta Lake Irrigation District in the Valley was conducted in order to determine available nitrogen and carbon sources in the Rio Grande Valley. It was observed that the Delta Lake Irrigation District had numerous fields of sorghum (*Sorghum* spp.) and sugar cane (*Saccharum officinarum*). Also observed was the presence of Georgia cane (*Arundo donax*), an invasive species found in riparian areas along the river and irrigation canals (Hodges 2010). According to Delta Lake Irrigation District Manager, Troy Allen (2010), instead of the traditional slash and burn method of control, Georgia cane is randomly cut-back, piled up, and then allowed to dry out in order to help control the species in the areas along the canals, thus relatively easily harvestable once dried. If a compost operation was implemented in the Valley, dried Georgia cane (*Arundo donax*) can be used as a substitute for the locally available woodchip material that is currently used in the compost operation and utilized as a carbon feedstock source in compost piles when available.

Objective 3

The third objective of this study was to evaluate if the high temperature(s) achieved in the composting process has the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks. The plant species of interest included water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*). The composting process kills plant pathogens and seeds if high

enough temperatures are obtained for long enough periods of time (Wiese *et al.* 1998; Dougherty 1999). Research has found that some seeds required temperatures of 120.0 to 180.0 degrees Fahrenheit (48.9 to 82.2 degrees Celsius) to be obtained and maintained for three to seven days for seed mortality to occur (Wiese *et al.* 1998).

Germination and Growth Tests

First, in order to observe and determine ideal germination and growth conditions for seeds and other propagules, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) plants were harvested from various areas of Spring Lake, the Aquatic Biology ponds, and banks of the San Marcos River during the summer and fall (while fruiting and flowering) seasons of 2010. Due to the difficulty of collecting a large amount of seeds in the field, plants were collected, observed, and separated by hand at the Agriculture Department's greenhouses at Texas State University-San Marcos. Otherwise, seeds and other propagules were collected from random samples and were stored in a refrigerator at the greenhouses (third floor of the Agriculture Building) until germination tests and oven mortality tests were implemented.

Research found that, in the United States, hydrilla are dioecious and female (Overholt *et al.* 2010; WSDE 2010a); nevertheless, hydrilla (*Hydrilla verticillata*) seeds were sought, even though there was no expectation of actually obtaining them. Of all of the hydrilla plants collected for this study, only nine flowers were found. These nine flowers were all observed and dissected and no seeds were found. The flowers were also observed to all be female due to the presence of stigmas and the absence of stamens, which supports the research that the type of hydrilla found in the United States are

dioieous and female (Overholt *et al.* 2010; WSDE 2010a). Furthermore, the hydrilla samples were randomly collected from areas of intense infestation, where dense tuber production, as well as, plentiful turions were observed. Therefore, since no seeds were obtained, tuber and turion propagules were collected from the hydrilla plants and these propagules were treated similarly to seeds for the laboratory tests. Also, since no seeds were found in the field, this study's results supported research that found that both monoecious and dioecious varieties of hydrilla utilize more energy towards tuber and turion production (Spencer and Anderson 1986; Netherland 1997).

Seeds and other propagules of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) were tested for germination rate(s) and optimal growing conditions through germination and growth tests. The germination and growth tests consisted of testing unscarified and scarified seeds, tubers, and turions, and included the use of a mini incubator chamber (Labnet Mini Incubator Model I 5110, Woodbridge, New Jersey). The incubator chamber was stored in research lab room 103 of the Agriculture Building at Texas State University-San Marcos.

Germination tests were conducted by placing the seeds in sterilized petri dishes containing two pieces of filter paper (growing media) moistened with distilled water to a depth equal to the thickness of the filter paper in order to replicate aquatic germination conditions. The scarified and unscarified seeds were placed in two separate petri dishes and then those petri dishes were placed in the incubator chamber. One hundred seeds, fifty scarified and fifty unscarified from each species [water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*)] were

tested separately. Scarified seeds in this study were chemically scarified using a 20% vinegar solution, which has been found to break or soften the seed coat without damaging the seed embryo while still encouraging germination (Patil and Dadlani 2009).

For hydrilla (*Hydrilla verticillata*), fifty tubers and fifty turions were placed on filter paper and held within beakers holding 600 milliliters of distilled water for complete submergence of propagules. The moisture level of the filter paper was maintained in each petri dish and beaker and allowed up to 14 days for radicle and root emergence and growth.

Research indicated that 80.0 degrees Fahrenheit (26.7 degrees Celsius) is an optimal temperature for germination of water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pista stratiotes*) seeds (Gopal 1987). Research found hydrilla (*Hydrilla verticillata*) tuber and turion production and growth to occur between temperatures of 70.0 to 85.0 degrees Fahrenheit (21.1 to 29.4 degrees Celsius), with optimum growth occurring at 77.5 degrees Fahrenheit (25.0 degrees Celsius). Therefore, the temperature in the incubator chamber was held the same, at 80.0 degrees Fahrenheit (26.7 degrees Celsius), for testing consistency. The temperature and moisture levels were monitored, maintained, and recorded daily.

Research indicated that germination should occur within 14 days for water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*) (Barrett 1980; Gopal 1987). Spencer and Anderson (1986) reported the noticeable growth of hydrilla (*Hydrilla verticillata*) tubers to have occurred within one week while in laboratory conditions. Research also found hydrilla turions to have produced roots and had fully developed into individual plants within 33 days under laboratory conditions (Netherland 1997). Yet,

Netherland (1997) observed both hydrilla tubers and turions, and reported that "sprouting of these propagules [was] often greater than 90% within a two week period" (p. 8). Therefore, after 14 days, viable turions should have emerging roots as well as stem growth even though they may not be fully developed (Netherland 1997).

After 14 days, germination and growth in the incubator chamber was observed and analyzed to determine the average germination rate amongst the scarified and unscarified treatments (greatest number of seeds germinated of those seeds tested) of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*), as well as growth rate of hydrilla (*Hydrilla verticillata*) tuber and turion propagules. Seeds were analyzed using a tetrazolium test. The tetrazolium test is a seed viability test and usually took approximately 30 minutes to conduct. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds were then imbibed by soaking them in water, and then in the biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC) to observe for staining of living tissue.

Water Hyacinth Germination and Viability Results

Of the fifty unscarified water hyacinth (*Eichhornia crassipes*) seeds, three seeds were observed to have radicle emergence on Day 8 of germination and growth tests. On Day 10, four more seeds were observed with radicle emergence and on Day 12, six more water hyacinth seeds were observed to have radicle emergence. By Day 14, thirteen more seeds were observed to have radicle emergence, for a total of 26 water hyacinth seeds observed germinating within 14 days. Therefore, the germination rate of unscarified water hyacinth (*Eichhornia crassipes*) seeds was found to be 52% (26/50). The other 24 ungerminated seeds were subjected to the tetrazolium tests, and tests

revealed that two water hyacinth (*Eichhornia crassipes*) seeds were viable. Therefore, seed germination and viability of unscarified water hyacinth seeds was 56% (28/50).

Scarification appeared to increase the germination rate of the seeds. Of the fifty scarified water hyacinth seeds, on Day 8, two seeds were observed with the presence of an emerging radicle, and on Day 10, six more seeds were observed to have radicle emergence. Another nine seeds were observed to have radicle emergence on Day 12. By Day 14, eighteen more seeds had germinated, for a total of 35 water hyacinth seeds germinating within fourteen days. The 15 ungerminated seeds were subjected to the tetrazolium tests and none were found to be viable. Germination and viability rate of scarified water hyacinth (*Eichhornia crassipes*) seeds was 70% (35/50).

Therefore, of the 100 unscarified and scarified water hyacinth seeds (*Eichhornia crassipes*), a total of 61 germinated for a total germination rate of 61% (61/100), giving water hyacinth a total seed germination and viability rate of 63% (63/100) after tetrazolium tests (Table 1). These results were consistent with Montoya and colleagues' (2012) findings, where 300 seeds were subjected to germination tests, and there was a reported 62% germination and viablility rate of water hyacinth (*Eichhornia crassipes*) seeds held for 14 days at a stable temperature of 80.0 Fahrenhiet (26.7 degrees Celsius) within the laboratory.

Table 1. Water hyacinth (*Eichhornia crassipes*) germination[z] and tetrazolium[y] test results conducted with scarified[x] and unscarified seeds in the study of the use of composting as a means of managing the invasive species.

Group	Germination[z] Rate	Germination[z] %	Tetrazolium[y] Test	% Viable	Total Germination & Viability %
Unscarified	26/50	52%	2/24	8.3%	(56/100) 56%
Scarified[x]	35/50	70%	0/15	0%	(35/100) 70%
Total	61/100	61%	2/39	5.1%	(63/100) 63%

[z]Germination tests were conducted using water hyacinth seeds placed on filter paper moistened with distilled water in petri dishes held at 80.0 degrees Fahrenheit (26.7 degrees Celsius) and were observed for 14 days for radical emergence.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds were then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, seed embryos that are respirating or alive will appear stained when soaked in TTC. Dead embryos will not turn red.

[x]Seeds were scarified by soaking them in a 20% vinegar solution for 30 minutes.

Water Lettuce Germination and Viability Results

Germination and growth of water lettuce (*Pistia stratiotes*) seeds were found to be consistent with literature research in that studies have reported the abundance of flowering, but observed seedlings were rarely found (Hall and Okali 1974). In addition, a study conducted by Habeck and Thompson (1997) found no seeds or seedlings produced in south Florida, where the conditions for germination of water lettuce are comparable to its suggested origin (Habeck and Thompson 1997). Furthermore, since water lettuce only produces one seed per plant, harvesting and collection of the required amount of seeds for this study was very time consuming and tedious. After fourteen days, of the 50 unscarified and 50 scarified water lettuce (*Pistia stratiotes*) seeds, none were observed to have the presence of an emerging radicle. Therefore, all 100 water lettuce seeds were then subjected to the tetrazolium test. Analysis of the seeds using the tetrazolium test

further revealed that all seeds were found to be non-viable; therefore, the germination and viability rates of water lettuce (*Pistia stratiotes*) seeds were 0% (0/100) (Table 2).

Table 2. Water lettuce (*Pistia stratiotes*) germination[z] and tetrazolium[y] test results conducted with scarified[x] and unscarified seeds in the study of the use of composting as a means of managing the invasive species.

Group	Germination[z] Rate	Germination[z] %	Tetrazolium[y] Test	% Viable	Total Germination & Viability %
Unscarified	0/50	0%	0/50	0%	(0/100) 0%
Scarified[x]	0/50	0%	0/50	0%	(0/100) 0%
Total	0/100	0%	0/100	0%	(0/100) 0%

[z]Germination tests were conducted using water lettuce seeds placed on filter paper moistened with distilled water in petri dishes held at 80.0 degrees Fahrenheit (26.7 degrees Celsius) and were observed for 14 days for radical emergence.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds were then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, seed embryos that are respirating or alive will appear stained when soaked in TTC. Dead embryos will not turn red.

[x]Seeds were scarified by soaking them in a 20% vinegar solution for 30 minutes.

Georgia Cane Germination and Viability Results

Germination and growth of Georgia cane (*Arundo donax*) seeds were found to be consistent with literature research in that studies reported that the seeds of Georgia cane are non-viable (Wijte *et al.* 2005). Furthermore, research conducted by Khudamrongsawat and colleagues (2004) stated "viable seeds have not been found in the United States" (p. 395). After fourteen days, of the 50 unscarified and 50 scarified Georgia cane (*Arundo donax*) seeds, none were observed to have the presence of an emerging radicle. Therefore, all 100 Georgia cane seeds were then subjected to the tetrazolium tests. Analysis of the seeds using the tetrazolium tests further revealed that

all seeds were found to be non-viable; therefore, the germination and viability rates of Georgia cane (*Arundo donax*) seeds were 0% (0/100) (Table 3).

Table 3. Georgia cane (*Arundo donax*) germination[z] and tetrazolium[y] test results conducted with scarified[x] and unscarified seeds in the study of the use of composting as a means of managing the invasive species.

Group	Germination[z] Rate	Germination[z] %	Tetrazolium[y] Test	% Viable	Total Germination & Viability %
Unscarified	0/50	0%	0/50	0%	(0/100) 0%
Scarified[x]	0/50	0%	0/50	0%	(0/100) 0%
Total	0/100	0%	0/100	0%	(0/100) 0%

[z]Germination tests were conducted using Georgia cane seeds placed on filter paper moistened with distilled water in petri dishes held at 80.0 degrees Fahrenheit (26.7 degrees Celsius) and were observed for 14 days for radical emergence.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds were then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, seed embryos that are respirating or alive will appear stained when soaked in TTC. Dead embryos will not turn red.

[x]Seeds were scarified by soaking them in a 20% vinegar solution for 30 minutes.

Hydrilla Tuber and Turion Growth Rate and Viability Results

One hundred samples of hydrilla (*Hydrilla verticillata*) propagules were subjected to growth tests within the incubator chamber including 50 tubers and 50 turions. Of the 50 tubers, three were observed to produce roots within six days and six more produced roots by Day 8. By Day 10, four more had root production, and on Day 12 another four had rooted. After fourteen days, observations found another 8 tubers to have produced roots for a total of 27 out of 50 tubers displaying root production for a growth rate of 54% (27/50). However, of the 27 tubers, eight were observed with root production, but otherwise were almost completely decomposed and, therefore, non-viable propagules (Figure 2). Furthermore, the 23 tubers that were observed not to produce roots were also

displaying early signs of decomposition, so all 50 tubers were subjected for analysis using the tetrazolium test. As a result, hydrilla tubers with adequate turgidity to be considered viable were found to have a viability rate of 38% (19/50).

Figure 2. Picture taken by the researcher of hydrilla (*Hydrilla verticillata*) tubers that were observed with root production, but otherwise almost completely decomposed and, therefore, non-viable propagules.

Decomposition of some of the tubers is debatable, but possibly due to the slightly higher temperature of the incubator at 80.0 degrees Fahrenheit (26.7 degrees Celsius), where optimum tuber growth was found to occur at 77.5 degrees Fahrenheit (25.0 degrees Celsius) (Netherland 1997). On the other hand, temperature might not be the culprit, since, hydrilla (*Hydrilla verticillata*) is known to be winter-hardy, withstanding temperatures as low as negative 20.2 degrees Fahrenheit (negative 29.0 degrees Celsius) and, as high as 95.0 degrees Fahrenheit (35.0 degrees Celsius) (Netherland 1997; Ramey 2001). However, this is the maximum temperature hydrilla has been found to tolerate and will not produce turions or tubers for further growth and distribution at this temperature (Netherland 1997). Therefore, for this study 80.0 degrees Fahrenheit (26.7 degrees Celsius) was within ideal temperatures for growth. However, these study results supported research that found that hydrilla plants held in water at 80.5 degrees Fahrenheit (27.0 degrees Celsius), with the water changed weekly, eventually decomposed which led

the hydrilla to being completely discarded after two to three weeks (Overholt *et al.* 2010). Consequently, the lack of water movement could potentially be responsible for the decomposition of some of the tubers. The researcher observed that in areas of the San Marcos River, hydrilla populations are primarily located just downstream of eddies and other meandering river structures, where there is an accumulation of rock and other materials on the riverbed subsurface – creating a more stable media for plant growth against the flowing current, but the current is slow enough for vegetative aquatic plant propagules and rock particles to have settled out of the water column. This supports research by Washington State Department of Ecology (2010b), where studies have shown that hydrilla actually grows faster and more aggressively in areas of flowing water. The addition of a pumping system or some type of water cycling system could have potential for future studies related to hydrilla.

Even though hydrilla turions seemed to be less susceptible when compared to the tubers held within the incubator chamber – where none were observed to be decomposing – the growth rate of the turions was essentially the same when compared to the growth rate of hydrilla tubers. Of the 50 hydrilla turions, only one had produced roots by Day 10 and one more was found to rooted on Day 12. After 14 days, four turions were observed to have stem and leaf growth as well as to have produced roots (Figure 3).

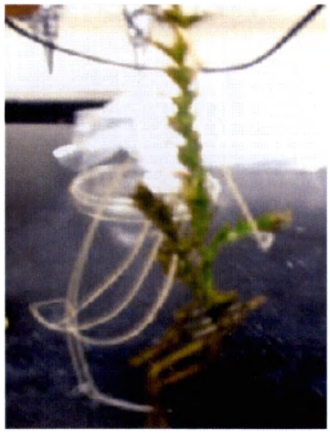

Figure 3. Picture taken by the research of a hydrilla (*Hydrilla verticilla*) turion observed to have stem and leaf growth, as well as to have produced roots.

Further analysis found twelve other turions with growth of stem and leaves without producing roots (Figure 4). However, since stem pieces can still produce roots at this stage, lack of root production at this stage does not necessarily suggest nonviable propagules (Netherland 1997). So, after 14 days the hydrilla turions were found to have a

Figure 4. Picture taken by the researchers of hydrilla (*Hydrilla verticillata*) turions with growth of stem and leaves without producing roots.

growth rate of 32% (16/50). The 34 turions observed not to display growth of any kind were subjected to the tetrazolium test and, when analyzed eight were found to be still viable, giving the turions a 23.5% viability rate. Therefore, hydrilla turions had a total growth and viability rate of 48% (24/50). Thus, tubers and turions of hydrilla (*Hydrilla verticillata*) were found to have a combined growth and viability rate of 43% (43/100) (Table 4).

Table 4. Hydrilla (*Hydrilla verticillata*) propagule growth[z] and tetrazolium[y] test results conducted with tubers and turions in the study of the use of composting as a means of managing the invasive species.

Group	Growth[z] Rate	Growth[z] %	Tetrazolium[y] Test	% Viable	Total Growth & Viability %
Tubers	27/50	54%	19/50	38%	(19/50) 38%
Turions	16/50	32%	8/34	23.5%	(24/50) 48%
Total	43/100	43%	27/84	32.1%	(43/100) 43%

[z]Growth tests were conducted using hydrilla tubers and turions placed on filter paper submerge in distilled water in 600 millileter beakers held at 80.0 degrees Fahrenheit (26.7 degrees Celsius) and were observed for 14 days for growth and root production.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. The propagules were tested by nicking the propagule to expose the tissue. The propagules were then soaked in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, propagules that are respirating or alive will appear stained when soaked in TTC. Dead tissue will not turn red.

It is interesting to note that the turions that produced roots were the four largest propagules and the twelve that grew stems and leaves but no roots were also the largest of the turion samples. This suggests that spread of hydrilla (*Hydrilla verticillata*) by turions is based on maturity, with size as an indicator of the developmental stage of carbohydrate (starch) storage of the propagule, which supports research conducted by Netherland (1997) in which he "[suggested] the smaller size of the turions limits the amount of storage reserve" for root production and "long-term survival" (p. 8).

Oven Mortality Tests Results

To determine the temperatures at which water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) seeds and other propagules were rendered non-viable, oven mortality tests were conducted utilizing various temperatures at which compost, seeds, and other propagules were held. The oven mortality tests were conducted in labratory room 103 of the Agriculture Building at Texas State University-San Marcos using three small Model

20AF Quincy Lab ovens (Chicago, Illinosis). Seeds and other propagules of interest were subjected to the oven mortality tests for three days in stabilized temperatures of either 120.0 degrees Fahrenheit (48.9 degrees Celsius), 135.0 degrees Fahrenheit (57.2 degrees Celsius), and 150.0 degrees Fahrenheit (65.6 degrees Celsius) and included 24 compost sample containers each weighing 140.0 grams. Each soil sample container contained separately: ten hydrilla (*Hydrilla verticillata*) tubers and turions, and ten scarified and ten unscarified seeds of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and Georgia cane (*Arundo donax*). The oven mortality tests were replicated five times and included a total of 120 compost samples. Three hundred water hyacinth (*Eichhornia crassipes*) seeds (150 scarified and 150 unscarified), 300 water lettuce (*Pistia stratiotes*) seeds (150 scarified and 150 unscarified), 300 Georgia cane (*Arundo donax*) seeds (150 scarified and 150 unscarified), 150 hydrilla (*Hydrilla verticillata*) tubers, and 150 hydrilla turions were incorporated into the tests.

Tetrazolium tests found after the first set of oven mortality tests that one scarified water hyacinth seed from the 120.0 degrees Fahrenheit (48.9 degrees Celsius) oven was still viable, giving water hyacinth seeds in that oven a mortality rate of 98%. However, the other 299 of 300 water hyacinth seeds held at any of the temperatures were found to be non-viable when analyzed using the tetrazolium test, resulting in an overall mortality rate for water hyacinth seeds of 99.7% (1/300) (Table 5). These results supported Montoya and colleagues (2012) study results, where two out of three hundred water hyacinth seeds were found to be still viable at 120.0 degrees Fahrenheit (48.9 degrees Celsius), for a 99.3% mortality rate of water hyacinth seeds at 120.0 degrees Fahrenheit (48.0 degrees Celsius).

Table 5. Water hyacinth (*Eichhornia crassipes*) oven mortality tests[z] and tetrazolium[y] test results conducted with scarified[x] and unscarified seeds in the study of the use of composting as a means of managing the invasive species.

Group	No. of Seeds	Tetrazolium[y] Test	% Viable	% Mortality
Unscarified				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Unscarified Total	150	0/150	0%	100%
Scarified[x]				
120.0°F/48.9°C	50	1/50	2%	98%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Scarified Total	150	1/150	0.7%	99.3%
Total	300	1/300	0.3%	99.7%

[z]Oven mortality tests were conducted using water hyacinth seeds placed in soil sample containers moistened with distilled water, held in each oven for three days, and then analyzed for viability/mortality.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds were then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, seed embryos that are respirating or alive will appear stained when soaked in TTC. Dead embryos will not turn red.

[x]Seeds were scarified by soaking them in a 20% vinegar solution for 30 minutes.

Of the 300 water lettuce (*Pistia stratiotes*) seeds, no viable seeds were found from any of the treatment temperatures when analyzed with tetrazolium tests (Table 6). This supports research that found that water lettuce spreads primarily through vegetative propagation and not by seeds (Weldon and Blackburn 1967). In nature, water lettuce flowers fall from the plant early leaving little time for pollination to occur and no viable seeds produced in almost all instances, even in the presence of abundant flowering plants (Weldon and Blackburn 1967).

Table 6. Water lettuce (*Pistia stratiotes*) oven mortality tests[z] and tetrazolium[y] test results conducted with scarified[x] and unscarified seeds in the study of the use of composting as a means of managing the invasive species.

Group	No. of Seeds	Tetrazolium[y] Test	% Viable	% Mortality
Unscarified				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Unscarified Total	150	0/150	0%	100%
Scarified[x]				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Scarified Total	150	0/150	0%	100%
Total	300	0/300	0%	100%

[z]Oven mortality tests were conducted using water lettuce seeds placed in soil sample containers moistened with distilled water, held in each oven for three days, and then analyzed for viability/mortality.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds are then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, seed embryos that are respirating or alive will appear stained when soaked in TTC. Dead embryos will not turn red.

[x]Seeds were scarified by soaking them in a 20% vinegar solution for 30 minutes.

Of the 300 Georgia cane (*Arundo donax*) seeds, no viable seeds were found when analyzed with tetrazolium test (Table 7). These results supported research that found Georgia cane (*Arundo donax*) reproduces primarily through vegetative reproduction of rhizomes, as well as studies that have reported that the seeds are non-viable (Wijte *et al.* 2005). According to Khudamrongsawat and colleagues (2004), "viable seeds have not been found in the United States" (p. 395).

Table 7. **Georgia Cane (*Arundo donax*) oven mortality tests[z] and tetrazolium[y] test results conducted with scarified[x] and unscarified seeds in the study of the use of composting as a means of managing the invasive species.**

Group	No. of Seeds	Tetrazolium[y] Test	% Viable	% Mortality
Unscarified				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Unscarified Total	150	0/150	0%	100%
Scarified[x]				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Scarified Total	150	0/150	0%	100%
Total	300	0/300	0%	100%

[z]Oven mortality tests were conducted using Georgia cane seeds placed in soil sample containers moistened with distilled water, held in each oven for three days, and then analyzed for viability/mortality.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Seed embryos were tested by nicking the seed coat to expose the embryo. The seeds are then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, seed embryos that are respirating or alive will appear stained when soaked in TTC. Dead embryos will not turn red.

[x]Seeds were scarified by soaking them in a 20% vinegar solution for 30 minutes.

Upon analysis of the 150 turions and 150 tubers of hydrilla (*Hydrilla verticillata*), the propagules decomposed when screened from compost samples and further analysis with the tetrazolium confirmed mortality of the vegetative propagules. Therefore, all hydrilla tubers and turions were rendered non-viable at temperatures of 120.0 degrees Fahrenheit (48.9 degrees Celsius) and above (Table 8). Literature review did not find any studies that researched specifically at what temperatures hydrilla tubers and turions were rendered non-viable, although, it was found that 95.0 degrees Fahrenheit (35.0 degrees Celsius) was the maximum temperature hydrilla can tolerate (Netherland 1977). However, higher temperatures inhibit tuber and turion production and growth as well (Netherland 1997).

Table 8. **Hydrilla (*Hydrilla verticillata*) oven mortality tests[z] and tetrazolium[y] test results conducted with tubers and turions in the study of the use of composting as a means of managing the invasive species.**

Group	No. of Propagules	Tetrazolium[y] Test	% Viable	% Mortality
Tubers				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Tuber Total	150	0/150	0%	100%
Turions				
120.0°F/48.9°C	50	0/50	0%	100%
135.0°F/57.2°C	50	0/50	0%	100%
150.0°F/65.6°C	50	0/50	0%	100%
Turion Total	150	0/150	0%	100%
Total	300	0/300	0%	100%

[z]Oven mortality tests were conducted using hydrilla tubers and turions placed separately in soil sample containers moistened with distilled water, held in each oven for three days, and then analyzed for viability/mortality.

[y]The tetrazolium test is a viability test that usually takes about 30 minutes to perform. Propagules were tested by nicking the propagule to expose the tissue. The propagules are then imbibed by soaking them in water, and then biochemical 2, 3, 5 triphenyl tetrazolium chloride (TTC). While TTC is initially colorless, it is converted to formazan red when living tissue is present. Therefore, propagules that are respirating or alive will appear stained when soaked in TTC. Dead tissue will not turn red.

Objective 4

The fourth objective of this study was to determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Compost was produced and stored at the Bobcat Blend composting site at the Muller Farm owned by Texas State University-San Marcos. At the compost site, a total of twelve compost piles were created using plants collected from the San Marcos River and surrounding area(s). Three piles contained 25% of each invasive species, which included water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*), independently; these three piles were replicated three times for a total of nine piles. Additionally, a compost pile was built containing 25% of all three species (water hyacinth, water lettuce, and hydrilla) simultaneously and was also

replicated three times. These twelve piles utilized approximately 5,760 pounds of water hyacinth, 6,000 pounds of water lettuce, and 4,272 pounds of hydrilla collected from areas of the San Marcos River.

In addition to these twelve piles, four more compost piles were planned for construction. However, only three more compost piles were created with plants collected from four different areas within the Rio Grande River Valley region. Of these three compost piles, one individual species compost pile containing 25% water hyacinth plants was created and one individual species compost pile containing 25% water lettuce plants was created. The researcher was unable to collect enough hydrilla plants from the Rio Grande River Valley area to construct an individual species compost pile. However, the researcher was able to collect enough hydrilla plants to build a combination pile utilizing 25% of all three species simultaneously. The harvestable hydrilla populations from the Rio Grande region was possibly due to the fact that, according to USDA entomologist Dr. Moran (2010), the United States Fish and Wildlife Department released a series of grass carp populations into the Rio Grande River during the past couple of years in order to help control hydrilla infestations (personal contact). The lack of harvestable hydrilla plant populations was also potentially due to the turbidity of the water, where the sunlight was unable to penetrate past the first six inches of the water column which limited not only the spread of the hydrilla to deeper areas but affected the structural growth of the hydrilla that was harvested, where growth was observed concentrated on stem growth and little to no leaves were produced (Figure 5).

Figure 5. Picture taken by the researcher of the stringing, diminished growth of the hydrilla (*Hydrilla verticillata*) plants harvested in the Rio Grande River Valley area.

These three piles utilized approximately 1,920 pounds of water hyacinth, 2,000 pounds of water lettuce, and 356 pounds of hydrilla collected from the Rio Grande River Valley region. In all, a total of 7,680 pounds of water hyacinth, 8,000 pounds of water lettuce, and 4,628 pounds of hydrilla were collected and utilized in this study – a total of 20,308 pounds of invasive plant species.

All compost piles incorporated other compost feedstocks, which included food waste and woodchips. The compost operation utilized food waste from the cafeterias on Texas State University-San Marcos campus in the compost feedstock blends. Some of the cafeteria food waste is processed through a grinder which made all of the food of an even "cole slaw" consistency, while some of the cafeteria waste consisted of whole food parts. The cafeteria food waste was used in all 15 compost piles constructed, where approximately 45,000 pounds of food waste was gathered for this study.

Locally and readily available shrub branches and trees waste pruned and managed by the Bartlett Tree Company were incorporated as a carbon source within the composting project. Therefore, size of chips was relatively consistent, but species of tree

waste varied, where in the Rio Grande Valley, woodchips could potentially be substituted with dried Georgia cane (*Arundo donax*) and/or sugar cane (*Saccharum officinarum*) if a composting operation was implemented in the region. Approximately 52,200 pounds of woodchips were used to construct the 15 compost piles for this study.

Various percentages of these feedstocks were tested and piles were modified as required to create ideal conditions for composting. The primary compost recipe applied to construct the piles consisted of 25% plant material, 25% food waste material, and 50% woodchips. However, when constructing the single species compost piles that were made of just hydrilla (*Hydrilla verticillata*), it was noticed when monitoring these piles, that additional nitrogen was needed in order for temperatures to reach the required 135.0 degrees Fahrenheit (57.2 degrees Celsius); therefore, the compost piles constructed with hydrilla plants had an altered compost recipe of 21% plant material, 33% food waste material, and 46% woodchips. The necessary addition of nitrogen could possibly be due to the fact that when these piles were built, the water lines busted during a freeze. Therefore, during the first two or three weeks after construction, the researcher was unable to maintain the moisture level via the addition of water, thus the addition of food waste. Otherwise, no modifications or alterations were made to the single species compost piles that were constructed using the invasive species water hyacinth (*Eichhornia crassipes*) or water lettuce (*Pistia stratiotes*). Additionally, no modifications or alterations were made to the combination piles that utilized all three species simultaneously.

Piles were turned every five to seven days, weather permitting, and composting was completed within approximately 30 days from time of construction. A moisture

level of 50 to 70% is ideal for composting to occur (Rynk *et al.* 1992), and in response to high environmental temperatures and lack of rain due to drought conditions, the moisture level of the piles was maintained via hand-watering approximately once a week. Moisture levels were measured with a 60-inch moisture meter (Compost Moisture Meter, ReoTemp Instrument Corporations), as well as with a "feel" test.

Compost was examined by sight and touch after 30 days (one month) to ensure that compost was decomposed and suitable for the curing stage. The curing stage occurs when there has been a sustained reduction in pile temperatures (50.0 to 105.0 degrees Fahrenheit/ 10.0 to 40.6 degrees Celsius) (Rynk *et al.* 1992). The compost was cured in the same piles where they were built. The curing stage generally takes approximately four to eight weeks (Rynk *et al.* 1992). Compost that is not allowed time for the curing process to occur can limit the availability of nitrogen to plants and introduce phytoxins that are harmful to plants (Rynk *et al.* 1992). After curing, compost piles consisting of the same invasive species recipe were merged together, which mixed the three smaller piles together to create one large pile of that compost recipe – for a total of four large piles of each invasive species recipe. From these large compost piles, compost was then screened using a quarter-inch screener, which allowed for the removal of larger particles and foreign materials.

Compost Quality Tests

Once the compost cured, samples of compost were collected to observe whether the composting process destroyed seeds and other propagules of each of the invasive species. Samples of compost can vary from location to location within the pile, and those collected for analysis must be representative of the entire material being analyzed

(Pennsylvania State University 2002). Therefore, the samples that were either analyzed by the researcher or sent to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program for testing assurance were composite samples of material collected from several locations and depths within the pile being sampled.

Twenty-five one gallon-sized samples were taken from each pile of cured compost of each compost recipe from plants collected in the San Marcos River area, for a total of 100 samples. Each compost sample was drawn by collecting one subsample from the peak of the compost pile in addition to subsamples from eight locations and at three different depths from the overall curing compost pile for a total of nine subsamples. Larger particles and any foreign material(s) were screened from the subsamples using a quarter-inch screener and then these subsamples were then combined to create composite five-gallon-sized samples. The samples were labeled appropriately to identify the corresponding cured compost pile from which they were gathered. Additionally, from the three compost piles made from the plants collected from the Rio Grande River Valley region, a total of 45 samples were collected and analyzed to observe whether the composting process destroyed seeds and other propagules of each of the invasive species. From each of these piles one-gallon-sized samples were collected from five locations and at three different depths within the piles for a total of 15 samples collected from each of the three Valley piles to create the representative composite sample.

To determine the effectiveness of composting at rendering seeds and other propagules non-viable, any water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) seeds and other propagules, were screened for from the finished cured compost using a consecutive series of screens. Since the

seeds and other propagules are very small (water hyacinth – 1.0 x 0.5 millimeters, water lettuce – 1.0 x 0.5 inches, Georgia cane – 0.1 x 0.1 millimeters, and hydrilla – 0.1 x 1.0 millimeters as well as 4.0 x 10.0 millimeter tubers and 3.0 x 8.0 millimeter turions) (Figure 6), it was suspected that they would be completely decomposed during the composting process (Mulholland-Olson 2004; WSDE 2010b; WSDE 2010d; Montoya *et al.* 2012).

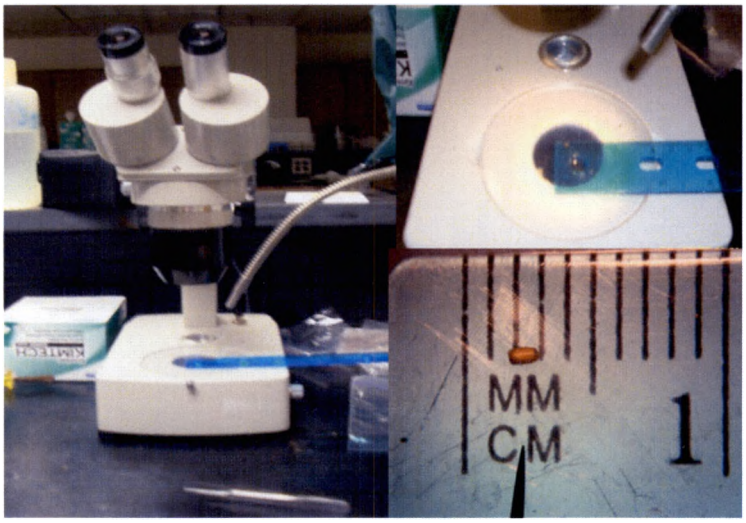

Figure 6. Equipment showing the size of a water hyacinth (*Eichhornia crassipes*) seed.

A consecutive series of screeners were used to screen down to the size of the species being analyzed. Any water hyacinth and water lettuce seeds and hydrilla propagules found were subjected to the tetrazolium test described previously, to determine if they were still viable or in fact rendered non-viable through the high temperatures achieved in the composting processes.

From the composite sample collected from the single species compost pile that consisted of water hyacinth (*Eichhornia crassipes*) plants harvested from areas in the San Marcos River, a total of 131 seeds or partial seeds were found. However, all water hyacinth seeds found were only identifyable with the use of a microscope (PARCO™

SPT Series, Model 10981, Westland, Michigan) since the seeds or portions of seed were so small. All of these partial seeds were, for the most part, effectively decomposed down into the embryo (Figure 7). Since, all of these partial seeds were, for the most part, effectively decomposed down into the embryo – this led the researcher to believe that any and all other water hyacinth seeds had been effectively and essentially destroyed beyond recognition, and therefore, non-viable. Analysis using the tetrazolium test confirmed that all 131 of the seeds were dead and, therefore, rendered non-viable through the high temperatures achieved in the compost piles.

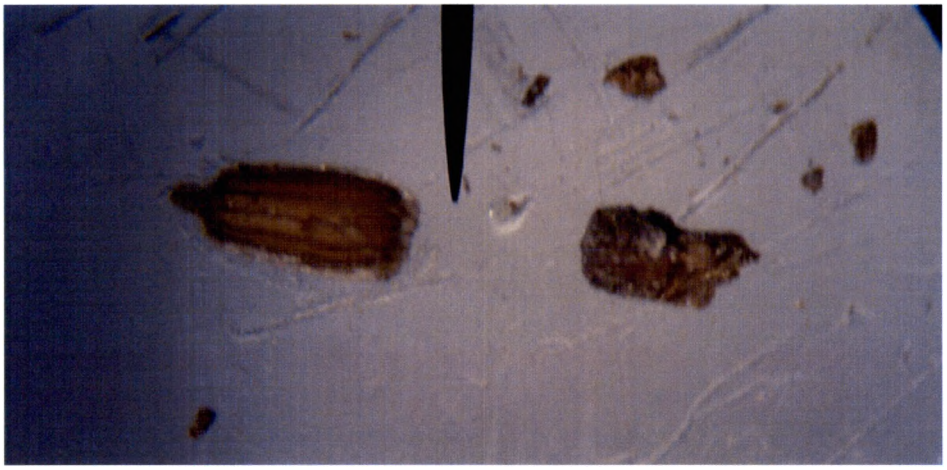

Figure 7. Picture taken by the researcher; comparison of a whole water hyacinth (*Eichhornia crassipes*) seed that was not exposed to any laboratory or field experiments (left seed) and a partial water hyacinth seed decomposed/destroyed from the composting process in the field experiments (right seed).

From the composite sample collected from the single species compost pile that consisted of water lettuce (*Pistia stratiotes*) plants harvested from areas in the San Marcos River, a total of three seeds where found. Analysis using the tetrazolium test revealed that all three seeds were non-viable. These results supports research that water lettuce seeds are non-viable, even in the presence of abundant flower plants (Weldon and Blackburn 1967).

From the composite sample collected from the single species compost pile that consisted of hydrilla (*Hydrilla verticillata*) plants harvested from areas in the San Marcos River, no tubers were found but a total of 31 turions where found. However, all turions found were difficult to identify, because some of the outer areas were slightly decomposed with the appearance of microorganism or macroorganism penetration and were also black in color – like they had been burned (Figure 8). Analysis using the tetrazolium test revealed that all 31 turions were non-viable. Similar to the growth and viability tests, the turions found were those of larger size, leaving the researcher to believe that all smaller turions, as well as tubers, were either destroyed beyond recognition or completely destroyed.

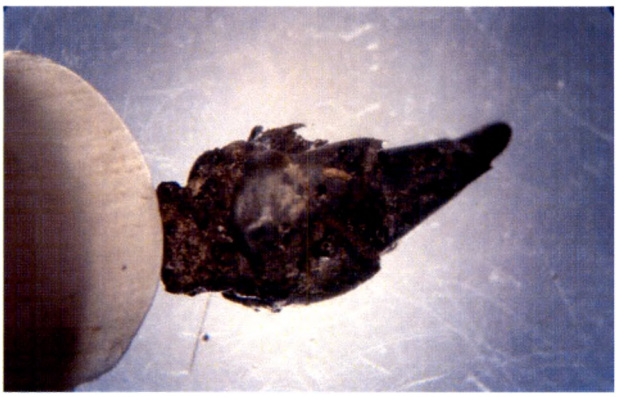

Figure 8. Picture taken by the researcher of a turion found, illustrating how some of the outer areas were slightly decomposed with the appearance of microorganism and/or macroorganism penetration and the blackness in color – like they had been burned.

From the composite sample collected from the combination compost pile that consisted of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) plants harvested from areas of the San Marcos River, the researcher found a total of 56 water hyacinth seeds, 2 water lettuce seeds, no hydrilla tubers, and 11 hydrilla turions. Analysis using the tetrazolium test revealed that all seeds and propagules were non-viable. Therefore, all seeds and propagules of water hyacinth

(*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) found in these piles were rendered non-viable through the high temperatures achieved in the composting process.

From the composite sample collected from the single species compost pile that consisted of water hyacinth (*Eichhornia crassipes*) plants harvested from areas in the Rio Grande River Valley, 24 seeds where found. All of the water hyacinth seeds found, were also only identifyable with the use of a microscope (PARCO™ SPT Series, Model 10981, Westland, Michigan) since they are so small and only partial seeds were found within these samples as well. All of these partial seeds were also, for the most part, effectively decomposed down into the embryo. Analysis using the tetrazolium test confirmed that all 24 of the seeds were non-viable. The decreased amount of seeds found in these samples of compost is potentially due to the lesser amount of flowering water hyacinth plants harvested from the areas in the Rio Grande River Valley.

From the composite sample collected from the single species compost pile that consisted of water lettuce (*Pistia stratiotes*) plants harvested from areas in the Rio Grande River Valley, only one seed was found. Analysis using the tetrazolium test revealed that this seed was non-viable. These results were similar to the results from the single species compost pile that consisted of water lettuce harvested from areas in the San Marcos River. The lack of finding water lettuce seeds within the samples of collected compost could potentially be due to the fragileness of the seeds, where they were either destroyed beyond recognition or completely destroyed by the high temperatures achieved in the composting process.

From the composite sample collected from the combination compost pile that consisted of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) plants harvested from areas of the Rio Grande River Valley, the researcher found a total of 17 water hyacinth seeds and 1 water lettuce seed, with no hydrilla tubers or turions found. Analysis using the tetrazolium tests revealed that all water hyacinth and water lettuce seeds were non-viable. Since no hydrilla tubers or turions were found, analysis using the tetrazolium tests was not applicable. Lack of hydrilla turions and tubers is possibly due to the poor condition of the plants harvested from the areas of the Rio Grande River Vally. Therefore, all seeds and propagules of water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) found in these piles were rendered non-viable through the high temperatures achieved in the composting process.

To determine if the compost produced from these invasive aquatic plants species is a high quality compost valuable to the horticultural and agricultural industries, in addition to the field sampling collections and analysis mentioned previously, four samples of the compost (one composite sample from each of the four invasive species' recipe compost piles made with plants collected from areas of the San Marcos River), were sent to the Agricultural Analytical Services Laboratory (Pennsylvania State University, University Park, Pennsylvania) for Certified Seal of Testing Assurance. For analysis by this certified composting laboratory, a total of 60 samples of compost were collected. From each compost pile, a total of 15 samples were collected at five different locations and from three depths at each location to create a composite sample (Pennsylvania State University 2002). Composite samples were collected from each pile

separately in order to analyze each compost pile feedstocks independently (See Appendix A for Compost Analysis Sampling and Mailing Procedure). At the Agricultural Analytical Services Laboratory, the samples were subjected to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program, which analyzed the compost on each of the following: pH, soluble salt content, and nutrient content [total carbon (C), total nitrogen (N), arsenic (As), cadmium (Cd), calcium (Ca), copper (Cu), lead (Pb), magnesium (Mg), mercury (Hg), molybdenum (Mo), nickel (Ni), phosphorous (P), potassium (K), selenium (Se), zinc (Zn), moisture content, organic matter content, bioassay (maturity)], as well as stability (respirometry), particle size, and weed seed(s) viability (Pennsylvania State University 2002; United States Composting Council 2010).

All compost samples analyzed by the Agricultural Analytical Services Laboratory's Seal of Testing Assurance Program (Pennsylvania State University, Univeristy Park, Pennsylvania), were determined as either within satisfactory to ideal levels for favorable compost and therefore, a valuable compost product within the agriculture and horticulture industries (See Appendix B-E for results of compost analysis of each invasive species compost pile).

CHAPTER V

DISCUSSION

Purpose

The purpose of this study was to investigate the effectiveness of a large-scale composting operation to manage various invasive plants found in the Rio Grande River, by rendering the seeds and other propagules non-viable, while producing a valuable agricultural and horticultural-based resource.

Objectives

The objectives of this study were:

1. To determine the aquatic plants in the Rio Grande River that are considered the most threatening invasive species.
2. To determine other nitrogen and carbon sources available in regions of the Rio Grande River Valley Basin that would be consistently available composting feedstock sources.
3. To evaluate if the high temperature(s) achieved in the composting process has the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks.
4. To determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Objective 1

The first objective of this study was to determine the aquatic plant(s) in the Rio Grande River that are considered the most threatening invasive species. There are many exotic and/or invasive species worldwide, and research found that water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) are invasive aquatic and riparian species that are particularly invasive and problematic in the Rio Grande River Valley area (TPWD 2009; WSDE 2010a; WSDE 2010c).

In the Rio Grande River Valley, observations and meetings with Delta Lake Irrigation District supervisors and USDA Research Entomologist, Dr. Patrick Moran (2010), verified that the three invasive aquatic species, water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*), were those that posed the biggest threats to the irrigation canals of the Rio Grande River. The invasive species, Georgia cane (*Arundo donax*), was observed in abundance in riparian areas along the river, as well as irrigation canals.

Objective 2

The second objective of this study was to determine other nitrogen and carbon sources available in regions of the Rio Grande Basin Valley, which would be consistently available composting feedstock sources. The Rio Grande Valley was found to be a major agricultural center for agribusiness and tourism, as well as one of the nation's largest producers of vegetables and other agricultural crops (Vigness and Odintz 2009). Agricultural vegetable waste, or essentially any type of food waste, acts as a nitrogen, as well as a moisture source within compost piles.

At the Delta Lake Irrigation District, the establishment of the invasive species Georgia cane (*Arundo donax*) was observed in riparian areas along the river and irrigation canals. The Georgia cane is cut, piled up, and allowed to dry, thus relatively readily and easily harvestable once dried. Therefore, if a compost operation was implemented in the Valley, dried Georgia cane (*Arundo donax*) can be used as a substitute for the locally available woodchip material that is currently used in the compost operation and utilized as a carbon feedstock source in compost piles when available.

Objective 3

The third objective of this study was to evaluate if the high temperature(s) achieved in the composting process has the potential to kill seeds and other propagules of the invasive aquatic plant species found in and along the Rio Grande River that are used as feedstocks. Research has found that the composting process kills plant pathogens and weed seeds if temperatures of 120.0 to 180.0 degrees Fahrenheit (48.9 to 82.2 degrees Celsius) are obtained and maintained for three to seven days (Wiese *et al.* 1998; Dougherty 1999).

At least 400 seeds from each species and 400 propagules (200 tubers and 200 turions) were needed for laboratory tests. The germination and growth tests included the use of a Labnet Mini Incubator chamber (Model I 5110, Woodbridge, New Jersey) held at 80.0 degrees Fahrenheit (26.7 degrees Celsius) for fourteen days and consisted of testing unscarified and scarified seeds, tubers, and turions for radicle and root emergence and/or growth.

Of the 100 unscarified and scarified water hyacinth (*Eichhornia crassipes*) seeds, a total of 61 germinated for a total germination rate of 61% (61/100), giving water hyacinth a total seed germination and viability rate of 63% (63/100) after tetrazolium tests. These results were consistent with Montoya and colleagues' (2012) findings, where 300 seeds were subjected to germination tests, and they reported a 62% germination and viablility rate of water hyacinth (*Eichhornia crassipes*) seeds held for 14 days at a stable temperature of 80.0 Fahrenheit (26.7 degrees Celsius) within the laboratory.

Germination and growth of water lettuce (*Pistia stratiotes*) seeds were found to be consistent with literature research in that studies have reported the abundance of flowering, but observed seedlings were rarely found (Hall and Okali 1974). Of the 50 unscarified and 50 scarified water lettuce seeds, none were observed to have the presence of an emerging radicle; therefore, all 100 water lettuce seeds were then subjected to the tetrazolium tests. Analysis of the seeds using the tetrazolium tests further revealed that all seeds were found to be non-viable; therefore, the germination and viability rates of water lettuce (*Pistia stratiotes*) seeds were 0% (0/100).

Germination and growth of Georgia cane (*Arundo donax*) seeds were found to be consistent with literature research in that studies reported that the seeds of Georgia cane are non-viable (Wijte *et al.* 2005). Of the 50 unscarified and 50 scarified Georgia cane seeds, none were observed to have the presence of an emerging radicle. Analysis of the 100 seeds using the tetrazolium tests further revealed that all seeds were found to be non-viable; therefore, the germination and viability rates of Georgia cane (*Arundo donax*) seeds were 0% (0/100).

Of the 50 hydrilla tubers tested, observations found a total of 27 out of 50 tubers displaying root production for a growth rate of 54% (27/50). However, of the 27 tubers, eight were observed with root production, but otherwise were almost completely decomposed and, therefore, non-viable propagules. Furthermore, the 23 tubers that were observed not to produce roots were also displaying early signs of decomposition, so all 50 tubers were subjected to tetrazolium tests. As a result, hydrilla tubers with adequate turgidity to be considered viable were found to have a viability rate of 38% (19/50).

Of the 50 hydrilla turions tested, 16 were observed to either have just stem and leaf growth or stem and leaf growth as well as root production. Therefore, hydrilla turions were found to have a growth rate of 32% (16/50). The 34 turions observed not to display growth of any kind were subjected to tetrazolium tests and, when analyzed, eight were found to be still viable, giving the turions a 23.5% viability rate. Therefore, hydrilla turions were found to have a total growth and viability rate of 48% (24/50). Thus, tubers and turions of hydrilla (*Hydrilla verticillata*) were found to have a combined growth and viability rate of 43% (43/100).

Oven Mortality Tests Results

To determine the temperatures at which water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), hydrilla (*Hydrilla verticillata*), and Georgia cane (*Arundo donax*) seeds and other propagules were rendered non-viable, oven mortality tests were conducted utilizing various temperatures at which compost, seeds, and other propagules were held. The oven mortality tests were replicated five times and a total of 300 water hyacinth (*Eichhornia crassipes*) seeds (150 scarified and 150 unscarified), 300 water lettuce (*Pistia stratiotes*) seeds (150 scarified and 150 unscarified), 300 Georgia cane

(*Arundo donax*) seeds (150 scarified and 150 unscarified), 150 hydrilla (*Hydrilla verticillata*) tubers, and 150 hydrilla turions were incorporated into the tests.

Tetrazolium tests found after the first set of oven mortality tests that one scarified water hyacinth seed from the 120.0 degrees Fahrenheit (48.9 degrees Celsius) oven was still viable, giving water hyacinth seeds in that oven a mortality rate of 98%. However, the other 299 of 300 water hyacinth seeds held at any of the temperatures were found to be non-viable when analyzed using the tetrazolium tests, resulting in an overall mortality rate for water hyacinth seeds of 99.7% (299/300). These results supported Montoya and colleagues (2012) study results, where two out of three hundred water hyacinth seeds were found to be still viable at 120.0 degrees Fahrenheit (48.9 degrees Celsius), for a 99.3% mortality rate of water hyacinth seeds held at 120.0 degrees Fahrenheit (48.0 degrees Celsius).

Of the 300 water lettuce (*Pistia stratiotes*) seeds, no viable seeds were found from any of the treatment temperatures when analyzed with tetrazolium tests. This supports research that found that water lettuce spreads primarily through vegetative propagation and not by seeds (Weldon and Blackburn 1967). In nature, water lettuce flowers fall from the plant early leaving little time for pollination to occur and no viable seeds produced in almost all instances, even in the presence of abundant flowering plants (Weldon and Blackburn 1967).

Of the 300 Georgia cane (*Arundo donax*) seeds, no viable seeds were found when analyzed with tetrazolium tests. These results supported research that found Georgia cane (*Arundo donax*) reproduces primarily through vegetative reproduction of rhizomes, in addition to studies that have reported that the seeds are non-viable and "viable seeds

have not been found in the United States" (Khudamrongsawat *et al.* 2004, p. 395; Wijte *et al.* 2005).

Upon analysis of the 150 turions and 150 tubers of hydrilla (*Hydrilla verticillata*), the propagules decomposed when screened from compost samples and further analysis with the tetrazolium confirmed mortality of the vegetative propagules. Therefore, all hydrilla tubers and turions were rendered non-viable at temperatures of 120.0 degrees Fahrenheit (48.9 degrees Celsius) and above. Literature review did not find any studies that researched specifically at what temperatures hydrilla tubers and turions were rendered non-viable, although it was found that 95.0 degrees Fahrenheit (35.0 degrees Celsius) was the maximum temperature hydrilla can tolerate (Netherland 1997).

The oven mortality tests determined that temperatures of at least 135.0 degress Fahrenheit (57.2 degrees Celsius) were necessary within the compost piles in order to render all of the seeds and propagules of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) non-viable, and allowed the researcher to continue the study and build compost piles containing these species. This methodology for determining the temperatures at which seeds and other propagules are rendered non-viable has the potential to be replicated using other invasive plant species and if they are thought to have the potential to be utilized within the composting process.

Objective 4

The fourth objective of this study was to determine if the compost produced from invasive aquatic plant species of the Rio Grande River is a high quality compost valuable to the horticultural and agricultural industries.

Compost was produced and stored at the Bobcat Blend composting site at the Muller Farm owned by Texas State University-San Marcos. At the compost site, twelve compost piles were created using approximately 5,760 pounds of water hyacinth (*Eichhornia crassipes*), 6,000 pounds of water lettuce (*Pistia stratiotes*), and 4,272 pounds of hydrilla (*Hydrilla verticillata*) collected from the San Marcos River.

In addition to these twelve piles, three more compost piles were created with plants collected from various areas within the Rio Grande River Valley region. These three piles utilized approximately 1,920 pounds of water hyacinth, 2,000 pounds of water lettuce, and 356 pounds of hydrilla collected from the Rio Grande River Valley region. In all, a total of 7,680 pounds of water hyacinth, 8,000 pounds of water lettuce, and 4,628 pounds of hydrilla was collected and utilized in this study – a total of 20,308 pounds of invasive plant species. All 15 compost piles incorporated other compost feedstocks, which included food waste and woodchips. Approximately 45,000 pounds of food waste and approximately 52,200 pounds of woodchips were utilized in this study. This study created an estimated 90 yards of compost, valued at approximately $2,500, from waste materials that would otherwise be considered troublesome (Frank 2011). This study demonstrates that the Delta Lake Irrigation Districts in the Rio Grande River Valley can harvest these invasive species as well sludge weeds from the irrigation canals and incorporate them into a composting operation, not only maintaining the irrigation canals but making a profit from the waste materials that would otherwise end up in landfills as trash.

Compost Quality Tests

Once the compost cured, samples of compost were collected to observe and determine whether the composting process destroyed seeds and other propagules of each of the invasive species. A total of 100 samples were taken from the cured compost of each compost recipe from plants collected in the San Marcos River area. From the three compost piles made from the plants collected from the Rio Grande River Valley region, a total of 45 samples were collected. A quarter-inch screener was used to screen larger particles and any foreign material(s) from the subsamples. The subsamples were combined to create composite samples, and then were labeled appropriately to identify the corresponding cured compost pile from which they were gathered. The samples were then screened using a consecutive series of screeners to screen down to the size of the species particle being analyzed at that time. Any water hyacinth and water lettuce seeds and hydrilla propagules found were subjected to the tetrazolium tests, to determine if they were still viable or in fact rendered non-viable through the high temperatures achieved in the composting processes.

From the composite sample collected from the single species compost pile that consisted of water hyacinth (*Eichhornia crassipes*), a total of 131 partial seeds were found. Analysis using the tetrazolium tests confirmed that all seeds found were dead and, therefore, rendered non-viable through the high temperatures achieved in the compost piles.

From the composite sample collected from the single species compost pile that consisted of water lettuce (*Pistia stratiotes*) plants harvested from areas in the San Marcos River, a total of three seeds where found. From the composite sample collected

from the single species compost pile that consisted of hydrilla (*Hydrilla verticillata*) plants harvested from areas in the San Marcos River, no tubers were found but a total of 31 turions where found. From the composite sample collected from the combination compost pile that consisted of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) plants harvested from areas of the San Marcos River, the researcher found a total of 56 water hyacinth seeds, 2 water lettuce seeds, no hydrilla tubers, and 11 hydrilla turions. From the composite sample collected from the single species compost pile that consisted of water hyacinth (*Eichhornia crassipes*) plants harvested from areas in the Rio Grande River Valley, 24 seeds where found. From the composite sample collected from the single species compost pile that consisted of water lettuce (*Pistia stratiotes*) plants harvested from areas in the Rio Grande River Valley, only one seed was found. From the composite sample collected from the combination compost pile that consisted of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) plants harvested from areas of the Rio Grande River Valley, the researcher found a total of 17 water hyacinth seeds and 1 water lettuce seed, with no hydrilla tubers or turions found. All seeds of water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) turions found from these samples were found non-viable when analyzed using the tetrazolium tests.

These results demonstrate that the invasive species water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) can be effectively managed by rendering the seeds and other propagules non-viable through the high temperatures achieved in the composting process. There are many countries that

have problematic invasive plant species, as well as poor soil in which to grow agricultural crops. Therefore, a basic composting operation would allow for the addition of compost which would potentially lead to greater agricultural productivity, while managing the invasive species.

To determine if the compost produced from the invasive species of interest is of high quality compost valuable to the agricultural and horticultural industries, four samples of the compost (one composite sample from each of the four invasive species' recipe compost piles), were sent to the Agricultural Analytical Services Laboratory (Pennsylvania State University, University Park, Pennsylvania) for Certified Seal of Testing Assurance. For analysis by this certified composting laboratory, a total of 60 samples of compost were collected (15 samples from each compost pile) to create the representative composite samples. All compost samples analyzed by the Agricultural Analytical Services Laboratory's Seal of Testing Assurance Program (Pennsylvania State University, Univeristy Park, Pennsylvania) were determined as being either within satisfactory to ideal levels for favorable compost and therefore, a valuable compost product within the agricultural and horticultural industries.

Therefore, results demonstrate that the large-scale composting of the invasive species water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) can be used to produce a nutrient rich resource for various applications within the agricultural and horticultural industries, while also effectively managing the invasive species. Additionally, with furthered experience in composting and creating compost, compost of a higher quality can potentially be produced by replicating this study.

Recommendations

Recommendations for future research includes the following:

- In future studies, pre- and post-tests of water quality variables such as electro conductivity and nitrate levels should be measured before and after each harvest in order to indicate positive or negative impacts on the water quality due to removal of the invasive species.
- Research should continue to investigate the potential of composting of other invasive plant species.
- Elephant ears (*Colocasia esculenta*) and Indian swampweed (*Hygrophilia polysperma*) are introduced aquatic species observed by the researcher in areas of the San Marcos River, and should be considered as potential species for future studies.
- Research investigating the various uses and applications of compost made from invasive species such as water lettuce (*Pistia stratiotes*) and hydrilla (*Hydrilla verticillata*), and especially water hyacinth (*Eichhornia crassipes*) because it absorbs contaminants in the water, should be tested and investigated.
- Further research should be conducted studying the economical and sustainable benefits of compost and compost management of invasive species such as water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*), rather than other management techniques such as chemical control methods.

APPENDIX A

COMPOST ANALYSIS: SAMPLING AND MAILING PROCEDURE

PENNSTATE
(814) 863-0841 Fax (814) 863-4540
Agricultural Analytical Services Laboratory
The Pennsylvania State University
University Park PA 16802

COMPOST ANALYSIS

Sampling and Mailing Procedure

The sample you collect for analysis must be representative of the entire material being analyzed. Piles of compost or feedstock often vary from place to place in the pile or windrow. Therefore, the sample sent for analysis should be a *composite sample* of material collected from several locations and depths within the windrow or pile being sampled. The number of sampling points will depend on the size and configuration of the pile or windrow. In most situations, material should be collected from *at least* 5 locations around the pile or windrow and from three depths at each location. Separate composite samples should be collected from different windrows or piles.

To collect a composite sample:

1. You will need a clean auger, soil probe, or spade, a clean 5 gal plastic bucket, a clean plastic tarp, and a 1-2 quart sample container (heavy plastic zip-loc bag, or wide mouth plastic bottle).
2. Proceed to the first sampling location and collect approximately 1 pint of material from near the surface of the windrow, another pint of material midway to the core of the windrow, and another pint of material from near the core of the windrow. Place the samples in a clean 5 gal plastic bucket.
3. Repeat this process at each of the sampling locations in the windrow or pile, adding the samples to the 5 gal bucket.
4. When you have completed sample collection at all sampling locations, thoroughly mix the material in the bucket being careful to avoid stratification of the different particle sizes. Depending on the consistency and volume of the sampled material, mixing can be facilitated by dumping the material on a plastic tarp and mixing thoroughly to ensure homogeneity prior to taking a composite sample for analysis.
5. Collect your composite sample from the mixed material. The volume of the composite sample needs to be 1 to 2 quarts depending on the tests you are requesting. Place the material in a suitable clean container for shipping. Label the container to identify the sample. Complete the compost sample analysis submission form. Mail the form, the labeled sample, and a check or money order for the appropriate amount payable to *The Pennsylvania State University.* Samples should be mailed directly to the laboratory as rapidly as possible.

APPENDIX B

WATER HYACINTH (*EICHHORNIA CRASSIPES*) VALUES FOR COMPOST QUALITY TEST VARIABLES FOR COMPOSITE SAMPLE ANALYZED BY A CERTIFIED QUALITY TESTING LABORATORY[z] IN THE STUDY OF THE USE OF COMPOSTING AS A MEANS TO MANAGE THE INVASIVE SPECIES.

Water hyacinth (*Eichhornia crassipes*) values for compost quality test variables for composite sample analyzed by a certified quality testing laboratory[z] in the study of the use of composting as a means to manage the invasive species.

SAMPLE ANALYTE	RESULTS (As is basis)	RESULTS (Dry weight basis)	NORMAL RANGE
pH[y]	7.8	—	5.0 – 8.5
SOLUBLE SALTS[x] (1:5 w:w)	1.2 mmhos/cm	—	1.0 – 10.0 mmhos/cm
SOLIDS[w]	65.0%	—	50.0 – 60.0%
MOISTURE[v]	35.0%	—	40.0 – 50.0%
ORGANIC MATTER[u]	18.8%	28.9%	30.0 – 70.0% (Dry weight)
TOTAL NITROGEN[t] (N)	0.6%	1.0%	0.5 – 2.5% (Dry weight)
ORGANIC NITROGEN[s]	0.6%	1.0%	0.5 – 2.5% (Dry weight)
AMMONIUM[r] (NH_4-N)	0.0003%	0.0005%	< 0.5%
CARBON[q] (C)	11.7%	18.0%	< 54.0%
CARBON:NITROGEN[p] (C:N Ratio)	18.10	18.10	< 20.0
PHOSPHORUS[o] (as P_2O_5)	0.34%	0.52%	n/a
POTASSIUM[n] (as K_2O)[2]	0.36%	0.56%	n/a
CALCIUM[m] (Ca)	6.95%	10.70%	n/a
MAGNESIUM[l] (Mg)	0.27%	0.42%	n/a
ARSENIC[k] (As)	4.7 mg/kg	7.3 mg/kg	< 41.0 mg/kg
CADMIUM[j] (Cd)	< 0.4 mg/kg	< 0.6 mg/kg	0.6 – 11.0 mg/kg
COPPER[i] (Cu)	15.5 mg/kg	23.8 mg/kg	3.6 – 762.0 mg/kg
LEAD[h] (Pb)	5.8 mg/kg	8.9 mg/kg	2.4 – 603.0 mg/kg
MERCURY[g] (Hg)	0.01 mg/kg	0.01 mg/kg	0.01 – 17.0 mg/kg
MOLYBDENUM[f] (Mo)	< 1.1 mg/kg	< 1.7 mg/kg	0.1 – 10.0 mg/kg

NICKEL[e] (Ni)	12.5 mg/kg	19.2 mg/kg	5.0 – 80.0 mg/kg
SELENIUM[d] (Se)	< 1.1 mg/kg	< 1.7 mg/kg	1.0 – 100.00 mg/kg
ZINC[c] (Zn)	43.4 mg/kg	66.8 mg/kg	38.0 – 1420.0 mg/kg
PARTICLE SIZE[b] (< 9.5 mm)	100.00%	—	% < 9.5 mm
EMERGENCE[a] (% of control)	100.00	—	> 90
SEEDLING VIGOR[zz] (% of control)	100.00	—	> 95
RESPIROMETRY (mg CO_2-C/g solids/day)[yy]	0.4	—	—
RESPIROMETRY (mg CO_2-C/g organic matter/day)[xx]	1.5	—	< 2 Very Stable 2 – 8 Stable

[z]Compost analysis was subjected to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program conducted at Pennsylvania State University's certified Agricultural Analytical Service Laboratory (Pennsylvania State University, University Park, Pennsylvania).

[y]The pH of finished compost was measured by making a slurry of compost and deionized water and then blended to a ratio of 1:5. The sample was then shaken for 20 minutes at room temperature to allow the salts to solubilize in the deionized water. The pH was then measured with an electrometric pH meter (TMECC 2002). The pH of the finished compost was within the upper range, which is slightly alkaline, but still within the normal pH range of finished compost (5.0 to 8.5).

[x]The soluble salts were measured by taking the electrical conductivity in a 1:5 (compost:water, weight ratio) slurry and was measured in units of millimhos/cm.

[w]The % solids was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[v]The % moisture was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[u]The % organic matter of the finished compost was measured by using the Loss-On-Ignition Organic Matter Method; which is a direct determination method that indicates organic matter content by quantifying the amount of solid material combusted relative to the original dried sample (TMECC 2002).

[t]The nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldahl Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[s]The organic nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[r]The ammonium nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[q]The total carbon content of the finished compost in this study was measured by the Combustion with CO_2 Detection method. This method uses a carbon analyzer (Leco CR-12) to determine total organic carbon in compost. The analyzer operates on the principle of total combustion of a sample in an oxygen-rich atmosphere of a 2500 degree Fahrenheit resistance furnace (TMECC 2002). The CO_2 produced by the combustion is swept into an oxygen stream through anhydrone tubes to scrub H_2O vapor from the stream (TMECC 2002). The CO_2 stream is then fed into the infrared detector and the amount of CO_2 produced is measured.

[p]The carbon:nitrogen ratio may be used as an indicator of compost stability and nitrogen (N) availability. C:N ratios of < 20 will mineralize or break-down organic N to inorganic (plant-available) N.

[o]The phosphorus content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the phosphorus content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universally accepted method to determine plant-available P in compost (TMECC 2002). To convert phosphorus (as P_2O_5) into elemental phosphorus (P), divide by 2.29 (TMECC 2002).

[n]The potassium content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the potassium content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universal method to determine plant-available K in compost (TMECC 2002).To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20 (TMECC 2002).

[m]The calcium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Calcium is a macronutrient and is taken up by plants as the calcium ion (Ca^{+2}), and is an essential part of cell wall structure and must be present for the formation of new cells (TMECC 2002).

[l]The magnesium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Magnesium is a macronutrient, taken up by plants as magnesium ions (Mg^{+2}) (TMECC 2002). Magnesium serves as a cofactor for many plant enzymes required for growth and is essential for photosynthesis (TMECC 2002).

[k]The arsenic content of the finished compost was determined by atomic absorption spectrophotometry (TMECC 2002). Arsenic found in compost is usually far below levels of concern (TMECC 2002).

[j]The cadmium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002).

[i]The copper content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Copper is a micronutrient essential in small quantities for plant growth (TMECC 2002). Copper in compost generally poses no human health risk (TMECC 2002).

[h]The lead content of the finished compost was determined by inductively coupled plasma (ICP) atomic emission spectroscopy (TMECC 2002). Lead is strongly bound in all types of soil (TMECC 2002). Lead taken up by plants is strongly immobilized in the roots and is not readily translocated to above ground plant parts (TMECC 2002).

[g]The mercury content of the finished compost was determined by Cold Vapor AAS Technique for Mercury in Compost (TMECC 2002). Mercury that is taken up by plants is immobilized in the roots and is not significantly translocated to the above ground plant portions (TMECC 2002).

[f]The molybdenum content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Molybdenum is an essential trace element for plant growth (TMECC 2002).

[e]The nickel content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Levels of nickel usually found in compost does not pose a risk to the food chain (TMECC 2002).

[d]The selenium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Selenium is a trace element that is generally nontoxic in the elemental form and is considered essential (TMECC 2002).

[c]The zinc content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Zinc is an essential micronutrient for plant growth (TMECC 2002).

[b]The % particle size content of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The % particle size indicated the percentage of particles present in the compost that are > 9.5 millimeters in size on an as is basis.

[a]Emergence (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 90 = Very Mature, 80 – 90 = Mature, < 80 = Immature.

[zz]Seedling Vigor (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 95 = Very Mature, 85 – 95 = Mature, < 85 = Immature.

[yy]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability.

[xx]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), which assumes optimal conditions for microbial activity are present including temperature, moisture, and nutrients and that toxic components that would inhibit microbial respiration are absent. Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability. Results of stability are based on mg CO_2-C/g organic matter/day. The interpretive index from the U.S. Compost Council Test Methods for the Examination of Composting and Compost Stability Ratings and General Characteristics indicates:

- **< 2 = Very Stable – well cured compost, no continued decomposition, no odors, and no potential for volatile fatty acid phytotoxicity and odor.**
- **2 – 8 = Stable – cured compost, odor production not likely, limited potential for volatile fatty acid phytotoxicity and odor, and minimal impact on soil carbon and nitrogen dynamics.**
- **8 – 15 = Moderately Unstable, Raw Compost – uncured compost, minimal odor production, moderate to high potential for volatile fatty acid phytotoxicity, and moderate potential for negative impact on soil carbon and nitrogen dynamics.**
- **15 – 40 = Raw Compost or Raw Organic Products – uncured compost, odor production likely, high potential for volatile fatty acid phytotoxicity and odor, and high potential for negative impact on soil carbon and soil nitrogen dynamics.**
- **> 40 = Raw Feedstocks, Unstable Material – raw, extremely unstable material, odor production expected, probably volatile fatty acid phytotoxicity with most materials, negative impact on soil carbon and nitrogen dynamics expected, and generally not recommended for use as compost.**

APPENDIX C

WATER LETTUCE (*PISTIA STRATIOTES*) VALUES FOR COMPOST QUALITY TEST VARIABLES FOR COMPOSITE SAMPLE ANALYZED BY A CERTIFIED QUALITY TESTING LABORATORY[z] IN THE STUDY OF THE USE OF COMPOSTING AS A MEANS TO MANAGE THE INVASIVE SPECIES.

Water lettuce (*Pistia stratiotes*) values for compost quality test variables for composite sample analyzed by a certified quality testing laboratory[z] in the study of the use of composting as a means to manage the invasive species.

SAMPLE ANALYTE	RESULTS (As is basis)	RESULTS (Dry weight basis)	NORMAL RANGE
pH[y]	7.7	—	5.0 – 8.5
SOLUBLE SALTS[x] (1:5 w:w)	1.41 mmhos/cm	—	1.0 – 10.0 mmhos/cm
SOLIDS[w]	63.7%	—	50.0 – 60.0%
MOISTURE[v]	36.3%	—	40.0 – 50.0%
ORGANIC MATTER[u]	25.6%	40.2%	30.0 – 70.0% (Dry weight)
TOTAL NITROGEN[t] (N)	0.9%	1.5%	0.5 – 2.5% (Dry weight)
ORGANIC NITROGEN[s]	0.9%	1.5%	0.5 – 2.5% (Dry weight)
AMMONIUM[r] (NH_4-N)	0.0044%	0.0069%	< 0.5%
CARBON[q] (C)	15.9%	25.0%	< 54.0%
CARBON:NITROGEN[p] (C:N Ratio)	17.20	17.20	< 20.0
PHOSPHORUS[o] (as P_2O_5)	0.42%	0.65%	n/a
POTASSIUM[n] (as K_2O)[2]	0.39%	0.61%	n/a
CALCIUM[m] (Ca)	6.42%	10.08%	n/a
MAGNESIUM[l] (Mg)	0.24%	0.38%	n/a
ARSENIC[k] (As)	3.8 mg/kg	5.9 mg/kg	< 41.0 mg/kg
CADMIUM[j] (Cd)	< 0.4 mg/kg	< 0.6 mg/kg	0.6 – 11.0 mg/kg
COPPER[i] (Cu)	14.2 mg/kg	22.3 mg/kg	3.6 – 762.0 mg/kg
LEAD[h] (Pb)	4.8 mg/kg	7.6 mg/kg	2.4 – 603.0 mg/kg
MERCURY[g] (Hg)	0.01 mg/kg	0.02 mg/kg	0.01 – 17.0 mg/kg
MOLYBDENUM[f] (Mo)	< 1.1 mg/kg	< 1.7 mg/kg	0.1 – 10.0 mg/kg

NICKEL[c] (Ni)	4.8 mg/kg	7.5 mg/kg	5.0 – 80.0 mg/kg
SELENIUM[d] (Se)	< 1.1 mg/kg	< 1.7 mg/kg	1.0 – 100.00 mg/kg
ZINC[c] (Zn)	39.0 mg/kg	61.2 mg/kg	38.0 – 1420.0 mg/kg
PARTICLE SIZE[b] (< 9.5 mm)	100.00%	—	% < 9.5 mm
EMERGENCE[a] (% of control)	100.00	—	> 90
SEEDLING VIGOR[zz] (% of control)	100.00	—	> 95
RESPIROMETRY (mg CO_2-C/g solids/day)[yy]	1.0	—	—
RESPIROMETRY (mg CO_2-C/g organic matter/day)[xx]	2.4	—	< 2 Very Stable 2 – 8 Stable

[z]Compost analysis was subjected to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program conducted at Pennsylvania State University's certified Agricultural Analytical Service Laboratory (Pennsylvania State University, University Park, Pennsylvania).

[y]The pH of finished compost was measured by making a slurry of compost and deionized water and then blended to a ratio of 1:5. The sample was then shaken for 20 minutes at room temperature to allow the salts to solubilize in the deionized water. The pH was then measured with an electrometric pH meter (TMECC 2002). The pH of the finished compost was within the upper range, which is slightly alkaline, but still within the normal pH range of finished compost (5.0 to 8.5).

[x]The soluble salts were measured by taking the electrical conductivity in a 1:5 (compost:water, weight ratio) slurry and was measured in units of millimhos/cm.

[w]The % solids was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[v]The % moisture was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[u]The % organic matter of the finished compost was measured by using the Loss-On-Ignition Organic Matter Method; which is a direct determination method that indicates organic matter content by quantifying the amount of solid material combusted relative to the original dried sample (TMECC 2002).

[t]The nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldahl Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[s]The organic nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[r]The ammonium nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[q]The total carbon content of the finished compost in this study was measured by the Combustion with CO_2 Detection method. This method uses a carbon analyzer (Leco CR-12) to determine total organic carbon in compost. The analyzer operates on the principle of total combustion of a sample in an oxygen-rich atmosphere of a 2500 degree Fahrenheit resistance furnace (TMECC 2002). The CO_2 produced by the combustion is swept into an oxygen stream through anhydrone tubes to scrub H_2O vapor from the stream (TMECC 2002). The CO_2 stream is then fed into the infrared detector and the amount of CO_2 produced is measured.

[p]The carbon:nitrogen ratio may be used as an indicator of compost stability and nitrogen (N) availability. C:N ratios of < 20 will mineralize or break-down organic N to inorganic (plant-available) N.

[o]The phosphorus content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the phosphorus content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universally accepted method to determine plant-available P in compost (TMECC 2002). To convert phosphorus (as P_2O_5) into elemental phosphorus (P), divide by 2.29 (TMECC 2002).

[n]The potassium content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the potassium content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universal method to determine plant-available K in compost (TMECC 2002). To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20 (TMECC 2002).

[m]The calcium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Calcium is a macronutrient and is taken up by plants as the calcium ion (Ca^{+2}), and is an essential part of cell wall structure and must be present for the formation of new cells (TMECC 2002).

[l]The magnesium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Magnesium is a macronutrient, taken up by plants as magnesium ions (Mg^{+2}) (TMECC 2002). Magnesium serves as a cofactor for many plant enzymes required for growth and is essential for photosynthesis (TMECC 2002).

[k]The arsenic content of the finished compost was determined by atomic absorption spectrophotometry (TMECC 2002). Arsenic found in compost is usually far below levels of concern (TMECC 2002).

[j]The cadmium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002).

[i]The copper content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Copper is a micronutrient essential in small quantities for plant growth (TMECC 2002). Copper in compost generally poses no human health risk (TMECC 2002).

[h]The lead content of the finished compost was determined by inductively coupled plasma (ICP) atomic emission spectroscopy (TMECC 2002). Lead is strongly bound in all types of soil (TMECC 2002). Lead taken up by plants is strongly immobilized in the roots and is not readily translocated to above ground plant parts (TMECC 2002).

[g]The mercury content of the finished compost was determined by Cold Vapor AAS Technique for Mercury in Compost (TMECC 2002). Mercury that is taken up by plants is immobilized in the roots and is not significantly translocated to the above ground plant portions (TMECC 2002).

[f]The molybdenum content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Molybdenum is an essential trace element for plant growth (TMECC 2002).

[e]The nickel content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Levels of nickel usually found in compost does not pose a risk to the food chain (TMECC 2002).

[d]The selenium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Selenium is a trace element that is generally nontoxic in the elemental form and is considered essential (TMECC 2002).

[c]The zinc content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Zinc is an essential micronutrient for plant growth (TMECC 2002).

[b]The % particle size content of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The % particle size indicated the percentage of particles present in the compost that are > 9.5 millimeters in size on an as is basis.

[a]Emergence (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 90 = Very Mature, 80 – 90 = Mature, < 80 = Immature.

[zz]Seedling Vigor (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 95 = Very Mature, 85 – 95 = Mature, < 85 = Immature.

[yy]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability.

[xx]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), which assumes optimal conditions for microbial activity are present including temperature, moisture, and nutrients and that toxic components that would inhibit microbial respiration are absent. Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability. Results of stability are based on mg CO_2-C/g organic matter/day. The interpretive index from the U.S. Compost Council Test Methods for the Examination of Composting and Compost Stability Ratings and General Characteristics indicates:

- < 2 = Very Stable – well cured compost, no continued decomposition, no odors, and no potential for volatile fatty acid phytotoxicity and odor.
- 2 – 8 = Stable – cured compost, odor production not likely, limited potential for volatile fatty acid phytotoxicity and odor, and minimal impact on soil carbon and nitrogen dynamics.
- 8 – 15 = Moderately Unstable, Raw Compost – uncured compost, minimal odor production, moderate to high potential for volatile fatty acid phytotoxicity, and moderate potential for negative impact on soil carbon and nitrogen dynamics.
- 15 – 40 = Raw Compost or Raw Organic Products – uncured compost, odor production likely, high potential for volatile fatty acid phytotoxicity and odor, and high potential for negative impact on soil carbon and soil nitrogen dynamics.
- > 40 = Raw Feedstocks, Unstable Material – raw, extremely unstable material, odor production expected, probably volatile fatty acid phytotoxicity with most materials, negative impact on soil carbon and nitrogen dynamics expected, and generally not recommended for use as compost.

APPENDIX D

HYDRILLA (*HYDRILLA VERTICILLATA*) VALUES FOR COMPOST QUALITY TEST VARIABLES FOR COMPOSITE SAMPLE ANALYZED BY A CERTIFIED QUALITY TESTING LABORATORY[z] IN THE STUDY OF THE USE OF COMPOSTING AS A MEANS TO MANAGE THE INVASIVE SPECIES.

Hydrilla (*Hydrilla verticillata*) values for compost quality test variables for composite sample analyzed by a certified quality testing laboratory[z] in the study of the use of composting as a means to manage the invasive species.

SAMPLE ANALYTE	RESULTS (As is basis)	RESULTS (Dry weight basis)	NORMAL RANGE
pH[y]	7.7	—	5.0 – 8.5
SOLUBLE SALTS[x] (1:5 w:w)	1.77 mmhos/cm	—	1.0 – 10.0 mmhos/cm
SOLIDS[w]	64.1%	—	50.0 – 60.0%
MOISTURE[v]	35.9%	—	40.0 – 50.0%
ORGANIC MATTER[u]	30.7%	47.8%	30.0 – 70.0% (Dry weight)
TOTAL NITROGEN[t] (N)	1.0%	1.6%	0.5 – 2.5% (Dry weight)
ORGANIC NITROGEN[s]	1.0%	1.6%	0.5 – 2.5% (Dry weight)
AMMONIUM[r] (NH_4-N)	0.0020%	0.0031%	< 0.5%
CARBON[q] (C)	17.7%	27.6%	< 54.0%
CARBON:NITROGEN[p] (C:N Ratio)	17.60	17.60	< 20.0
PHOSPHORUS[o] (as P_2O_5)	0.42%	0.65%	n/a
POTASSIUM[n] (as K_2O)[2]	0.46%	0.71%	n/a
CALCIUM[m] (Ca)	6.53%	10.19%	n/a
MAGNESIUM[l] (Mg)	0.25%	0.39%	n/a
ARSENIC[k] (As)	2.9 mg/kg	4.5 mg/kg	< 41.0 mg/kg
CADMIUM[j] (Cd)	< 0.4 mg/kg	< 0.6 mg/kg	0.6 – 11.0 mg/kg
COPPER[i] (Cu)	12.1 mg/kg	18.9 mg/kg	3.6 – 762.0 mg/kg
LEAD[h] (Pb)	4.6 mg/kg	7.2 mg/kg	2.4 – 603.0 mg/kg
MERCURY[g] (Hg)	0.01 mg/kg	0.01 mg/kg	0.01 – 17.0 mg/kg
MOLYBDENUM[f] (Mo)	< 1.2 mg/kg	< 1.9 mg/kg	0.1 – 10.0 mg/kg

NICKEL[e] (Ni)	4.0 mg/kg	6.2 mg/kg	5.0 – 80.0 mg/kg
SELENIUM[d] (Se)	< 1.2 mg/kg	< 1.9 mg/kg	1.0 – 100.00 mg/kg
ZINC[c] (Zn)	41.5 mg/kg	64.8 mg/kg	38.0 – 1420.0 mg/kg
PARTICLE SIZE[b] (< 9.5 mm)	100.00%	—	% < 9.5 mm
EMERGENCE[a] (% of control)	100.00	—	> 90
SEEDLING VIGOR[zz] (% of control)	100.00	—	> 95
RESPIROMETRY (mg CO_2-C/g solids/day)[yy]	1.6	—	—
RESPIROMETRY (mg CO_2-C/g organic matter/day)[xx]	3.2	—	< 2 Very Stable 2 – 8 Stable

[z]Compost analysis was subjected to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program conducted at Pennsylvania State University's certified Agricultural Analytical Service Laboratory (Pennsylvania State University, University Park, Pennsylvania).

[y]The pH of finished compost was measured by making a slurry of compost and deionized water and then blended to a ratio of 1:5. The sample was then shaken for 20 minutes at room temperature to allow the salts to solubilize in the deionized water. The pH was then measured with an electrometric pH meter (TMECC 2002). The pH of the finished compost was within the upper range, which is slightly alkaline, but still within the normal pH range of finished compost (5.0 to 8.5).

[x]The soluble salts were measured by taking the electrical conductivity in a 1:5 (compost:water, weight ratio) slurry and was measured in units of millimhos/cm.

[w]The % solids was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[v]The % moisture was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[u]The % organic matter of the finished compost was measured by using the Loss-On-Ignition Organic Matter Method; which is a direct determination method that indicates organic matter content by quantifying the amount of solid material combusted relative to the original dried sample (TMECC 2002).

[t]The nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldahl Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[s]The organic nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[r]The ammonium nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[q]The total carbon content of the finished compost in this study was measured by the Combustion with CO_2 Detection method. This method uses a carbon analyzer (Leco CR-12) to determine total organic carbon in compost. The analyzer operates on the principle of total combustion of a sample in an oxygen-rich atmosphere of a 2500 degree Fahrenheit resistance furnace (TMECC 2002). The CO_2 produced by the combustion is swept into an oxygen stream through anhydrone tubes to scrub H_2O vapor from the stream (TMECC 2002). The CO_2 stream is then fed into the infrared detector and the amount of CO_2 produced is measured.

[p]The carbon:nitrogen ratio may be used as an indicator of compost stability and nitrogen (N) availability. C:N ratios of < 20 will mineralize or break-down organic N to inorganic (plant-available) N.

[o]The phosphorus content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the phosphorus content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universally accepted method to determine plant-available P in compost (TMECC 2002). To convert phosphorus (as P_2O_5) into elemental phosphorus (P), divide by 2.29 (TMECC 2002).

[n]The potassium content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the potassium content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universal method to determine plant-available K in compost (TMECC 2002). To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20 (TMECC 2002).

[m]The calcium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Calcium is a macronutrient and is taken up by plants as the calcium ion (Ca^{+2}), and is an essential part of cell wall structure and must be present for the formation of new cells (TMECC 2002).

[l]The magnesium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Magnesium is a macronutrient, taken up by plants as magnesium ions (Mg^{+2}) (TMECC 2002). Magnesium serves as a cofactor for many plant enzymes required for growth and is essential for photosynthesis (TMECC 2002).

[k]The arsenic content of the finished compost was determined by atomic absorption spectrophotometry (TMECC 2002). Arsenic found in compost is usually far below levels of concern (TMECC 2002).

[j]The cadmium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002).

[r]The copper content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Copper is a micronutrient essential in small quantities for plant growth (TMECC 2002). Copper in compost generally poses no human health risk (TMECC 2002).

[h]The lead content of the finished compost was determined by inductively coupled plasma (ICP) atomic emission spectroscopy (TMECC 2002). Lead is strongly bound in all types of soil (TMECC 2002). Lead taken up by plants is strongly immobilized in the roots and is not readily translocated to above ground plant parts (TMECC 2002).

[g]The mercury content of the finished compost was determined by Cold Vapor AAS Technique for Mercury in Compost (TMECC 2002). Mercury that is taken up by plants is immobilized in the roots and is not significantly translocated to the above ground plant portions (TMECC 2002).

[f]The molybdenum content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Molybdenum is an essential trace element for plant growth (TMECC 2002).

[e]The nickel content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Levels of nickel usually found in compost does not pose a risk to the food chain (TMECC 2002).

[d]The selenium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Selenium is a trace element that is generally nontoxic in the elemental form and is considered essential (TMECC 2002).

[c]The zinc content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Zinc is an essential micronutrient for plant growth (TMECC 2002).

[b]The % particle size content of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The % particle size indicated the percentage of particles present in the compost that are > 9.5 millimeters in size on an as is basis.

[a]Emergence (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 90 = Very Mature, 80 – 90 = Mature, < 80 = Immature.

[zz]Seedling Vigor (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 95 = Very Mature, 85 – 95 = Mature, < 85 = Immature.

[yy]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability.

[xx]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), which assumes optimal conditions for microbial activity are present including temperature, moisture, and nutrients and that toxic components that would inhibit microbial respiration are absent. Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability. Results of stability are based on mg CO_2-C/g organic matter/day. The interpretive index from the U.S. Compost Council Test Methods for the Examination of Composting and Compost Stability Ratings and General Characteristics indicates:

- < 2 = Very Stable – well cured compost, no continued decomposition, no odors, and no potential for volatile fatty acid phytotoxicity and odor.
- 2 – 8 = Stable – cured compost, odor production not likely, limited potential for volatile fatty acid phytotoxicity and odor, and minimal impact on soil carbon and nitrogen dynamics.
- 8 – 15 = Moderately Unstable, Raw Compost – uncured compost, minimal odor production, moderate to high potential for volatile fatty acid phytotoxicity, and moderate potential for negative impact on soil carbon and nitrogen dynamics.
- 15 – 40 = Raw Compost or Raw Organic Products – uncured compost, odor production likely, high potential for volatile fatty acid phytotoxicity and odor, and high potential for negative impact on soil carbon and soil nitrogen dynamics.
- > 40 = Raw Feedstocks, Unstable Material – raw, extremely unstable material, odor production expected, probably volatile fatty acid phytotoxicity with most materials, negative impact on soil carbon and nitrogen dynamics expected, and generally not recommended for use as compost.

APPENDIX E

COMINATION OF WATER HYACINTH (*EICHHORNIA CRASSIPES*), WATER LETTUCE (*PISTIA STRATIOTES*), AND HYDRILLA (*HYDRILLA VERTICILLATA*) VALUES FOR COMPOST QUALITY TEST VARIABLES FOR COMPOSITE SAMPLE ANALYZED BY A CERTIFIED QUALITY TESTING LABORATORY[z] IN THE STUDY OF THE USE OF COMPOSTING AS A MEANS TO MANAGE THE INVASIVE SPECIES.

Combination of water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*), and hydrilla (*Hydrilla verticillata*) values for compost quality test variables for composite sample analyzed by a certified quality testing laboratory[z] in the study of the use of composting as a means to manage the invasive species.

SAMPLE ANALYTE	RESULTS (As is basis)	RESULTS (Dry weight basis)	NORMAL RANGE
pH[y]	7.9	—	5.0 – 8.5
SOLUBLE SALTS[x] (1:5 w:w)	1.21 mmhos/cm	—	1.0 – 10.0 mmhos/cm
SOLIDS[w]	70.1%	—	50.0 – 60.0%
MOISTURE[v]	29.9%	—	40.0 – 50.0%
ORGANIC MATTER[u]	20.3%	29.0%	30.0 – 70.0% (Dry weight)
TOTAL NITROGEN[t] (N)	0.7%	1.1%	0.5 – 2.5% (Dry weight)
ORGANIC NITROGEN[s]	0.7%	1.1%	0.5 – 2.5% (Dry weight)
AMMONIUM[r] (NH_4-N)	0.0017%	0.0024%	< 0.5%
CARBON[q] (C)	13.2%	18.8%	< 54.0%
CARBON:NITROGEN[p] (C:N Ratio)	17.80	17.80	< 20.0
PHOSPHORUS[o] (as P_2O_5)	0.33%	0.48%	n/a
POTASSIUM[n] (as K_2O)[2]	0.45%	0.64%	n/a
CALCIUM[m] (Ca)	6.88%	9.81%	n/a
MAGNESIUM[l] (Mg)	0.31%	0.45%	n/a
ARSENIC[k] (As)	5.3 mg/kg	7.6 mg/kg	< 41.0 mg/kg
CADMIUM[j] (Cd)	< 0.4 mg/kg	< 0.6 mg/kg	0.6 – 11.0 mg/kg
COPPER[i] (Cu)	15.8 mg/kg	22.6 mg/kg	3.6 – 762.0 mg/kg
LEAD[h] (Pb)	6.4 mg/kg	9.2 mg/kg	2.4 – 603.0 mg/kg
MERCURY[g] (Hg)	0.01 mg/kg	0.02 mg/kg	0.01 – 17.0 mg/kg
MOLYBDENUM[f]	< 1.2 mg/kg	< 1.7 mg/kg	0.1 – 10.0 mg/kg

(Mo)			
NICKEL[e] (Ni)	5.9 mg/kg	8.4 mg/kg	5.0 – 80.0 mg/kg
SELENIUM[d] (Se)	< 1.2 mg/kg	< 1.7 mg/kg	1.0 – 100.00 mg/kg
ZINC[c] (Zn)	43.2 mg/kg	61.6 mg/kg	38.0 – 1420.0 mg/kg
PARTICLE SIZE[b] (< 9.5 mm)	100.00%	—	% < 9.5 mm
EMERGENCE[a] (% of control)	100.00	—	> 90
SEEDLING VIGOR[zz] (% of control)	100.00	—	> 95
RESPIROMETRY (mg CO_2-C/g solids/day)[yy]	0.7	—	—
RESPIROMETRY (mg CO_2-C/g organic matter/day)[xx]	1.9	—	< 2 Very Stable 2 – 8 Stable

[z]Compost analysis was subjected to the Compost Tests for U.S. Compost Council's Seal of Testing Approval Program conducted at Pennsylvania State University's certified Agricultural Analytical Service Laboratory (Pennsylvania State University, University Park, Pennsylvania).

[y]The pH of finished compost was measured by making a slurry of compost and deionized water and then blended to a ratio of 1:5. The sample was then shaken for 20 minutes at room temperature to allow the salts to solubilize in the deionized water. The pH was then measured with an electrometric pH meter (TMECC 2002). The pH of the finished compost was within the upper range, which is slightly alkaline, but still within the normal pH range of finished compost (5.0 to 8.5).

[x]The soluble salts were measured by taking the electrical conductivity in a 1:5 (compost:water, weight ratio) slurry and was measured in units of millimhos/cm.

[w]The % solids was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[v]The % moisture was measured by weighing a sample and then drying it at 70 (+/- 5) degrees Celsius and then re-weighed. The remaining dry solids fraction represented the total solids, and the evaporated fraction represented the % moisture (TMECC 2002).

[u]The % organic matter of the finished compost was measured by using the Loss-On-Ignition Organic Matter Method; which is a direct determination method that indicates organic matter content by quantifying the amount of solid material combusted relative to the original dried sample (TMECC 2002).

[t]The nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldahl Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[s]The organic nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[r]The ammonium nitrogen content of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), specifically the Total Kjeldhal Nitrogen Semi-Micro Kjeldahl technique (TMECC 2002).

[q]The total carbon content of the finished compost in this study was measured by the Combustion with CO_2 Detection method. This method uses a carbon analyzer (Leco CR-12) to determine total organic carbon in compost. The analyzer operates on the principle of total combustion of a sample in an oxygen-rich atmosphere of a 2500 degree Fahrenheit resistance furnace (TMECC 2002). The CO_2 produced by the combustion is swept into an oxygen stream through anhydrone tubes to scrub H_2O vapor from the stream (TMECC 2002). The CO_2 stream is then fed into the infrared detector and the amount of CO_2 produced is measured.

[p]The carbon:nitrogen ratio may be used as an indicator of compost stability and nitrogen (N) availability. C:N ratios of < 20 will mineralize or break-down organic N to inorganic (plant-available) N.

[o]The phosphorus content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the phosphorus content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universally accepted method to determine plant-available P in compost (TMECC 2002). To convert phosphorus (as P_2O_5) into elemental phosphorus (P), divide by 2.29 (TMECC 2002).

[n]The potassium content of the finished compost in this study was measured by digesting an air-dried, milled sample and determining the potassium content using inductively coupled plasma emission spectrospy (ICP) (TMECC 2002). There is no universal method to determine plant-available K in compost (TMECC 2002). To convert potassium (as K_2O) into elemental potassium (K), divide by 1.20 (TMECC 2002).

[m]The calcium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Calcium is a macronutrient and is taken up by plants as the calcium ion (Ca^{+2}), and is an essential part of cell wall structure and must be present for the formation of new cells (TMECC 2002).

[l]The magnesium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Magnesium is a macronutrient, taken up by plants as magnesium ions (Mg^{+2}) (TMECC 2002). Magnesium serves as a cofactor for many plant enzymes required for growth and is essential for photosynthesis (TMECC 2002).

[k]The arsenic content of the finished compost was determined by atomic absorption spectrophotometry (TMECC 2002). Arsenic found in compost is usually far below levels of concern (TMECC 2002).

[j]The cadmium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002).

[i]The copper content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Copper is a micronutrient essential in small quantities for plant growth (TMECC 2002). Copper in compost generally poses no human health risk (TMECC 2002).

[h]The lead content of the finished compost was determined by inductively coupled plasma (ICP) atomic emission spectroscopy (TMECC 2002). Lead is strongly bound in all types of soil (TMECC 2002). Lead taken up by plants is strongly immobilized in the roots and is not readily translocated to above ground plant parts (TMECC 2002).

[g]The mercury content of the finished compost was determined by Cold Vapor AAS Technique for Mercury in Compost (TMECC 2002). Mercury that is taken up by plants is immobilized in the roots and is not significantly translocated to the above ground plant portions (TMECC 2002).

[f]The molybdenum content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Molybdenum is an essential trace element for plant growth (TMECC 2002).

[e]The nickel content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Levels of nickel usually found in compost does not pose a risk to the food chain (TMECC 2002).

[d]The selenium content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Selenium is a trace element that is generally nontoxic in the elemental form and is considered essential (TMECC 2002).

[c]The zinc content of the finished compost was determined by the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Zinc is an essential micronutrient for plant growth (TMECC 2002).

[b]The % particle size content of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The % particle size indicated the percentage of particles present in the compost that are > 9.5 millimeters in size on an as is basis.

[a]Emergence (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 90 = Very Mature, 80 – 90 = Mature, < 80 = Immature.

[zz]Seedling Vigor (% of control) of the finished compost was determined by using the methodologies specified in the U.S. Compost Council Test Methods for the Examination of Composting and Compost (TMECC 2002). The bioassay test provides a screen for the presence of phytotoxins in compost based on seedling emergence and seedling vigor relative to a control and provides an assessment of compost maturity. The Test Methods for the Examination of Composting and Compost (2002) provides the following Maturity Indicator Ratings based on this tests: > 95 = Very Mature, 85 – 95 = Mature, < 85 = Immature.

[yy]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002). Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability.

[xx]Respirometry of the finished compost was determined by using the methodologies specified in the Test Methods for the Examination of Composting and Compost (TMECC 2002), which assumes optimal conditions for microbial activity are present including temperature, moisture, and nutrients and that toxic components that would inhibit microbial respiration are absent. Respirometry: Carbon Dioxide (CO_2) Evolution Rate provides a measurement of the relative microbial activity in a compost and, hence can be used as an estimate of compost stability. Results of stability are based on mg CO_2-C/g organic matter/day. The interpretive index from the U.S. Compost Council Test Methods for the Examination of Composting and Compost Stability Ratings and General Characteristics indicates:

- < 2 = Very Stable – well cured compost, no continued decomposition, no odors, and no potential for volatile fatty acid phytotoxicity and odor.
- 2 – 8 = Stable – cured compost, odor production not likely, limited potential for volatile fatty acid phytotoxicity and odor, and minimal impact on soil carbon and nitrogen dynamics.
- 8 – 15 = Moderately Unstable, Raw Compost – uncured compost, minimal odor production, moderate to high potential for volatile fatty acid phytotoxicity, and moderate potential for negative impact on soil carbon and nitrogen dynamics.
- 15 – 40 = Raw Compost or Raw Organic Products – uncured compost, odor production likely, high potential for volatile fatty acid phytotoxicity and odor, and high potential for negative impact on soil carbon and soil nitrogen dynamics.
- > 40 = Raw Feedstocks, Unstable Material – raw, extremely unstable material, odor production expected, probably volatile fatty acid phytotoxicity with most materials, negative impact on soil carbon and nitrogen dynamics expected, and generally not recommended for use as compost.

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