EVALUATION OF *EUCALYPTUS* INVASIVENESS IN FLORIDA AND METHODS FOR DIRECT CONTROL

By

KIMBERLY A. LORENTZ

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2013

To all of my family, who are most precious to me

ACKNOWLEDGMENTS

I want to express my gratitude to my advisor, Dr. Patrick Minogue, for his enthusiasm, for sharing his wealth of knowledge and for the material and instructive support that he provided. I am also thankful to my committee members, Drs. Kimberly Bohn, Donald Rockwood and Jason Ferrell, for the care with which they reviewed this thesis, and for their valuable guidance along the way. Insightful advice given by Drs. Anna Osiecka, Luke Flory and Damian Adams has also been a great help in the development of this research. I extend many thanks to James Colee, IFAS statistics, and Dr. Dwight Lauer, Silvics Analytic, for their assistance with statistical analyses in this thesis. The assistance with germination testing that Jim Aldrich provided was greatly appreciated. I also thank Luke Wright, Seth Wright and Daniela Chevasco for assisting with data collection and for their pleasant company that made long days in the field go by quickly. I am forever grateful for the companionship of my fellow graduate students and friends at the University of Florida, as we moved through this learning experience together. It has been a pleasure getting to know and collaborating with the many great people in the School of Forest Resources and Conservation. Most of all, loving thanks to my parents, Rick and Kathy Lorentz, and to Marie Lorentz and Johnny Grebenc for their unwavering support and encouragement throughout this journey.

TABLE OF CONTENTS

page

APPENDIX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ae	Acid equivalent
ai	Active ingredient
AMPC	Aminocyclopyrachlor
ANOVA	Analysis of variance
APHIS	Animal and Plant Health Inspection Service
BSD	Basal stem diameter
BTU	British thermal unit
C	Celsius
cm	Centimeter
d	Day
DBH	Diameter at breast height
df	Degrees of freedom
FAC	Florida Administrative Code
FAO	Food and Agriculture Organization of the United Nations
ft	Foot
FTE	Frost tolerant *Eucalyptus*
g	Gram
gly	Glyphosate
ha	Hectare
HSD	Honestly significant difference
IFAS	University of Florida Institute of Food and Agricultural Sciences
imaz	Imazapyr
L	Liter
M	Mean

m	Meter
MAT	Months after treatment
mg	Milligram
ml	Milliliter
mm	Millimeter
mo	Month
N	North
n	Sample size
NISC	National Invasive Species Council
NOAA	National Oceanic and Atmospheric Administration
NPS	National Park Service
REGWQ	Ryan-Einot-Gabriel-Welsch multiple range test
RSB	Roundtable on Sustainable Biofuels
SE	Standard error
SU	Suflonylurea herbicides
spp.	Species
triclo	Triclopyr
US	United States
USDA	United States Department of Agriculture
v/v	Volume per volume ratio
W	West
WRA	Weed Risk Assessment
YAT	Years after treatment

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

EVALUATION OF *EUCALYPTUS* INVASIVENESS IN FLORIDA AND METHODS FOR DIRECT CONTROL

By

Kimberly A. Lorentz

May 2013

Chair: Patrick Minogue
Major: Forest Resources and Conservation

Potential invasiveness is a concern for *Eucalyptus* species, which are being planted in the southeastern US for landscaping mulch and possible bioenergy crops. The purpose of this research was to evaluate the potential invasiveness of *Eucalyptus* species that are under consideration for large-scale planting and to improve practices for control of *Eucalyptus*. Surveys for natural recruitment within and proximate to seed bearing stands at two Florida locations found no *Eucalyptus* seedlings. Seed addition studies then examined the potential for seedling emergence and survival among *Eucalyptus amplifolia*, *Eucalyptus camaldulensis* and *Eucalyptus grandis* relative to various disturbance levels, seeding densities and vegetation community types within and proximate to the mature *Eucalyptus* stands. Greater survival was found for *E. camaldulensis* compared to the other species. Greater emergence was observed under disturbed conditions and within *Eucalyptus* communities. Overall, emergence of added seed was very low (0.0 to 0.32%) and no seedlings survived more than 13 weeks. Experiments were also conducted to compare aminocyclopyrachlor (AMCP), a new herbicide, to the standard imazapyr and triclopyr herbicide treatments for control of 29-month-old *Eucalyptus benthamii*. Basal bark applications of a 120 g ae L^{-1} AMCP

product formulation at 5% v/v in methylated soybean oil carrier resulted in 97–99% crown reduction of *E. benthamii,* and generally provided greater control than the standard 240 g ae L^{-1} imazapyr product formulation at 28.1% v/v or the 480 g ae L^{-1} triclopyr ester product formulation at 75% v/v, when assessed at 6 and 12 mo after treatment. Basal frill applications of 120 g ae L^{-1} AMCP product formulation at 12.5% v/v in water resulted in 100% crown reduction of *Eucalyptus* and greater control than the standard 240 g ae L^{-1} imazapyr product formulation at 7.8% v/v or 360 g ae L^{-1} triclopyr amine product formulation at 50% v/v treatments when evaluated 6 and 12 mo after treatment. These data indicate that under specific favorable conditions *Eucalyptus* spp. seedlings may establish within or proximate to planted stands in Florida, but the overall likelihood of establishment is low and complete control of unwanted *Eucalyptus* can be provided by AMCP herbicide at low rates.

CHAPTER 1

EUCALYPTUS AS BIOENERGY FEEDSTOCK IN THE SOUTEASTERN UNITED STATES: INVASION RISK ASSESSMENT, MANAGEMENT PRACTICES AND POLICIES

Introduction

Since the announcement of the US renewable energy initiative in 2006, forewarning of the high risk for biomass bioenergy crops to escape from cultivation and invade unmanaged areas has become prevalent throughout literature on renewable energy sustainability (Raghu et al. 2006; Barney and DiTomaso 2008; Buddenhagen et al. 2009; Richardson and Blanchard 2011). Numerous authors advocated that the precautionary principle should be applied in the adoption of bioenergy policies (Chimera et al. 2010; Davis et al. 2010; Witt 2010; McCormick and Howard 2013). These authors suggested that predictive risk assessment and ‘polluter pays’ legal mechanisms should be key components of these policies in order to minimize negative environmental impacts of invasive species. While a precautionary decision rule is attractive from an ecological perspective, historically invasive species policy in the US has allowed for risk, in part because policy decisions must be made using scientific information that is inherently complex and difficult to attain. How to effectively manage potentially invasive species that are economically valuable and whose management is complicated by many different stakeholders and government agencies remains uncertain. Studies of conflicts over *Acacia* and *Pinus* invasions in South Africa and throughout the world contributed valuable insight into policy and management solutions for invasive forestry species (van Wilgen et al. 2011, 2012; Wilson et al. 2011a). In the southeastern US, potential invasiveness recently emerged as concern for *Eucalyptus* and several research efforts addressed invasion risk.

Members of the diverse *Eucalyptus* genus (family *Myrtaceae*) are now among the most common exotic trees throughout the world, although they are controversial wherever they occur outside of their native Australia. Commercial *Eucalyptus* plantings in the southeastern US are increasing in both area and biomass production to provide feedstock for an emerging bioenergy industry. Of the approximately 90 *Eucalyptus* species that have been introduced to North America (Paine et al. 2010), several exhibit the desired features for low-cost delivered biomass relative to biomass productivity, rotation length, establishment and maintenance, harvesting and storage (Gonzalez et al. 2011). *Eucalyptus* can currently be produced in the southeastern US at a cost per BTU that is competitive with coal (Dougherty and Wright 2012), and research is under way to further improve the cellulosic conversion process and other aspects of the supply chain (Gonzalez et al. 2011). Additionally, transgenic and conventional breeding programs have developed cultivars with superior cold tolerance that hold promise for plantings beyond the current frost-limited northern range boundary of central Florida (Hinchee et al. 2011).

While *Eucalyptus* species have the potential to make a substantial contribution to bioenergy production in the southeastern US, consideration must be given to reports of invasiveness elsewhere, since history of invasiveness has been shown to correctly predict invasion probability in a new introduced range 90 percent of the time (Panetta 1993). Naturalization (reproducing and maintaining populations without human help) has been reported for 40 *Eucalyptus* species including *E. globulus* in California and Hawaii, and *E. camaldulensis, E. robusta* and *E. saligna* in many regions (Rejmánek and Richardson 2011). Further contributing to concern is the likeness between many traits which are selected to maximize biofuel crop yield and the ecological traits of successful invasive species (Raghu et al. 2006; Barney and DiTomaso 2008; NISC 2009). Many *Eucalyptus* species possesses these shared traits, which include rapid accumulation of biomass, perennial growth form, prolific seed production, low disease, pest resistance, and tolerance to drought and low soil fertility (Booth 2012). An additional risk factor is that an estimated 1.09 million ha of new short rotation woody crops may be planted in order to meet proposed Renewable Portfolio Standards in Florida alone (Hodges et al. 2010), resulting in high potential production and release of seed propagules.

Little data have focused on the costs related to invasions of non-native trees introduced through plantation forestry (Dodet and Collet 2012). Correspondingly, the economic and ecological impacts of introductions of *Eucalyptus* in the southeastern US have not been directly quantified. Data are absent likely because it is inherently difficult to predict costs associated with an introduction (Parker et al. 1999; Anderson et al. 2004) although certain potential costs may be anticipated based on studies from other locations where *Eucalyptus* are invasive. For example, increased wildfire intensity and frequency is a chief concern for areas that are considering large-scale planting of *Eucalyptus* because ignition of *Eucalyptus* foliage significantly increased the severity of a 1991 fire in the Oakland-Berkeley hills in California that cost over $1 billion (Pagni 1992). Concern has also been raised over the reports that *Eucalyptus* have caused desiccation of streams and groundwater such that nearby crop productivity was reduced in areas of east Africa (FAO 2010b; Senbeta et al. 2010). In South Africa, *Eucalyptus* invasions in two watersheds lead to 6.0 and 9.4% reductions in natural river flows and the corresponding costs of control programs to prevent these losses were $US 4.1 and 6.6 million, and would rise to $278.0 and 11.1 million if areas became fully invaded (Le Maitre et al. 2002). Ecological impacts of *Eucalyptus* have been observed in California, where dense monocultures formed, thereby altering ecosystem structure, function and native faunal composition and density (Sax 2002).

Although anecdotal evidence supports the potential invasiveness and high costs associated with *Eucalyptus* introduction in the southeastern US, *Eucalyptus* have been remarkably unobtrusive to date. *Eucalyptus* have been grown for mulch in central Florida for more than 40 years without significant problems, although four species (*E. camaldulensis*, *E. grandis*, *E. robusta* and *E. torelliana*) are reported to have established (Wunderlin and Hansen 2008). This pattern is consistent with a degree of success at the global scale that is orders of magnitude less than that of other introduced trees (Rejmánek and Richardson 2011). Some authors suggested that *Eucalyptus* species are safe choices for restoration projects in Brazil (da Silva et al. 2011) and are non-invasive forestry alternatives to problematic *Pinus* and *Acacia* species (Dodet and Collet 2012). Some authors hypothesized that *Eucalyptus* have a relatively poor invasive ability because seeds are short lived and have low viability and because there is high seedling mortality due to herbivory (Li et al. 2003; Becerra and Bustamente 2008; da Silvia et al. 2011). Other possible limiting factors include susceptibility to pathogens and fungi (Rejmánek and Richardson 2011a) and intolerance to competition (Adams et al. 2003; Garau et al. 2009). Recent studies conducted to improve herbicide site preparation or post-planting options to combat high mortality in plantation establishment

(Blazier et al. 2012; Osiecka and Minogue 2011) offer supporting evidence for the sensitivity of *Eucalyptus* seedlings.

Eucalyptus ecology is still marked by many gaps in knowledge and contradictions meriting significant future research. The goal of the following literature synthesis was to frame the steps that can be taken to address potential environmental problems while this uncertainty remains. Existing and potential risk assessment and management practices for controlling invasive spread of *Eucalyptus* in the southeastern US are reviewed and implications of alternative policies for non-native bioenergy crops are discussed subsequently. Information about effective management practices and policies for invasive forestry species was obtained from published literature identified by searching several scientific databases for keywords including: *Eucalyptus*, *Acacia*, *Pinus*, invasive, bioenergy, Weed Risk Assessment, management, policy and sustainability. Management practices for *Eucalyptus* are presented with respect to four commonly recognized phases of intervention: prevention, containment, control, and management and restoration of the affected ecosystems (Table A-1). Practices could have also been organized relative to phases in a plant's life cycle (e.g., Wilson et al. 2011a) or supply chain stages (e.g., McCormick and Howard 2013). However, a framework of intervention phases that generally corresponded with steps in the invasion process (i.e., introduction, establishment, spread and impact) was favorable for adequately conveying the importance of certain strategies such as mitigation of invasion impacts, which are not exclusively connected to a specific stage in *Eucalyptus* life cycle or in the supply chain.

Risk Management Practices for *Eucalyptus*

Prevention

Prevention, which can be described as avoidance through appropriate risk assessments and quarantine enforcement (McCormick and Howard 2013), is often considered the most cost-effective approach for dealing with biological invasions (Leung et al. 2002). Accordingly, several authors have urged that tools such as the Australian Weed Risk Assessment (WRA) and quarantined experimental introductions be conducted to predict invasion risk posed by bioenergy crops (Barney and DiTomaso 2008; Chimera et al. 2010; Davis et al. 2010; Gordon et al. 2011; Flory et al. 2012). The WRA uses 49 ‘yes’ or ‘no’ questions about a species’ current weed status in other parts of the world, climate and environmental preferences, and biological attributes, to conclude if a species should be accepted, rejected or evaluated further for introduction (Pheloung et al*.* 1999) (Table A-1). Conclusions from the WRA are used to identify low-risk species for which a supply chain should be developed and high-risk species that should not be considered as bioenergy feedstock candidates (Dodet and Collet 2012; Gordon et al. 2012). It is an exceptionally valuable tool because it has been demonstrated to correctly identify invaders 90 percent of the time and non-invaders 70 percent of the time (Gordon et al. 2008) and an assessment can typically be completed in five to eight hours with little cost (Daehler et al. 2004).

Rejmánek and Richardson (2011) argued that there is a particular need for tools such as the WRA to evaluate *Eucalyptus* and other groups that are widely planted but so far have proportionately low incidence of invasiveness. Accordingly, *Eucalyptus* invasion risk was recently evaluated for 38 of the most commercially important species using the WRA (Gordon et al. 2011, 2012). A study adjusted for the national scale concluded that 15 of these *Eucalyptus* species have low risk of invasion, 14 have high risk and 9 require further information (Gordon et al. 2012). When the WRA was modified to reflect Florida's climate to assess the invasion risk of three *Eucalyptus* species, it concluded that *E. grandis* and *E. camaldulensis* posed high invasion risk and that further information was required for *E. amplifolia* (Gordon et al. 2011). While these results are useful, this approach to assessing *Eucalyptus* invasion risk is limited because assessments need to be performed for each candidate genotype and cultivar individually, because plant structure and function, hence invasive potential, can vary widely within a species (Casler et al. 2004; Flory et al. 2012). Life history information specific to new Freeze Tolerant *Eucalyptus* (FTE) lines (ArborGen, Ridgeville, SC) is still largely unknown and evaluation of these cultivars using the WRA is not possible. Additionally, concerns over assessor subjectivity and inconsistency (Davis et al. 2011, Gordon et al. 2008) and difficulties regarding the interpretation of the WRA questions for biomass crops (Barney and DiTomaso 2008, Flory et al. 2012) must be weighed. Questions also remain about how to treat the nine *Eucalyptus* species that fall into the 'evaluate further' category.

While qualitative risk assessment by the WRA has been developed extensively, existing empirical research to investigate *Eucalyptus* invasion risk is limited. The only instance of empirical research in the southeastern US consisted of an observational study in which surveys for natural recruitment were conducted within and proximate to *Eucalyptus* stands (Callaham et al. 2013). These authors detected limited establishment of *E. amplifolia*, *E. grandis* and *E. robusta* seedlings in modified land use types at latitudes south of 27° N. Other examples of empirical research include two experiments that found limited invasive potential of *E. saligna*, *E. grandis* and *E. urograndis* in Brazil, (da Silva et al. 2011; Emer and Fonseca 2010). Notably, no studies reported the use of experiments to test *Eucalyptus* invasiveness in the southeastern US. While broad application experiments as part of a bioenergy crop screening protocol has been proposed as a way to inform proposed regulatory restrictions (Davis et al. 2010), experimental tests for are still rare. This is possibly due to uncertainty about how to translate response variables such as seedling survival and growth into meaningful measures of risk that can be easily interpreted by policymakers (Table A-1). The absence of standardized procedures or agency mandate for the high cost and oversight of experimental evaluations (Flory et al. 2012) have also limited the effectiveness of this approach.

Containment

Containment refers to management practices that decrease the likelihood of spread from a site (McCormick and Howard 2013). One potentially very effective containment approach for managing *Eucalyptus* invasion risk is trait selection during breeding (Table A-1). The likelihood of spread can be reduced by decreasing fecundity or by increasing the age to maturity, although the later method may negatively influence productivity (Gordon et al. 2012). This strategy was successfully implemented in other taxonomic groups, including sterile clones of *Pinus* species used in South Africa and triploid hybrid *Leucaena* in Hawaii (Richardson 1998). Likewise, elimination of seed production is thought to be a feasible goal in *Eucalyptus* (Gordon et al. 2012), and elimination of pollen production has already been accomplished in the transgenic hybrid, *E. grandis x E. urophylla* (AGEH427) (Hinchee et al. 2011). The ability to ensure containment of genetically modified trees through sterility is significant because it eliminates the need for costly, imprecise and complex ecological research to understand and predict the impacts of spread (FAO 2010a). However, the major limitation to this approach is that the effectiveness of containment technology is uncertain due to relatively novel use in forestry (FAO 2010a). Genetic research is also costly (Wang and Brummer 2012), and progress in the commercial development of genetically modified organisms is slow under the current stringent regulatory structure (Strauss and Viswanath 2011). Further consideration must also be given to the acceptability of genetically modified trees (Dodet and Collet 2012) and the possibility of controversy over intellectual property concerns similar to those that have arisen for agriculture (FAO 2010a).

Contrary to the use of trait selection to hinder potential for spread, a key possible risk management step is disallowing the development of trees that may be harder to control due to genetic modifications that confer herbicide tolerance or insect resistance (Table A-1). Examples of hazardous traits exist in Australian developed *E. camaldulensis* that is resistant to chrysomelid beetles and tolerant to broad-spectrum glufosinate-ammonium herbicide (Harcourt et al. 2000), although this cultivar has not been introduced in the United States. However, the USDA Animal and Plant Health Inspection Service (APHIS) has permitted field trials and received petitions to deregulate *Eucalyptus* cultivars that have altered cold tolerance, fertility, lignin biosynthesis, growth rate, or selectable markers (Harfouche et al. 2011). Whether cold tolerance and increased growth rate are traits that would increase vigor in a way that promotes invasiveness is a matter that requires more research.

In addition to containment practices using genetic technology, other practices that create of temporal boundaries to dispersal may also decrease the movement of propagules. Harvesting trees before seed maturation could significantly reduce the possibility of spread (Table A-1). Harvesting within six months of floral onset is recommended for conventionally bred cold tolerant *E. grandis*, which the WRA predicted to have high invasion risk (Flory et al. 2012). However, observance of this recommendation is not compulsory under existing regulations of biomass plantings (FAC 2008) and the degree to which this recommended practice has been executed is unknown. Growers may oppose this management practice because flowering and seed maturation may occur earlier than the optimal harvest age. For example, *E. grandis* is known to flower as early as 2 to 3 years after germination (Hodgson 1976), but the age of maximum sustained yield is 2.7 to 2.9 years with even longer optimum economic rotation lengths (Langholtz et al. 2005). Moreover, *Eucalyptus* are often managed to produce several coppice harvests within a rotation and information about the effect of coppicing on flowering and seed production is not readily available. Harvesting relative to unpredictable seed development would make it difficult for growers to make sound economic decisions, which require prior knowledge of the length of each stage of the rotation (Langholtz et al. 2005).

The use of physical barriers to dispersal such as buffer zones around plantings is generally recommended (Barney and DiTomaso 2008; Flory et al. 2012) and is required in the state of Florida under a unique biomass planting permitting system (FAC 2008) (Table A-1). Two strategies exist regarding buffer zones: establish a stable vegetation community to impede seedling establishment, or maintain fallow areas by regular burning, or mechanical or chemical control to disturb seedlings and to enhance detection of small-sized individuals through monitoring (Ledgard 2001). While maintenance of buffer zones is enforceable through random checks, uncertainty exists about how large the barrier needs to be in order to prevent seed dispersal into proximate susceptible communities (Flory et al. 2012). The Institute of Food and Agricultural Sciences (IFAS) of the University of Florida recommends that a 75 ft barrier be established, while Florida law requires only a 25 ft barrier (FAC 2008; Flory et al. 2012). However, evidence regarding patterns of seed dispersal suggests that neither of these sizes may be adequate. Seed dispersal occurs primarily via wind and seeds are deposited within a radius of twice the tree or canopy height (Cremer 1977), which is approximately 100 ft for *E. grandis* that are known to grow up to 49 ft in height by 3.5 years of age (Rockwood et al. 2006). The possibility of seed dispersal beyond this area may also be increased due hurricane force winds, which are theoretically able to transport the fine seeds of confamilial *Melaleuca quinquenervia* a maximum distance of 7.1 kilometers, contributing to increases in populations following hurricanes (Browder and Schroeder 1981).

The establishment of production areas as monoclonal plantings can also serve as a physical barrier to contain spread (Flory et al. 2012) (Table A-1). Different cultivars should remain separate in order to decrease risk of hybridization, which may have a positive effect on invasive ability through increased vigor (Lee 2002). Single large blocks of *Eucalyptus* would also have comparatively less plantation border than dispersed plantings, resulting in less adjoining area that could receive wind-dispersed seed. Decreasing the amount of plantation border is also critical because these areas have been hypothesized to be more vulnerable to colonization by volunteer seedlings (da Silva et al. 2011). Additionally, growers might embrace the practice of monoclonal plantings for *Eucalyptus*, considering the numerous benefits that pure culture provides including simplicity of management, predictability of yield and potentially increased yield compared to seedling-based plantings in which trees may have non-uniform growth (DeBell and Harrington 1993; Zalesny et al. 2011). A significant disadvantage of this practice is increased risk of total loss due to disease when entire stands are composed of identical genetic stock (DeBell and Harrington 1993).

Regular cleaning of seeds and plant material from harvesting equipment is another generally recommended practice for limiting dispersal of bioenergy crops (NISC 2009), although it has not been argued for in the context of *Eucalyptus* (Table A-1). It is reasonable to predict that grower willingness to participate in a cumbersome management activity may be low, especially when budgetary limitations and other higher impact priorities of regulatory agencies make enforcement impractical. Another shortcoming of this practice is that it only addresses a minor dispersal vector and does not affect wind dispersal of seeds. Additionally, as more money and time is spent cleaning equipment, efficiency decreases and thus there are diminishing returns to cleaning (Leung et al. 2005).

Control

Additional management practices that aim to decrease the spread of an already established invader represent a shift from the previously discussed proactive practices, to a reactive approach. Removal of *Eucalyptus* has proven to be challenging due to their habit of mass sprouting from the base and roots in response to injury (NPS 2006). There has been experimentation with a wide variety of control methods, many of which remain complex, expensive and inefficient (NPS 2006). Mechanical control treatments which are generally considered the least damaging control method for surrounding environments (Bean and Russo 1989), were applied in management projects and experienced varying levels of success (Table A-1). Burning of stumps and coppice sprouts was an ineffective method of control (Bean and Russo 1989; Little and van den Berg 2006). Stump grinding, in which stumps were ground down to two feet below the ground surface (Bean and Russo 1989, NPS 2006), was used in management projects although regrowth sometimes occurred. This method was also notably labor intensive and costly (NPS 2006). Stump light deprivation (tarping), in which thick plastic was stapled to the stump and the stump was then buried under mulch was also used to stop regrowth (NPS 2006), and moderately increased mortality was observed when tarping was combined with herbicide treatment (Bean and Russo 1989).

Whereas mechanical control is advantageous for some conservation objectives, chemical control using herbicides have generally been the most successful method for controlling *Eucalyptus* (Table A-1). Recommended chemical methods include cut-stump, basal bark, or basal frill applications of triclopyr, imazapyr or glyphosate herbicides, though complete control of regrowth is not typically provided by single applications of herbicides using standard protocols (Bachelard et al. 1965; Bossard et al. 2000; Moore 2002; Little 2003; Little and van den Berg 2006). Results of previous research and specific research needs for improving *Eucalyptus* control by herbicide treatments are further described in Chapter 3 of this thesis.

Biological control (biocontrol), the control of invasive species populations through the introduction of predators or pathogens, is a management option that is typically considered unacceptable when a species has economic value (Hoffmann et al. 2011), especially considering the costly and complicated research (Hobbs and Humphries 1995) and the lengthy regulatory process for approval of biocontrol agents (Montgomery 2011). While biocontrol was deemed an unlikely option for *Eucalyptus* (Rejmánek and Richardson 2011) and no published methods were found regarding the intentional release of biocontrol agents for *Eucalyptus*, some authors have speculated that biocontrol may have essentially occurred through accidental introductions of *Eucalyptus*-harming insect pests in California (Paine et al. 2011) (Table A-1). Options for biocontrol candidates exist among the 15 different Australian *Eucalyptus*-feeding insect species from at least four different feeding guilds (2 borer species, 3 leaf-eating beetle species, 4 gall wasp species and at least 8 psyllid species) that have been introduced into California, Florida and Hawaii (Paine et al. 2011). If biocontrol is sought as a management strategy, biocontrol programs for invasive *Acacia* in South Africa can serve as successful models that balance social and ecological concerns. Biocontrol agent selection in South Africa focused on agents that only attack the flower buds, flowers or seed pods, to minimize the impact on commercial production, but also reduce the costs of follow-up management and spread rates (Wilson et al. 2011b). Similarly, potential biocontrol efforts for *Eucalyptus* in the southeastern US might look to the galling wasp, *Quadrastichodella nova*, which has been reported to infest seed capsules (Paine et al. 2011).

Impact Management

Long-term management practices for mitigating the ecological impacts of invaders have improved vastly in the past decade and are now viewed as useful and financially viable supplements to prevention and containment practices (Simberloff et al.

2012). A number of practices may be used to minimize the impacts of *Eucalyptus* cultivation before and during commercial production. Buffer zones around plantings, mentioned previously for the purpose of limiting seed dispersal, can also provide surface water and wildfire protection by limiting the proximity of trees to waterways and by establishing a firebreak around the stand (Booth 2012; Flory et al. 2012) (Table A-1). The impacts of wildfires can also be minimized by reducing the density of the fuel load, which accumulates below trees through the annual shedding of bark and limbs (NPS 2006), by harvesting trees before they begin to shed bark abundantly and by controlling the understory shrub layer (Goodrick and Santurf 2012). While addressing surface water depletion and fire hazard is relatively straightforward, some ecosystem impacts that are consequential but are not readily detected (Simberloff et al. 2012) (Table A-1). Impacts such as reduced biodiversity and habitat loss, which are not easily estimated through economic valuation, and which are inherently incompatible with the presence of the large-scale *Eucalyptus* plantings, may have significant hidden costs. These costs are also known to rise as mitigation is delayed, so it is important that the long-term management of invasion impacts be viewed as the last option after unsuccessful prevention or containment (Simberloff et al. 2012).

Implications of Policy Alternatives

There are many ways that *Eucalyptus* invasion risk can be managed, although no single option is ideal in a biological, economic and social sense and many require considerable further research. Challenge lies ahead in deciding what practices should be considered a priority (Flory et al. 2012). Consideration also needs to be given as to what kind of regulatory techniques should be used to implement policies regarding the management of non-native biomass crops and who should assume the burden of management. Various authors have suggested that importers, developers and growers who are responsible for introducing potentially invasive crops such as *Eucalyptus* should be responsible for damages to the environment (i.e., ‘polluter pays’ principle) rather than allowing that burden to be borne by tax payers or neighboring private landowners who are affected (Buddenhagen et al. 2009; Bradley et al. 2010; Chimera et al. 2010; Davis et al. 2010; Witt 2010; McCormick and Howard 2013). However, Florida is the only state thus far that has adopted any legal authority governing the uses of non-native species in biofuel production (Environmental Law Institute 2010), and this novel regulation employs only containment and impact management approaches as they pertain to growers (FAC 2008). While the cost of field testing is assumed by the developer in the case of genetically modified crops such as FTE *Eucalyptus* regulated by APHIS, field testing to determine negative environmental impacts is not required for traditionally bred non-native biomass crops including most of the *Eucalyptus* species currently being considered for feedstocks. Chimera et al. (2010) proposed that stringent protocols analogous to those for testing of genetically modified organisms should be developed for all biomass crops and that the expense of conducting such evaluations be the responsibility of the developer. However, stakeholders may be resistant to the adoption of more rules governing the cultivation of biomass crops, which may threaten the viability of a market that already has low profit margins. Additionally, biomass crop regulations have been unsuccessful elsewhere in the world. For example, laws that assigned growers of invasive Acacia species the responsibility of controlling seed spread failed because most landowners and growers had insufficient resources and there was a lack of commitment to prosecute offenders (van Wilgen et al. 2012). This historical ineffectiveness of regulations may diminish support for the implementation of similar rules in the US at a time when many new growers are entering the market.

Relatively less discussion has emerged about the potential utility of a softer approach involving informal social control of non-native bioenergy crops, although such an approach may be valuable considering the many previously reviewed uncertainties over the environmental risks associated with *Eucalyptus* culture. Informal control through voluntary third party certification programs and promotion of sustainable forest management practices have long been important policy tools in the forestry sector (Ramensteiner and Simula 2002). Certification systems, which were developed to address public concerns related to deforestation and biodiversity loss, are well equipped to develop sustainability criteria for mitigating invasion risk of biomass crops, and to fulfill such criteria through proper enforcement and verification mechanisms (Lewandowski 2006). Indicative of this is a recent study finding that 10 of 17 existing agricultural or forestry certification schemes already included some criteria related to invasive species (Scarlat and Dallemand 2011). A criterion to prevent invasive bioenergy feedstock species from spreading outside the operation site is also included in the Roundtable on Sustainable Biofuels (RSB) certification system, which was launched in 2011 as the first certification specifically for biofuel supply chains (RSB 2010). Inclusion of this criterion in certification schemes provides a way to persuade landowners to plant cultivars that are predicted to present a low invasion risk, to maintain appropriate buffer zones, to control and monitor for escaped seedlings, and to carry out impact management practices. Certification schemes may also act as a vehicle for stakeholder education and knowledge sharing about how to improve the economic and ecological efficiency of such protocols (Gootee et al. 2012). Most importantly, informal control is not bound by public officials who are constrained by the election cycle and local interests, or by lengthy legal processes, which are cumbersome barriers to change for regulations. Consequently, informal control allows greater flexibility to respond to new developments in scientific information, which is critical for managing *Eucalyptus* in light of existing gaps in knowledge.

Conclusion and Needed Research

Eucalyptus species are considered potentially invasive species and sources of environmental problems in various regions where they have been introduced, so recent concerns about potential invasiveness in the southeastern US demand attention. While regulation and ‘polluter pays’ techniques that require containment strategies by the producer are widely recommended, they are certainly not the only answer or even the best answer in an economic or social sense. Minimizing invasion risk could also be achieved through informal relationships, which place a lesser burden on the biomass producer and represent a flexible approach that is desirable under uncertain risk. As with the management of any invasive species, success is likely to require a landscape-level response that integrates a wide range of management practices implemented by different stakeholders at different stages of the invasion process. Studies made significant progress in the identification of risk factors and the specific level of risk posed by certain *Eucalyptus* species. However, further evidence is needed to improve the confidence of existing predictions. Management tools have also identified and include genetic, physical and temporal containment methods as well as herbicide and biocontrol techniques, although research is also needed to improve the effectiveness of many of these methods. The purpose of the research that is described in the subsequent chapters of this thesis was to further evaluate the potential invasiveness of *Eucalyptus* species being considered for large-scale planting in Florida and to improve practices for control of *Eucalyptus* using a new herbicide.

CHAPTER 2
POTENTIAL *EUCALYPTUS* INVASIVENESS IN FLORIDA'S NATIVE AND MODIFIED PLANT COMMUNITIES

Introduction

Invasion risk is shaped not only by species life history traits but also by stochastic processes and local interactions with the biotic and abiotic features of ecosystems in the new range that can greatly affect the population's performance (Hulme 2011; Minton and Mack 2010). These complex processes and relationships are often non-linear and affected by temporal lag effects and positive feedbacks (Hulme 2011). Therefore it is difficult to predict invasions based on qualitative information using literature-based tools like the WRA. While the WRA offers a useful starting point, more quantitative tests are needed to evaluate the many species and taxa of biofuel crops that are being proposed for introduction (Barney and DiTomaso 2008; Chimera et al. 2010; Davis et al. 2010; Hulme 2011). Several studies have used experiments to assess factors contributing to invasion risk (Myers 1983; Minton and Mack 2010; Davis et al. 2011). Recent work synthesized these past experimental tests of invasion potential and summarized the key elements of a standardized protocol for testing invasion risk (Flory et al. 2012). Key recommendations were that controlled introductions should be performed at multiple sites that represent the communities that are susceptible to invasion (Ewel et al. 1999; Parker and Kareiva 1996) and that factors important for establishment and performance such as disturbance, founder population size and timing of introduction be assessed as experimental variables (Flory et al. 2012).

Previous observational and literature based studies (e.g., Callaham et al. 2013; Gordon et al. 2012) yielded valuable insights about what *Eucalyptus* species have a high risk of becoming invasive and what areas might be at risk of colonization by

Eucalyptus seedlings. However, experimental evaluation of invasion risk was needed to understand invasion risk relative to complex ecological processes and to resolve differing conclusions from observational and literature based work about the likelihood of introduced *Eucalyptus* species becoming invasive. Our study heeded the recent recommendations for experimental tests of invasion risk (Flory et al. 2012) and aimed to identify the invasive potential of three *Eucalyptus* species and to determine conditions conducive to naturalization in different vegetation communities. Of particular interest for *Eucalyptus*, were the effects of seed density and site disturbance on seedling emergence and survival. Disturbances such as vegetation removal were important to take into account because they have long been recognized as possible important drivers of invasion (Elton 1958) and have been suggested to be a prerequisite for *Eucalyptus* establishment (Wevill and Read 2010). High propagule has also been observed as one of the mechanisms responsible for tipping the balance to invasion (Gordon 2011; Hobbs & Humphries 1995) and it is thought to be an especially important factor for *Eucalyptus* population performance. *Eucalyptus* propagule pressure can potentially be very high considering that seed rain from mature *Eucalyptus* can be up to 4,000 seeds m^{-2} (Richardson and Rejmánek 2011).

The overarching objective of this study was to evaluate the potential invasiveness of three *Eucalyptus* species being considered for large-scale planting in Florida to supply bioenergy and fiber through two quantitative approaches.

1. Site surveys were conducted to determine the abundance and height distribution of *Eucalyptus* seedling recruitment within seed bearing stands and in the proximate plant communities where seed dispersal may occur. Plant communities included upland hardwood forest, non-grazed pasture, intensively site-prepared forest land and abandoned forest road.

2. Seed addition studies were conducted to determine the relative potential for seedling emergence and survival among *E. amplifolia*, *E. camaldulensis* and *E. grandis* sown at two seed densities and for disturbed and non-disturbed conditions in the understory of reproductively mature *Eucalyptus* and in each of the aforementioned plant communities proximate to the *Eucalyptus* stands.

Materials and Methods

Study Areas

Separate studies were conducted in the proximity of reproductively mature *Eucalyptus* stands at two locations in Florida. The Gainesville location was an *E. amplifolia* seed orchard on the University of Florida campus (29°37'36" N, 82°21'32" W) at approximately 23 m elevation. *Eucalyptus* were planted in this 0.7 ha stand at various times from 1992 to 1997. There had not been any vegetation management in the two years prior to initiating this study. The stand is adjacent to native upland hardwood forest and an abandoned forest road. The Quincy location is a 0.9 ha *E. amplifolia* progeny test planted in 1999 at the North Florida Research and Education Center, south of the city of Quincy (30°32'32" N, 84°35'25" W) at approximately 73 m elevation. This stand is adjacent to non-grazed pasture and intensively site prepared forest land. The intensively site prepared forest land previously supported a 13-year-old eastern cottonwood (*Populus deltoides,* W. Bartram ex Marshall) clone test harvested in January 2012 and was site prepared for planting *Eucalyptus* in March 2012 by the rake/pile/burn/disk method (Lowery and Gjerstad 1991). *Eucalyptus amplifolia* stands at both locations have relatively wide spacing between trees and some direct sunlight in the understory, potentially fostering seedling establishment (Booth 2013). The Gainesville location has a temperate climate with highest temperatures in July (mean 27 C), lowest temperatures in January (mean 13 C), an average annual extreme minimum temperature of -6.7 to -3.9 C (USDA hardiness zone 9a) and 125 cm average annual precipitation (NOAA 2002; USDA 2012a). The Quincy location has highest temperatures in July (mean 27 C), lowest temperatures in January (mean 10 C), an annual average extreme minimum temperature of -9.4 to -6.7 C (USDA hardiness zone 8b) and 143 cm average annual precipitation (NOAA 2002; USDA 2012a).

Site Surveys

In May 2012, line transect sampling was used to identify natural recruitment of *E. amplifolia* seedlings from the mature *Eucalyptus* trees. At each study location, 1 m^2 plots (72 Gainesville, 238 Quincy) were evaluated using a sampling frame placed every 10 m on line transects established 20 m apart across the narrow dimension of the stand. Line transects extended 60 m from the stand's edge into the adjacent communities. *Eucalyptus* seeds are typically dispersed by wind within an estimated radius equal to twice the canopy or tree height (Cremer 1977), and 60 m is approximately twice the canopy height of these *E. amplifolia* trees in these stands.

Seed Addition Studies

Germination testing

Germination capacity of *Eucalyptus* species is highly variable, ranging from 11% to 98% (USDA 2008). Prior to initiating the seed addition experiment, the expected germination *E. amplifolia*, *E. camaldulensis* and *E. grandis* seed stocks were determined by measuring the mean number of germinating seed per gram for each species, so that the amounts seed added into plots had an equal number of seeds that were expected to germinate (Table 2-1). *Eucalyptus* seeds are typically 1–3 mm long and weigh less than 0.5 mg up to 2.0 mg depending on species (Rejmánek and Richardson 2011). Seeds are also mixed with chaff (inert material) in seed capsules and the proportion of chaff by weight can range from 5:1 to 30:1 (USDA 2008). Therefore, the use of weight-specific expected germination number was preferable because the small size of *Eucalyptus* seeds and the intermixed chaff made it difficult to count individual seeds accurately. Using a protocol adapted from previously established guidelines for *Eucalyptus* germination testing (Boland 1986; USDA 2008), expected germination was evaluated in a controlled environment growth chamber. Growth chamber conditions corresponded to average minimum (20.1 C) and maximum (32.5 C) temperature conditions (NOAA 2002) and 14 hr photoperiod for June in north-central Florida (Naval Meteorology and Naval Command 2012). Light was provided with the higher temperature for 14 hr followed by the lower temperature without light for 10 hr. While some alpine *Eucalyptus* species require cold-moist stratification to break dormancy (Boland 1986), the three species used in this study are not known to be among these. Eight 0.05 g samples of each seedlot were tested for each species. All materials that were exposed to seeds during preparation of the samples were autoclaved to reduce the likelihood of microbial growth. Samples were placed in 9 cm glass Petri dishes containing two thicknesses of filter paper as a substrate. Three ml of sterile distilled water was applied to the filter paper using a glass pipette. Seeds were dispersed evenly over the filter paper, and the Petri dishes were sealed with paraffin film to prevent samples from desiccating. A germination count was made after 5 d and every 2 d thereafter. During each count, normal and abnormal germinated seeds were counted and removed from the dish. A normal *Eucalyptus* seedling had a healthy radicle, hypocotyl and cotyledons. It was recommended that germination tests for *E. grandis* last 14 d, and while there was not specific information available about the test duration for *E. amplifolia* and *E. camaldulensis*, 10 to 21 d is generally suitable for most species (Boland 1986). Thus, the final count was made at 14 d and the total number of normal germinated seeds in each sample was used to calculate the average number of germinating seed per g for each seedlot.

Experimental design

The potential for *Eucalyptus* seedling establishment and survival in common native or modified vegetation communities was evaluated through a seed addition approach similar to da Silva et al. (2011). Sampling plots (Figure 2-1) were established within the understory of the *E. amplifolia* stand and in two proximate communities at both study locations, resulting in 63 plots at each location. At the Gainesville location, proximate communities included an upland native hardwood forest and an abandoned forest road. At the Quincy location, proximate communities included a non-grazed pasture and an intensively prepared site for *Eucalyptus* planting, as described above. The characteristics of the communities studied are presented in Tables 2-2 and 2-3.

In each vegetation community, seeds from *E. amplifolia*, *E. camaldulensis* and *E. grandis* were placed on separate nested paired plots to examine the effects of disturbance and seeding density for each species (Figure 2-1). Adjacent 0.75 m^2 paired plots were randomly left non-disturbed or were disturbed through removal of the vegetation layer and soil scarification with a rake. In the center of each of the paired plots, two nested 0.25 m x 0.25 m paired subplots were randomly assigned to addition of amounts of seed expected to result in either 500 or 1000 germinating seeds m^{-2}. These levels were chosen to reflect the hypothesized intermediate and maximum levels that occur naturally based on estimates of actual seed rain in the literature (Virtue and Melland 2003). The 0.625 m^2 non-seeded areas outside of the smaller nested paired seeded subplots within the disturbed or non-disturbed treatments were used to observe

Eucalyptus seedling establishment from mature trees. Randomly distributed paired plots were replicated eight times in each vegetation community type for *E*. *amplifolia* and *E. grandis,* and only five times for *E. camaldulensis*, due to a shortage of seed. Seeds were sown on June 7, 2012 in Quincy and on June 8, 2012 in Gainesville, following 2.0 cm and 13.1 cm of rain (tropical storm event) in the previous 24 hours at each location, respectively (IFAS 2012).

Seedling assessments

A census was taken at 2, 4, 6, 8, 11, 14, 17, 21 and 25 weeks after treatment (WAT) with seed to determine emergence and survival of *Eucalyptus* seedlings. Newly emerged seedlings at each census were counted and marked with uniquely colored toothpicks to distinguish them from new seedlings in subsequent censuses. Surviving seedlings of each emergence date group were counted at each census so that the approximate length of time that seedlings survived could be determined.

Statistical analysis

Seedling emergence. Statistical analyses were performed using SAS v. 9.3 (SAS Institute, Cary, NC). Because cumulative *Eucalyptus* seedling counts from the periodic plot assessments were generally too low to evaluate the effect of seeding density treatments, emergence data were combined for high and low density seeded subplots within the disturbed or non-disturbed treatments. Emergence data were analyzed as the proportion of seeds that emerged relative to the expected number of germinating seeds m^{-2} (e.g., 62 seeds expected to germinate in high density subplot + 31 seeds expected to germinate in low density subplot = 93 seeds expected to germinate in paired subplots). A generalized linear model (PROC GLIMMIX) with a binomial distribution and a logit link function was used to determine the effect of treatments on percent emergence and to estimate mean percent emergence for treatment combinations, using data from both study locations. This approach has been used for determining effects of treatments on very low proportion germination data in similar experimental designs (Humber and Hermanutz 2011; Carillo-Gavilán et al. 2012). Vegetation community, species, disturbance, disturbance by species and species by vegetation community were considered fixed effects. The three-way interaction was dropped from the model because it was not significant. The random effect of plot within each community could not be estimated and was dropped from the model due to nonconvergence when this effect was included. The significance level for all tests was $\alpha = 0.05$.

An additional analysis was performed to capture the effect of disturbance, community and location experimental variables on *Eucalyptus* seedling emergence due to recruitment by the mature *Eucalyptus*. Since seedling emergence was very close to zero in seed addition subplots and because seed rain from the *Eucalyptus* canopy could not be excluded from these parts of the plot, total emergence counts for seeded and non-seeded areas were combined. Because variation across plots could not be estimated, analyses were performed on the total number of emerged seedlings summed across seeded and non-seeded areas of the 21 0.75 m^2 disturbance treatment plots (total 15.75 m^2) within each of the various vegetation communities at the two locations. In order for the model to converge, vegetation communities that had zero emerged seedlings were not included in this analysis. A generalized linear model (PROC GLIMMIX) with a Poisson distribution and a log link was used because the total number of seeds in plots was unknown. Vegetation community (nested within location) and disturbance were treated as fixed effects. Contrasts were used to compare combinations of factor level means at $\alpha = 0.05$.

Seedling survival. Data for the time that each seedling was first observed to when it was last observed were used to calculate the minimum and maximum possible time of survival for each of the 62 seedlings that germinated. The middle value of this range was assigned to each seedling to approximate time of survival. The significance of species, disturbance and vegetation community for time of survival were each evaluated for survival values classified as a categorical response (< 1 month, 1 to 2 months, > 2 months) using Pearson's chi-squared tests under the Proc Freq procedure in SAS. Analyses were performed for the Gainesville and Quincy locations pooled together because the difference in time of survival between locations was not significant. The time of survival by species analysis were performed to compare seeding treatments in subplots and in whole plots to capture species effects considering the possibility of movement of the added seed.

Results and Discussion

Site Surveys

The 1 m^2 sampling plots in the *Eucalyptus* stands and surrounding communities included various types of ground cover including grasses, vines, forbs, shrubs, trees, bare mineral soil and leaf litter (Tables 2-4 and 2-5). No *Eucalyptus* seedlings were found in any of the 72 plots at the Gainesville study location or in the 238 plots at the Quincy location. The lack of natural *Eucalyptus* recruitment observed in these surveys is consistent with results of similar studies in which natural recruitment of *Eucalyptus* seedlings were found in only 4 out of 16 surveys within and proximate to *Eucalyptus* stands in the Florida (Callaham et al. 2013). Similar surveys in Brazil found recruitment within *Eucalyptus* stands, but not in adjacent pine communities (Emer and Fonseca 2010).

Seed Addition Studies

Seedling emergence in seeded subplots

The probability of *Eucalyptus* seedling emergence in seed addition subplots was very low overall (0.0– 0.32%) (Tables 2-4 and 2-5). Mean percent emergence was generally higher within disturbed (0.0001%) than in non-disturbed areas (0.000001%) (Table 2-4). The highest percent emergence for the three tested *Eucalyptus* species was observed for *E. camaldulensis* (0.00028%) (Table 2-4). The Gainesville *Eucalyptus* stand had the highest percent emergence (0.0005%) among the various vegetation communities studied (Table 2-4). Correspondingly, the vegetation community by species combination that yielded the highest percentage of emerged seedlings was *E. camaldulensis* in the Gainesville *Eucalyptus* stand (0.32%) (Table 2-5). However, differences in the probability of emergence for disturbance, species and vegetation community factors were not significant. Da Silva et al. (2011) reported similarly low levels of *Eucalyptus* emergence in seed addition studies in Brazilian plant communities. When approximately 5,000 total seeds were added, only 111 seedlings were observed across 5 periodic censuses (2.22% emergence). In their study, new seedlings were not distinguished from survivors, so the actual number of seedlings that emerged may have been lower if they inadvertently counted survivors as additional seedlings in subsequent censuses.

Seedling emergence in non-seeded areas

Eucalyptus seedlings were observed in non-seeded subplots beginning in early August, approximately eight weeks after the studies were initiated. The random distribution of seedlings throughout the plots and the presence of open *Eucalyptus* seed capsules on the ground in plots at this same time suggested seedlings likely occurred in non-seeded areas and seeded subplots as a result of recruitment from the mature *Eucalyptus* trees. While significant treatment effects were not detected for emergence in seed addition subplots, significant effects were detected in the analysis that summed the total number of emerged *Eucalyptus* seedlings across seeded and non-seeded areas to capture treatment effects on recruitment by the mature trees.

Location and Community. No seedlings ever emerged in the native hardwood forest in Gainesville or in the intensively prepared land or non-grazed pasture communities in Quincy. The total number of emerged *Eucalyptus* seedlings in the remaining vegetation communities varied significantly by community ($F_{2,2} = 22.55$, $P = 0.043$). Contrasts revealed that *Eucalyptus* seedling emergence was significantly greater in plots in the *Eucalyptus* stand in Gainesville (52 seedlings) compared to the stand in Quincy (7 seedlings) ($F_{1,2} = 24.81$, $P = 0.038$). Both communities had more seedling emergence than forest road. Research by others has also shown greater recruitment for *Eucalyptus* seedlings within *Eucalyptus* stands compared to other vegetation communities. Emer and Fonseca (2010) found that transplanted seedlings survived for an average of 9.3 months within a *Eucalyptus* stand, but only survived 2.7 to 5.3 months in other communities. These authors conducted surveys for natural recruitment of *Eucalyptus* seedlings that also revealed the same trend. Emer and Fonseca (2010) reported that *Eucalyptus* seedling recruitment was not found in surveys of native pine forests and pine plantations, but did occur within mature *Eucalyptus* stands. Likewise, in their surveys adjacent to *Eucalyptus* stands, Callaham et al. (2013)

found 65 seedlings in *Eucalyptus* stands, but only 13 in neighboring wetland, four in pasture, two in pine, one in open forest and zero elsewhere (lawn, roadside, disturbed soil etc.). While these experiments cannot fully explain the mechanism driving the decreased success outside of *Eucalyptus* stands, other authors hypothesized that seed rain is not a limiting factor and that seedling mortality in native communities is related to plant community richness, plant abundance, soil fertility (Emer and Fonseca 2010) and also light availability (Booth 2013). In our experiment, mean total emergence including natural recruitment in plots was high as 28.3 thousand seedlings ha^{-1} for one combination of factor levels (Table 2-6). These data support the hypothesis seed rain is not a limiting factor for *Eucalyptus* establishment.

While this experiment did not provide information about the specific drivers of greater *Eucalyptus* seedling emergence in the Gainesville *Eucalyptus* stand compared to the Quincy stand, possible influential factors include soil composition, canopy cover and weather near the beginning of the experiments. While both Gainesville and Quincy had similar mean temperatures (24.9 C and 24.7 C) throughout the first month of the seed addition studies, a tropical storm event caused 13.3 cm of rainfall in the week following seeding treatments in Gainesville, while only 1.8 cm of rainfall occurred in Quincy. Although there was not conclusive evidence to evaluate the specific drivers of location differences for this study, the results of Callaham et al. (2013) indicated that invasiveness might be related to latitude. In their surveys in and around *Eucalyptus* stands throughout the southeastern US, they reported that no *Eucalyptus* seedling recruitment was seen north of 27° N, which is approximately the southern limit of the range where freezing conditions are expected to occur annually (USDA 2012a). The results of these experiments, which were located at 29° N (Gainesville) and 30° N (Quincy), support their conclusion that 27° N may represent a latitudinal threshold for establishment.

Disturbance. The effect of disturbance on *Eucalyptus* seedling emergence due to recruitment by mature *Eucalyptus* was also significant ($F_{1,2}$ =24.77, P = 0.038), with greater emergence occurring under disturbed conditions. The effect of disturbance is evident when *Eucalyptus* seedling emergence was compared for disturbance treatments within communities on a per hectare basis. The generalized linear model estimated that disturbed treatments in the Gainesville *Eucalyptus* stand had a mean seedling emergence equivalent to 15.0 to 53.5 thousand seedlings ha^{-1} (95 % confidence interval), while emergence in this same community without disturbance was 1.1 to 20.2 thousand seedlings ha^{-1} (Table 2-6). Likewise in Quincy, mean seedling emergence was estimated to be between 738 and 19.7 thousand seedlings ha^{-1} under disturbed conditions but was notably lower under undisturbed conditions (635 to 5.2 thousand seedlings ha^{-1}).This trend was expected considering that disturbance improved germination of *Eucalyptus* seedlings in other studies (da Silva et al. 2011). This result supports the suggestion of Wevill and Read (2010) that rare disturbances such as fire or flooding which suppress competition are necessary for *Eucalyptus* to establish.

Seedling survival

Most seedlings survived less than one month, although a few lived up to approximately 3 months (Figures 2-2 and 2-3). Pearson's chi-squared tests found no significant effects for plant community or disturbance on the time of survival. Species was not a significant factor when evaluated for seeded subplots. However, when the effect of species on survival was evaluated for whole plots at both locations together, species emerged as a significant factor affecting survival (χ^2 = 9.72, 1 df, n = 58, P = 0.045). While all 3 species had a similar number of seedlings that survived up to 2 months, only seedlings in *E. camaldulensis* plots survived for more than 2 months (Figure 2-4). Similar to these results, Emer and Fonseca (2010) also found that *Eucalyptus* seedlings that were transplanted into various native and modified communities were short lived, surviving only 2.7 to 9.3 months. The significantly greater longevity of *E. camaldulensis* whenever differences were detected, in combination with a highest percent emergence observed in the seed addition studies, may indicate that *E. camaldulensis* does have a relatively greater ability to establish than the other species tested. These results may support previous research that suggested *E. camaldulensis* has high potential for invasiveness. Gordon et al. (2012) determined that *E. camaldulensis* has a Weed Risk Assessment (WRA) score of 18, representing a high invasion risk level and tying with *E. globulus* as the *Eucalyptus* species with the highest score out of the 38 species evaluated. Additionally, Rejmánek and Richardson (2011) reported that *E. camaldulensis* is the most widespread *Eucalyptus* species and has naturalized in 16 regions.

Conclusion

The results of this study are valuable because the potential for emergence and survival of *Eucalyptus* was tested in natural conditions that include the complex biotic and abiotic environmental components that can influence invasion success. The most potential for invasiveness was demonstrated for *E. camaldulensis* seedlings, in disturbed conditions and within *Eucalyptus* stands when compared to other levels of respective variables. However, the probability of *Eucalyptus* seedling emergence in seed addition subplots was very low overall (0.0 to 0.32%). While mean total emergence due to recruitment by the mature *Eucalyptus* was estimated to be as 28.3 thousand seedlings ha^{-1} (95% CI: 15.0 to 53.5 thousand seedlings ha^{-1}) in the most favorable treatment combination for emergence, no seedlings survived longer than 13 weeks. Similarly, the two site surveys found no *Eucalyptus* seedlings resulting from natural recruitment. Before making broad conclusions about the invasive potential of *Eucalyptus,* consideration needs to be given to the fact that seeding treatments and site surveys were performed only once and may not necessarily reflect the continual propagule pressure of seed rain falling over entire seasons for multiple years. The scope of the seed addition studies in this study must also be considered, in that conclusions about invasiveness should be restricted to the studied conditions and *Eucalyptus* species tested. While it is not possible to guarantee that treatment combinations that demonstrated limited success in these studies will never result in higher levels of seedling establishment, the trends observed in these studies are consistent with evidence from previous empirical research, which did not support a particularly extensive invasive ability of *Eucalyptus* species.

Table 2-1. Expected germination was evaluated in a controlled environment growth chamber using eight 0.05 g samples of the seedlot for each species. The total number of normal germinated seeds in each sample was used to calculate the mean number of germinating seed per g for each seedlot. The amounts of seed that were added into low and high seeding density subplots, which were expected to result in 500 and 1,000 germinating seeds m^{-2}, were calculated.

Eucalyptus species	Mean number of germinating seeds g^{-1} seedlot	Mass of seeds added in low seeding density subplots	Mass of seeds added in high seeding density subplots
E. grandis	655.1	0.048	0.095
E. amplifolia	92.3	0.339	0.678
E. camaldulensis	388.0	0.081	0.161

Table 2-2. Vegetation community characteristics at the Gainesville, Florida, study location.

Characteristics	*Eucalyptus amplifolia* seed orchard	Abandoned forest road	Native hardwood forest
Soil series [a]	Blitchton sand	Lochloosa fine sand	Blitchton sand
Canopy cover [b]	74.89 ± 1.86 %	64.03 ± 4.27 %	98.25 ± 0.18 %
Predominant vegetation	*Albizia julibrissin* *Eucalyptus amplifolia* *Liquidambar styraciflua* *Quercus nigra* *Quercus virginiana* *Smilax auriculata* *Vitis* sp.	*Dichondra carolinensis* *Quercus nigra* *Rubus* sp. *Smilax auriculata*	*Parthenocissus quinquefolia* *Pinus taeda* *Quercus virginiana* *Smilax auriculata* *Toxicodendron radicans* *Ulmus americana*

[a] USDA 2012. Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm. Accessed: January 10, 2013.
[b] Means and standard errors for canopy cover measured over the center of each treatment plot.

Table 2-3. Vegetation community characteristics at the Quincy, Florida, study location.

Characteristics	*Eucalyptus amplifolia* progeny test	Intensively prepared land for *Eucalyptus* planting	Non-grazed pasture
Soil series [a]	Dothan-Furquay complex	Dothan Furquay complex	Dothan Furquay complex
Canopy cover [b]	90.38 ± 0.71 %	0.00 ± 0.00%	0.00 ± 0.00%
Predominant vegetation	*Diospyros virginiana* *Eucalyptus amplifolia* *Ligustrum sinense* *Populus deltoides* *Rubus* sp.	*Eupatorium capillifolium* *Ipomoea coccinea* *Ipomoea cordatotriloba* *Oxalis stricta* *Passiflora incarnata* *Portulaca pilosa* *Senna obtusifolia* L.	*Paspalum urvillei* *Paspalum laeve* *Oxalis stricta*

[a] USDA 2012. Web Soil Survey. http://websoilsurvey.nrcs.usda.gov/app/HomePage.htm. Accessed: January 10, 2013.
[b] Means and standard errors for canopy cover measured over the center of each treatment plot.

Table 2-4. Mean percent *Eucalyptus* seedling emergence after seeds of three *Eucalyptus* species were added into disturbance treatment plots within three communities at each of the two Florida study locations in June 2012. Seedling emergence was based on the total number of emerged seedlings that were counted in periodic plot assessments over 25 weeks following seed addition. Mean percent emergence is presented for disturbance, species and vegetation community variables. There were no significant differences in the probability of emergence for these main effects at $\alpha = 0.05$.

Variable	*Eucalyptus* seedlings emerged
	- - - - - - - - % - - - - - - - -
Disturbance	
Disturbed	0.000102
Non-disturbed	0.000001
Eucalyptus species	
E. amplifolia	0.000000
E. camaldulensis	0.000228
E. grandis	0.000023
Vegetation community	
Gainesville	
Eucalyptus stand	0.000499
Forest road	0.015400
Native hardwood forest	0.000000
Quincy	
Eucalyptus stand	0.000316
Non-grazed pasture	0.000000
Intensively prepared land	0.000000

Table 2-5. Mean percent *Eucalyptus* seedling emergence after seeds of three *Eucalyptus* species were added into disturbance treatment plots within three communities at each of the two Florida study locations in June 2012. Seedling emergence was based on the total number of emerged seedlings that were counted in periodic plot assessments over 25 weeks following seed addition. Mean percent emergence is presented for location, vegetation community and species treatment combinations. There were no significant differences in the probability of emergence for these combinations of factor levels at $\alpha = 0.05$.

Treatment combinations			*Eucalyptus* seedlings emerged
Location	Vegetation community	*Eucalyptus* species	
			- - - - - - - - - - - %- - - - - - - - - - - -
Gainesville	*Eucalyptus* stand	*E. amplifolia*	0.000000
Gainesville	*Eucalyptus* stand	*E. camaldulensis*	0.323500
Gainesville	*Eucalyptus* stand	*E. grandis*	0.126700
Gainesville	Forest road	*E. amplifolia*	0.000446
Gainesville	Forest road	*E. camaldulensis*	0.129000
Gainesville	Forest road	*E. grandis*	0.063400
Gainesville	Native hardwood forest	*E. amplifolia*	0.000000
Gainesville	Native hardwood forest	*E. camaldulensis*	0.000000
Gainesville	Native hardwood forest	*E. grandis*	0.000000
Quincy	*Eucalyptus* stand	*E. amplifolia*	0.000446
Quincy	*Eucalyptus* stand	*E. camaldulensis*	0.193700
Quincy	*Eucalyptus* stand	*E. grandis*	0.000000
Quincy	Non-grazed pasture	*E. amplifolia*	0.000000
Quincy	Non-grazed pasture	*E. camaldulensis*	0.000000
Quincy	Non-grazed pasture	*E. grandis*	0.000000
Quincy	Intensively prepared land	*E. amplifolia*	0.000000
Quincy	Intensively prepared land	*E. camaldulensis*	0.000000
Quincy	Intensively prepared land	*E. grandis*	0.000000

Table 2-6. Population means for *Eucalyptus* seedling emergence across both seeded and non-seeded areas of 21 disturbed and 21 non-disturbed treatment plots within each of the various vegetation communities at two Florida study locations. Since emergence was very close to zero in seed addition subplots, these means reflect recruitment by mature *Eucalyptus* trees. Population means for seedling emergence and 95% confidence intervals are also expressed on a per hectare basis for each treatment combination. Treatment combinations are presented in order from greatest emergence to least emergence.

Treatment combinations [a]			*Eucalyptus* seedlings emerged [b, c]	Seedling emergence ha^{-1}		
Location	Vegetation community	Disturbance		Mean [c]	Lower 95% CL	Upper 95% CL
Gainesville	*Eucalyptus* stand	Disturbed	45	28299	14976	53476
Gainesville	*Eucalyptus* stand	Non-disturbed	7	4717	1100	20223
Quincy	*Eucalyptus* stand	Disturbed	6	3810	738	19663
Gainesville	Forest road	Disturbed	3	2177	250	18927
Quincy	*Eucalyptus* stand	Non-disturbed	1	635	78	5182
Gainesville	Forest road	Non-disturbed	1	363	29	4546

[a] Not including non-grazed pasture, intensively prepared land and native hardwood forest communities that had zero emerged seedlings.

[b] Total area of plots = 15.75 m^2.

[c] Estimated population means from a generalized linear model.

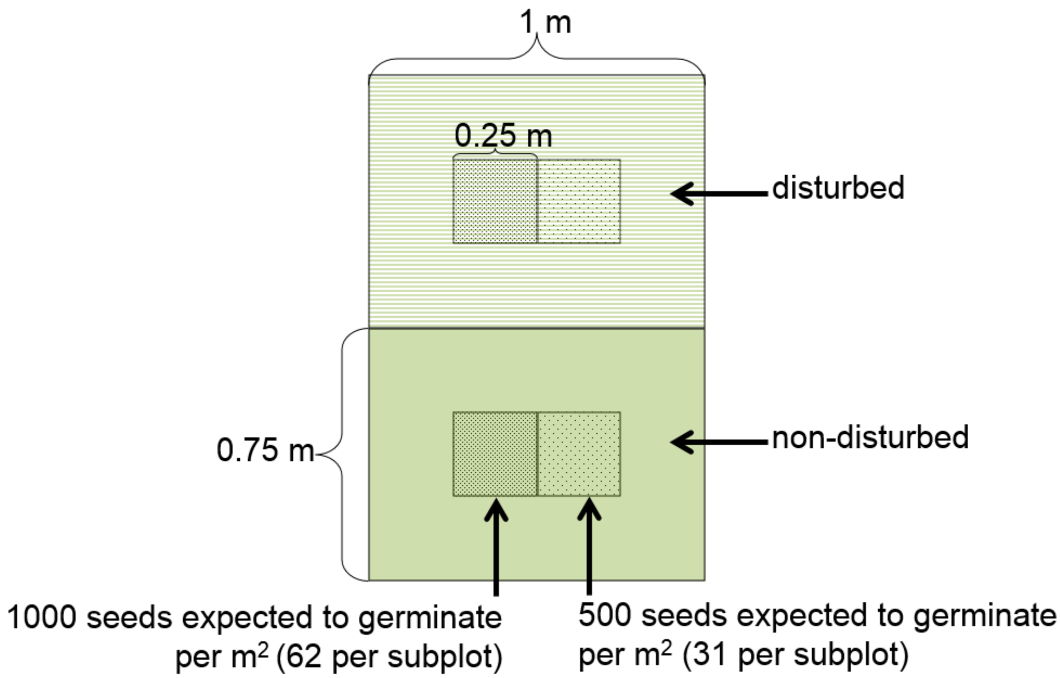

Figure 2-1. Treatment plot dimensions and possible randomly assigned disturbance treatments within whole plots (1 x 1.5 m) is shown. Possible randomly assigned seeding density subplot treatments and non-seeded areas used in the seed addition studies are also shown.

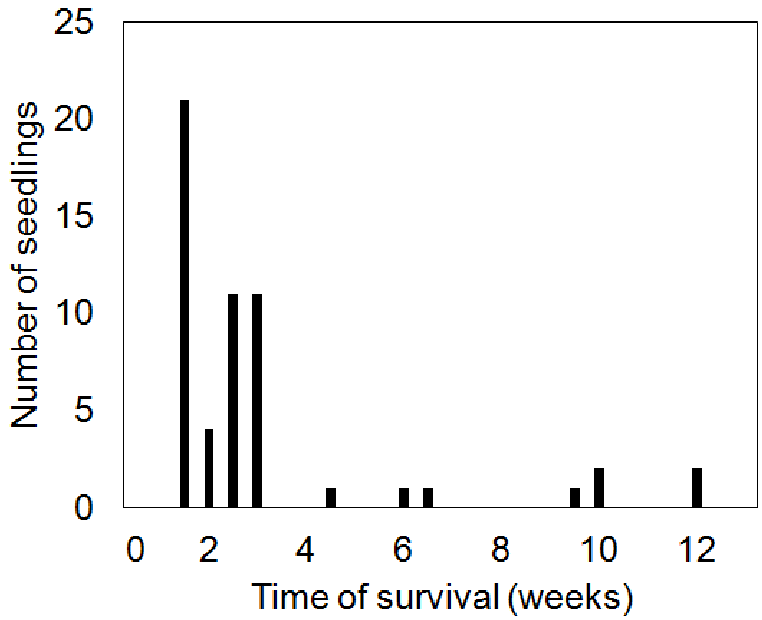

Figure 2-2. Seedling longevity for the Gainesville, Florida study location. No seedlings survived more than 12 weeks.

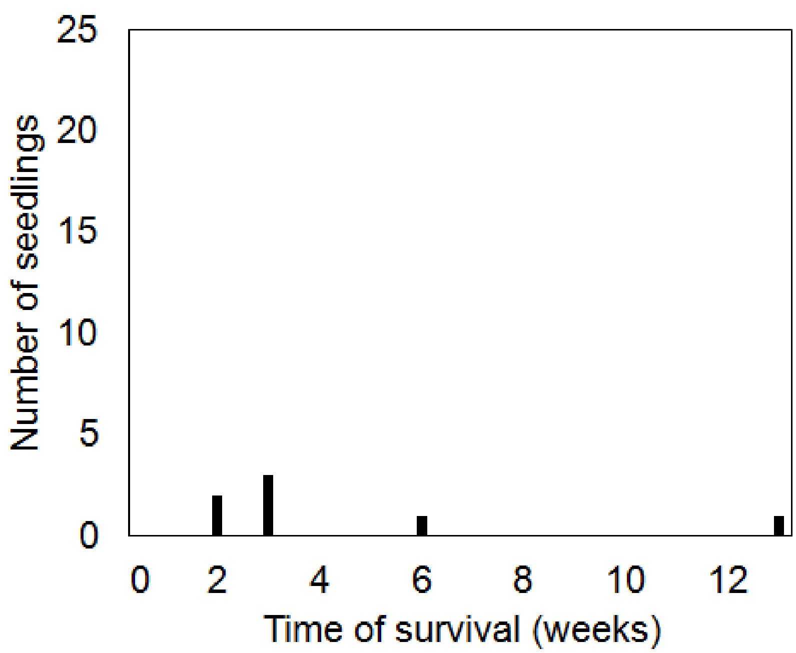

Figure 2-3. Seedling longevity for the Quincy, Florida study location. No seedlings survived more than 13 weeks.

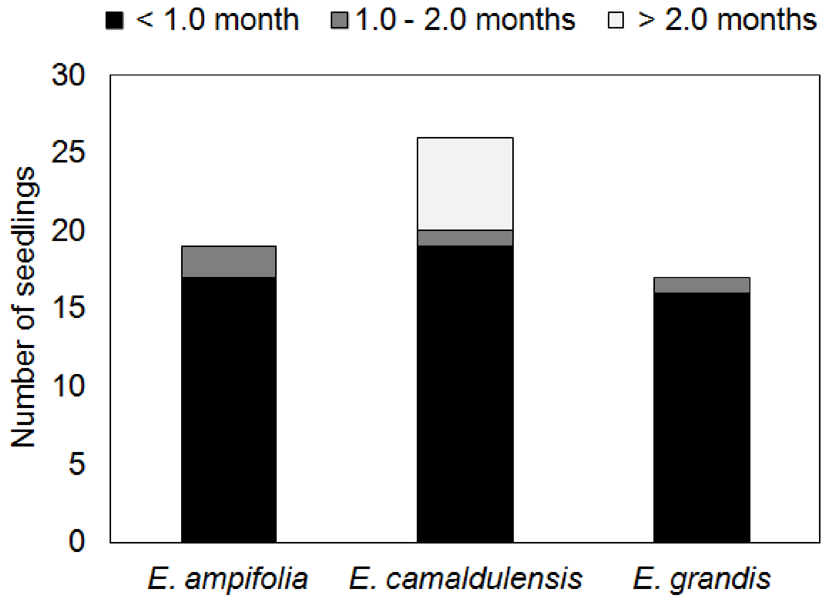

Figure 2-4. Time of *Eucalyptus* seedling survival by species for all seedlings at both Quincy and Gainesville study locations in Florida. No seedlings survived more than 13 weeks.

CHAPTER 3

COMPARISON OF AMINOCYCLOPYRACHLOR TO STANDARD HERBICIDES FOR CONTROL OF *EUCALYPTUS*

Introduction

The Invasive Species Advisory Committee, a group of non-federal experts and stakeholders which was established to provide advice on invasive species related issues to the interagency National Invasive Species Council (Federal Register 1999), made nine recommendations for federal biofuels programs to minimize the risk of bioenergy crop escape into the surrounding environment. Their recommendations included the need to establish protocols for rapid removal of bioenergy crops, should they disperse into surrounding areas or become abandoned and unwanted populations (NISC 2009). Herbicides are a relatively effective and inexpensive tool that can be used to manage *Eucalyptus* seedlings occurring from natural recruitment or for the removal of abandoned stands of mature trees. To prepare for potential management of invasive *Eucalyptus* in the southeastern US, effective chemical control methods need to be established.

Herbicides for *Eucalyptus* Control

The existing recommendations regarding methods for chemical control of *Eucalyptus* are inexact. They generally entail cut stump, basal frill (also known as cut stem treatment), or basal bark applications using concentrated herbicide solutions or emulsions containing the active ingredients triclopyr, imazapyr, or glyphosate. Individual stem treatments using these broad-spectrum herbicides are preferred for their ease of use and targeted application. Triclopyr and glyphosate may be applied to the stems of target vegetation with minimal impact to nearby vegetation, because these herbicides are not readily absorbed from the soil by roots (Senseman 2007). However, imazapyr is a soil active herbicide, thus injury to non-target vegetation is more likely (Little and Shaner 1991).

In *Eucalyptus* removal projects in Marin County, California, triclopyr and imazapyr had the best results, and cut-stump applications of 80.0% Garlon® 4, or 100.0% Garlon® 3A, Stalker®, or Roundup® were recommended (Bossard et al. 2000). Moore (2008) suggested that a lower rate (50.0%) of glyphosate or triclopyr is sufficient for basal bark, basal frill, or cut stump applications. One study reported that very low rates of picloram (formulated as Tordon® 22K) in partial and complete basal frills may provide satisfactory control (Bachelard et al.1965), however, control using these relatively low concentrations of picloram was not consistent in all study areas or at all times. Additional trials with 12.5% Tordon 101 mixture (containing 240 g ae L^{-1} 2,4-D amine plus 65 g ae L^{-1} picloram) as a basal frill treatment to different *Eucalyptus* species resulted in 90-100% mortality, although “flashback” (suspected translocation through natural root grafts) was a serious issue, as 30 m tall non-treated trees were strongly affected. In general, complete control of *Eucalyptus* is rarely achieved through the use of single herbicide treatments and reapplication to control sprouting is often necessary (Bachelard et al. 1965; Morze 1971; Bossard et al. 2000; Little 2003; Little and van den Berg 2006), potentially taking up to three herbicide treatments to completely prevent resprouting (Bossard et al. 2000). Furthermore, variable levels of tolerance among different species and the varying effectiveness of different application timings for commonly used herbicides make it difficult to make recommendations (Bachelard et al. 1965; Morze 1971). Few recommendation sources consider tree size and vigor, which are also important for the success of herbicide treatments (Morze

1971). Research was needed to develop herbicide prescriptions for controlling *Eucalyptus* using basal frill and basal bark approaches, to refine the dose response for different tree diameters and to determine the impact to non-target vegetation.

Aminocyclopyrachlor for Control of Woody Plants

Aminocyclopyrachlor (AMCP) is a new herbicide being developed by DuPont for use in non-crop areas such as rights-of-way, turf, range and natural areas (E.I. du Pont de Nemours and Company 2009). A pyrimidine carboxylic acid herbicide, it is structurally similar to pyridine carboxylic acid herbicides such as aminopyralid, picloram and triclopyr. It is believed that synthetic auxin mode of action is employed, interfering with normal plant growth (USDA 2012b). AMCP has good selectivity to established cool- and warm-season perennial grasses (Westra et al. 2009) and shows other positive stewardship attributes including low volatility, the absence of bioaccumulation in animals, and low toxicity to terrestrial and aquatic organisms (E.I. du Pont de Nemours and Company 2009). It has shown excellent control at rates as low as 140 g ae ha^{-1} for many species including herbaceous weeds resistant to ALS inhibitors, triazines and glyphosate (Turner et al. 2009). AMCP has also demonstrated control at low rates for many woody plants in recent trials (Appendix B), indicating its potential as an alternative to currently recommended herbicide treatments for control of *Eucalyptus* trees.

Eucalyptus benthamii (Maiden et Cambage) is among the more promising species for wide-scale planting in the southeastern US because of its cold hardiness, success in out-plantings under a variety of conditions and fast growth rates (Zalesny et al. 2011). In particular, the ability of this species to withstand cold weather could contribute to its potential invasiveness at a geographic scale. In September 2011, three studies were initiated in 29-month-old *E. benthamii* plantations to evaluate the control provided by the new herbicide AMCP. The specific objectives of this study were as follows.

- Compare the effectiveness of four rates of AMCP to operational standard treatments, imazapyr and triclopyr, for control of various diameter classes of *Eucalyptus* trees using basal bark and basal frill stem applications.
- Describe the rate-response of AMCP for control of various diameter classes of *Eucalyptus* using basal frill and basal bark applications.
- Determine the effects of herbicide treatments to nearby non-target *Eucalyptus* trees.

Materials and Methods

Study Areas

Three experiments were installed on adjacent 0.2 ha *E. benthamii* plantations established in 2009 at the University of Florida, North Florida Research and Education Center, south of Quincy (30°32′48″ N, 84°35′52″ W) at approximately 73 m elevation. This location has a temperate climate with highest temperatures in July (mean 27 C), lowest temperatures in January (mean 10 C) and 143 cm average annual precipitation (NOAA 2002). The prevalent soil series in both plantations is Orangeburg fine sandy loam, but soils at the North plantation site were highly eroded, to the extent that the typic sandy surface horizon was absent (hereafter the two sites are referred to as eroded and non-eroded). Across the two sites, 960 seedlings had little winter dieback or mortality prior to the establishment of this study. Trees were generally smaller on the eroded site, but there was also high variability in size within both sites. As a part of a separate study (Osiecka and Minogue 2011), trees received different levels of competition control during the establishment year. This resulted in an array of tree sizes, ranging from less than 1 to 10 m in height and from 4 to 20 cm in basal stem diameter (BSD) at groundline, when measured prior to treatment in late October 2011.

Basal Bark Treatments and Experimental Design

Identical experiments were conducted in each of the sites using a randomized complete block design. One hundred and forty randomly selected healthy trees were ranked according to basal diameter and sequentially divided into 20 groups of seven trees each. The seven similarly sized trees of each group were randomly assigned one of seven herbicide treatments (Table 3-1), resulting in 20 replicates of each herbicide treatment across the entire range of tree diameters. Non-treated buffer trees were included in the design so that no treated tree was next to another treated tree. AMCP (120 g ae L^{-1}, in the form of DPX MAT28-159, DuPont, Wilmington, DE) was tested as a basal bark treatment at four rates (5, 10, 20 and 40% v/v formulated material). Since AMCP is a new herbicide for which few guidelines exist, the range of treatment rates was selected based on evidence from testing across other woody plants (Edwards and Beck 2011; Wilson et al. 2011b; Yeiser et al. 2011; J. Ferrell, University of Florida and M. Link, DuPont, personal communications, May, 2011).

Other basal bark treatments included imazapyr (isopropylamine salt of imazapyr, 240 g ae L^{-1}, in the form of Stalker®, BASF, Research Triangle Park, NC), triclopyr (butoxyethyl ester, 480 g ae L^{-1}, in the form of Garlon® 4 Ultra, Dow AgroSciences, Indianapolis, IN) and a non-herbicide treated check (applied seed oil carrier only). The formulated imazapyr product was applied at the mid-range of prescribed label rates for thinline basal and stem applications (28.1% v/v) (BASF Corporation 2008), as previous experience indicated this rate was more than adequate. Formulated triclopyr ester product was applied at the highest prescribed label rate for thinline basal bark treatment

(75% v/v) (Dow AgroSciences LLC 2008). All herbicides were thoroughly mixed with 100% methylated soybean oil, alkylphenol ethoxylate, as the carrier (M.O.C., Helena, Collierville, TN). Five ml of herbicide/oil mixture per 2.5 cm basal stem diameter (BSD) were applied to the base of trees from 30 cm height to the groundline using a syringe. Preliminary testing indicated this volume generally sufficient to wet the stem completely from 30 cm height to the ground line for the various diameter classes studied. Herbicide treatments were applied on November 4 and 5, 2011, and no rainfall occurred within 48 hr following application.

Basal Frill Treatments and Experimental Design

Basal frill application is recommended for trees that are greater than 5 cm in diameter at breast height (Miller 2010). Because few large trees were present in the eroded site, this experiment was performed only at the non-eroded site. As in the basal bark studies, healthy trees with suitable diameters were ranked by BSD and sequentially divided into eight groups of seven trees with like diameters. The seven treatments (Table 3-2) were assigned in a randomized complete block design with eight replications. A hatchet was used to make cup-like downward incisions at 30 cm above the groundline. One cut was made per 2.5 cm BSD. Cuts were evenly spaced around the stem circumference. A syringe was used to apply one ml of a water and herbicide mixture to each cut. Trees in the non-herbicide treated check were cut with a hatchet in the same manner as other treatments, but no herbicides were applied.

AMCP was applied at four rates (12.5, 25, 50 and 100% formulated material). Herbicide concentrations tested were higher than in the basal bark treatments to accommodate the small volume of mixture that must be contained within each cut, but the actual amount of active ingredient applied to each tree was comparable to the various rates tested in basal bark treatments (Table 3-1). Standard treatments for *Eucalyptus* control included imazapyr (Stalker®) and triclopyr (triclopyr triethylamine salt, 360 g ae L^{-1}, in the form of Garlon® 3A, Dow AgroSciences) applied at the respective recommended label rates of 7.81% and 50.0% v/v (BASF Corporation 2008, Dow AgroSciences LLC 2003).

Tree Assessments

For all trees in both sites, pre-treatment stem diameter was measured to the nearest millimeter at ground level (BSD) and at breast height, 137 cm from groundline (DBH), in October 2011. Pre-treatment stem height was measured to the nearest centimeter using a height pole. In order to quantify stem dieback or growth, the live height was measured again 12 months after treatment (MAT). Additionally, at 2, 6 and 12 MAT12 MAT, each tree was assessed for percent crown reduction. As defined by Miller and Glover (1991), this standard variable is an ocular estimate of reduction relative to the pre-treatment condition, and includes stem dieback, leaf necrosis and defoliation. Estimates were made to the nearest five percent for values between 0 and 10%, and 90 and 100%; and to the nearest 10 percent for values between 10 and 90%. Additionally, phytotoxicity symptoms (mortality, foliar necrosis, defoliation, red foliage, chlorosis, stem sap flow, epinasty, basal sprouting and adventitious buds on stems) were noted as present or not present for each tree at 2, 6 and 12 MAT.

Statistical Analysis

Stem live height and crown reduction

For each experiment, SAS Proc GLM (SAS v. 9.3, SAS Institute, Cary, NC) determined differences between treatment groups for change in live stem height at 12 MAT. Tukey’s HSD test was used to compare means at $\alpha = 0.05$. Crown reduction responses were primarily near to the fixed limits of 0 and 100%. Homogeneity of variances and normality could not be achieved by standard data transformations (arcsine, arcsine square root and log), so traditional parametric and non-parametric tests could not be used to determine if there were differences between treatment groups. Instead, Welch's analysis of variance (ANOVA) on ranked data was performed using SAS PROC GLM to determine if there were differences between treatments in each of the three experiments. The Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) was performed to compare treatment means at $\alpha = 0.05$. These procedures are recommended for analysis of data that is simultaneously heteroscedastic and non-normal, and they have been shown to provide good power and acceptable control for type I error rates (Cribbie et al. 2007).

Phytotoxicity symptoms

For each of the three experiments, Fisher's exact tests were used to evaluate the significance of herbicide treatment for binary present/absent phytotoxicity responses (red foliage, chlorosis, epinasty, basal sprouting and adventitious buds on stems) that had not already been evaluated by percentage data. Red foliage and chlorosis were only evaluated for trees that did not show 100% crown reduction because of the mutual exclusivity of these conditions.

Aminocyclopyrachlor rate-response

Data from all three experiments were combined to model the rate-response of AMCP relative to tree size using SAS Proc Logistic. Crown reduction was evaluated as the binomial response of mortality where trees were considered dead only when there was 100% crown reduction. Logistic regression models examined the relationship between the probability of mortality and basal stem diameter (BSD) or diameter at breast height (DBH), AMCP concentration, application method and the interactions between these explanatory variables. Logistic regression used logit transformation of probability, P, called the “log odds,” denoted by θ, as the response variable where $\theta = \mathrm{Log}\left(\frac{\mathrm{P}}{1-\mathrm{P}}\right)$. The models fitted had the general form

$$\theta = \beta_0 + \beta_1(\mathrm{D}) + \beta_2(\mathrm{C}) + \beta_3(\mathrm{A}) + \beta_4(\mathrm{C})(\mathrm{D}) \qquad \text{3-1}$$

where A was a dummy variable for application method, D was diameter (DBH or BSD), C was the AMCP concentration and β_x values were the specific parameters of the model. Similar to the methods of Bergerud (1988), a final model was chosen using backward conditional selection starting with a full model and dropping insignificant explanatory variables and interactions one at a time.

Herbicide impacts on non-target trees

As a measure of the potential for flashback at various distances from treated trees, symptoms of phytotoxicity were compared for non-treated and buffer trees. Buffer trees were those directly adjacent to treated trees (1.5 m away), whereas non-treated check trees were randomly assigned with the herbicide treatments to trees that were not adjacent to other treated trees (> 1.5 m away). Non-treated trees in the basal frill study were cut but were grouped with non-treated trees from the basal bark experiment for this analysis. For each assessment date, buffer trees were compared to non-treated trees using Fisher’s exact test for absent/present responses. Crown reduction and stem height were compared for all trees in non-treated and buffer groups using the methods described previously for each response variable. It was not possible to determine the impacts to herbaceous vegetation and grasses due to the highly variable vegetation patterns throughout the sites and lack of data for a reference state of the vegetation when the herbicide treatments were applied.

Results and Discussion

Eucalyptus Control using Basal Bark Treatments

Crown reduction

Welch's ANOVA on ranked data revealed a highly significant ($P < 0.0001$) effect of basal bark treatments on crown reduction at 2 and 6 MAT for both sites, and at 12 MAT on the non-eroded site. Differences in crown reduction due to treatments were not significant at 12 MAT on the eroded site, although all treatments had significantly greater crown reduction than the non-treated check (Table 3-3).

The three highest AMCP rates resulted in 100% crown reduction in all assessments at both sites. The lowest rate of AMCP only differed from the higher rates initially, when at 2 MAT it resulted in less crown reduction (82%) on the non-eroded site, which had larger trees. However, by 6 MAT, 100% crown reduction was observed for nearly all of the trees that were treated with AMCP, regardless of rate, tree size, or site. This complete control was achieved using AMCP at 0.03 to 0.24 g ae per 2.5 cm BSD. No other studies were found regarding the use of basal bark herbicide treatments for the control of *Eucalyptus*, but these results indicate that this method of application is effective for *Eucalyptus* control using AMCP.

Standard herbicide treatments always resulted in significantly less crown reduction than AMCP treatments, except at 6 and 12 MAT on the eroded site (Table 3-3). Here, the population was comprised of small, less vigorous trees, which were perhaps more susceptible to herbicides. Mean crown reduction for imazapyr treated trees increased from 62% to 100% on the eroded site and from 42% to 91% on the non-eroded site between 2 and 12 MAT, indicating that symptoms of imazapyr injury are slow to develop, as is commonly reported for herbicides with an amino acid synthesis inhibiting mode of action (Gunsolus and Curran 1999). Likewise, symptoms were also slow to appear for triclopyr ester, with mean crown reduction increasing from 26% to 97% on the eroded site and 4% to 86% on the non-eroded site between 2 and 12 MAT. Triclopyr ester was consistently the least effective herbicide in both basal bark experiments.

Stem live height

The overall effect of basal bark treatment on live stem height at 12 MAT was highly significant at both sites ($P < 0.0001$). At the eroded site, all herbicide treatments resulted in stem reduction whereas trees in the non-treated check grew 335 cm on average (Figure 3-1). All herbicide treatments on the eroded site were significantly different from the non-treated check, but there were no significant differences among herbicide treatments. At the non-eroded site, all rates of AMCP and the imazapyr treatment resulted in significantly greater stem reduction compared to triclopyr ester or the non-treated check, but reduction did not differ among AMCP and imazapyr treatments (Figure 3-1).

Phytotoxicity symptoms

For trees that did not have 100% crown reduction (143 trees at 2 MAT, 66 trees at 6 MAT and 60 trees at 12 MAT), significant relationships between phytotoxicity symptoms and basal bark herbicide treatments were only detected for the study on the non-eroded site. Phytotoxic symptoms were most common for the standard imazapyr and triclopyr ester treatments, because AMCP treatments resulted in a high incidence of complete crown reduction, and this symptom also occurred more quickly than with the standard treatments. Red foliage was more common (P = 0.014) at 2 MAT for triclopyr ester (44%) and imazapyr (17%) treatments than for the AMCP treatments, which did not result in red foliage symptoms. Treatment also had a significant effect (P = 0.002) on the occurrence of stem sap flow at 2 MAT. At this assessment, stem sap flow was observed for 20% of the trees treated with triclopyr ester, and for 20% and 5% of the trees treated with the two lowest rates of AMCP, respectively. Treatment had a highly significant effect (P = 0.002) on adventitious budding on stems, a known symptom of imazapyr in angiosperms (Osiecka and Minogue 2012) at 12 MAT, when it was observed at nodes on the trunks of 20% of imazapyr treated *Eucalyptus* trees. The incidences of epinasty and basal sprouting were rare and did not differ by herbicide treatment at any time in either study. Basal sprouting was only observed on one triclopyr ester treated tree in each study at 12 MAT.

The effectiveness of AMCP treatments observed in these studies are consistent with those of Wilson et al. (2011b) who reported effective control by AMCP basal bark treatments for other fast growing woody plants such as Russian olive (*Elaeagnus angustifolia* L.) and salt cedar (*Tamarix* sp.). Edwards and Beck (2011) also reported similar results for Russian olive, although their trials were notably different because they applied approximately 30 ml per 2.5 cm of stem diameter, while 5 ml per 2.5 cm BSD was applied in our study. Although a higher herbicide rate may be required for control of Russian olive as compared to *Eucalyptus*, it appears that other studies have generally not investigated the lowest rates necessary to achieve control of some woody plants. Future studies of woody plant control with AMCP should examine lower rates than used in our study.

Eucalyptus Control using Basal Frill Treatments

Crown reduction

The overall effect of basal frill herbicide treatment on crown reduction was highly significant ($P < 0.0001$). However, there was not a significant difference in crown reduction among the AMCP rates tested at any assessment, and by 6 MAT all 30 trees treated with AMCP had 100% crown reduction (Table 3-4). While AMCP treatments were significantly more effective at reducing live crown than triclopyr amine (49%) and imazapyr (19%) treatments at 2 MAT, by 6 MAT there was no difference between triclopyr amine (80% at 6 MAT and 81% at 12 MAT) and any of the AMCP rates (all 100%). Whereas imazapyr treatments resulted in significantly greater crown reduction than the non-treated check, imazapyr remained the least effective herbicide throughout the study, resulting in 58% mean crown reduction by 12 MAT.

In comparing the two application methods studied for the assessment 12 MAT, the mean crown reduction resulting from basal frill applications of imazapyr (58%) and triclopyr amine (81%) were qualitatively less than basal bark applications of imazapyr (91–100%) and triclopyr ester (97–100%). This trend contrasts the conclusion of Bossard et al. (2000) that application of triclopyr and imazapyr herbicides to the foliage or stems of sprouts is less effective than application directly to the vascular cambium immediately following cutting. This contrasting trend may have arisen because Bossard et al. (2000) applied herbicides at 80–100% v/v for both application methods, whereas relatively lower rates were applied for basal frill applications in of these herbicides our study.

Stem live height

The overall effect of basal frill herbicide treatment on stem height at 12 MAT was highly significant ($P < 0.0001$) and all herbicide treatments resulted in height reduction from pre-treatment values due to stem dieback, whereas the non-treated check grew 425 cm(Figure 3-2). There were no differences among the AMCP rates, but all AMCP treatments resulted in significantly greater stem dieback than either of the standard treatments.

Phytotoxicity symptoms

The overall effect of treatment was significant ($P = 0.0196$) for the presence of red foliage symptoms only at 2 MAT (results not shown). However, at this assessment it was commonly observed in all herbicide treatments, with the exception of the three highest AMCP rates, which had already resulted in 100% crown reduction. Treatment had a significant effect ($P = 0.014$) on stem sap flow, and was most common for imazapyr (44%) and triclopyr amine (71%) treatments. Similar to the basal bark experiment, treatment had a significant effect ($P = 0.0149$) on the presence of adventitious buds on stems at 12 MAT, whereby 33.3% of trees treated with imazapyr had this symptom. Adventitious buds were not observed for trees in any other treatment. Although treatment effects were not significant, chlorosis was most common at 6 MAT when it was observed for 50% of the frilled non-herbicide treated check trees, 11% of trees treated with imazapyr, and 14% of trees treated with triclopyr amine. Epinasty or basal sprouting were not significantly affected by treatment nor were they common symptoms at any assessment. Basal sprouting was observed for only one triclopyr amine treated tree and one imazapyr treated tree at 12 months following basal frill treatment.

Whereas no other literature reporting results for AMCP basal frill application for the control of woody plants was found, these new results demonstrate that it is an effective alternative to more labor intensive cut-stump applications, which have been the focus of several studies (Edwards and Beck 2011; Wilson et al. 2011b; Yeiser et al. 2011). With respect to triclopyr amine basal frill treatments, control of *E. benthamii* in this study differed from results described by Little and van den Berg (2006) who applied the same formulation of triclopyr amine at 3% v/v in basal frill treatments to *E. macarthurii* trees and reported mortality of 83-90% of trees when evaluated nine months after treatment. Our study found that only 57% of triclopyr amine treated trees had complete mortality by 12 MAT, even though a much higher herbicide rate (50% v/v) was used. This difference in control may indicate differential susceptibility for these two *Eucalyptus* species, or possibly the need for continued assessments in our study to determine if greater levels of mortality will eventually occur.

Aminocyclopyrachlor Rate Response Relative to Tree Diameter at Breast Height

A significant model (Wald χ^2 = 29.82, 4 df, P < 0.0001) was developed that accurately predicts the actual mortality response observed for 161 out of 189 trees in this study (Table 3-5). The following logistic regression equation was used to predict the minimum concentration of AMCP (120 g ae L^{-1} AMCP formulation), C, required for a desired probability of mortality at 2 MAT, P, of *E. benthamii* of a given DBH, D, using basal bark or basal frill applications:

$$\mathrm{C} = \frac{\mathrm{Log}\left(\frac{\mathrm{p}}{1-\mathrm{p}}\right) - 2.9302 + 1.1556(\mathrm{A}) + 0.3946(\mathrm{D})}{0.1908 - 0.0159(\mathrm{D})} \qquad \text{3-2}$$

where application method, A,is dummy coded so that basal bark = 1 and basal frill = 0. For example, the concentration of AMCP required to achieve 90% control of *E. benthamii* given by

$$\mathrm{C} = \frac{-0.2084+0.3946(\mathrm{D})}{0.1908-0.0159(\mathrm{D})} \qquad \text{for basal bark applications} \qquad (3\text{-}3)$$

$$\mathrm{C} = \frac{-1.4360+0.3946(\mathrm{D})}{0.1908-0.0159(\mathrm{D})} \qquad \text{for basal frill applications} \qquad (3\text{-}4)$$

Even though application method was not a significant predictor, it was included in the model because it increased the percent concordant. A logistic regression model was not fitted for mortality at 6 and 12 MAT because only one out of 199 trees treated with AMCP did not have 100% crown reduction at that point in time. This tree was one of the largest in the study (13.2 cm BSD) and received the treatment with the lowest AMCP rate as a basal bark application using the lowest AMCP rate tested. Although complete mortality did not occur for the tree within the study period, at 6 MAT 45% of the crown was reduced by 45%and by 12 MAT crown reduction increased to 75%.

The model developed in this study is the first that considers tree size for the control of *Eucalyptus*. These results demonstrate that tree diameter and application method have an important relationship with rate response that should be considered when prescribing herbicide treatments. When applying AMCP to *Eucalyptus*, Figures 3-4 and 3-5 developed from the logistic regression model can be used as a guide to select the lowest dosage for which death is likely to occur (predicted probability of death >50%) as early as two months after treatment, for a given stem diameter.

Impacts to Non-target Vegetation

There was no significant difference in crown reduction when buffer trees (those directly adjacent to treated trees) and non-treated check trees were compared. While non-treated trees grew 377 cm and buffer trees only grew 337 cm, this difference in growth was not statistically significant. The most frequently observed phytotoxicity symptoms for both buffer and non-treated check trees were foliar necrosis, defoliation and chlorosis (Table 3-6). Fisher's exact test found a significant probability that the presence of foliar necrosis was greater for buffer trees than for non-treated check trees at 6 MAT ($P = 0.0236$). However, no mortality was observed in either group at 2 MAT, and by 6 MAT mortality occurred in only one percent of buffer trees, but not in any non-treated check trees. At 12 MAT, two of the five buffer trees that appeared to have 100% crown reduction at 6 MAT had sprouted new basal shoots. The large or medium buffer trees (5-15 cm BSD) that died were all neighbored on at least three sides by AMCP treated trees, and small trees (<5 cm BSD) were bordered by one or two AMCP treated trees. This suggests that absorption of this herbicide from the soil by roots, or perhaps herbicide uptake through root grafts, may be of concern when making applications in very close proximity to desirable trees. Other symptoms observed included epinasty, a symptom characteristic of the growth regulator herbicides triclopyr and AMCP. Various symptoms in buffer trees and non-treated checks began to appear by 6 MAT, but remained uncommon and differences were insignificant between buffer and non-treated trees throughout the study period.

While it was not possible to quantify impacts to herbaceous vegetation in this study, no obvious symptoms of injury such as necrosis or chlorosis were observed for herbaceous vegetation near treated stems. Moreover, at 6 MAT, several newly established pine and oak seedlings were discovered throughout the *Eucalyptus* understory. While there have been concerns about the effects of AMCP on non-target trees and vegetation (USDA 2012b), these results suggest that risk injury to non-target vegetation may be reduced when low rate, low volume directed applications are used.

Conclusion

Using a model for the predicted probabilities of mortality responses for a range of AMCP doses as a function of stem diameter and basal bark and basal frill herbicide treatment methods (Figures 3-4 and 3-5, Table 3-5), we can determine the appropriate dosage for any desired level of mortality at 2 MAT. At 6 and 12 MAT, the lowest AMCP rates tested provided complete or nearly complete control of *E. benthamii* and often provided significantly greater control than the standard triclopyr and imazapyr treatments. At 12 MAT, no basal sprouting was observed for AMCP treated trees, indicating long-term control. Mortality was observed for less than 1% of non-treated check trees that were within 1.5 m of herbicide treated trees in this study. These results suggest that AMCP should be considered for new labeling for control of undesirable *Eucalyptus* trees. Based on this research, 0.03 g ae AMCP per 2.5 cm basal stem diameter (BSD) is effective as a basal stem treatment and 0.015 g ae per 2.5 cm BSD is effective for basal frill treatment to control *Eucalyptus* trees. Since these treatments gave complete control with basal frill treatment and near complete control with basal bark treatment when evaluated at 12 MAT, future research should examine the efficacy of lower herbicide rates for *Eucalyptus* control.

Although these studies tested AMCP rate response and the effects of tree diameter in comparison to the efficacy of standard herbicides, the potentially important effects of application timing and *Eucalyptus* species susceptibility were not determined.

This would require experiments across multiple seasons for those *Eucalyptus* species most commonly grown, such as *E. camaldulensis, E. grandis, E. urograndis, E. amplifolia,* and others. Future research regarding species susceptibility could look to the work of Morze (1971) which identified susceptible and resistant *Eucalyptus* species for picloram. Despite all factors that could affect herbicide treatment success not being evaluated, the strong control of *E. benthamii* in this study, with minimal injury to non-target trees, represents significant progress in the development of effective herbicide prescriptions for *Eucalyptus* management and indicates that future research with different species and timings would be worthwhile.

Table 3-1. Treatments tested in the basal bark studies for *Eucalyptus benthamii* control in Florida. All herbicide treatments were applied in methylated soybean oil carrier. Five ml of the herbicide in oil mixtures were applied per 2.5 cm basal stem diameter (BSD), from 30 cm stem height to groundline.

Herbicide	Formulation tested	Formulation concentration	Applied formulation concentration in oil carrier	Basal stem diameter specific dose
		g ae L^{-1}	% v/v	g ae per 2.5 cm BSD
AMCP [a]	DPX MAT28-159	120	5.0	0.030
AMCP	DPX MAT28-159	120	10.0	0.060
AMCP	DPX MAT28-159	120	20.0	0.120
AMCP	DPX MAT28-159	120	40.0	0.240
Imazapyr	Stalker®	240	28.1	0.337
Triclopyr ester	Garlon® 4 Ultra	480	75.0	1.800
Non-treated check [a]	–	–	–	–

[a] AMCP is aminocyclopyrachlor.
[b] Methylated soybean oil alone was applied to the non-herbicide treated check.

Table 3-2. Treatments tested in the basal frill studies for *Eucalyptus benthamii* control in Florida. One cut was made per 2.5 cm basal stem diameter (BSD). Cuts were evenly spaced around the stem circumference at 30 cm above the groundline. All herbicide treatments were diluted in water. One ml of herbicide mixtures was applied to each cut.

Herbicide	Formulation tested	Formulation concentration	Applied formulation concentration in water	Basal stem diameter specific dose
		g ae L^{-1}	% v/v	g ae per 2.5 cm BSD
AMCP	DPX MAT28-159	120	12.5	0.015
AMCP	DPX MAT28-159	120	25.0	0.030
AMCP	DPX MAT28-159	120	50.0	0.060
AMCP	DPX MAT28-159	120	100.0	0.120
Imazapyr	Stalker®	240	7.8	0.019
Triclopyr amine	Garlon® 3A	360	50.0	0.180
Non-treated check [a]	–	–	–	–

[a] AMCP is aminocyclopyrachlor.
[b] The non-treated check was cut without herbicide treatment.

Table 3-3. Crown reduction of *Eucalyptus benthamii* at 2, 6 and 12 months after treatment (MAT) with diameter specific basal bark herbicide applications at the eroded and non-eroded study sites. Crown reduction was determined as an ocular estimate of reduction relative to the pre-treatment condition, and included stem dieback, leaf necrosis and defoliation.

			Crown reduction [a]					
			2 MAT		6 MAT		12 MAT	
Herbicide	Applied formulation conc. in oil carrier	Basal stem diameter specific dose	Eroded	Non-eroded	Eroded	Non-eroded	Eroded	Non-eroded
	(% v/v)	g ae per 2.5 cm BSD	- - - - - -	- - - - - -	- - - - - -	(%)	- - - - - -	- - - - - -
AMCP [b]	5.0	0.030	86 ± 5 a	82 ± 7 a	100 ± 0 a	97 ± 3 a	100 ± 0 a	99 ± 1 a
AMCP	10.0	0.060	98 ± 2 a	98 ± 2 b	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
AMCP	20.0	0.120	100 ± 0 a	99 ± 1 b	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
AMCP	40.0	0.240	100 ± 0 a	99 ± 1 b	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a
Imazapyr	28.1	0.337	62 ± 8 b	42 ± 9 c	98 ± 1 a	83 ± 6 b	100 ± 0 a	91 ± 5 b
Triclopyr ester	75.0	1.800	26 ± 8 c	4 ± 2 d	92 ± 5 a	73 ± 9 b	97 ± 2a	86 ± 6 b
Non-treated check	–	–	1 ± 0 d	3 ± 2 d	1 ± 1 b	3 ± 1 c	0 ± 0 b	3 ± 1 c

[a] Treatment means (M ± SE) within a column followed by the same letter are not significantly different according to the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) on ranked data at $\alpha = 0.05$.

[b] AMCP is aminocyclopyrachlor.

Table 3-4. Crown reduction of *Eucalyptus benthamii* at 2, 6 and 12 months after treatment (MAT) with diameter specific basal frill herbicide applications at the non-eroded study site. Crown reduction was determined as an ocular estimate of reduction relative to the pre-treatment condition, and included stem dieback, leaf necrosis and defoliation.

Herbicide	Applied formulation conc. in water	Basal stem diameter specific dose	Crown reduction [a] 2 MAT	6 MAT	12 MAT
	(% v/v)	g ae per 2.5 cm BSD	- - - - - - - - - - - (%)	- - - - - - - - - - -	
AMCP [b]	12.5	0.015	91 ± 9 a	100 ± 0 a	100 ±0 a
AMCP	25.0	0.030	100 ± 0 a	100 ± 0 a	100 ±0 a
AMCP	50.0	0.060	99 ± 2 a	100 ± 0 a	100 ± 0 a
AMCP	100.0	0.120	100 ± 0 a	100 ± 0 a	100 ± 0 a
Imazapyr	7.8	0.019	19 ± 12 bc	49 ± 11 b	58 ± 11 b
Triclopyr amine	50.0	0.180	49 ± 16 b	80 ± 14 a	81 ± 10 a
Non-treated check	–	–	3 ± 2 c	0 ± 0 c	2 ± 1 c

[a] Treatment means (M ± SE) within a column followed by the same letter are not significantly different according to the Ryan-Einot-Gabriel-Welsch multiple range test (REGWQ) on ranks at $\alpha = 0.05$.
[b] AMCP is aminocyclopyrachlor.

Table 3-5. Logistic regression model variables for aminocyclopyrachlor (AMCP) rate response in *Eucalyptus benthamii* mortality at two months after treatment as a function of stem diameter at 137 cm height (diameter breast height, DBH).

Predictor	β	SE β	Wald χ^2	df	P
Intercept	2.3902	1.1183	4.5678	1	0.0326*
DBH (cm)	-0.3946	0.1376	8.2263	1	0.0041*
AMCP concentration [a]	0.1908	0.0612	9.7122	1	0.0018*
Application method [b]	-1.1556	0.7264	2.5308	1	0.1116
DBH × AMCP concentration	-0.0159	0.0075	4.5769	1	0.0324*

[a] The formulation used (DPX MAT28-159) contained 120 g ae L^{-1} AMCP.
[b] Basal bark is the reference level (i.e. application method = 1 for basal bark, application method = 0 for basal frill)
* Significant predictor at $\alpha = 0.05$

Table 3-6. Percentage of buffer (n = 411) and non-treated (n = 48) *Eucalyptus benthamii* trees that displayed symptoms of herbicide injury at 2, 6 and 12 months after treatment (MAT) in a study using aminocyclopyrachlor, imazapyr and triclopyr herbicides. Buffer trees were adjacent to treated trees

(1.5 m away). Non-treated trees were not adjacent to treated trees (> 1.5 m away).

	2 MAT		6 MAT		12 MAT	
Symptom	Buffer	Non-treated	Buffer	Non-treated	Buffer	Non-treated
	- -		- - - - % - - - -		- -	
Mortality	0	0	1	0	1	0
Necrosis	8	10	28*	13*	17	19
Defoliation	5	10	13	8	16	10
Red foliage	4	2	9	13	3	6
Chlorosis	7	10	15	25	4	0
Stem sap flow	0	0	1	0	0	0
Epinasty	0	0	2	2	7	6
Basal sprouting	0	0	1	2	1	0
Stem adventitious buds	0	0	0	0	0	0

* Significant differences at α = .0.05 between buffer and non-treated groups using Fisher's exact test.

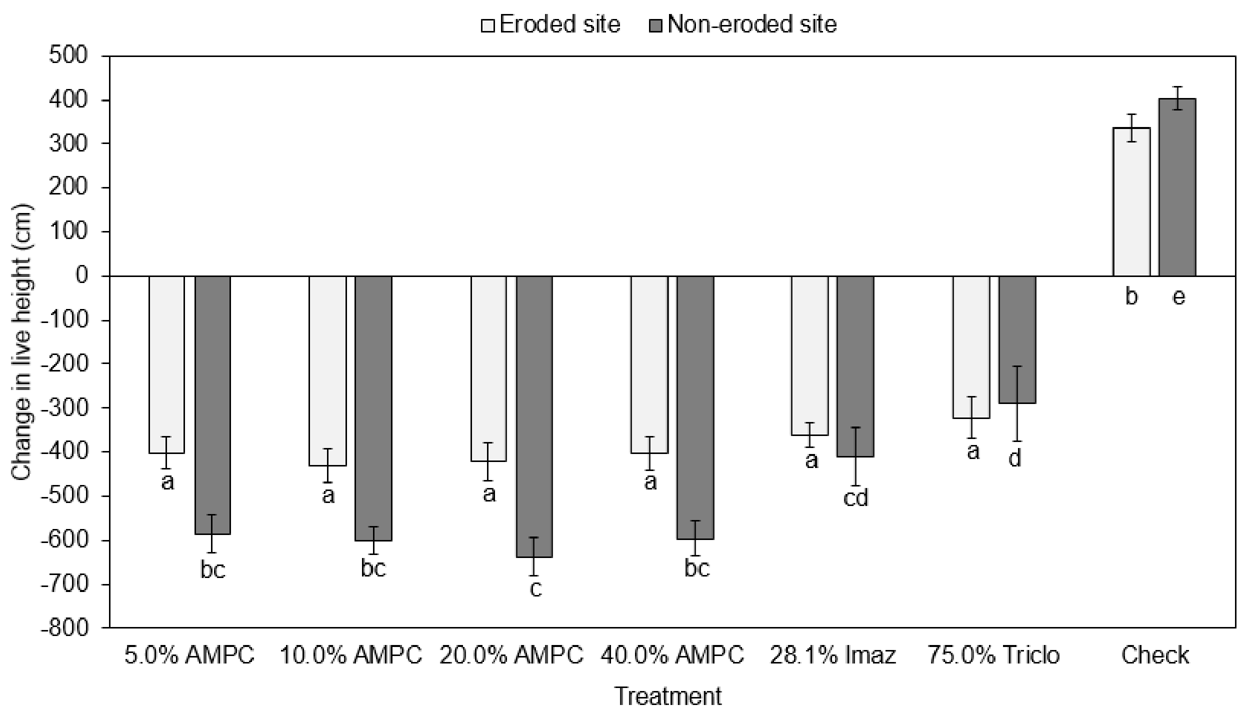

Figure 3-1. Change in *Eucalyptus benthamii* live stem height from pre-treatment values to 12 months after diameter specific basal bark treatments at the eroded (lighter bars) and non-eroded (darker bars) study sites. Treatments

included various concentrations of 120 g ae L^{-1} aminocyclopyrachlor (AMCP), 240 g ae L^{-1} imazapyr (imaz) and 480 g ae L^{-1} triclopyr ester (triclo) in methylated soybean oil carrier, as compared to a non-herbicide treated check receiving oil carrier alone. Error bars show the standard error of the mean. Treatments within each site that are noted with the same letter are not significantly different according to Tukey's HSD at $\alpha = 0.05$.

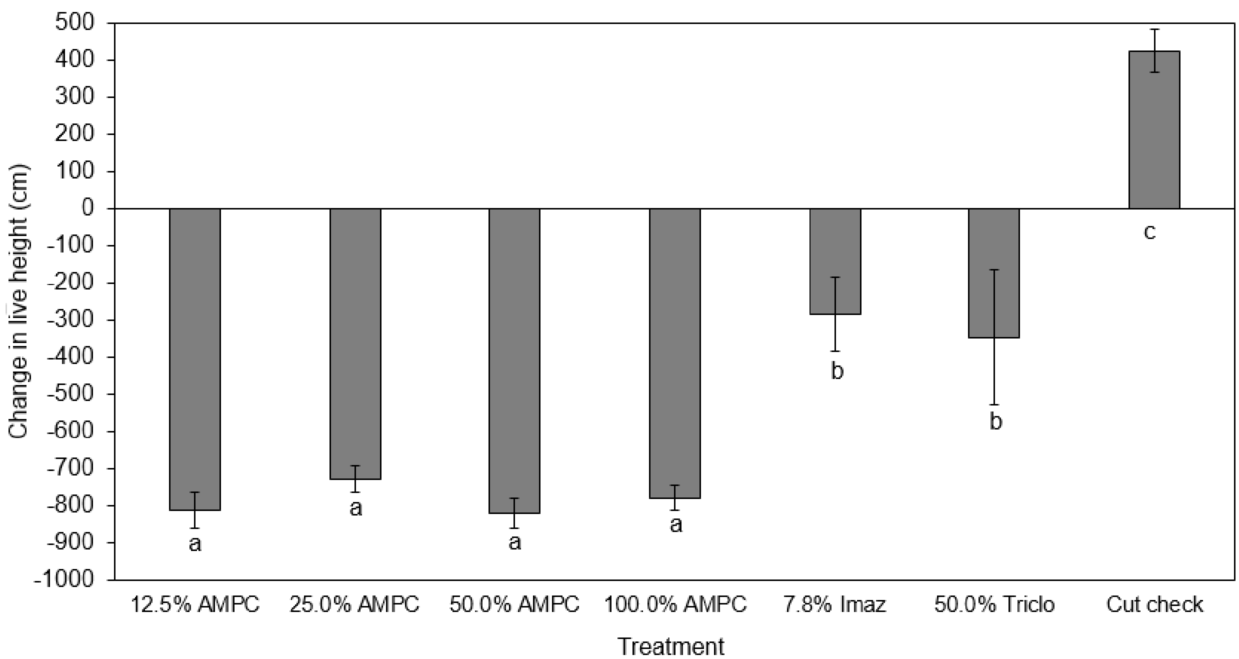

Figure 3-2. Change in *Eucalyptus benthamii* live stem height from pre-treatment values to 12 months after diameter specific basal frill treatments at the non-eroded study site. Treatments included various concentrations of 120 g ae L^{-1} aminocyclopyrachlor (AMCP), 240 g ae L^{-1} imazapyr (imaz) and 360 g ae L^{-1} triclopyr amine (triclo) in water carrier, as compared to a basal frilled non-herbicide treated check. Error bars show from the standard error of the mean. Treatments with the same letter are not significantly different according to Tukey's HSD at $\alpha = 0.05$.

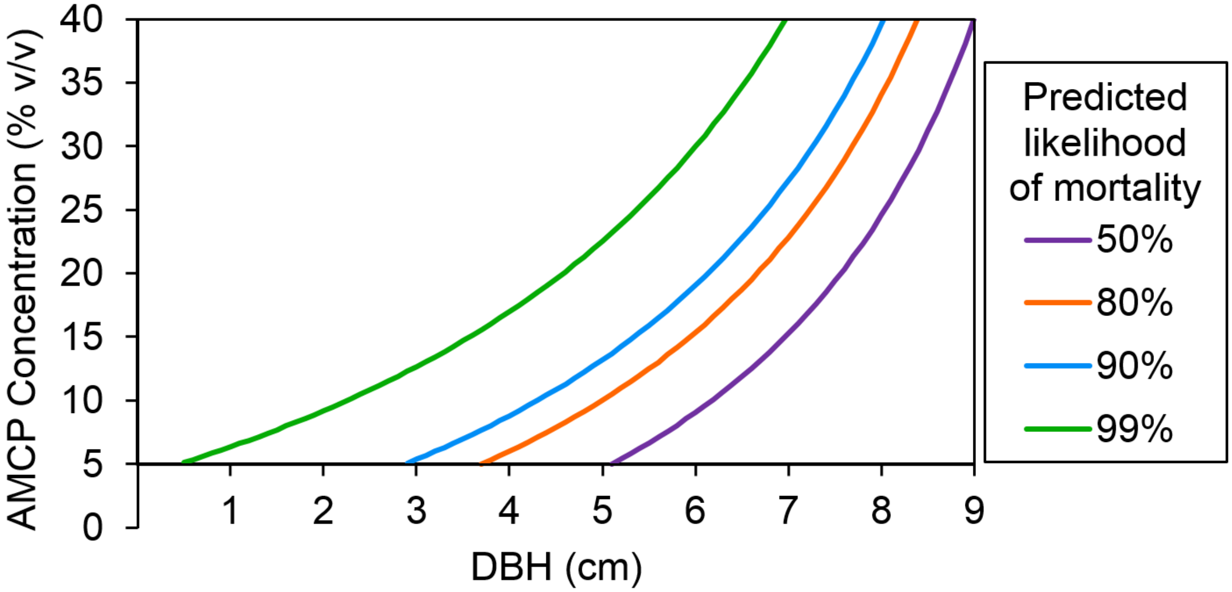

Figure 3-3. Relationship between *Eucalyptus benthamii* stem diameter at breast height (DBH, measured at 137 cm height) and applied concentration of 120 g ae L^{-1} aminocyclopyrachlor (AMCP) in methylated soybean oil carrier for the predicted likelihood of mortality at two months after diameter specific basal bark treatment, as determined using a logistic regression model. The AMCP concentrations that were tested ranged from 5% to 40%.

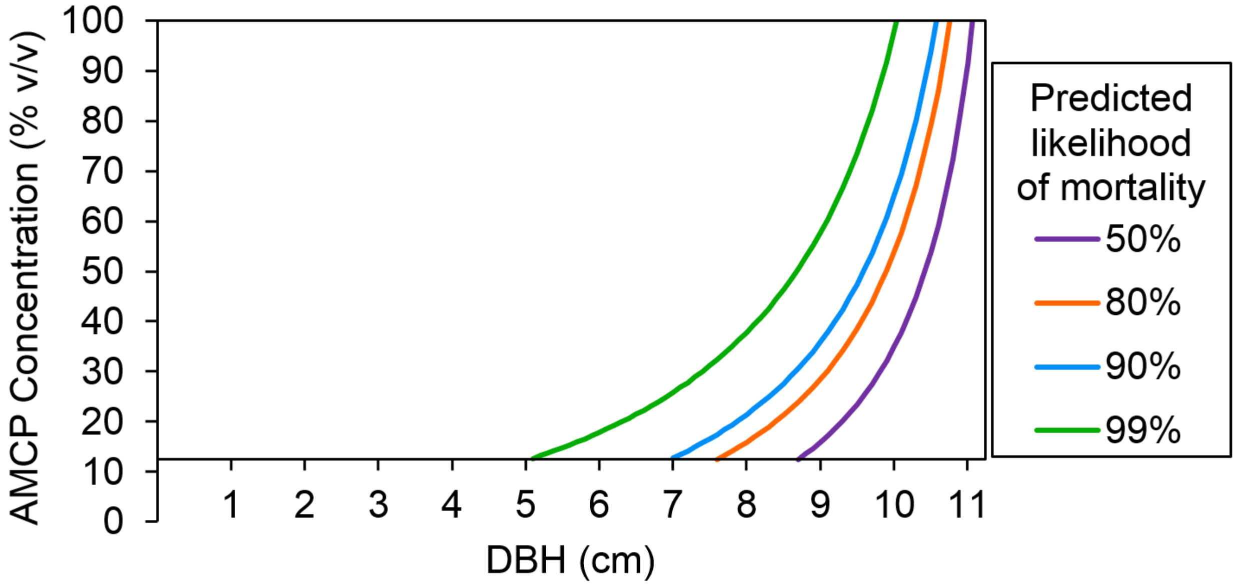

Figure 3-4. Relationship between *Eucalyptus benthamii* stem diameter at breast height (DBH, measured at 137 cm height) and applied concentration of 120 g ae L^{-1} aminocyclopyrachlor (AMCP) diluted in water for the predicted likelihood of mortality at two months after diameter specific basal frill treatment, as determined using a logistic regression model. The AMCP concentrations that were tested ranged from 12.5% to 100%.

CHAPTER 4
SUMMARY AND IMPLICATIONS FOR MANAGEMENT AND FUTURE RESEARCH

As hardy, fast growing grasses, trees and oil crops are being evaluated for an emerging bioenergy market, understanding the environmental issues associated with large-scale exotic bioenergy plantings has become a priority. In particular, the potential for invasiveness has emerged as a critically important issue. This thesis included two research approaches to examine the likelihood of important *Eucalyptus* species becoming invasive. Another component of the thesis research identified a new herbicide that is highly effective in controlling unwanted or invasive *Eucalyptus.*

In a study of the potential invasiveness of *Eucalyptus* in the southeastern US, the occurrence of *Eucalyptus* seedling recruitment within two geographically separate seed bearing *E. amplifolia* stands, and also in native and modified plant communities proximate to the stands, no *Eucalyptus* recruitment was found. The other study, was the first experiment to examine invasive potential of *Eucalyptus* in the southeastern US. Using an approach similar to the approach used in Brazil by da Silva et al. (2011), this study evaluated the invasive potential of three commercially important *Eucalyptus* species within the seed bearing *E. amplifolia* stands and in their proximate native and modified vegetation community types. The combined research findings demonstrated that *Eucalyptus* establishment and survival were generally low at the northern Florida locations, although greater success was sometimes observed for *E. camaldulensis* seedlings, in disturbed conditions and within *Eucalyptus* stands. These results support conclusions by Gordon et al. (2011, 2012) and suggest that caution is warranted regarding the cultivation of *E. camaldulensis* and concerning practices that might increase disturbance near *Eucalyptus* stands. The implications of this research are of particular relevance for the common practice of establishing a bare soil or vegetative buffer zone around *Eucalyptus* plantings to mitigate their spread. The demonstrated role of disturbance in facilitating *Eucalyptus* seedling recruitment suggests that bare soil buffer zones should be discontinued, and that a stable perennial plant community should be established instead. Considering that greater success was observed for *Eucalyptus* in Gainesville, the study location with higher rainfall and lower latitude, further experiments are also recommended for exploring the possibility for invasion in southern Florida and across sites with the widely varying rainfall patterns.

In the second component of this thesis, the potential for effective *Eucalyptus* control by a promising new herbicide chemistry was evaluated and compared to standard applications of imazapyr and triclopyr herbicides. Rate-response models were developed to predict the lowest effective AMCP rate for managing trees of various diameter sizes by two mo after treatment. Basal bark applications of 120 g ae L^{-1} AMCP at 5% v/v in methylated soybean oil resulted in 97–99% crown reduction of *Eucalyptus* and generally provided greater control than the standard 240 g ae L^{-1} imazapyr or 480 g ae L^{-1} triclopyr ester at 6 and 12 mo after treatment for all diameter classes. Similarly, basal frill applications of 120 g ae L^{-1} AMCP at 12.5% v/v in water resulted in 100% crown reduction of *Eucalyptus* and greater control than the standard imazapyr or 360 g ae L^{-1} triclopyr amine treatments at 6 and 12 mo after treatment for all diameter classes. In addition to the positive implications of these results for *Eucalyptus* control in the southeastern US, this research may be useful to land managers in other regions, especially in California and in South Africa where extensive *Eucalyptus* removal projects are ongoing.

The results of this research are valuable because implications regarding both invasion ecology and management are considered, thus contributing to a more comprehensive knowledge of the potential environmental challenges posed by *Eucalyptus* culture than research in either field would have allowed alone. Although the definitive outcome of widespread *Eucalyptus* introductions in the southeastern US cannot be determined, together the two components of this research suggest that the level of invasiveness demonstrated by *E. camaldulensis*, *E. amplifolia* and *E. grandis* is not overwhelming for northern and central Florida, and invading seedlings or unwanted populations could be effectively controlled using AMCP herbicide treatments.

APPENDIX A
POTENTIAL INVASION RISK MANAGEMENT PRACTICES FOR *EUCALYPTUS*

Table A-1. Practices to evaluate and manage potential invasiveness of *Eucalyptus* in the Southeastern US with respect to four commonly recognized phases of intervention for managing invasive species including prevention, containment, control and management of the impacted ecosystem. Supporting references for management practices and their advantages and limitations are indicated in parentheses.

Intervention type	Management practice	Goal of Practice	Advantages of practice	Limitations of practice
Prevention	Weed Risk Assessment (WRA) (1, 2)	Identify low/high risk taxa	Known level of accuracy (3). Widely tested (3). Time and cost efficient (4).	Cannot assess novel cultivars (5). Imprecision in context of biomass crops (5, 6). Assessor bias. Ambiguous ‘evaluate further’ conclusion.
	Experimental introductions (5, 7 – 9)	Identify low/high risk taxa and conditions favorable to growth, spread	Ability to assess novel cultivars (5). Can assess species for which WRA concludes ‘evaluate further’ (5, 9).	A novel tool in invasive context (5). Time and cost of experiments (5). Inexperience translating performance metrics into meaningful measures of invasion risk (5).
Containment	Selection for sterility or decreased fertility (10)	Reduce/ eliminate probability of seed dispersal	Replaces the need for complex ecological research to evaluate spread and predict impacts (11).	Costly high-risk research (12). Complex regulatory process (12). Intellectual property concerns (11). Effectiveness uncertain due to novel use in forestry (11). Only attempted in one cultivar (14).
	Avoid trait selection that complicates invasive control	Maintain ability to control escaped *Eucalyptus*	Little up-front cost.	Unknown if cold tolerance confers vigor that increases invasiveness. Does not affect traditionally bred trees.
	Harvest before seed maturation (5, 10)	Prevent seed dispersal	Potentially elimination of seed dispersal. Feasible to enforce.	Unpredictable seed maturation. Possible economic losses if harvest must occur before optimum economic rotation length (15).
	Buffer zones around plantings (5, 10, 16)	Reduce seed establishment.	Feasible to enforce maintenance of barrier. Low cost.	Disagreement over barrier size (5, 16) Does not account for long-distance dispersal due to hurricanes (17). Unknown level of compliance for monitoring.
	Clean harvesting equipment (18)	Reduce seed dispersal.	Reduces risk of spread from easily identifiable transportation vector.	Impractical enforcement. Decreasing efficiency over time (19). Does not affect natural dispersal.

Table A-1. Continued.

Intervention type	Management practice	Goal of Practice	Advantages of practice	Limitations of practice
	Monoclonal planting blocks (5)	Reduce hybridization. Decrease area vulnerable to seed dispersal.	Simple management, predictable and potentially increased yield from monoclonal plantings (20, 22)	Increased risk of severe losses from disease. Negative impacts on soil and biodiversity (20, 22).
Control	Mechanical control (22–24)	Removal of escaped seedlings	May be less injurious to environment compared to chemical control (22)	Labor intensive and costly (23). Unlikely elimination of regrowth (22–24).
	Chemical control (23–29)	Removal of escaped seedlings	Better control compared to mechanical methods.	Reapplication often necessary (23–26). Imprecise herbicide prescriptions (25, 28).
	Biocontrol (seeds) (30)	Reduce rate of spread	Growers can protect crops from damage. Self-perpetuating barrier to spread.	Lengthy regulatory process (31), costly to identify biocontrol agents (32), inability to protect seed supply (33), possible introduction of pathogens (34)
	Biocontrol (plants)	Reduce vigor and population size	Self-perpetuating barrier to spread	Lengthy regulatory process (31), costly to identify biocontrol agents (32), inability to protect crops from damage (33), possible introduction of pathogens (34)
Impact management	Ground and surface water protection (5, 10)	Reduce adverse ecological impacts	Planting away from waterways also reduces seed dispersal.	Limited water availability to *Eucalyptus* may negatively affect yield.
	Wildfire protection (5, 10, 35)	Reduce adverse ecological impacts	Bare soil fire break also reduces dispersal and establishment.	Labor and cost to reduce accumulated fuel load (23)
	Mitigate other ecological impacts	Reduce adverse ecological impacts	Management for key affected species may provide benefits to non-target species.	Impacts may not be easily quantified (36). May be incompatible with profitable *Eucalyptus* cultivation.

Supporting references: (1) Gordon et al. 2011; (2) Gordon et al. 2012; (3) Gordon et al. 2008; (4) Daehler et al. 2004; (5) Flory et al. 2012; (6) Barney and DiTomaso 2008; (7) da Silva et al. 2011; (8) Emer and Fonseca 2010; (9) Davis et al. 2010; (10) Booth 2012; (11) FAO 2010a; (12) Wang and Brummer 2012; (13) Strauss and Viswanath 2011; (14) Hinchee 2011; (15) Langholtz et al. 2005; (16) FAC 2008; (17) Browder and Schroeder 1981; (18) NISC 2009; (19) Leung et al. 2005; (20) DeBell and Harrington 1993;(21) Zalesny et al. 2011; (22) Bean and Russo 1989; (23) NPS 2006; (24) Little and van den Berg (2006); (25) Bossard et al. 2000; (26) Bachelard et al. 1965; (27) Little 2003; (28) Moore 2002; (29) Morze 1971; (30) Wilson et al. 2011b; (31) Montgomery 2011; (32) Hobbs and Humphries 1995; (33) van Wilgen et al. 2011; (34) Hoffmann et al. 2011; (35) Goodrick and Santurf 2012; (36) Simberloff et al. 2012.

APPENDIX B
AMINOCYCLOPYRACHLOR FOR CONTROL OF WOODY PLANTS: LITERATURE REVIEW

Table B-1. A list of reported research for the control of woody plant species using aminocyclopyrachlor (AMCP) herbicides is given below. The rate of AMCP tested, other herbicides included, methods of herbicide application and the outcome for control of each taxa are described as they were reported in the original references.

Tested taxa	AMCP rate [a]	Other herbicides in tank-mix [b]	Application method	Reported outcome	Reference
Acacia sp.	–	SU	Foliar	Excellent control	Rick et al. 2009
	–	–	Broadcast	Control	Alford et al. 2012
Acer negundo	–	SU	Foliar	Excellent control	Rick et al. 2009
Acer rubrum	–	SU	Foliar	Excellent control	Rick et al. 2009
	–	–	Foliar	Variable control	Ezell et al. 2012
	–	Imaz	Foliar	Excellent control	Ezell et al. 2012
Acropitlon repens	140 g ai ha^{-1}	–	Pre-emergence	26–37% control	Sebastian et al. 2011
Albizia julibrissin	8.75–70 g ha^{-1}	–	Foliar	53–100 % control 1 MAT	Koepke-Hill et al. 2012
Artesima spp.	180 g ai ha^{-1}	–	Broadcast	Growth reduced ≥81%	Hergert et al. 2011
Baccharis halimifolia	280 g ai ha^{-1}	–	Foliar	95–100% control	Ezell et al. 2012
	280 g ai ha^{-1}	Metsulfuron	Foliar	95–100% control	Ezell et al. 2012
Carya sp.	280–350 g product ha^{-1}	–	Foliar	70–80% control	Ezell et al. 2012
Celtis laevigata	–	SU	Foliar	Excellent control	Rick et al. 2009
Celtis occidentalis	–	SU	Foliar	Excellent control	Rick et al. 2009
Diospyros virginiana	280–350 g product ha^{-1}	–	–	> 90% control	Ezell et al. 2012
Elaeagnus angustifolia	140–280 g ai ha^{-1}	–	Foliar	99% control 1 YAT	Wilson et al. 2011b
	280 g ai ha^{-1}	Metsulfuron	Foliar	99% control 1 YAT	Wilson et al. 2011b
	5% v/v	–	Basal bark	99% control 1 YAT	Wilson et al. 2011b
	5–25% v/v	–	Cut stump	Sig. diff. from check	Edwards and Beck 2011
	15% v/v	–	Basal bark	Effective	Edwards and Beck 2011
Fraxinus sp.	–	–	Foliar	Control	Turner et al. 2009
	–	–	–	Poor control	Ezell et al. 2012

Table B-1. Continued.

Tested taxa	AMCP rate [a]	Other herbicides in tank-mix [b]	Application method	Reported outcome	Reference
Ilex vomitoria	2.5–15% v/v	–	Cut stump	97–99% control 1.5 YAT	Yeiser et al. 2011
	10% v/v	Triclo	Cut stump	100% control 540 DAT	Yeiser et al. 2011
Kochia scoparia	140–315 g ai ha^{-1}	SU	Foliar	Excellent control 1-12 MAT	Turner et al. 2009
Lantana camara	200 g ai ha^{-1}	–	Broadcast	98-100% 1 YAT	Ferrell et al. 2012
Liquidambar styraciflua	2.5–15% v/v	–	Cut stump	73–81% control 1.5 YAT	Yeiser et al. 2011
	10% v/v	Triclo	Cut stump	83% control 1.5 YAT	Yeiser et al. 2011
	–	–	Foliar	Poor control	Ezell et al. 2012
	–	Imaz	Foliar	Good/excellent control	Ezell et al. 2012
Morella cerifera	–	Triclo	–	Marginal control	Ezell and Yeiser 2010
Pinus sp.	–	–	–	Excellent control	Ezell and Yeiser 2010
Prosopis gradnulosia	–	–	Broadcast	Control	Alford et al. 2012
Prosopis sp.	–	SU	Foliar	Excellent control	Rick et al. 2009
Quercus alba	280–350 g product ha^{-1}	–	Foliar	Very good/excellent control	Ezell et al. 2012
	280–350 g product ha^{-1}	Imaz or Gly	Foliar	Very good/excellent control	Ezell et al. 2012
Quercus rubra	–	–	Foliar	Variable control	Ezell et al. 2012
	–	Imaz or Gly	Foliar	> 75% control	Ezell et al. 2012
Rhamnus cathartica	–	–	Foliar	Control	Turner et al. 2009
Rhus sp.	–	–	Foliar	Control	Ezell et al. 2012
Robinia sp.	–	–	Foliar	Control	Turner et al. 2009
Rosa multiflora	–	–	Foliar	Control	Turner et al. 2009
Sarcobatus	329 g ai ha^{-1}	–	Broadcast	≤ 50% mortality	LaFantasie et al. 2012
Symphoricarpos occidentalis	126 g ai ha^{-1}	Metsulfuron	Foliar	99% control 1 YAT	Wilson et al. 2011b
Tamarix spp.	5% v/v	–	Basal bark	99% control 1 YAT	Wilson et al. 2011b
	280 g ai ha^{-1}	–	Foliar	33% control 1 YAT	Wilson et al. 2011b
Triadica sebifera	2.5–15% v/v	–	Cut stump	77– 100% control 1.5 YAT	Yeiser et al. 2011
	10% v/v	Triclo	Cut stump	100% control 1.5 YAT	Yeiser et al. 2011

[a] A dash indicates that application rate was not described in the study report.
[b] Product formulations of glyphosate (gly), imazapyr (imaz), triclopyr (triclo), metsulfuron methyl (metsulfuron), or sulfonylurea herbicides (SU). A dash indicates no other herbicides were used in the tank mixture.

LITERATURE CITED

Adams, P., C. Beadle, N. Mendham, and P. Smethurst. 2003. The impact of timing and duration of grass control on growth of a young *Eucalyptus globulus* Labill. plantation. New Forest. 26:147–165.Alford, C. M., J. H. Meredith, E. P. Castner, and C. Medlin. 2012. Aminocyclopyrachlor: A New Active for Brush Control in Range and Pasture. Proc. West. Soc. Weed Sci. 65: 61-62. [Abstract]

Anderson, M. C., H. Adams, B. Hope, and M. Powell. 2004. Risk Assessment for Invasive Species. Risk Anal. 24:787–793.

Bachelard, E. P., A. Sarfaty, and P.M. Attiwill. 1965. Chemical Control of Eucalypt Vegetation. Aust. For. 29:181–191.

Barney, J. N., and J. M. DiTomaso. 2008. Nonnative Species and Bioenergy: Are We Cultivating the Next Invader? BioScience 58:1–7.

BASF Corporation. 2008. Stalker® herbicide product label. Research Triangle Park, NC. 9 p.

Bean, C., and M. J. Russo. 1989. Environmental Stewardship Abstract for *Eucalyptus globulus*. Arlington, VA: The Nature Conservancy. 21 p.

Becerra, P. I., and R. O. Bustamente. 2008. The effect of herbivory on seedling survival of the invasive exotic species *Pinus radiata* and *Eucalyptus globulus* in a Mediterranean ecosystem of Central Chile. Forest. Ecol. Manag. 256:1573–1578.

Bergerud, W.A. 1988. Dose-Response Models for Stand Thinning with the “Ezject” Herbicide Injection System. Victoria, BC, Canada: Ministry of Forests and Lands Research Note 102. 28 p.

Blazier, M. A., J. Johnson, E. L. Taylor, and B. Osbon. 2012. Herbicide site preparation and release options for *Eucalyptus* plantation establishment in the western gulf. Pages 19–23 in J. R. Butnor, ed. Proceedings of the 16th Biennial Southern Silvicultural Research Conference. Asheville, NC: USDA, Forest Service, Southern Research Station e-Gen. Tech. Rep. SRS-156.

Boland, D. J. 1986. Testing and Storage of *Eucalyptus* and *Acacia* Seed. Pages 75–94 in R. D. Ayling, B.R.T. Seward, eds., Proceedings of a Workshop on Seed Handling and Eucalypt Taxonomy. Harare, Zimbabwe.

Booth, T. H. 2013. Eucalypts and Their Potential for Invasiveness Particularly in Frost-Prone Regions. Int. J. For. Res. doi:10.1155/2012/837165.

Bossard, C. C., J. M. Randall, and M. C. Hoshovsky. 2000. Invasive Plants of California’s Wildlands. Berkeley, CA: University of California Press.www.cal-ipc.org. Accessed: January 22, 2013.

Bradley, B. A., D. M. Blumenthal, D. S. Wilcove, and L. H. Ziska. 2010. Predicting plant invasions in an era of global change. Trends Ecol. Evol. 25:310–18.

Browder, J. A., and P. B. Schroeder. 1981. Melaleuca seed dispersal and perspective on control. Pages 17–21 in R. K. Geiger, ed., Proceedings of the Melaleuca Symposium. Ft. Myers, Florida: Florida Dept. of Agriculture and Consumer Services, Division of Forestry, Tallahassee.

Buddenhagen, C. E., C. Chimera, and P. Clifford. 2009. Assessing Biofuel Crop Invasiveness: A Case Study. PLoS ONE 4:1–6.

Callaham Jr., M. A., J. A. Stanturf, W. J. Hammond, D. L. Rockwood, E. S. Wenk, and J. J. O'Brien. 2013. Survey to Evaluate Escape of *Eucalyptus* spp. Seedlings from Plantations in Southeastern USA. Int. J. For. Res. doi:10.1155/2013/946374.

Carillo-Gavilán, A., J. M. Espelta, and M. Vilà. 2012. Establishment constraints of an alien and a native conifer in different habitats. Biol. Invasions 13:1279–1289.

Casler, M. D., K. P. Vogel, C. Taliaferro and R. L. Wynia. 2004. Latitudinal adaptation of switchgrass populations. Crop. Sci. 44:293–303.

Chimera, C. G., C. E. Buddenhagen, and P. M. Clifford. 2010. Biofuels: the risks and dangers of introducing invasive species. Biofuels 1:785–596.

Cremer K. W.1977. Distance of seed dispersal in Eucalypts estimated from seed weights. Aust. For. Restor. 7:225–228.

Cribbie, R. A., R. R. Wilcox, C. Bewell, and H. J. Keselman. 2007. Tests for Treatment Group Equality When Data are Nonnormal and Heteroscedastic. J. Mod. Appl. Statistical Method. 6:117–132.

da Silva, P.H.M., F. Poggiani, A. M. Sebbenn, and E. S. Mori. 2011. Can *Eucalyptus* invade native forest fragments close to commercial stands? Forest Ecol. Manag. 26:2075–2080.

Daehler, C. C., J. S. Denslow, S. Ansari, and H. C. Kuo. 2004. A Risk-Assessment System for Screening out Invasive Pest Plants from Hawaii and Other Pacific Islands. Conserv. Biol. 18:360–368.

Davis, A. S., R. D. Cousens, J. Hill, R. N. Mack, D. Simberloff, and S. Raghu. 2010. Screening bioenergy feedstock crops to mitigate invasion risk. Frontiers Ecol. Environ. 8:533–539.

Davis, P. B., F. D. Menalled, R.K.D. Peterson, and B. D. Maxwell. 2011. Refinement of weed risk assessments for biofuels using *Camelina sativa* as a model species. J. Appl. Ecol. 48:989–997.

DeBell, D. S., and C. A. Harrington. 1993. Deploying genotypes in short-rotation plantations: Mixtures and pure cultures of clones and species. Forest. Chron. 69:705–713.

Dodet, M., and C. Collet. 2012. When should exotic forest plantation tree species be considered as an invasive threat and how should we treat them? Biol. Invasions 14:1765–1778.

Dougherty, D., and J. Wright. 2012. Silviculture and economic evaluation of Eucalypt plantations in the southern US. BioResources 7:1994–2001.

Dow AgroSciences LLC. 2003. Garlon® 3A herbicide product label. LOES No. 010-00084. Indianapolis, IN. 9 p.

Dow AgroSciences LLC. 2008. Garlon® 4 Ultra herbicide product label. LOES No. 010-02127. Indianapolis, IN. 9 p.

Edwards, R. J., and K. G. Beck. 2011. Control of Russian Olive Through Cut Stump and Basal Bark Herbicide Applications. Proc. West. Soc. Weed Sci. 64:70–71. [Abstract]

E.I. du Pont de Nemours and Company. 2009. DuPont DPX-MAT28 herbicide. Technical Bulletin No. K15023. Wilmington, DE. 7 p.

Emer, C., and C. R. Fonseca. 2010. Araucaria Forest conservation: mechanisms providing resistance to invasion by exotic timber trees. Biol. Invasion 13:189–202.

Environmental Law Institute. 2010. Status and Trends in State Invasive Species Policy: 2002-2009. Washington, D.C. http://100thmeridian.org/documents/ELI_Invasive_Species_State_Policy_Report_05_2010.pdf. Accessed: December 6, 2012.

Ewel, J. J., D. J. O'Dowd, J. Bergelson, C. C. Daehler, C. M. D'Antonio, L. D. Gómez, D. R. Gordon, R. J. Hobbs, A. Holt, K. R. Hopper, C. E. Hughes, M. LaHart, R.R.B. Leakey, W. G. Lee, L. L. Loope, D. H. Lorence, S. M. Louda, A. E. Lugo, P. B. McEvoy, D. M. Richardson, and P. M. Vitousek. Deliberate introductions of species: research needs. Bioscience 49:619–30.

Ezell A. W., R. Turner, and J. L. Yeiser. 2012. Use of Aminocyclopyrachlor for forestry site preparation in the southeastern U.S. Pages 120–121 in J. R. Butnor, ed., 2012. Proceedings of the 16th Southern Silvicultural Research Conference. Asheville, NC: USDA, Forest Service, Southern Research Station e-Gen. Tech. Rep. SRS-156.

Ezell, A. and J. L. Yeiser. 2010. Aminocyclopyrachlor (MAT28) for brush control. http://conference.ifas.ufl.edu/aw10/presentations/Wed/Session%20B/1340%20Ferrell.pdf. Accessed: January 23, 2012. [Presentation]

[FAC] Florida Administrative Code. 2008. 5B-57.011 Biomass Plantings. Pages 6 – 7 in Introduction or Release of Plant Pests, Noxious Weeds, Arthropods, and Biological

Control Agents. Gainesville, FL: Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry.

[FAO] Food and Agriculture Organization of the United Nations. 2010a. Forests and Genetically Modified Trees. Rome, Italy: FAO. 240 p.

[FAO] Food and Agriculture Organization of the United Nations. 2010b. Eucalyptus in East Africa socio-economic and environmental issues. Planted Forests and Trees Working Papers. Rome, Italy: FAO. 30 p.

Federal Register. 1999. Executive Order 13112 of February 3, 1999–Invasive Species. Federal Register, Feb 8, 1999. 64(25):6183-6186.

Ferrell, J., B. Sellers, and E. Jennings. 2012. Herbicidal Control of Largeleaf Lantana (*Lantana camara*). Weed Technol. 26:554–558.

Flory, S. L., K. A. Lorentz, D. R. Gordon, and L. E. Sollenberger. 2012. Experimental approaches for evaluating the invasion risk of biofuel crops. Environ. Res. Lett. doi:10.1088/1748-9326/7/4/045904.

Garau, A. M., C. M. Ghersa, J. H. Lemcoff, and J. J. Barañao. 2009. Weeds in *Eucalyptus globulus* subsp. *maidenii* (F. Muell) establishment: effects of competition on sapling growth and survivorship. New Forest. 37:251–264.

Gonzalez, R., T. Treasure, J. Wright, D. Saloni, R. Phillips, R. Abt, and H. Jameel. 2011. Exploring the potential of *Eucalyptus* for energy production in the Southern United States: Financial analysis of delivered biomass. Part I. Biomass Bioenerg. 35:755–766.

Goodrick, S. L., and J. A. Stanturf. 2012. Evaluating Potential Changes in Fire Risk from *Eucalyptus* Plantings in the Southern United States. Int. J. For. Res. doi:10.1155/2012/680246.

Gootee, R. S., E. P. Weber, K. Blatner, M. Carroll, and D. Baumgartner. 2012. Regulation, Knowledge Transfer, and Forestry Policy Implementation: Different Strokes for Different Folks? Sustain. Ag. Res. 155–65.

Gordon, D. R., D. A. Onderdonk, A. M. Fox, and R. K. Stocker. 2008. Consistent accuracy of the Australian weed risk assessment system across varied geographies. Divers. Distrib. 14:234–242.

Gordon, D. R., S. L. Flory, A. L. Cooper, and S. K. Morris. 2012. Assessing the Invasion Risk of *Eucalyptus* in the Unites States using the Australian Weed Risk Assessment. Int. J. For. Res. doi:10.1155/2012/203768.

Gordon, D.R., K. J. Tancig, D. A. Onderdonk, and C. A. Gantz. 2011. Assessing the invasive potential of biofuel species proposed for Florida and the United States using the Australian Weed Risk Assessment. Biomass Bioenerg. 35:74–79.

Gunsolus, J. L., and W. S. Curran. 1999. Herbicide Mode of Action and Injury Symptoms. North Central Regional Publication 377.

Harcourt, R. L., J. Kyozuka , R. B. Floyd, K. S. Bateman, H. Tanaka, V. Decroocq, D. J. Llewellyn, X. Zhu, W. J. Peacock, and E. S. Dennis. 2000. Insect- and herbicide-resistant transgenic eucalypts. Mol. Breeding 6:307–315.

Harfouche, A., R. Meilan, and A. Altman. 2011. Tree genetic engineering and applications to sustainable forestry and biomass production. Trends. Biotechnol. 29:9–17.

Hergert, H. J., B. Mealor, R. D. Mealor, and A. D. Kniss. 2011. Seedling Response of 27 Native Species and 2 Exotic Weeds to Aminocyclopyrachlor. Proc. West. Soc. Weed Sci. 64:22. [Abstract]

Hinchee, M., C. Zhang, S. Chang, M. Cunningham, W. Hammond, and N. Nehra. 2011. Biotech *Eucalyptus* can sustainably address society's need for wood: the example of freeze tolerant *Eucalyptus* in the southeastern U.S. BMC Proc. 5 (Suppl 7):I24.

Hobbs, R. J., and S. E. Humphries. 1995. An integrated approach to the ecology and management of invasive plants. Conserv. Biol. 9:761–770.

Hodges, A. W., T. J. Stevens, and M. Rahmani. 2010. Economic Impacts of Expanded Woody Biomass Utilization on the Bioenergy and Forest Products Industries in Florida. Sponsored project final report to FDACS Division of Forestry. Gainesville, FL: University of Florida, IFAS, Food and Resource Economics Department. 36 p.

Hodgson, L. M. 1976. Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden at JDM Keet Forest Research Station formally Zomerkomst Forest Research Station. S. Afr. For. J. 97:18–28.

Hoffmann, J. H., V. C. Moran, and B. W. van Wilgen. 2011. Prospects for the biological control of invasive *Pinus* species (Pinaceae) in South Africa. Afr. Entomol. 19:393–401.

Hulme, P. E. 2011. Weed risk assessment: a way forward or a waste of time? J. Appl. Ecol. 49: 10–19.

Humber, J. M., and L. Hermanutz. 2011. Impacts of non-native plant and animal invaders on gap regeneration in a protected boreal forest. Biol. Invasions 13:2361–2377.

[IFAS] Institute of Food and Agricultural Sciences. 2012. Florida Automated Weather Network. fawn.ifas.ufl.edu/. Accessed: January 10, 2013.

Jenkins, P. T. 2012. Invasive animals and wildlife pathogens in the United States: the economic case for more risk assessments and regulation. Biol. Invasions doi:10.1007/s10530-012-0296-8.

Koepke-Hill, R., G. R. Armel, J. T. Brosnan, G. K. Breeden, J. J. Vargas, and T. C. Mueller. 2012. Control of Silk Tree (*Albizia julibrissin*) with Aminocyclopyrachlor and Other Herbicides. Weed Sci. 60:345–349.

Kull, C. A., and J. Tassin. 2012. Australian acacias: useful and (sometimes) weedy. Biol. Invasions doi:10.1007/s10530-012-0244-7.

LaFantasie, J. J., B. A. Mealor, and A. R. Kniss. 2012. Black Greasewood Community Response to Aminocyclopyrachlor. Proc. West. Soc. Weed Sci. 65: 25–26. [Abstract]

Langholtz, M., D. R. Carter, D. L. Rockwood, J.R.R. Alavalapati, and A. Green. 2005. Effect of dendroremediation incentives on the profitability of short-rotation woody cropping of *Eucalyptus grandis*. Forest Policy Econ. 7:806–817.

Le Maitre, D. C., B. W. van Wilgen, C. M. Gelderblom, C. Bailey, R. A. Chapman, and J. A. Nel. 2002. Invasive alien trees and water resources in South Africa: case studies of the costs and benefits of management. For. Ecol. Manag. 160: 143–159.

Ledgard, N. 2001. The spread of lodgepole pine (*Pinus contorta*, Dougl) in New Zealand. For. Ecol. Manag.141:43–57.

Lee, C. E. 2002. Evolutionary genetics of invasive species. Trends Ecol. Evol. 17:386–391.

Leung, B., D. M. Lodge, D. Finnoff, J. F. Shogren, and D. Lodge. 2005. Managing invasive species: Rules of thumb for rapid assessment. Ecol. Econ. 55:24–36.

Lewandowski, I., and A. Faaij. 2006. Steps towards the development of a certification system for sustainable bio-energy trade. Biomass Bioenerg. 30:83–104.

Li, J., J. A. Duggin, C. D. Grant, and W. A. Loneragan. 2003. Changes in the early survival of *Eucalyptus blakelyi* in grasslands of the New England Tablelands, NSW, Australia. Forest Ecol. Manag.173:319–334.

Little, D. L. and D. L. Shaner. 1991. Absorption and translocation of the imidazolinone herbicides. Pages 53–69 in D. L. Shaner and S. L. O'Connor, eds., The Imidazolinone Herbicides. CRC Press, Boca Raton, FL. Little, K. M. 2003. Killing *Eucalyptus grandis* cut stumps after multiple coppice rotations in the KwaZulu-Natal midlands, South Africa. S. Afr. For. J. 199:7–13.

Little, K. M., and G. J. van den Berg. 2006. First rotation *Eucalyptus macarthurii* cut stump control in KwaZulu-Natal. S. Afr. For. J. 207:15–20.

Lockwood, J., M. Hoopes, and M. Marchetti. 2007. Invasion Ecology. Malden, MA: Blackwell Publishing. p 219.

Lockwood, J.L.L., D. Simberloff, M. L. McKinney, and B. Von Holle. 2001. How many, and which, plants will invade natural areas? Biol. Invasions 3:1–8.

Low, T. 2012. Australian acacias: weeds or useful trees? Biol. Invasions 14:2217–2227.

Lowery, R. F., and D. H. Gjerstad. 1991. Chemical and mechanical site preparation. Pages 251-262 in M. L. Duryea, and P. M. Dougherty, eds., Forest Regeneration Manual. Dordrecht, Netherlands: Kluwer Academic Publishers.

McCormick, N., and G. Howard. 2013. Beating back biofuel crop invasions: Guidelines on managing the invasion risk of biofuel developments. Renew. Energ. 49:263–266.

Miller, J. H., and G. R. Glover, eds. 1991. Standard Methods for Forest Herbicide Research. Champaign, IL: Southern Weed Science Society. 68 p.

Miller, J. H., S. T. Manning, and S. F. Enloe. 2010. A Management Guide for Invasive Plants in Southern Forests. Asheville, NC: USDA, Forest Service, Southern Research Station e-Gen. Tech. Rep. SRS-156.120 p.

Minton, M. S., and R. N. Mack. 2010. Naturalization of plant populations: the role of cultivation and population size and density. Oecologia 164: 399–409.

Montgomery, M. E. 2011. Understanding Federal Regulations as Guidelines for Classical Biological Control Programs. Pages 25–42 in B. Onken, and R. Reardon, eds., Implementation and Status of Biological control of the Hemlock Wooly Adelgid. USDA, Forest Service Publication FHTET-2011-04.

Moore, K. 2008. Eradicating *Eucalyptus*, *Acacia* and Other Invasive Trees. Capitola, CA: Wildlands Restoration Team. 5 p.

Morze, J. 1971. Chemical control of *Eucalyptus* species. For. S. Afr. 12:49–53.

Myers, R. L. 1983. Site susceptibility to invasion by the exotic tree *Melaleuca quinquenervia* in southern Florida. J. Appl. Ecol. 20: 645–648.

Naval Meteorology and Oceanography Command. 2012. Complete Sun and Moon Data for One Day: U.S. Cities and Towns. Stennis Space Center, MS. http://aa.usno.navy.mil/data/docs/RS_OneDay.php. Accessed: May 15, 2012.

[NISC] National Invasive Species Council. 2009. Biofuels: Cultivating Energy, not Invasive Species. Washington, DC: National Invasive Species Council Paper 11. 4 p.

[NOAA] National Oceanic and Atmospheric Administration. 2002. Monthly Station Normals of Temperature, Precipitation, and Heating and Cooling Degree Days 1971–2000. In Climatography of the United States No. 81.08–Florida. Asheville, NC: NOAA National Climatic Data Center and National Environmental Satellite, Data, and Information Service. http://cdo.ncdc.noaa.gov/climatenormals/clim81/FLnorm.pdf. Accessed: June 10, 2011.

[NPS] National Park Service 2006. Eucalyptus, a Transcontinental Legacy. Fire management, Resource Protection, and the Challenges of Tasmanian Blue Gum.

Washington, DC: US Department of the Interior, NPS. http://biomass.forestguild.org/casestudies/1001/Eucalyptus.pdf. Accessed: December 6, 2012.

Osiecka, A. and P. J. Minogue. 2011. Preliminary results: Development of selective herbicide treatments for establishment of *Eucalyptus urograndis* (FTE) and *Eucalyptus benthamii* plantations. Quincy, FL: Institute of Food and Agricultural Sciences, North Florida Research and Education Center Research Report 2011-01.14 p.
Osiecka, A. and P. J. Minogue. 2012. Selective Herbicides for Bald Cypress Restoration and Cultivation. Weed Tech. 26:460–468.

Pagni, P. J. 1993. Causes of the 20 October 1991 Oakland Hills Conflagration. Fire Safety J. 2:331–339.

Paine, T. D., M. J. Steinbauer, and S. A. Lawson. 2011. Native and Exotic Pests of *Eucalyptus*: A Worldwide Perspective. Annu. Rev. Entomol. 56:181–201.

Panetta, F. D. 1993. A system for assessing proposed plant introductions for weed potential. Plant. Prot. Q. 8:10–14.

Parker, I. M., D. Simberloff, W. M. Lonsdale, K. Goodell, M. Wonham, P. M. Kareiva, M. H. Williamson, B. Von Holle, P. B. Moyle, J. E. Byers, and L. Goldwasser. 1999. Impact : Toward a Framework for Understanding the Ecological Effects of Invaders. Biol. Invasions 1:3–19.

Parker, I. M., and P. Kareiva. 1996. Assessing the risks of invasion for genetically engineered plants: acceptable evidence and reasonable doubt. Biol. Conserv. 78:196–208.

Raghu, S., R. C. Anderson, C. C. Daehler, A. S. Davis, R. N. Wiedenmann, D. Simberloff, and R. N. Mack. 2006. Adding Biofuels to the Invasive Species Fire? Science 313:1742.

Pheloung, P. C., P. A. Williams, and S. R. Halloy. 1999. A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. J. Environ. Manage. 57:239–251.

Ramensteiner, E., and M. Simula. 2002. Forest certification – an instrument to promote sustainable forest management? J. Env. Manage.67:87–98.

Rejmánek, M., and D. M. Richardson. 2011. Eucalypts. Pages 203–209 in D. Simberloff, and M. Rejmánek, eds., Encyclopedia of Biological Invasions. Berkeley, CA: University of California Press.

Richardson, D. M. 1998. Forestry trees as invasive aliens. Conserv. Biol. 12:18–26.

Richardson, D. M., and M. Rejmánek. 2011. Trees and shrubs as invasive alien species - a global review. Divers. Distrib. 17:788–809.

Richardson, D. M., and R. Blanchard. 2011. Learning from our mistakes: minimizing problems with invasive biofuel plants. Curr. Opin. Environ. Sustain. 3:36–42.

Rick, S. K., R. G. Turner, J. R. Pitts, E. Hidalgo, and J. C. Claus. 2009. Aminocyclopyrachlor Blend Products for Brush and Weed Control on Utility and Roadside Rights-of-Way. North Central Weed Sci. Soc. Proc. 64:129. [Abstract]

Rockwood, D., D. Carter, M. Langholtz, and J. Stricker. 2006. *Eucalyptus* and *Populus* short rotation woody crops for phosphate mined lands in Florida USA. Biomass Bioenerg. 30:728–734.

[RSB] Roundtable on Sustainable Biofuels 2010. RSB Principles and Criteria for Sustainable Biofuel Production. RSB-STD-01-001 Version 2.0. http://rsb.org/pdfs/standards/11-03-08-RSB-PCs-Version-2.pdf. Accessed: December 6, 2012.

Sax, D. F. 2002. Equal diversity in disparate species assemblages: a comparison of native and exotic woodlands in California. Global. Ecol. Biogeog. 11:49–57.

Scarlat, N., and J.F. Dallemand. 2011 Recent developments of biofuels/bioenergy sustainability certification: A global review. Energ. Policy 39:1630–1646.

Sebastian, J. R., J. G. Beck, S. Nissen, D. Sebastian, and S. Rodgers. 2011. Native Species Establishment on Russian Knapweed Infested Rangeland Following Pre-Plant Herbicides Applications. Proc. West. Soc. Weed Sci. 64:16. [Abstract]

Senbeta, F., A. Seyoum, and T. Woldemariam. 2010. Is *Eucalyptus* farming a blessing or a curse in the central highlands of Ethiopia? Analysis from a farmer's perspective. Pages 160-170 in W. Tadesse, E. Tolosana, and R. López, eds., *Eucalyptus* Species Management, History, Status and Trends in Ethiopia. Proceedings from the Congress held in Addis Ababa.

Senseman, S. A., ed. 2007. Herbicide Handbook. Weed Science Society of America. Lawrence, KS. Pp. 244-245, 360.

Simberloff, D., J. L. Martin, P. Genovesi, V. Maris, D. A. Wardle, J. Aronson, F. Courchamp, B. Galil, E. García-Berthou, M. Pascal, P. Pyŝek, R. Sousa, E. Tabacchi, and M. Vilà. 2012. Impacts of biological invasions: what's what and the way forward. Trends Ecol. Evol. 1578:1–9.

Strauss, S. H., and V. Viswanath. 2011. Field trials of GM trees in the USA: activity and regulatory developments. BMC Proc. 5 (Suppl 7):O61.

Turner R. G., J. R. Pitts and D. D. Ganske. 2009. Aminocyclopyrachlor blend products for vegetation management on railroad and utility sites. Proc. North Central Weed Sci. Soc. 64:130. [Abstract]

[USDA] United States Department of Agriculture, Agricultural Research Service. 2012a. Plant Hardiness Zone Map. http://planthardiness.ars.usda.gov/PHZMWeb/. Accessed: January 10, 2013.

[USDA] United States Department of Agriculture, Forest Service. 2008. Agriculture Handbook 727: The Woody Plant Seed Manual. Pp. 504–512.

[USDA] United States Department of Agriculture, Forest Service. 2012b. Aminocyclopyrachlor Human Health and Ecological Risk Assessment Final Report. Morgantown, WV: Forest Health Technology Enterprise Team SERA TR-056-01-03a. 212 p.

van Wilgen, B. W., C. Dyer, J. H. Hoffmann, P. Ivey, D. C. Le Maitre, J. L. Moore, D. M. Richardson, M. Rouget, A. Wannenburgh, and J.R.U. Wilson. 2011. National-scale strategic approaches for managing introduced plants: insights from Australian acacias in South Africa. Divers. Distrib. 17:1060–1075.

van Wilgen, B. W., G. G. Forsyth, D. C. Le Maitre, A. Wannenburgh, J.D.F. Kotzé, E. van den Berg, and L. Henderson. 2012. An assessment of the effectiveness of a large, national-scale invasive alien plant control strategy in South Africa. Biol. Cons. 148:28–38.

Virtue, J. G., and R. L. Melland. 2003. The Environmental Weed Risk of Revegetation and Forestry Plants. Adelaide, South Australia: Department of Water, Land and Biodiversity Conservation Report DWLBS2003/02. 184 p.

Wang, Z., and C. E. Brummer. 2012. Is genetic engineering ever going to take off in forage, turf and bioenergy crop breeding? Ann. Bot. -London doi:10.1093/aob/mcs027.

Westra, P., S. Nissen, D. Shaner, B. Lindenmayer, and G. Brunk. 2009. Invasive Weed Management with Aminocyclopyrachlor in the Central Great Plains. Proc. Weed Sci. Soc. Am. 49:407. [Abstract]

Wevill, T., and J. Read. 2010. Fine-scale patterns in the distribution of semi-arid tree species at Wyperfeld National Park, southeastern Australia – the potential roles of resource gradients vs. disturbance. J. Arid Environ. 74:482–490.

Williamson, M. 1993. Invaders, weeds and the risk from genetically manipulated organisms. Experentia. 49:219–224.

Wilson, J.R.U., C. Garifo, M. R. Gibson, M. Arianoutsou, B. B. Bakar, S. Baret, L. Celesti-Grapow, J. M. DiTomaso, J. Dufour-Dror, C. Kueffer, C. A. Kull, J. H. Hoffman, F.A.C. Impson, L. L. Loope, E. Marchante, H. Marchante, J. L. Moore, D. J. Murphy, J. Tassin, A. Witt, R. D. Zenni, and D. M. Richardson. 2011a. Risk assessment, eradication, and biological control: global efforts to limit Australian acacia invasions. Divers. Distrib. 17:1030–1046.

Wilson, R., G. Sbatella, G. and S. Young. 2011b. New Herbicides for Managing Invasive Plants in Range, Pasture, and Riparian Areas. Lincoln, NE: University of Nebraska Crop Production Clinics. 2 p.

Witt, A.B.R. 2010. Biofuels and invasive species from an African perspective - a review. GCB Bioenerg. 2:321–329.

Wunderlin, R. P., and B. F. Hansen. 2008. Atlas of Florida Vascular Plants. Tampa, FL: Institute for Systematic Botany, University of South Florida. http://florida.plantatlas.usf.edu/. Accessed: December 6, 2012.

Yeiser, J. L., M. Link and J. Grogan. 2011. Screening Cut-Stump Control of Chinese Tallowtree, Sweetgum, and Yaupon with Aminocyclopyrachlor. Pages 389–393 in J. R. Butnor, ed., Proceedings of the16th Biennial Southern Silvicultural Research Conference. Asheville, NC: USDA, Forest Service, Southern Research Station e-Gen. Tech. Rep. SRS-156.

Zalesny, R. S., R. W. Cunningham, R. B. Hall, J. Mirck, and D. L. Rockwood, J. Santurf, and T. A. Volk. 2011. Woody Biomass from Short Rotation Energy Crops. Pages 27–63 in J. Zhu, ed., Sustainable Production of Fuels, Chemicals, and Fibers from Forest Biomass. Washington , DC: American Chemical Society.

BIOGRAPHICAL SKETCH

Kimberly Lorentz was born and raised in Cleveland, Ohio. She received her Bachelor of Science degree in biology from the University of Akron in 2011. As an undergraduate research assistant there, she contributed to projects in the fields of genetics, biomechanics, animal behavior and plant ecology. In the summer of 2010, she took an in internship at the Rocky Mountain Biological Laboratory in Colorado, where she completed research for her undergraduate honors thesis that compared nectar and pollen rewards for pollinators across native and invasive confamilial plants. Her time at Rocky Mountain Biological Laboratory inspired her to enroll in the graduate program at the University of Florida to further pursue her interest in invasive plant ecology and management. Kimberly received her Master of Science in forest resources and conservation from the University of Florida in the spring of 2013.