Multi-scale population genetic analysis of cogongrass (*Imperata cylindrica*) in the southeastern United States: introduction history, range expansion, and hybridization

By

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Biological invasions are a significant area of research due to perceived negative environmental and economic impacts. The study of biological invasions has identified three broad components involved in successful invasions: propagule pressure, abiotic and biotic conditions. Propagule pressure is the product of introduced propagules and the frequency of those introductions, and is considered a driver in all stages of invasion. Data to quantify propagule pressure is often unavailable, and therefore, this research approached genetic information to produce estimates of propagule pressure in the successful invasion of cogongrass (*Imperata cylindrica*) in the United States.

The following research utilized molecular methodologies to estimate genetic diversity and to infer historical introductions. Population genetic analyses were conducted for the purpose of estimating extant population-level genetic diversity at multiple-scales to first address documented cogongrass introduction(s) into this country, followed by an exploration of substantial range expansion into seven states across the region. Lastly, this research explicitly seeks evidence to support interspecific
hybridization between cogongrass and a co-occurring congeneric (*Imperata brasiliensis*) having occurred. Aggressive range expansion by cogongrass has been attributed to such a hybridization event, but has not yet been specifically tested.

Historical accounts of foreign introduction of cogongrass propagules identify two separate introductions of distinct source material made into Mississippi and Alabama. Localized population genetic analysis of these two states found substantial genetic variability within and among cogongrass populations, and supported a two-introduction scenario of distinct genetic source propagules establishing and subsequently intermixing. Enlarging the geographic scale of study incorporated five additional U.S. states currently experiencing and managing cogongrass invasion. Considerable genetic variability was found within and among the seven states surveyed. It was found that range expansion was unequal across the range, and that the most distant states tested were not genetically isolated from source populations, suggesting a possible anthropogenic role. Focusing solely on Florida where two congeners overlap ranges, morphology, and ecology, this population genetic analysis failed to detect significant evidence to support interspecific hybridization. Collectively, these investigations explored genome-level dynamics during invasion by a noteworthy invasive grass in an effort to better understand the process of biological invasions.
DEDICATION

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Invasive species are defined as organisms that are transported and introduced from one location to another, successfully establish, spread from the site of introduction, and cause a perceived negative impact (Richardson et al. 2000; Kolar & Lodge 2001). The perceived, negative impact can be environmental, economic, or some combination of factors that are undesired (Richardson et al. 2000; Pimentel et al. 2005). Some well-known invasive species include: the zebra mussel (*Dreissena polymorpha*) detrimentally affecting the Great Lakes region (Johnson & Carlton 1996; MacIsaac 1996), kudzu (*Pueraria montana* var. *lobata*) throughout the southern United States (U.S.) (Pappert et al. 2000; Sun et al. 2005), spotted knapweed (*Centaurea maculosa*) in montane areas of the Western U.S. (Tyser & Key 1988; Ridenour & Callaway 2001), the brown tree snake (*Boiga irregularis*), which has decimated native bird populations on Guam (Wiles et al. 2003), and Dutch Elm disease (*Ophiostoma novo-ulmi*) devastating elm tree populations in Europe and North America (Brasier & Buck 2001; Desprez-Loustau et al. 2007). Such diversity in invasive species biology and ecology has made generalizing biological invasions unpredictable, leaving many systems susceptible (Mooney & Cleland 2001; Lee 2002; Simberloff et al. 2005).

Perceived increases in invasive species occurrence have been attributed to a variety of reasons, including increased global connectivity and trade, which physically
transports non-native organisms (Levine & D’Antonio 2003; Lockwood et al. 2005; Meyerson & Mooney 2007; Hulme 2009). This smaller world, in combination with publicized concern about invasive species (e.g., Asian Carp) and improvements in public educational outreach, has increased overall awareness of invasive species and the potential effects on ecosystem services and the role global climate change might have facilitating or limiting invasive species occurrence and frequency, and vice versa (MacDougall & Turkington 2005; Hellman et al. 2008; Pejchar & Mooney 2009).

Negative impacts of invasive organisms are perceived through adverse effects on human interests, such as the costs associated with management and mitigation of invasive organisms, as well as reduced or modified access to and enjoyment of outdoor recreation (both aquatic and terrestrial) (Pimentel et al. 2005; Pejchar & Mooney 2009). The effects of invasion and non-native organisms proliferating and spreading into new areas can also negatively affect native/natural communities, including a diversity of plants and animals, which are significantly reduced, threatened, and/or conserved (Wiles et al. 2003; Simberloff et al. 2005; Meyerson & Mooney 2007).

Academic interest in invasive species, and the systems in which they occur, has also increased reflecting this growing trend (Lockwood et al. 2007). Though a significant portion of research focuses on prediction and management of invasive species, a shift is occurring to also utilize invasive species and invaded systems as laboratories for exploring the process and dynamics of invasion and evolution (Mooney & Cleeland 2001; Lee 2002; Novack & Mack 2005). These ideas address the role of evolution in invasion success; a result of changes in allele frequencies within populations causing populations to genetically differentiate from one another over time. During the invasion
process, genetic material is introduced and recombined which may facilitate or limit invasion success (Lee 2002; Ward et al. 2008; Whitney & Gabler 2008). Abundant genetic diversity is not necessarily required for an invasive species’ success (Tsutsui et al. 2000; Poulin et al. 2005), however, it is thought that increased propagule pressure can generate greater genetic variation to facilitate successful establishment allowing a population to posses a greater range of adaptability to a novel environment (Lockwood et al. 2005). Therefore, it is possible for invasion to be studied through population genetic analysis of invasive populations, where such studies seek to gain a better understanding of biological invasions, and the ability to predict which and why some are successful.

This work aims to conduct a population genetic analysis of a successful invader, cogongrass (Imperata cylindrica (L.) Raeuschel\(^1\)), found in seven states across the southeastern region of the U.S. This chapter first presents the framework and ideas of biological invasions relevant to the studies in this work, followed by an exploration of the potential role(s) of genetic variation and recombination contributing to the biology and ecology of invasions. Relevant discussion of the biology and ecology of cogongrass is provided in the next section, followed by brief overviews of research data chapters. This chapter concludes by stating the overarching goals and significance of this research with regard to cogongrass invasion, as well as what gains may be made in the general study of invasive species and biological invasions.

\(^1\) Imperata cylindrica (L.) Raeuschel is the current accepted botanical nomenclature for cogongrass and includes the previous accepted nomenclature Imperata cylindrica (L.) P. Beauv. (Integrated Taxonomic Information System).
Biological Invasions

Upwards of 29 hypotheses seek to explain invasion-associated phenomena: where organisms are transported and introduced, where they establish, and, to be successful invaders, where and how they spread beyond areas of introduction (Kolar & Lodge 2001; Catford et al. 2009). In addition, a perceived negative impact is necessary for a species to be considered invasive (Richardson et al. 2000). Negative impacts of biological invasions include associated economic costs, which surpassed $120 billion (US) dollars annually in the U.S. alone (Pimentel et al. 2005). Impacts of invasions in natural and managed areas have affected industries such as agriculture, fisheries, and silviculture, have negatively affected conservation of endangered and endemic species (and the areas in which they occur), and have reduced the quality of and access to recreational opportunities on private and public land (Pimentel et al. 2005).

Biological invasions - the process, the invading organism, and the susceptible system - have generated many important ecological questions and hypotheses, but a generalized and accepted framework remains elusive. Numerous models and hypotheses regarding invasion are available (see: Shea & Chesson 2002; Coulatti & MacIsaac 2004; Richardson & Pyšek 2006). Biological invasions, especially plant invasions, can be broadly studied through three main components: propagule pressure, abiotic conditions, and biotic interactions (Meyerson & Mooney 2007; Ward et al. 2008; Catford et al. 2009). These three major mechanisms (of many) are inclusive of most hypotheses in invasion biology, and are in concordance with the invasion process, where successful transport, introduction, establishment, spread, and impact, must occur (Richardson et al. 2000; Kolar & Lodge 2001; Coulatti & MacIsaac 2004; Catford et al. 2009).
Propagule pressure is a function of dispersal limitation and can be thought of as the number of individuals transported and introduced (Lonsdale 1999; Lockwood et al. 2005; Eppstein & Molofsky 2007). Non-native invasive individuals can be transported both purposefully and inadvertently. Pressure may be increased through introduction of higher numbers of individuals per introduction, multiple introductions increasing the frequency of introduction, and also through greater quantities of genetic information (i.e., higher genetic diversity) per introduction. Propagule pressure has been suggested as a significant factor affecting successful introduction and establishment (Lonsdale 1999; Lockwood et al. 2005; Coulatti et al. 2006; Meyerson & Mooney 2007). Without successful introduction and establishment, a potentially invasive species would be unable to spread and cause a perceived negative impact (Richardson et al. 2000; Kolar & Lodge 2001; Catford et al. 2009). In addition, Catford et al.’s (2009) model shows that propagule pressure is a primary driver of most stages and secondary driver in all the remaining stages of invasion. In their model, propagule pressure is also the only factor that is directly modifiable by human activities. The significance of propagule pressure throughout the invasion process implies the amount of introduced genetic variation and the frequency of introductions increasing variation are important factors in the determination of the success of biological invasions. These conceptual ideas will follow throughout the rest of this work.

Genetic diversity underlying invasive potential

The study of invasion dynamics increasingly incorporates genetic and molecular information (Lee 2002). Genetic variation is the source for evolution to occur, and being as such, lack of variation and natural selection can limit invasive potential and impact
Genetic tools can provide invasion ecologists a better understanding of how introduced species interact with native communities and ecosystems. This would also include how genetic diversity, or lack thereof, affects a founder population’s ability to overcome the ecological filters or stages introduced organisms must overcome for invasion to be considered successful (Richardson et al. 2000; Coulatti & MacIsaac 2004). Differential levels of genetic variation within and among populations may also indicate in which stage of invasion populations are found and which ecological barriers might limit a particular plant invasion. Mechanisms such as a genetic bottleneck, a founder effect, relaxed selection, or novel genomic recombination can limit or stimulate the processes of evolution, thereby potentially affecting the speed and success of an invasion (Huey et al. 2005; Lavergne & Molofsky 2007; Dlugosch & Parker 2008).

Most colonizing species are thought to pass through a genetic bottleneck that diminishes genetic variation within the founding population(s) (Nei et al. 1978; Luikart et al. 1998; Dlugosch & Parker 2008). The rate of population growth after a genetic bottleneck directly affects genetic variation and may possibly generate rapid adaptive evolution in isolation from conspecifics (Huey et al. 2005; Lavergne & Molofsky 2007). Contemporary reviews on founder effects suggest some implications of population dynamics and structure on genetic variation within invasive species (Luikart et al. 1998; Ouborg et al. 1999; Dlugosch & Parker 2008). Clonal or partially asexual organisms can tolerate losses of variation greater than those that rely solely on outcrossing modes of reproduction (Ellstrand & Roose 1987; Pappert et al. 2000). On the other hand, it is also possible that clonal organisms maintain advantageous phenotypes once selected upon
(Lavergne & Molofsky 2007). If a loss of genetic variability occurs, either within or across populations, short and long-term limitations may become evident in an invading population’s response to new environments (Nei et al. 1978; Dlugosch & Parker 2008). A significant loss of genetic variation, possibly through a hybridization event or a selective sweep, does not necessarily diminish invasive potential, and can still allow for significant spread and impact (Amsellem et al. 2000; Tsutsui et al. 2000; Poulin et al. 2005; Salmon et al. 2005).

Alternatively, a few immigrant propagules may already contain a high degree of genetic variability, such as a representative genetic subsample of the species from the entire native range introduced into a localized area in a new range; in this case, the expected effects of a genetic bottleneck may be reduced or averted by the introduced propagules forming a founding population (Novack & Mack 2005). With plant species, sometimes the number of founder individuals per introduction may not be known. Propagule pressure is the product of the number of individuals introduced and the frequency of introductions during invasion (Lonsdale 1999; Eppstein & Molofsky 2007). Unfortunately, those data are often unavailable. Therefore, I propose that the amount of genetic variation and population-level diversity may serve as a proxy for the number of individuals introduced. Genetic diversity data, combined with information gleaned about frequency of introductions, may facilitate estimation of propagule pressure, providing actual data toward estimating this significant factor in biological invasions (Lockwood et al. 2005).

Additional introductions increase the number and frequency of introduced propagules, thereby increasing propagule pressure in the invasion process. Multiple
introductions can be from the same parent source of propagules, or can be from populations of the same species separated by physical distance and/or barriers. Multiple introductions can therefore, result in interbreeding populations established by individuals from geographically separate areas that would otherwise not have intermingled (Novack & Mack 2005). The intermixing of previously isolated genotypes, from multiple regions of a single species, can result in increased genetic diversity above that of the parent populations across the native range resulting in recombination of genotypes (Lavergne & Molofsky 2007). Through recombination, new phenotypes may arise from genomic rearrangement, resulting in characters that may impart fitness benefits such as adaptive flexibility to new conditions in a newly encountered environment (Lee 2002). Through intraspecific examination of an important invasive species affecting the Southeast, I aim to quantify genetic diversity within and among populations, population structure, and infer population relationships. The genetic data set should provide evidence to allow for inferences to be made regarding the number of introductions and potentially, the amount of genetic diversity present today as a product of substantial variation upon introduction(s).

**Cogongrass, the system**

Cogongrass (*Imperata cylindrica* (L.) Raeusch.) is a perennial, C4, invasive grass with a widespread global distribution, essentially cosmopolitan in tropical and subtropical regions (Hubbard et al. 1944; Bryson & Carter 1993; Schilling et al. 1997). It has been a top-ranked weedy species since the late twentieth century, and continues to earn that negative reputation (Holm et al. 1977). Cogongrass is listed as a Federal Noxious Weed in the U.S. (USDA-APHIS, Federal Noxious Weed Program), and it is presently found
and actively managed in at least seven states. The use of molecular information to infer partitioning of genetic variation and population structure may help to explain and/or predict the ongoing and rapid spread of this species in the U.S.

Despite having been considered as a potential forage grass, cogongrass has been found undesirable for this purpose due to the high silica accumulation in its leaf tissues (Tabor 1952; Dickens 1974; MacDonald 2004). Cogongrass also detrimentally affects forestry, conservation, wildlife management, and effectively excludes other plant species, forming dense monotypic stands (Bryson & Carter 1993; MacDonald 2004; Miller 2007). This is especially significant in silviculture and efforts at restoring or conserving natural habitats. Cogongrass litter drastically alters fire regimens, and resulting fires can kill young trees (e.g., Pinus sp.), eliminate wildlife, and negatively impact community biodiversity and ecosystem functioning (Lippincott 2000; Howard 2005; Miller 2007). From its extensive rhizomes, cogongrass emerges as the sole survivor to recover and further dominate the community (Lippincott 2000; Howard 2005; Holly & Ervin 2006).

Cogongrass also is a prolific annual seed producer, with rights-of-ways along highways providing excellent corridors for wind dispersal of viable seed (Willard et al. 1990; Holly & Ervin 2007; Holly 2008). Cogongrass is phenotypically plastic, with considerable variation in leaf and inflorescence shape across a broad range of environments (Al-Jaboory & Hassawy 1980; Bryson et al. 2010). The ecology of cogongrass characterizes it as a highly invasive species, and it is known to cause significant economic damage in areas where it is well established.

Cogongrass inhabits the tropical and sub-tropical regions of the globe (Hubbard et al. 1944; Bryson & Carter 1993; MacDonald 2004), and it is not expected to expand its
range into latitudes with cooler temperatures. Presently, the most limiting factor in the geographic spread of cogongrass in the U.S. is cooler temperatures of more northern latitudes (Patterson et al. 1980; Wilcut et al. 1988). However, a red, cold-tolerant, horticultural cultivar (var. *rubra*) of the species is available and sold through nurseries in the U.S. This variety is more cold- and shade-tolerant than invasive wild-type (green) populations in the south (Tu 2002; Cseke & Talley 2012). Present distribution of var. *rubra* places it in every state in the U.S., and it also has a demonstrated capacity to produce seed (Cseke & Talley 2012). It is unknown if this horticultural variety is reproductively isolated, so the potential may exist for future hybridization with invasive populations found in the South. The possibility of introgression of cold- and shade-tolerance into invasive populations may pose a serious risk of broad, nationwide expansion of cogongrass (Tu 2002; Cseke & Talley 2012).

The known cogongrass introductions into the U.S. originated from Japan and the Philippines: the first report of cogongrass introduction is of a shipment of Satsuma oranges arriving in the Mobile Bay, Alabama (AL) locale in 1912, where the grass had been used as packing material. The subsequent documented introduction into the southern Gulf Coast is reported to have been purposeful, into McNeil, Mississippi (MS) in 1921, for forage and soil conservation efforts (Tabor 1949, 1952; Dickens 1974; Dozier et al. 1998). Therefore, at least two geographically isolated populations from Asia (Philippines and Japan) are known to have been introduced into the Gulf Coast region of the U.S. Today, cogongrass has spread throughout the southeastern region of the U.S., in the states of Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, Tennessee, and Texas.
Each of these states possesses a different invasion history with cogongrass. For example, only Alabama and Mississippi are known to have received direct introductions from Asia, whereas Florida received introductions from established populations in Mississippi and Alabama for the purpose of forage cropping. At the time of this introduction into Florida, a congener (*Imperata brasiliensis* Trin.) was already considered introduced and naturalized, presumably from South America (Hall 1998). This has led some to believe that the invasion dynamics in the Southeast are primarily the result of ‘hybrid swarms’ between cogongrass (*I. cylindrica*) and the co-occurring congener (*I. brasiliensis*, Howard 2005). Both species are listed Federal Noxious Weeds with U.S. range overlap in Florida, with shared morphology and ecology. Cogongrass and Brazilian satintail are morphologically similar, with little differences; these species delimited by a single morphological character in flower structure, based on the presence of a bi-staminate flower (*I. cylindrica*) or single staminate flower (all other *Imperata* species) (Gabel 1982; Welker & Longhi-Wagner 2012). The native range for cogongrass spans the tropical and sub-tropical areas of the Old World, whereas the native range for Brazilian satintail is restricted to the tropical and subtropical areas of the New World. Ecologically, both species are considered ruderal, and are managed weeds in their native range (Hubbard et al. 1944; Gabel 1982; Welker & Longhi-Wagner 2012). Intraspecific hybridization, defined as genetic mixing between previously isolated populations of the same species, is also one documented vehicle through which genetic diversity of invasive plant species may be increased during the process of invasion (Genton et al. 2005). The documented introductions of geographically isolated parent material into MS and AL indicate that these two states posses the longest invasion history with cogongrass, and
that mixing of previously isolated genomes may have facilitated this successful invasion in the U.S. The propagule pressure for the founding populations of cogongrass are unknown, but it may be possible to estimate this in terms of number of introductions and amount of genetic variation present in the U.S.

Secondary invasions, or the iterative cycle of invasion as a species spreads into new areas, have occurred in the U.S. This spread into other southern states is the result of secondary founding populations, presumably from initial source populations. These propagules can be wind dispersed, as the biology of cogongrass suggests, but is also strongly affected by anthropogenic transport, thereby altering the strength and frequency of cogongrass propagule pressure during secondary invasion into new areas (Holzmueller & Jose 2011).

This work, therefore, aims to conduct population genetic analysis to detect the number of genetic groups or types in the states that received direct introductions either providing support for documented introductions, or not. Determination of genetic admixture between groups may be inferred, which may support the role of intraspecific hybridization. Inclusion of regional sampling aims to address secondary invasions from a population genetic perspective for the purpose of gaining insight into secondary invasion dynamics and how genetic information is spread during invasive range expansion. Finally, this work aims to preliminarily address if genetic evidence is present supporting interspecific hybridization between congeneres (*I. cylindrica* and *I. brasiliensis*) in Florida, generating increased genetic diversity and contributing toward invasive spread.
Local intraspecific genetic variation (Chapter II)

*Imperata cylindrica* is phenotypically plastic and can be morphologically variable among populations, resulting in ecotypes (Al-Jaboory & Hassawy 1980; Bryson et al. 2010). In Taiwan, ecotypic variation has been explored both phenotypically and molecularly, discovering significant diversity and differentiation among cogongrass populations (Cheng & Chou 1997; Chou & Tsai 1999). It is reasonable to infer that invasive populations occurring in the U.S. can also demonstrate significant genetic differentiation and population structure because this species was introduced into the region nearly a century ago.

The literature states that two separate introductions of cogongrass were made into the U.S. from geographically isolated parts of Asia. These introductions were made into the Gulf Coast region both temporally and spatially close together (Grand Bay, AL, 1912 and McNeil, MS, 1921). A population genetic analysis of Mississippi (MS) and Alabama (AL) may reveal genetic signatures of the introductions and may determine patterns of genetic variation over a localized spatial scale. From the patterns of genetic variation within and among populations, inferences can be made about how cogongrass has spread since those documented introductions into MS and AL.

If the presence of multiple introductions into the region were a significant factor in cogongrass invasion, then one would expect to detect two different genetic groups. Significant admixture between introduced types would result in genetic homogenization and little to no population structure. Because cogongrass possesses the capacity to reproduce clonally, some have hypothesized that within-population variation would be low, but among population differences would be high. A previous study conducted by
Capo-chichi et al. (2008) found high genetic diversity within populations near one site of introduction (Mobile Bay area, AL), but failed to detect isolation-by-distance population structure as had been hypothesized by the authors. The lack of concordance in their data led the authors to invoke interspecific hybridization with *I. brasiliensis* as a factor confounding the genetic signal and creating the resultant patterns of variation. Those results, in combination with the knowledge that cogongrass was first introduced into MS and AL (providing almost a century of potential genetic change), allows for the inference that these two states have experienced cogongrass invasion for the longest period of time. However, I also suggest the contribution of intraspecific hybridization, rather than interspecific, between two introduced sets of invading propagules, from different parent material, as the mechanism by which cogongrass has successfully averted the impact of a contemporary genetic bottleneck and the subsequent consequences of reduced genetic variation in novel environments.

**Interspecific variation in Florida (Chapter III)**

Two species within the genus *Imperata* are reported to co-occur within the state of Florida (Hall 1978, 1998). Both cogongrass (*Imperata cylindrica* (L.) Raeuschel) and Brazilian satintail (*Imperata brasiliensis* Trin.) are federally listed noxious weeds in the U.S. The delimitations for these two species are primarily based on morphology; however, many morphological characters of the two overlap, making differentiating between the two species difficult (Bryson & Carter 1993). The primary character to distinguish *I. cylindrica* from *I. brasiliensis* is the presence of two anthers in the former, with only one anther present in the latter (Gabel 1982). Arguments regarding the presence and number of anthers have been published, and these also invoke interspecific
hybridization, based on the presence of individuals with both one and two anthers within a single population in Florida (Hall 1978, 1998).

With regard to invasion, interspecific hybridization event(s) have been invoked as a significant contributing factor in the invasiveness and spread of *I. cylindrica* into much of the southeastern U.S. via ‘hybrid swarms’ (Howard 2005). In this section of research, I seek to address whether a genetic signal of interspecific hybridization can be detected in populations sampled throughout Florida, and to address if significant genetic differentiation between these two species exists in Florida. The alternative hypothesis would suggest that interspecific hybridization has not occurred and that individuals identified as *I. cylindrica* and *I. brasiliensis* may not be genetically different species, *per se*.

**Regional intraspecific genetic variation (Chapter IV)**

Inclusion of five more states in the southeast region of the U.S. will provide information on how genetic variation within and among populations contributes toward the invasion process across a broader spatial scale of the cogongrass invasion. This portion of the study includes the states of: Florida (FL), Georgia (GA), Louisiana (LA), South Carolina (SC), and Texas (TX), in addition to MS and AL. All seven states are currently experiencing significant cogongrass invasion and actively managing present populations based on available funding.

Long-distance dispersal of cogongrass propagules is often assisted through human activities, such as contaminated heavy equipment, vehicles, and through escaped propagules from horticulture. I anticipate that heavily infested areas, such as populations in MS and AL, source material into other states, and that human activities would
unequally transport propagules and would generate erratic geographic patterns of genetic information. At the regional level, I hypothesize that outlier populations or occurrences on the leading edge of the invasion may possess a genetic signature that strongly resembles that of more heavily infested areas near the site(s) of introduction and/or indicative of intraspecific hybridization (in MS and AL). Considering the rapidity of secondary founder population establishment (<30 years), lack of nearby conspecifics with which to outcross, and continuous influx of propagules from sourcing locales, I do not expect to see a strong isolation-by-distance relationship of genetic and geographic data. Though an isolation-by-distance relationship is possible, such a relationship is more probable in natural species and populations, where human activities are less influential. I do, however, expect that outlier populations will be diversity depauperate, as compared with, but also related to, populations in heavily infested areas, resulting from one or a combination of the following factors: genetic bottleneck, increased distance to neighboring populations for sexual recombination increasing genetic distance, the number of propagules introduced (propagule pressure), and/or active management which may reduce biomass, reproductive output, and genetic diversity. This kind of information can be intended for determination of genetic group(s) or type(s) primarily responsible for the bulk of the geographic spread. Such information may contribute to large-scale, concerted regional management, and/or toward a detection of resistance to management practices.

Finally, this study aims to test the wave hypothesis, as presented by Miller (2011) specific to cogongrass invasion, and if it can be supported through genetic analysis. Miller hypothesized that the MS-AL border represents the epicenter of invasion and is
geographically located between the two sites of cogongrass introduction. McNeil, MS, is located in Pearl River County in the coastal physiographic region of the state, while Grand Bay, AL, is located near Mobile Bay, AL, also located on the coast bordering the Gulf of Mexico (Figure 3.1, Chapter III).

Goals and significance of research

The goals of this research are to address population-level genetic variation, at multiple geographic scales, in contemporary *I. cylindrica* invasion in the southeastern region of the U.S. This work will measure levels of genetic diversity and patterns of genetic variation to quantify extant variation and to identify supportive genetic evidence for the number of introductions as an estimate of propagule pressure. As an invading organism, cogongrass has successfully passed through the stages of transport, introduction, establishment, spread, and impact. But, at the population level, each new population must also complete this invasion process to become a persisting invader population in a new area. Thus, my work attempts to estimate propagule pressure from genetic analysis, to infer strength at the time of initial introduction(s), but also the estimated strength and role of propagule pressure, in terms of available genetic variation and frequency of introduction, in secondary invasions occurring at the population level during range expansion and invasive spread. It is also important to consider hybridization events, both inter- and intraspecific, as they may suddenly increase or reduce genetic variation, thus affecting measures of propagule pressure.

This ecological genetics approach seeks to identify key genetic patterns to inform introduction history and patterns of spread in the novel range, along with addressing the role of hybridization, intra- and/or interspecific, and population level responses to
differing management and control efforts across the invaded range (Ward et al. 2008). The goals of my research are to 1) determine the number of introductions, 2) quantify extant genetic variation at a local and regional scale, and 3) address both intra- and interspecific hybridization in the invasive cogongrass system in the U.S. This is accomplished via the population genetic study of cogongrass on local to regional scales, to test whether patterns support documented introduction history and to determine if genetic variation is highest near sites of earliest introduction, and the role of intraspecific hybridization between two previously isolated populations from a geographically broad native range, in this case East and Southeast Asiatic archipelagos. Next, this work addresses the partitioning of genetic variation among populations sampled in the U.S. invaded range. Patterns of genetic information and clustering may inform how secondary invasions occur, such as identifying a sourcing genetic group or geographic locale. There is the expectation that populations at the leading edge of invasion will possess the lowest genetic diversity as compared to founding populations in the states of MS and AL. These founding secondary invasions at the invading front would likely be the most recent introductions, and part of this work seeks to determine if spatial patterns of infestation mirror patterns of genetic information across the invaded region (Miller 2007). Finally, this work tests genetic material and interspecific hybridization claims between two congeneric species that share ranges, ecology, and morphology: *I. cylindrica* and *I. brasiliensis*, in Florida.

Each invasion is complex, often context-dependent, and therefore, represents a unique combination of factors and mechanisms (Catford et al. 2009). This research hopes to achieve the development of an approach to address one significant component of
invasions: propagule pressure inferred from genetic information. Invasive organisms in their invaded range can vary spatially (e.g., extent of spread) and temporally (e.g., time since introduction). Through appropriate sampling and molecular marker selection, studies that incorporate population-level genetic analyses will be an effective means of obtaining adequate representation of propagule pressure by elucidating the frequency and strength of the initial introduction. Furthermore, these studies can further our understanding of invasions as they continue to occur, where the amount of genetic variation introduced during ‘secondary introductions’ as an invader spreads affects the overall speed and scale of the invasion. My hope is that through interdisciplinary studies such as this, in research and in the field, I may contribute towards the development of an applicable, inclusive, and generalized framework in which to study the why and how of biological invasions.
Literature Cited


CHAPTER II

POPULATION GENETIC ANALYSIS OF COGONGRASS (IMPERATA CYLINDRICA) POPULATIONS IN MISSISSIPPI AND ALABAMA: IS INVASION SUCCESS A PRODUCT OF MULTIPLE INTRODUCTIONS AND INTRASPECIFIC HYBRIDIZATION?

Abstract

Cogongrass (Imperata cylindrica (L.) Raeuschel) is warm-season, perennial grass considered invasive throughout the Gulf Coast and southeastern region of the United States (U.S.). Documented introduction history available in the literature attributes two sources of parent material from isolated populations in Asia: Japan and the Philippines. These introductions were made separately and directly into Alabama and Mississippi, respectively. This study is a population genetic analysis of cogongrass in the two states that were alleged to receive direct introductions of cogongrass propagules, and would have been the first established populations in the United States. Estimating the number of introductions and extant genetic diversity in an invasive species can indicate the strength of propagule pressure historically, as well as the potential strength of propagule pressure for concurrent secondary invasions in a new range. Molecular analysis utilized amplified fragment length polymorphism (AFLP) markers for 21 populations comprised of 388 individuals, sampled from Alabama and Mississippi. Within-population genetic
diversity varied among sampled cogongrass populations (percentage of polymorphic loci, 4-32%; heterozygosity, 0.013-0.051), with significant partitioning of within-population genetic variation accounting for the bulk of genetic variation (66%). The remaining molecular variation (34%) is partitioned among-populations across Mississippi and Alabama (i.e., significant genetic differentiation among populations). Two genetic groups (MS-type and AL-type) were detected by STRUCTURE and supported by principal coordinates analysis. Significant lack of genetic similarity also was found between the two detected genetic groups (F_{ST} = 0.534, P <0.001), with the spatial pattern of distribution of populations providing molecular support for a two-introduction scenario as reported in the literature of cogongrass introduction history. This study also detected a contact population, defined as a site where these two genetic groups have physically met within a single population. This contact population is located in south-central Mississippi. This, in conjunction with substantial admixture between genetic groups, has provided support for intraspecific hybridization between previously isolated conspecific origin populations that were introduced into geographic and temporal proximity. These results support intraspecific hybridization occurrence between genetic groups, and where cogongrass populations in Mississippi and Alabama may continue to experience substantial admixture and/or introgression of beneficial traits through hybridization events. Thus, cogongrass invasion in Mississippi and Alabama is the likely product of multiple introductions with support for intraspecific hybridization contributing toward increases in genetic variation across the region.
Introduction

A recent synthesis suggested three broad mechanisms involved in biological invasions: propagule pressure, abiotic characteristics, and biotic interactions (Catford et al. 2009). In their proposed framework, referred to as a PAB-model, Catford et al. (2009) aimed to streamline redundancy in hypotheses that attempt to explain and predict successful invasions. Propagule pressure (P) is broadly defined as product of the number of founding individuals (or propagules) and the frequency of introductions into an area (Lockwood et al. 2005; Eppstein and Molofsky 2007). The two other major components of the PAB-model include abiotic factors (A) and biotic interactions (B) in the new environment. Dissimilarities in abiotic conditions between ranges may restrict establishment of an exotic population reducing the probability of successful invasion, whereas similarities in conditions may facilitate establishment and/or subsequent spread (Richardson et al. 2000). Abiotic characteristics contributing to successful invasion include beneficial differential resource availability and allocation as well as the role of disturbance in facilitating invasion (Rejmánek & Richardson 1996; Davis et al. 2000). Biotic interactions between the invading population and new above and below-ground communities may limit or facilitate successful establishment and spread, as proposed in varying models, such as the enemy release hypothesis, novel weapons hypothesis, invasional meltdown hypothesis, and the evolution of increased competitive ability (Blossey & Nötzold 1995; Simberloff & Holle 1999; Keane & Crawley 2002; Callaway & Ridenour 2004). In concert, propagule pressure, abiotic characteristics of the novel environment, and biotic interactions with other organisms are proposed in the PAB-model to more generally explain and predict biological invasions (Catford et al. 2009).
The early stages of invasion are directed largely by the strength of propagule pressure, which can be a significant determinant and predictor of establishment and spread into undesired areas (Lonsdale 1999; Lockwood et al. 2005; Coulatti et al. 2006). In Catford et al.’s (2009) PAB-model, propagule pressure is the only factor that is directly influenced by humans and involved, either primarily or secondarily, in every process of invasion. Development of a method to estimate propagule pressure is one step towards defining its role and action during the various stages of invasion. By better understanding the component that is modifiable by humans, human activities may then be modified to reduce propagule pressure such as to prevent, reduce, or stop biological invasions. This work aims to incorporate population genetic approaches with ecological theory specific to biological invasions to approximate propagule pressure in a noteworthy North American invasive plant by: 1) inferring an estimate of propagule introduction from existing genetic diversity; and 2) providing evidence to infer the number of introduction(s). In concert, addressing with molecular tools the number of genotypes or diversity introduced and the frequency of introduction of varied parent material, provides information about propagule pressure that is generally otherwise unavailable.

The study species for this work, commonly known as cogongrass (*Imperata cylindrica* (L.) Raeuschel), is a perennial, invasive grass with a widespread global distribution. It is essentially cosmopolitan in the tropical and subtropical regions of the world and found on every continent except Antarctica (Hubbard et al. 1944; Bryson and Carter 1993). Documented introductions of cogongrass into the U.S. report east and southeast Asia as origins for parent material: 1) in 1912, propagules from Japan were inadvertently introduced into Grand Bay, AL, and 2) in 1921, propagules were introduced
into McNeil, MS, from the Philippines purposefully for forage (Tabor 1949, 1952). Though numerous other introductions of cogongrass from Asia or elsewhere may have occurred, published documentation for other introductions is currently unavailable. Since its introduction into this country, cogongrass has earned Federal Noxious Weed Status, with specific permitting required to allow its cultivation and transport on American soil. It is presently found and actively managed in nine states located in the southern region of the U.S., and may possess the adaptive potential to spread elsewhere throughout the nation (Bryson & Carter 1993; MacDonald 2004; Miller 2007; Lucardi, personal observations).

Capo-chichi et al. (2008) found high genetic diversity within (but not as much among) cogongrass populations near one site of known introduction in and around Mobile Bay, AL. Populations that possess higher genetic variation can experience fitness benefits due to a greater range of adaptability, fecundity, or other traits that allow the population to withstand broader environmental conditions, potentially due to a more plastic genome (Genton et al. 2005; Salmon et al. 2005; Lavergne & Molofsky 2007). Phenotypic plasticity among populations has also been observed in ecotypes of cogongrass in the Old World range (Al-Jaboory and Hassawy 1980; Cheng & Chou 1997), as well as in ecotypes in the U.S. (Bryson et al. 2010). These studies demonstrate evident phenotypic variability in cogongrass populations in the native and the invaded range, suggesting genetic mechanisms underlying phenotypic population differentiation. Demonstrated phenotypic and genetic variability at the population level in cogongrass indicates population genetic analysis as a potential tool to elucidate patterns of genetic partitioning that may have contributed to successful invasion of this species.
The intent of this study was to determine the presence of population structure in and among invasive cogongrass populations in the two states that have received direct, documented introductions. In this study, I quantify extant genetic diversity within and among 21 cogongrass populations in the states of MS and AL. These population genetic analyses used highly reproducible, dominant genetic markers, known as amplified fragment length polymorphisms (AFLPs). AFLPs are arbitrarily amplified dominant markers and were selected for several reasons: no a priori sequence information was necessary, we were able to sample the entire genome simultaneously, and the technique is considered to be highly reproducible and practical (in cost and data generation) in population genetic studies such as this (Bussell et al. 2005; Campbell et al. 2003; Meudt and Clarke 2007). Previous AFLP analyses performed by Capo-chichi, et al., (2008) on cogongrass populations from around one site of documented introduction in Mobile Bay, AL, utilized two selective primer combinations. This study utilized six selective primer combinations to improve capture of genetic variation and to avoid introduction of error or bias, such as homoplasy, as it has been shown that greater numbers of detected fragments over fewer selective primer combinations increases the potential strength of homoplasious errors/biases in the generated AFLP dataset (Koopman & Gort 2004). A different dominant molecular marker, known as random amplified polymorphic DNA (RAPDs), was used by Cheng and Chou (1997) on Taiwanese populations of cogongrass. Their study revealed significant genetic variation, correlated with phenotypic variation among Taiwanese ecotypes.

It was expected that high genetic variability would be found across populations in MS and AL. This hypothesis was based on the two documented introductions into the
U.S. from previously isolated Asian island nations. Within-population diversity is expected to be low due the ability of cogongrass to reproduce asexually through resilient, belowground rhizomes (Holly & Ervin 2006); however, previous research, utilizing AFLP makers, observed the majority of cogongrass genetic diversity partitioned within-populations (Capo-chichi et al. 2008). Multiple introductions from previously isolated parent material (i.e., Philippines and Japan) may also have contributed toward significant population structuring among populations, as has been supported in other invasive plant species (e.g., O’Hanlon et al. 1999; Walker et al. 2003; Genton et al. 2005).

Introductions of genetically different genetic parent material(s) into a localized geographic area within a short period of time (<10 years) sets the stage for secondary contact and substantial admixture between previously isolated groups, resulting in intraspecific hybridization of establishing invasive populations. Events such as hybridization, resulting from multiple introductions, during the invasion process may increase overall genetic diversity for the invading species in the naïve range, increasing the species’ capacity for survival, establishment, secondary spread, and adaptation (Ellstrand & Schierenbeck 2000; Lee 2002; Hughes et al. 2008). Identification of distinct genetic groups would allow for subsequent targeting of specific populations that are key sources of propagules and genetic information in the establishment phase of secondary invasions (i.e., in the ongoing spread of cogongrass throughout the region).

**Materials and Methods**

**Sampling**

Live leaf tissues were collected in the field from cogongrass populations in Mississippi and Alabama, during the spring and summer of 2009. Each tiller was
assumed representative of an “individual” or a ramet in the located patch or population (while acknowledging that individual patches may have arisen from only one to a few colonizing propagules resulting in few genets comprising a population). Populations were identified as contiguous patches of cogongrass, often occurring as circular patches in open areas or as long, narrow patches along roadside rights-of-way. Tissues from individual leaves were collected systematically from the edges and the middle of sampled populations. Aboveground leaf tissues were stored in individually labeled plastic bags in a cooler. Tissues then were dried in the lab by placing tissues in silica gel with color indicator. Because cogongrass is a listed Federal Noxious Weed, all sampling was conducted with approval by the U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine (Permit #: P526P-12-00211, P526-080721-005).

A total of 180 individuals were sampled from 11 MS populations: four from Desoto National Forest (Jones, Greene, and Wayne Counties), four from Bienville National Forest (Scott, Jasper, and Smith Counties), and three from around the Biloxi metro area of the Mississippi Coast (Harrison County). Ten populations were sampled from Alabama for a total of 208 individuals. Six populations were sampled in the vicinity of Mobile Bay (Baldwin, Mobile, and Washington Counties), two from the Talladega National Forest (Hale County), one from West Alabama (Sumter County), and one from Auburn, AL (Lee County). The sampling from these 21 populations generated the tissue set for genetic analysis (N=388; Figure 2.1). Minimum geographic distance between populations was 1-km, with the exception of paired populations each in AL (AL-2 and AL-3) and MS (MS-9 and MS-10), which were sampled from what appeared to be
large contiguous patches in excess of 4-km$^2$. Distances between populations may be visualized in Figure 2.1. Along with leaf tissues, habitat type (e.g., right-of-way, forested, residential, commercial, etc.), location coordinates (global positioning system [GPS], WGS 1984), and approximate patch size (area) were documented.

**Tissue processing and molecular methods**

Extraction of DNA utilized a modified NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue (Life Technologies, Carlsbad, CA, USA). Approximately 1-cm$^2$ of cogongrass tissue was aseptically transferred into a 2-ml microcentrifuge tube. Samples were fully disrupted utilizing a Retsch mixer mill and then processed using the NucPrep chemistry for DNA extraction (NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue, P/N 4333959). Purified DNA were transferred into sterile, individually labeled tubes and frozen (-20 °C) until analysis (-80 °C for long-term storage).

Amplified fragment length polymorphism (AFLPs) analysis utilized a modified protocol for capillary electrophoresis based on technique and methodology developed by Vos et al. (1995). AFLPs are dominant, arbitrarily amplified DNA markers, and a PCR-based methodology that is utilized to detect polymorphisms in the genome. This methodology is for scanning the entire genome simultaneously, highly reproducible, and applicable for a wide array of investigations in population genetics, ecology, and shallow evolutionary studies (Bonin et al. 2007; Meudt & Clarke 2007). It is considered ‘highly sensitive’ and is known to detect slight differences within species where little genomic study has previously occurred, taking advantage of the absence of a requirement for sequence information (Campbell et al. 2003; Meudt & Clarke 2007).
Extracted, purified DNA underwent digestion by restriction enzymes, ligation of linking primers, followed by pre-selective amplification to generate fragments of interest, and then, a selective cycle to amplify fragments into a marker set to detect polymorphisms within the genome among DNA samples. Restriction digest of individual genomic DNA was achieved in 25μl reactions incubated at 37 ℃ for 2 hours in a thermal cycler, finalized by denaturing of enzymes by heating samples to 70 ℃ for 15 minutes. Restriction digest enzymes and reagents utilized per reaction were: 1 μl of EcoRI (20,000 U/ml, 5’-G^AATT, 3’CTTAA^G; New England BioLabs, Ipswich, MA, USA), 1 μl of MseI (10,000 U/ml, 5’-T^TAA, 3’-AAT^T; New England BioLabs, Ipswich, MA, USA), 2.5 μl of included 10X NEBuffer4, 0.25 μl of 100 μg/ml BSA, 10 μl of individually purified genomic DNA, and 10.25 μl of sterile water.

Eco AFLP linkers were annealed in a thermal cycler by heating to 95 ℃ for 5 minutes and cooled to 5 ℃ over 30 minutes using the following reagents: 1 μl of Eco Linker 1 (100 μM, 5’-CTC GTA GAC TGC CC), 1 μl of Eco Linker 2 (100 μM, 5’-AAT TGG TAC GCA GTC TAC), 90 μl of TE buffer (10 mM Tris & 1 mM EDTA), and 108 μl sterile water. Mse AFLP linkers were annealed in a thermal cycler by heating to 95 ℃ for 5 minutes and cooled to 5 ℃ over 30 minutes using the following reagents: 10 μl of Mse Linker 1(100 μM, 5’-GAC GAT GAG TCC TGA G), 10 μl of Mse Linker 2 (100 μM, 5’-TAC TCA GGA CTC AT), 90 μl of TE buffer (10 mM Tris & 1 mM EDTA), and 90 μl sterile water. Annealed linkers are stored frozen at -20 ℃ until use for ligation of linkers.

Ligation of Eco and Mse linkers were conducted in 20 μl reactions comprised of: 1 μl each of Eco and Mse Linker (as annealed above), 1 μl T4 DNA Ligase enzyme and 2
µl of included 10X T4 DNA Ligase Reaction Buffer (New England BioLabs, Ipswich, MA, USA), 10 µl of individually digested DNA, and 5 µl sterile water. Ligation reactions were conducted in a thermal cycler at 16 °C overnight or at 37 °C for 3 hours. Individual ligated reactions were stored at -80 °C to prevent degradation.

Pre-selective 20 µl polymerase chain reaction (PCR)-conditions for amplification were: 1 µl each of Eco+A (10 µM, 5’-GAC TGC GTA CCA ATT CA) and Mse+C (10 µM, 5’-GAT GAG TCC TGA GTA AC) primers, 2.5 µl of dNTPs (0.2 mM dATP, dCTP, dGTP, and dTTP; Deoxynucleotide Solution Set, New England BioLabs, Ipswich, MA, USA), 4 µl 5X GoTaq™ FlexiBuffer (Promega Corp., Madison, WI, USA), 1.2 µl MgCl2 (Promega Corp., Madison, WI, USA), 3 µl of individually ligated DNA, 7.1 µl sterile water, and 0.2 µl GoTaq™ DNA polymerase (5u/µl, Promega Corp., Madison, WI, USA). Pre-selective amplifications consisted of an initial denaturing step of 94 °C for 1 minute, 30-cycles of 30 seconds at 94 °C, 1 minute at 56 °C, and 1 minute at 72°C, and followed by final annealing for 2 minutes at 72 °C.

For selective amplification, pre-selective amplification products are individually diluted 1:20 with sterile water. Each combination of selective Mse and fluorescent Eco selective primers is a separate PCR amplification. Selective amplification for all individuals was achieved in 20 µl reactions consisting of: 1 µl each of Mse (5µM) and fluorescent Eco (1 µM) selective primer (see: Table 2.1), 2.5 µl of dNTPs (0.2 mM dATP, dCTP, dGTP, and dTTP; Deoxynucleotide Solution Set, New England BioLabs, Ipswich, MA, USA), 4 µl 5X GoTaq™ FlexiBuffer (Promega Corp., Madison, WI, USA), 3.7 µl MgCl2 (Promega Corp., Madison, WI, USA), 3 µl of diluted pre-selective amplification product, 4.6 µl sterile water, and 0.2 µl GoTaq™ DNA polymerase (5u/µl,
Selective amplifications consisted of an initial denaturing step of 94 °C for 2 minutes, 10-cycles of 30 seconds at 94 °C, 30 seconds at 65 °C, and 1 minute at 72°C (reducing annealing temperature by 1 °C/cycle), 30-cycles of 30 seconds at 94 °C, 30 seconds at 56 °C, and 1 minute at 72°C, and finished with 30 seconds at 72 °C. In total, six selective primer pair combinations were applied to the individuals in this study (Table 2.1); therefore each individual underwent six different PCR amplifications.

The selective primers were fluorescently tagged such that product from multiple combinations of DNA primers could be analyzed at once. Three different fluorescently tagged products (1.5 μl of each) were combined per well with a single-stranded, fluorescent ROX-1000 size standard (0.25 μl; MapMarker (50-1000), BioVentures, Inc., Murfreesboro, TN, USA) and fixed with formamide (10 μl, Hi-Di™, Life Technologies, Carlsbad, CA, USA). Pooled fragment products were run on an ABI 3730 capillary sequencer at the Arizona State University DNA Lab (Tempe, AZ, USA) with positive and negative control sample replicates. Positive control replicates consisted of one individual randomly selected at the beginning of the study and followed each set and step of molecular analysis throughout to determine reproducibility. Negative control replicates consisted of reagents alone, and no genomic DNA to check for cross-contamination among individual samples. Standard error of positive control replicates suggests reproducibility of the AFLP methodology (SE = 0.004).

**Data management and analysis**

Fragment data were digitally visualized in GeneMarker® (SoftGenetics, LLC, State College, PA, USA), and data were exported into a general text format for input to
Excel 2007 (Microsoft Corporation, Redmond, WA, USA). Fragments were sorted based on migration size (basepairs) and auto-scored utilizing an independently developed procedure (Lucardi and Walker, unpublished methodology) that utilizes both Excel 2007 and PASW v.18.0 (SPSS, IBM Corporation, Armonk, NY, USA). This procedure sorted fragments, and differences were scored with a 0.3-basepair threshold. Polymorphisms were identified as scored (or ‘called’) alleles/bands that did not occur in all individuals. Scoring of polymorphisms sorted detected fragments and determined scored bands as polymorphic or not. Data matrices were created from scored fragment data and auto-populated over several steps in both software programs. Matrices were coded ‘0’ for absence and ‘1’ for presence. Detected polymorphic loci less than 200 basepairs in length were removed from statistical analyses in an effort to avoid potential effects of fragment-size homoplasy, due to disproportionate number of smaller fragments produced by AFLPs (Koopman & Gort 2004; Bonin et al. 2007). Homoplasy is the result of co-migrating bands during electrophoresis that are not of the same physical locus in the genome and lack homology. Homoplasious biases influence errors in allele frequency detection, generally toward overestimation, generating erroneous heterozygosity estimates and underrepresentation of genetic differentiation between subpopulations (Meudt & Clarke 2007; Caballero et al. 2008).

This dataset detected a large number of informative AFLP markers, from several selective primer combinations. Population genetic diversity metrics, as presented here, estimate allele frequencies from dominant (presence-absence) data generated by AFLP analyses, and are subject to Hardy-Weinberg equilibrium assumptions. These assumptions can reduce accuracy in allele frequency estimations from dominant data, but
reliable results for comparative study can be achieved with adequate population sampling and sufficient number of primer sets, which generate a large number of detected polymorphic loci (Mariette et al. 2002; Bonin et al. 2007; Meudt & Clarke 2007).

Data conversions of presence-absence matrices for input into population genetic software programs (such as, STRUCTURE and Arlequin, below) utilized the R-package, AFLPdat source script (Ehrich 2006). Genetic diversity within populations was assessed on the number of bands and private bands, the percentage of polymorphic loci, expected heterozygosity (biased (H_e) and unbiased (UH_e)) based on Hardy-Weinberg expectations (Nei 1978), and Shannon’s Information/Diversity Index (I), serving as a coefficient of similarity, for each of the 21 populations (GenAlEx 6.3, Peakall and Smouse 2006). Cogongrass is able to reproduce asexually via belowground rhizomes. The number of unique multi-locus genotypes per population would therefore contribute toward more accurate assessments of genetic diversity within populations. Determination of the number of different genotypes present in each population utilized the “Clones” function within AFLPdat, with a corresponding error parameter, which is the product of an error rate and the number of markers (Ehrich 2006). Standard error among positive control replicates served as the error rate for clonal diversity analysis. This function within AFLPdat estimates genotype diversity (Nei 1987), the effective number of genotypes (Parker 1979), and Nei’s gene diversity (1987).

This intraspecific examination also evaluated among-population genetic variation and structuring. For the purpose of ascertaining genetic variation among populations, and how much genetic distance exists between each population, this study utilized Nei’s pairwise unbiased genetic distances (Nei 1972, GenAlEx v.6.3) and population pairwise
FST (Arlequin v.3.5). STRUCTURE v.2.3.3 (Pritchard et al. 2000) was released in 2009 (http://pritch.bsd.uchicago.edu/structure.html). This program was utilized to infer the number of clusters that best fit and are most biologically appropriate for the data without *apriori* information applied to the number or structure of expected clusters within the dataset. Since the most appropriate number of clusters was initially unknown, duplicate runs of $K=1$-$7$ were performed, where admixture ancestry model was applied with a burnin of 10,000 and 50,000 MCMC (Markov Chain Monte Carlo) (Pritchard et al. 2000). Based on non-genetic evidence, such as the introduction history of cogongrass, a $K=2$ is expected, however, multiple iterations of $K=1$-$7$ were run to determine if that expectation was supported. To infer the most appropriate number of clusters ($K$), this study utilized the method developed by Evanno et al. (2005), to detect the second order rate of change in likelihood values, based on the difference between likelihood values generated with each $K$, from each STRUCTURE run. The difference between likelihood values and the second order rate of change was then plotted and allowed for a $\Delta K$ to be determined suggesting the most likely number of clusters appropriate for the dataset.

Additional analysis of population structure was assessed through principal coordinates analysis (PCA, GenAlEx v.6.3) of genetic covariance with data standardization among all individuals. Two different analyses of molecular variation (AMOVA, Excoffier et al. 1992) were performed in Arlequin v.3.5 (Excoffier and Lischer 2010): first, between populations based on collection location data (State-level), and second, between individuals that were assigned to differing clusters from preliminary STRUCTURE analysis.
Results and Discussion

Within-population Genetic Diversity

AFLP analysis detected 850 polymorphic loci among sampled cogongrass individuals in MS and AL (N=388). Over 300-400 AFLP loci are generally recommended for the detection of potential selection signatures, and/or greater than 200 polymorphic loci for intraspecific population genetic analyses (Bonin et al. 2007). The detected number of polymorphic bands also exceeds another recommended minimum of “500 AFLP loci” for intraspecific examination of an outcrossing species (Mariette et al. 2002; Bonin et al. 2007; Meudt & Clarke 2007). The greater the number of detected AFLP loci (<500) can improve accuracy of diversity estimates, and estimates within and among-population genetic diversity derived from AFLPs are comparable to other molecular markers, such as microsatellites (Mariette et al. 2002; Nybom 2004).

Polymorphic bands that occur in only one population and are not detected in any other population are referred to as private bands. The number of private bands detected per population ranged from none-detected, to 127 private bands. Percentage of polymorphic loci per population ranged from 4%-32% with a mean of 14% (standard error ± 2%). Heterozygosity, a measure of genetic diversity, was averaged over each population. Mean expected heterozygosity (H_e) of the 21 cogongrass populations in this study ranged from 0.013-0.049, while mean unbiased expected heterozygosity (UH_e) ranged from 0.013-0.051. The overall average mean expected heterozygosity (H_e) was found to be 0.028 (standard error ± 0.001), and overall average mean unbiased expected heterozygosity (UH_e) was found to be 0.030 (standard error ± 0.001). Shannon’s Information Index (I), ranged from 0.020-0.088, with an overall mean of 0.047 (standard
error ± 0.001). These data are summarized in Table 2.2. Overall, genetic diversity is relatively similar among all populations sampled (percentage of polymorphic loci <35%, $H_e$ and $UH_e \leq 0.050$, $I < 0.1$). The detection of low overall genetic diversity within populations, however, does not necessarily limit population expansion in other invasive species (see: Amsellem et al. 2000; Tsutsui et al. 2000) and does not appear to have limited cogongrass population expansion in the U.S.

Population AL-4, located in Washington Co., AL, possessed the greatest genetic diversity among sampled populations, with the highest heterozygosity and Shannon’s Information Index ($H_e/UH_e = 0.049/0.051$, $I=0.088$), the most polymorphic bands detected (283), and 127 of those detected were private bands. This population is proximally located near the AL/MS border. Together, these data suggest AL-4 is the most diverse population in this study. Lower genetic distances between AL-4 and most other populations may also suggest that AL-4 may be sourcing genetic material. On the other hand, this population may also be subject continuous propagule rain, which is the frequent introduction of new propagules, and may subsequently hybridize.

Cogongrass invasion and rapid spread in a localized area are characterized to arise from resilient and rapidly growing rhizomes (Bryson & Carter 1993; Holly & Ervin 2006). The number of clones (or genetically identical individuals) per population was calculated for the 21 populations (N=388) included in this study, using an error parameter of four bands. The frequency of unique genotypes per population equals sample size, with the exception of populations MS-6, 7, 8, 9, 10, 11, and AL-2, 7, 8, 9, and10 (Table 2.3). The overall number of genotypes among all individuals tested resulted in a
reduction to 349, and an observed value of 318 for the overall effective number of genotypes; also a reduction from the total number of sampled individuals.

Data from clonal analysis for two populations provide evidence for substantial reduction in genotype diversity (<0.90) relative to other populations in the study. These reductions are observed in population MS-8 (0.711) and MS-11 (0.867), where the number and effective number of genotypes for these two populations are also reduced. Genetic diversity for MS-8 and MS-11 may therefore be overestimated due to clonal effects, where Nei’s gene diversity (1987) for each population is 0.022 and 0.023, respectively. However, the remaining 19 populations maintain high genotypic diversity (>0.90) supporting sexual reproduction and outcrossing occurrence. (Kreivi et al. 2005). Genetic diversity estimates, such as heterozygosity, for the bulk of the analyzed cogongrass populations are comprised of mostly unique individuals, and are therefore, considered reliable. Individual cogongrass patch expansion has been thought to rely heavily on asexual rhizomatous growth; however, genotype analyses suggest that high diversity among sampled individuals are not primarily the product of clonal reproduction.

Among-population Genetic Variation

Population pairwise genetic distances and genetic similarities, or lack thereof, provide supporting data for two genetic groups, one located in central MS and the other containing coastal MS and AL populations. The two observed groups based on these data provide support for a two-introduction scenario. Coastal MS populations (MS-9, 10, and 11) sampled from the Biloxi metro-area resulted in very low genetic distances and pairwise FST between one another, but resulted in intermediate values when compared to some central MS and AL populations. Multiple intraspecific hybridization events can
reduce the signal of two separate introductions of genetically distinct parent material; however, based on the resultant patterns, these populations may have resulted from hybrid founders of originally genetically distinct sources.

Nei’s pairwise unbiased genetic distances between populations ($D$) ranged from 0.002 to 0.058 (Table 2.3; Nei 1978). The greatest genetic distance was found between MS-3 and MS-9 ($D = 0.058$); similar genetic distance values are also found between MS-3 and MS-10 ($D = 0.056$), MS-2 and MS-9 ($D = 0.053$), MS-3 and MS-11 ($D = 0.052$), and between MS-1/MS-9 and MS-2/MS-10 ($D = 0.051$), indicative of consistent genetic distance among populations within MS. The highest genetic distances found were between the populations located in central MS and populations sampled from the MS coast, near Biloxi (MS-9, 10, 11). This pattern of genetic distances, in concert with genetic diversity, suggests that central MS populations (MS-1, 2, 3, 4, 5, and 6) are more genetically distant from populations located in coastal MS and all AL populations sampled in this study.

Genetic dissimilarity was found among pairwise population F$S_T$ values. The greatest genetic dissimilarity observed is between MS-8 and AL-8 ($F_{ST} = 0.816$; see Figure 2.1). Population MS-11, sampled near Biloxi, MS, is genetically dissimilar ($F_{ST} \geq 0.4$) to all populations with the exception of AL-1, AL-2, and MS-10. This pattern may suggest that MS-11 and MS-10 were founded from a single introduction, or that one sourced the other, and are from the same source propagule pool as the populations AL-1 and AL-2. Populations MS-9 and MS-10 are more genetically similar ($F_{ST} = 0.022$), than MS-9 and MS-11 ($F_{ST} = 0.486$). This similarity between MS-11 and other populations can support intraspecific hybridization between MS and AL groups.
Overall, observed pairwise $F_{ST}$ values between all populations show that central MS populations (MS-1 thru 6) are genetically similar to one another, with greater differentiation between those and all other populations (Table 2.4). Pairwise population matrix of $F_{ST}$ values ranged from 0.022-0.816, and all pairwise values are significant ($P < 0.05$) (Table 2.4). The most genetically similar populations are MS-9 and MS-10 ($F_{ST} = 0.022$), MS-3 and MS-4 ($F_{ST} = 0.074$), AL-4 and AL-5 ($F_{ST} = 0.037$), and AL-8 and AL-9 ($F_{ST} = 0.055$). Each pair co-occurs in the same county (see Table 2.2 and Figure 2.1), such that a trend can be observed between high genetic similarity (low $F_{ST}$) and county location. This lack of genetic differentiation of populations within the same county may indicate that intra-county maintenance (of rights-of-way) may contribute toward the spread of cogongrass propagules within individual counties.

Low genetic distances between geographic distance populations may suggest long-distance gene flow, as observed between population AL-9 (near Mobile Bay) and AL-10 (in Lee County, near Auburn University) ($D = 0.002$). Similar patterns of low genetic distance are also observed between AL-3 (Talladega NF, Hale County) and AL-4 (Washington County) ($D = 0.003$), and between AL-1 (Sumter County) and AL-2 (Talladega NF, Hale County) ($D = 0.004$). Populations AL-2 and AL-3 are both located in the Talladega National Forest, and these populations co-occur where AL-2 was sampled in and around an abandoned logging deck at the top of a hill, and AL-3 was sampled on the new unimproved road leading to the abandoned logging deck. However, the genetic distance between AL-1 and AL-4 ($D = 0.016$) is similar to genetic distance between AL-2 and AL-3 ($D = 0.015$). Cogongrass propagules were likely introduced during road construction and silviculture activities observed in Talladega NF. The
evident logging operation required new, unimproved road construction, and both
appeared to be relatively recent (<12 months). Observed patterns suggest that
populations sampled in the Talladega National Forest may be the product of two separate
introductions from other populations located elsewhere in AL (see Figure 2.1, Table 2.2
and Table 2.4).

A significant and slight positive correlation was found via linear regression
between population pairwise $F_{ST}$ values and geographic distances separating sampled
populations ($R^2=0.09807, P<0.0001$, Figure 2.2). However, this relationship is weak, and
does not provide strong evidence in support of an isolation-by-distance scenario among
these populations. The loose correlation between genetic differentiation and geographic
distances in Figure 2.2, suggest that genetic relatedness among this invasive organism is
probably the product of a more erratic contributing factor, such as human-assisted
transport of propagules, supported by the degree of scatter among data points. Therefore,
it is not expected to find significant relationships between geographic locations of
documented sites of introduction and genetic signals of sampled populations. Inferences
of population structure are primarily based on genetic distances, population
differentiation, and partitioning of genetic variation.

**Population Structure**

STRUCTURE analysis inferred two groups ($K = 2$) as the most likely number of
clusters in the dataset (Figure 2.3; N=388 from 21 populations). This STRUCTURE
analysis also shows admixture present between the two detected clusters. Admixture was
present in both clusters and was more significant in some populations than others (e.g.,
AL-6). The degree of genetic admixture and observed patterns of genetic diversity
between the two detected groups also provides support for the potential of intraspecific hybridization contributing toward success of this invasive species in this region (O’Hanlon et al. 1999).

Principal coordinates analysis (PCA) on the same genetic dataset also resulted in two clusters: one well-grouped cluster and another less organized cluster, with some intermediate individuals (Figure 2.4). The first two axes in the PCA explain nearly 70% of the variation of the same genetic dataset. The break between the two clusters in STRUCTURE and the PCA is further supported by genetic diversity indices as presented earlier. In Figure 2.3, the first cluster (black) includes populations MS-1, 2, 3, 4, 5, and individuals from population MS-6. The second cluster (white) includes populations MS 7, 8, and 9, all populations sampled in AL, and some individuals from population MS-6. Principal coordinates analysis (PCA) assigned individuals into two clusters as well (Figure 2.4), and the results are concordant with those from the STRUCTURE analysis. Spatial distribution (Figure 2.1) of the first cluster (MS-group) shows those populations are geographically contained to MS, more specifically, in the Bienville National Forest and the northwest portion of the Desoto National Forest, based on this study’s sampling. The second cluster (AL-group) includes the rest of the populations sampled from the Desoto NF, coastal MS populations, and all sampled AL populations. The cluster comprised by individuals identified as belonging to the AL-group is more scattered (Figure 2.4), suggesting more variation among individuals of the AL-group. The MS-group is more tightly clustered, possessing less variation within the cluster. This may indicate reduced genetic variability with low levels of subsequent introgression from AL-group populations.
Analyses of molecular variance (AMOVA) were conducted to test the degree of genetic similarity between populations located in MS and AL, and another to test between the two inferred genetic groups, based on STRUCTURE and PCA results (Table 2.5.A and B). When populations are grouped by state of origin (MS or AL), analysis of molecular variance (AMOVA) revealed significant genetic partitioning (Table 2.5.A): 54% of genetic variation partitioned within populations, 33% among populations and within groups, and 10% partitioned among groups (defined as MS or AL; $F_{ST} = 0.435, P < 0.001$). However, an AMOVA conducted among groups defined by genetic data derived from STRUCTURE and PCA, resulted in a reduction in the amount of molecular variation partitioned within-populations (50%), and also among populations and within genetic groups (22%). However, a substantial increase in population structure was observed, where the amount of molecular variation among groups increased to greater than 27% ($F_{ST} = 0.499, P < 0.001$). This lack of genetic similarity between the two genetic groups supports two potential sources of parent material, with some admixture. The location for this admixture is likely along the southern AL/MS border (Figure 2.1, Table 2.4, Figure 2.3, Figure 2.4). These data suggest two genetically different groups among sampled populations of *I. cylindrica* in MS and AL, without introduction of *a priori* biases about population structure. These results are in concordance and support geographic-genetic population structuring of cogongrass populations as sampled in MS and AL. The population structuring is directed more by genetic identity rather than state of origin or geographic location.

Resulting partitioning of molecular variation resulted in similar partitioning as published by Capo-chichi et al. (2008), who also found the bulk of genetic variation
within, rather than among, cogongrass populations. This pattern where the bulk of
genetic variation is partitioned within populations can also observed in other studies on
outcrossing grasses (buffalograss, Huff et al. 1993; crested wheatgrass, Mellish et al.
2002; smooth bromegrass, Li et al. 2006). Cogongrass, being a known perennial obligate
outcrossing species (Bryson & Carter 1993; Gabel 1982), experiences significant sexual
recombination among genetically different individuals, then retaining this accumulated
variation within populations (Nybom 2004). This pattern may also be amplified by non-
equilibrium conditions generated by multiple introductions of parent material, whether
genetically similar or not, reducing genetic population structuring (Genton et al. 2005).
Observed within-population variation supports substantial admixture in terms of
intraspecific hybridization (e.g., among progeny of the original Philippine and Japanese
introductions) as another potential mechanism leading to increases in within-population
diversity (Kolbe et al. 2004).

**Contact Population**

Interpretation of population structure data indicated that both groups (AL-group
and MS-group) co-occur within a population identified as MS-6. This population is
located in Greene Co., MS. Individual tissue samples were collected along two sides of a
right-of-way (ROW), bordering private land within the Desoto NF acquisition boundary.
When this population was sampled, individuals were collected first from the north ROW
and subsequently from the south ROW (Figure 2.4). Principal coordinates analysis also
assigned 12 of the 20 individuals from MS-6 within the MS-group cluster, and the
remaining eight individuals within the AL-group cluster (Figure 2.3), in concordance
with the sampling as stated above. More specifically, the eight individuals collected from

50
the south ROW cluster tightly with other individuals from populations located in Desoto NF (MS-7, 8) and the MS Gulf coast (MS-9, 10, 11). This population (MS-6) represents a single likely ‘contact population’ where the two detected genetic groups have physically met, and provides support for contemporary intraspecific hybridization in cogongrass.

Conclusions

AFLP analysis of cogongrass populations from across two southern states that received direct, documented introductions of propagules from Asia, found variable genetic diversity among the 21 sampled populations. The mean percentage of polymorphic loci, among all populations in this study, is lower than reported values in other recent studies also utilizing AFLP analysis on grass species (Li et al. 2006; Nissar et al. 2010; Baba et al. 2012). Average heterozygosity was also reduced in comparison to other plant species analyzed by dominant molecular markers, regardless of demographic life history characteristics, including the type of breeding system, breadth of range, dispersal strategy, or successional status; however, comparison of $F_{ST}$ and $F_{ST}$ analogs across those same variables for plants analyzed with dominant markers, found greater population structure in the partitioning of molecular variation among groups in this study of cogongrass than other similar plant species (long-lived perennials, all geographic ranges, mixed breeding system, and wind dispersed; see: Nybom 2004). This pattern shows that cogongrass populations are comparatively less diverse genetically than other plant species. Reductions in cogongrass genetic variability may be due to lower numbers of founding propagules, reduced frequency of introduction, or the lack of time since founding for populations to accrue and retain genetic diversity over time. Many of the
cogongrass populations sampled may then be relatively young and may still be influenced by a genetic bottleneck and/or lack of proximal, genetically distinct populations with which to outcross. The diversity that is found in cogongrass populations is primarily partitioned within-populations; among-population variation is enough to result in significant and substantial population structure among detected genetic groups ($F_{ST}=0.499$ between MS and AL-types).

The results of this AFLP study, from varied analyses, are consistent for two genetic groups; one, the MS-type, is currently localized to central Mississippi (among areas sampled for this study), whereas the AL-group includes all sampled populations in Alabama and coastal Mississippi (Figure 2.1). Concordant results from analyses combined with the spatial distribution of detected genetic groups support a two-introduction scenario as suggested by the literature (Tabor 1949, 1952). Two introductions of parent material were made from isolated populations in Asia, where propagules from Japan were introduced into the vicinity of Mobile Bay, AL (1912), and propagules from the Philippines were introduced into McNeil, MS (1921). The MS-type (MS-1 through most of 6) is genetically distant and differentiated from populations assigned to the AL-type. However, admixture between the two types is present.

The geographic localization of the MS-type indicates that it may be related to the Philippine introduction, but identification of source parent material(s) is not addressed in this study. Sampling for this study was primarily limited to public land (i.e., National Forests), and limited broader spatial examination of the occurrence of these genetic groups. More intensive sampling of additional populations throughout MS and AL, in addition to reference material from Asia, may suggest whether or not the central MS-
group is the direct result of a separate introduction of parent material. Direct sampling of populations in and around McNeil, MS, may also address the origin of MS-group parental material and determine the degree of relatedness to a separate Philippine introduction more directly. However, even with direct sampling of population(s) occurring in McNeil, MS, genetic support may not be present, as there is the possibility that the parent signal, if present in McNeil, MS, may be swamped by introductions of various genetic materials during the time since introduction. Phylogenetic analysis with parent material from Japan and the Philippines may be able to more clearly elucidate genetically based relationships and provide more information with regard to origin sites of cogongrass, and its multiple-introduction history.

From this study, coastal Mississippi populations were found to genetically cluster within the AL-group. These populations on the Mississippi coast are also genetically similar to populations in west-central Alabama. These occur geographically between and north of the two sites of initial introduction. Coastal MS populations are least genetically distant from population in west central Alabama. These results suggest these populations may have sourced one another and/or originated from the same, possibly hybridized, propagule pool.

This genetic analysis of cogongrass populations in MS and AL found the majority of genetic variation partitioned within populations consistent with an obligate outcrossing and perennial species, but with significant population structure among two distinct genetic groups. This study also found the presence of a contact population (MS-6), where the two genetic groups detected in this study have physically met. The presence of admixture of genetic material between the two detected genetic groups in this study
support intraspecific hybridization having occurred; in the case of this contact population (MS-6), the physical meeting of two distinct genetic types suggests such an event will likely occur again.

Studies on other invasive plant species affecting the U.S. have suggested multiple introductions driving high genetic diversity within and among populations of the same species (Pappert et al. 2000; Baker & Dyer 2011). For *Microstegium vimineum* populations in Virginia, another invasive grass affecting the East and Southeast, AFLP analysis found higher within-population genetic diversity, where percentage of polymorphic loci per population, and on average, are comparatively higher than those values for cogongrass populations in this study (possibly due to differing mating systems and life history); by addressing population structure, comparatively similar population structure were found, suggesting multiple introductions and secondary contact between differing lineages of *M. vimineum* (Baker & Dyer 2011).

Propagules are the transported, viable individuals involved in invasions; propagule pressure is the product of the number of propagules and the frequency of introductions (Lockwood et al. 2005). Propagule pressure is considered to be one of the most significant contributing factors capable of defining a founding population’s success or failure as a nascent invasive species (Eppstein & Molofsky 2007; Catford et al. 2009). Cogongrass invasion in the U.S. has reached the stages of substantial range expansion, and its perceived negative impact across the invaded range is considered threatening to native and managed systems (Bryson & Carter 1993, MacDonald 2004; Miller 2007). If “increased propagule pressure will serve to increase genetic variability in an incipient non-native population, and thereby increases the chances of establishment” (Lockwood et
al. 2005), then genetic diversity, and the events that increase or limit such diversity within and among populations, are a function of propagule pressure and influences the chances of successful establishment, range expansion, and perceived impact. Therefore, quantification of genetic diversity in populations of cogongrass in the states of documented introduction begins to reveal the quantity of extant genetic diversity and to infer the historical processes that have affected the success of this invasion. Components of propagules, such as intrinsic genetic variability, may be one vehicle in which to quantify propagule pressure of an invasion, especially due to data on propagule pressure being rarely available or reliable (Ward et al. 2008).

A large quantity of introduced genetic variability may be considered a function of the number of propagules at the time of introduction, providing a more chances of success through a greater range of adaptability and/or selection for beneficial traits (Lockwood et al. 2005). The introduction of substantial genetic diversity can better equip an invading population to successfully establish and can promote a self-sustaining population in a short duration without lag phases due to genetic bottlenecks or other such founding effects (Dlugosch & Parker 2008). Alternatively, limited genetic variation upon introduction, coupled with the effects of a genetic bottleneck would therefore reduce the potential successful establishment and subsequent spread, characteristic of successful invasions (Sakai et al. 2001; Lee 2002). Changes in genetic variation via novel genetic recombination, such as intraspecific hybridization events, can contribute toward successful invasion (Ellstrand and Schierenbeck 2000; Hughes et al. 2008; Vellend et al. 2010). These inferences also provide some support for relatively rapid evolution within ecological time scales during invasions (Sakai et al. 2001; Lee 2002; Ward et al. 2008).
Comparatively low genetic variation within and among cogongrass populations have not limited aggressive range expansion and prevalent negative impacts. However, molecular data supporting intraspecific hybridization between genetically differentiated groups, in addition to an already ruderal and adaptable nature, contribute toward explaining its rapid expansion during the last century. Therefore, invasion success is partially a product of multiple introductions and the amount of introduced and/or generated genetic variation through hybridization events, in the context of propagule pressure (Coulatti et al. 2006; Catford et al. 2009).
Table 2.1 Six AFLP selective amplification primer combinations utilized.

<table>
<thead>
<tr>
<th>MseI Primer</th>
<th>Fluorescent Dye-Labeled EcoRI Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MseI-CAT</td>
<td>EcoRI-ACT-FAM</td>
</tr>
<tr>
<td>MseI-CTA</td>
<td>EcoRI-AGG-HEX</td>
</tr>
<tr>
<td>MseI-CTG</td>
<td>EcoRI-AGC-NED</td>
</tr>
<tr>
<td>MseI-CTT</td>
<td>EcoRI-ACT-FAM</td>
</tr>
<tr>
<td>MseI-CTC</td>
<td>EcoRI-AGG-HEX</td>
</tr>
<tr>
<td>MseI-CAC</td>
<td>EcoRI-AGC-NED</td>
</tr>
</tbody>
</table>

NOTE: Fluorescent dye-labeled selective primers are denoted by “EcoRI-primer-[Axx]-[dye]. Each fluorescent dye is visualized as a different color for fragment analysis: FAM (blue), HEX (green), NED (yellow or black).
Table 2.2  Genetic Diversity Indices with Population Location Information.

<table>
<thead>
<tr>
<th>Population</th>
<th>County, State</th>
<th>Other Location Information</th>
<th>Number of individuals (n)</th>
<th>Number of Bands Detected</th>
<th>Number of Private Bands</th>
<th>Percentage Polymorphic Loci</th>
<th>$H_e \pm SE$</th>
<th>$UH_e \pm SE$</th>
<th>Shannon's Information Index (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-1</td>
<td>Jasper Co., MS</td>
<td>Bienville NF</td>
<td>20</td>
<td>159</td>
<td>17</td>
<td>17%</td>
<td>0.035 ± 0.036 ± 0.058 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-2</td>
<td>Smith Co., MS</td>
<td>Bienville NF</td>
<td>20</td>
<td>136</td>
<td>10</td>
<td>14%</td>
<td>0.035 ± 0.037 ± 0.056 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-3</td>
<td>Scott Co., MS</td>
<td>Bienville NF</td>
<td>20</td>
<td>150</td>
<td>7</td>
<td>15%</td>
<td>0.041 ± 0.043 ± 0.064 ± 0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-4</td>
<td>Scott Co., MS</td>
<td>Bienville NF</td>
<td>10</td>
<td>113</td>
<td>3</td>
<td>11%</td>
<td>0.032 ± 0.035 ± 0.050 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-5</td>
<td>Jones Co., MS</td>
<td>Bienville NF</td>
<td>20</td>
<td>225</td>
<td>36</td>
<td>26%</td>
<td>0.035 ± 0.036 ± 0.058 ± 0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-6</td>
<td>Greene Co., MS</td>
<td>DeSoto NF</td>
<td>20</td>
<td>200</td>
<td>48</td>
<td>23%</td>
<td>0.048 ± 0.051 ± 0.079 ± 0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-7</td>
<td>Wayne Co., MS</td>
<td>DeSoto NF</td>
<td>20</td>
<td>88</td>
<td>10</td>
<td>10%</td>
<td>0.016 ± 0.017 ± 0.029 ± 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-8</td>
<td>Wayne Co., MS</td>
<td>DeSoto NF</td>
<td>20</td>
<td>83</td>
<td>6</td>
<td>9%</td>
<td>0.013 ± 0.013 ± 0.024 ± 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-9</td>
<td>Harrison Co., MS</td>
<td></td>
<td>10</td>
<td>48</td>
<td>6</td>
<td>5%</td>
<td>0.018 ± 0.019 ± 0.027 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-10</td>
<td>Harrison Co., MS</td>
<td></td>
<td>10</td>
<td>41</td>
<td>2</td>
<td>4%</td>
<td>0.013 ± 0.014 ± 0.020 ± 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS-11</td>
<td>Harrison Co., MS</td>
<td></td>
<td>10</td>
<td>64</td>
<td>10</td>
<td>7%</td>
<td>0.015 ± 0.017 ± 0.026 ± 0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-1</td>
<td>Sumter Co., AL</td>
<td></td>
<td>20</td>
<td>141</td>
<td>25</td>
<td>16%</td>
<td>0.025 ± 0.026 ± 0.045 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-2</td>
<td>Hale Co., AL</td>
<td>Talladega NF</td>
<td>20</td>
<td>218</td>
<td>34</td>
<td>26%</td>
<td>0.043 ± 0.045 ± 0.077 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-3</td>
<td>Hale Co., AL</td>
<td>Talladega NF</td>
<td>20</td>
<td>99</td>
<td>15</td>
<td>11%</td>
<td>0.022 ± 0.023 ± 0.037 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-4</td>
<td>Washington Co., AL</td>
<td></td>
<td>20</td>
<td>283</td>
<td>127</td>
<td>32%</td>
<td>0.049 ± 0.051 ± 0.088 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-5</td>
<td>Washington Co., AL</td>
<td>Frank Boykin WMA</td>
<td>20</td>
<td>109</td>
<td>0</td>
<td>10%</td>
<td>0.025 ± 0.026 ± 0.040 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-6</td>
<td>Baldwin Co., AL</td>
<td></td>
<td>20</td>
<td>123</td>
<td>4</td>
<td>12%</td>
<td>0.031 ± 0.032 ± 0.050 ± 0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-7</td>
<td>Baldwin Co., AL</td>
<td></td>
<td>20</td>
<td>91</td>
<td>0</td>
<td>10%</td>
<td>0.022 ± 0.023 ± 0.036 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-8</td>
<td>Mobile Co., AL</td>
<td></td>
<td>20</td>
<td>85</td>
<td>1</td>
<td>9%</td>
<td>0.019 ± 0.020 ± 0.031 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-9</td>
<td>Mobile Co., AL</td>
<td></td>
<td>20</td>
<td>74</td>
<td>12</td>
<td>8%</td>
<td>0.016 ± 0.017 ± 0.026 ± 0.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL-10</td>
<td>Lee Co., AL</td>
<td>Auburn</td>
<td>28</td>
<td>177</td>
<td>67</td>
<td>20%</td>
<td>0.025 ± 0.026 ± 0.046 ± 0.004</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Overall Total or Average**

|               | 388 | 850 | 14% ± 2% | 0.001 | 0.001 | 0.001 |

± standard error (SE)

**NOTE:** Population information, genetic diversity indices ($H_e$=expected heterozygosity, $UH_e$=unbiased expected heterozygosity, $I$=Shannon’s Information index, with per population) for 21 sampled cogongrass populations in Mississippi and Alabama. Overall totals and averages are in **bold.**
Table 2.3  Clonal Diversity Analysis based on AFLP Multilocus Data.

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of individuals (n)</th>
<th>Number of Genotypes</th>
<th>Genotype Diversity</th>
<th>Effective Number of Genotypes</th>
<th>Nei’s Gene Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-1</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.036</td>
</tr>
<tr>
<td>MS-2</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.037</td>
</tr>
<tr>
<td>MS-3</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.043</td>
</tr>
<tr>
<td>MS-4</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>10</td>
<td>0.035</td>
</tr>
<tr>
<td>MS-5</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.053</td>
</tr>
<tr>
<td>MS-6</td>
<td>20</td>
<td>18</td>
<td>0.984</td>
<td>15.385</td>
<td>0.052</td>
</tr>
<tr>
<td>MS-7</td>
<td>20</td>
<td>17</td>
<td>0.979</td>
<td>14.286</td>
<td>0.019</td>
</tr>
<tr>
<td>MS-8</td>
<td>20</td>
<td>10</td>
<td>0.711</td>
<td>3.077</td>
<td>0.022</td>
</tr>
<tr>
<td>MS-9</td>
<td>10</td>
<td>9</td>
<td>0.978</td>
<td>8.333</td>
<td>0.021</td>
</tr>
<tr>
<td>MS-10</td>
<td>10</td>
<td>9</td>
<td>0.978</td>
<td>8.333</td>
<td>0.015</td>
</tr>
<tr>
<td>MS-11</td>
<td>10</td>
<td>7</td>
<td>0.867</td>
<td>4.545</td>
<td>0.023</td>
</tr>
<tr>
<td>AL-1</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.026</td>
</tr>
<tr>
<td>AL-2</td>
<td>20</td>
<td>18</td>
<td>0.989</td>
<td>16.667</td>
<td>0.049</td>
</tr>
<tr>
<td>AL-3</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.023</td>
</tr>
<tr>
<td>AL-4</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.051</td>
</tr>
<tr>
<td>AL-5</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.026</td>
</tr>
<tr>
<td>AL-6</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>20</td>
<td>0.032</td>
</tr>
<tr>
<td>AL-7</td>
<td>20</td>
<td>19</td>
<td>0.995</td>
<td>18.182</td>
<td>0.024</td>
</tr>
<tr>
<td>AL-8</td>
<td>20</td>
<td>15</td>
<td>0.963</td>
<td>11.765</td>
<td>0.023</td>
</tr>
<tr>
<td>AL-9</td>
<td>20</td>
<td>17</td>
<td>0.979</td>
<td>14.286</td>
<td>0.018</td>
</tr>
<tr>
<td>AL-10</td>
<td>20</td>
<td>20</td>
<td>0.963</td>
<td>14</td>
<td>0.032</td>
</tr>
<tr>
<td>Overall</td>
<td>388</td>
<td>349</td>
<td></td>
<td></td>
<td>318.858</td>
</tr>
</tbody>
</table>

NOTE: Population identifiers, sample size, and resulting clonal diversity from multilocus AFLP data, including number of different genotypes detected, genotypic diversity, effective number of genotypes, and Nei’s gene diversity for each of the 21 analyzed populations. Overall totals and averages are in **bold**.
Table 2.4  
Pairwise population matrix of Nei’s unbiased genetic distances and pairwise population F_{ST} matrix.

### Pairwise Population Matrix of F_{ST}

<table>
<thead>
<tr>
<th></th>
<th>MS-1</th>
<th>MS-2</th>
<th>MS-3</th>
<th>MS-4</th>
<th>MS-5</th>
<th>MS-6</th>
<th>MS-7</th>
<th>MS-8</th>
<th>MS-9</th>
<th>MS-10</th>
<th>AL-1</th>
<th>AL-2</th>
<th>AL-3</th>
<th>AL-4</th>
<th>AL-5</th>
<th>AL-6</th>
<th>AL-7</th>
<th>AL-8</th>
<th>AL-9</th>
<th>AL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-1</td>
<td>0.0</td>
<td>0.152</td>
<td>0.116</td>
<td>0.124</td>
<td>0.206</td>
<td>0.166</td>
<td>0.551</td>
<td>0.438</td>
<td>0.505</td>
<td>0.362</td>
<td>0.487</td>
<td>0.442</td>
<td>0.415</td>
<td>0.481</td>
<td>0.540</td>
<td>0.505</td>
<td>0.605</td>
<td>0.577</td>
<td>0.598</td>
<td>0.584</td>
</tr>
<tr>
<td>MS-2</td>
<td>0.099</td>
<td>0.0</td>
<td>0.139</td>
<td>0.186</td>
<td>0.223</td>
<td>0.183</td>
<td>0.554</td>
<td>0.454</td>
<td>0.518</td>
<td>0.387</td>
<td>0.513</td>
<td>0.469</td>
<td>0.460</td>
<td>0.511</td>
<td>0.557</td>
<td>0.530</td>
<td>0.602</td>
<td>0.575</td>
<td>0.600</td>
<td>0.588</td>
</tr>
<tr>
<td>MS-3</td>
<td>0.099</td>
<td>0.011</td>
<td>0.074</td>
<td>0.170</td>
<td>0.173</td>
<td>0.543</td>
<td>0.401</td>
<td>0.512</td>
<td>0.328</td>
<td>0.513</td>
<td>0.448</td>
<td>0.429</td>
<td>0.504</td>
<td>0.548</td>
<td>0.490</td>
<td>0.627</td>
<td>0.591</td>
<td>0.620</td>
<td>0.599</td>
<td>0.610</td>
</tr>
<tr>
<td>MS-4</td>
<td>0.008</td>
<td>0.010</td>
<td>0.092</td>
<td>0.112</td>
<td>0.412</td>
<td>0.302</td>
<td>0.379</td>
<td>0.265</td>
<td>0.411</td>
<td>0.248</td>
<td>0.313</td>
<td>0.351</td>
<td>0.400</td>
<td>0.395</td>
<td>0.459</td>
<td>0.422</td>
<td>0.440</td>
<td>0.440</td>
<td>0.432</td>
<td>0.459</td>
</tr>
<tr>
<td>MS-5</td>
<td>0.009</td>
<td>0.014</td>
<td>0.008</td>
<td>0.163</td>
<td>0.273</td>
<td>0.183</td>
<td>0.311</td>
<td>0.212</td>
<td>0.414</td>
<td>0.334</td>
<td>0.298</td>
<td>0.274</td>
<td>0.314</td>
<td>0.326</td>
<td>0.366</td>
<td>0.316</td>
<td>0.284</td>
<td>0.278</td>
<td>0.366</td>
<td></td>
</tr>
<tr>
<td>MS-6</td>
<td>0.014</td>
<td>0.011</td>
<td>0.017</td>
<td>0.013</td>
<td>0.008</td>
<td>0.555</td>
<td>0.449</td>
<td>0.525</td>
<td>0.388</td>
<td>0.518</td>
<td>0.476</td>
<td>0.471</td>
<td>0.511</td>
<td>0.557</td>
<td>0.527</td>
<td>0.611</td>
<td>0.588</td>
<td>0.602</td>
<td>0.591</td>
<td>0.607</td>
</tr>
</tbody>
</table>

**NOTE:** All population pairwise F_{ST} values are significant (P<0.05). Population pairwise values are among 21 sampled Mississippi and Alabama cogongrass populations. Population identifiers are in **bold**. Shaded values are genetic distances and F_{ST} values between MS-1 through MS-6.
Table 2.5  Results from analysis of molecular variation (AMOVA) using $F_{ST}$.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Percentage of variation</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Groups</td>
<td>1</td>
<td>622.01</td>
<td>10.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>19</td>
<td>2916.34</td>
<td>33.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within populations</td>
<td>367</td>
<td>4776.53</td>
<td>54.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>387</td>
<td>8314.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F_{ST} = 0.435 (P<0.001), F_{SC} = 0.370 (P<0.001), F_{CT} = 0.104 (P<0.001)$


B.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Percentage of variation</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Groups</td>
<td>1</td>
<td>1251.15</td>
<td>27.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>19</td>
<td>2287.20</td>
<td>22.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Within populations</td>
<td>367</td>
<td>4776.53</td>
<td>50.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>387</td>
<td>8314.87</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F_{ST} = 0.499 (P<0.001), F_{SC} = 0.310 (P<0.001), F_{CT} = 0.275 (P<0.001)$

NOTE: (A) AMOVA groups defined based on state of location (MS or AL political boundaries), (B) AMOVA Group 1 defined as cluster 1 (MS-type) and group 2 defined as cluster 2 (AL-type) as inferred from STRUCTURE analysis. Greater $F_{ST}$ is observed when groups are tested based on genetic information (B) than when defined by political boundaries (A).
Figure 2.1  Map with 21 marked cogongrass (*I. cylindrica*) populations sampled from Mississippi and Alabama (US).

NOTE: Areas shaded gray represent National Forests acquisition boundaries. Square symbols represent populations that genetically identify as “MS-type”; circle symbols represent populations that genetically identify as “AL-type”. Star symbol represents population MS-6, identified as a contact population where two genetic groups have physically met. Each population marker is labeled with its population identifier, (N=388).
Figure 2.2  Correlation between population pairwise geographic distances and pairwise population $F_{ST}$ values.

NOTE: Slightly positive, significant ($R^2=0.098$, $P<0.001$). Geographic distances are straightline. All pairwise population $F_{ST}$ values are significant ($P<0.005$).
Figure 2.3  Bar plot from STRUCTURE analysis assuming two populations ($K=2$) among samples from MS and AL.

NOTE: A total of 21 populations from MS and AL were analyzed using STRUCTURE. Population identifier labels along the bottom horizontal axis; individuals are represented by vertical bars and horizontally organized by population. Cluster 1 (“MS-type”) is predominantly black and cluster 2 (“AL-type”) is predominantly white, with admixture present between the two groups. Each bar represents an individual. Each cluster is represented by a different color. Different colors (black or white) represent the frequency of AFLP loci assigned to clusters, where some individuals are completely assigned to one cluster or another, while others show mixed ancestry.
Figure 2.4  Principal coordinates analysis (PCA) of genetic covariance (with data standardization) of cogongrass individuals in MS and AL.

NOTE: A total of 388 individuals from 21 populations were analyzed. Individuals from MS-6 occur in both clusters. The first two axes explain 69.11% of the variation in this dataset. Individuals from MS-1, 2, 3, 4, and 5, form one cluster in the upper-right quadrant. Individuals from MS-7, 8, 9, 10, and 11, form a tight cluster in the upper-left quadrant and co-occur with individuals from AL populations. This is considered part of the larger, scattered cluster comprised of all AL populations.
Figure 2.5 Satellite imagery of inferred contact population where two genetic groups co-occur (Population MS-6).

NOTE: Twenty individuals were collected from this site, located on private land within the Desoto National Forest acquisition boundary, MS. Ten individuals collected from the north right-of-way, and two individuals from the northeast corner of the intersection clustered within the “MS-type” group (white line). Eight individuals collected from the south right-of-way clustered within the “AL-type” group (black line).


CHAPTER III
COGONGRASS IN FLORIDA: DOES MOLECULAR EVIDENCE SUPPORT TWO DIFFERENT SPECIES AND INTERSPECIFIC HYBRIDIZATION WITH BRAZILIAN SATINTAIL?

Abstract

A single, ephemeral morphological character in flower morphology taxonomically differentiates cogongrass (*Imperata cylindrica*) from Brazilian satintail (*Imperata brasiliensis*) known to co-occur in the Southeast U.S., with speculation concerning interspecific hybridization occurring between these two species generating ‘hybrid swarms’ contributing toward aggressive cogongrass invasion throughout the region. This study is meant to determine whether a genetic signal of two different species and/or evidence to support interspecific hybridization can be detected. Sampling included invasive *I. cylindrica* populations from throughout FL (n=66 individuals), while *I. brasiliensis* populations were sampled solely from isolated and non-expanding populations located in municipal parks within Miami-Dade County, in South FL (n=63 individuals). Genetic analysis utilized amplified fragment length polymorphisms (AFLPs). Genetic diversity measures found relatively similar diversity within groups denoted as one of the species: *I. cylindrica* (8-26% polymorphic loci; $U_{He} = 0.033$ to 0.082; Shannon’s Information index = 0.042 to 0.116) or *I. brasiliensis* (7-39% polymorphic loci; $U_{He} = 0.020$ to 0.051; Shannon’s Information index = 0.029 to 0.134).
Analysis of molecular variance (AMOVA) partitioned the majority of detected variation within populations (86%), while 8% of the variation was partitioned between *I. cylindrica* and *I. brasiliensis* ($F_{ST} = 0.135$, significant at $P<0.05$). STRUCTURE analysis could not conclusively determine a biologically relevant number of genetic groups, resulting in failure to reject the null hypothesis for one group to explain this dataset. Population pairwise $F_{ST}$ values resulted in greater genetic dissimilarity between populations grouped within *I. brasiliensis*, than between populations assigned to *I. brasiliensis* and *I. cylindrica*, or between populations of *I. cylindrica*, alone. The observed pattern of genetic variation and partitioning of that variation, as detected in this study, suggest that populations located in South FL that are currently considered *I. brasiliensis*, are not supported to be a separate species by this genetic analysis. This further suggests that populations sampled in this study are not necessarily different species, but are also from the same genetic parent material(s). Furthermore, admixture clearly has occurred between populations in FL, and substantial population structure was not found in support of the hypothesis for two congeneric species having hybridized. Therefore, the impressive cogongrass spread throughout much of the region appears not to have resulted from interspecific hybridization.

**Introduction**

Cogongrass is a highly invasive and problematic species affecting the majority of the southeastern region of the United States (U.S.) (Bryson & Carter 1993; MacDonald 2004). Cogongrass (*Imperata cylindrica* (L.) Raesusch.) introductions from Japan and the Philippines into the region have been documented in the literature (Tabor 1949, 1952).
The aggressive invasive growth and spread of cogongrass has made management and containment of range expansion difficult (MacDonald 2004; Miller 2007). The characteristic invasive ecology of cogongrass has been attributed, in part, to interspecific hybridization with co-occurring congenic(s), such as Brazilian satintail (Howard 2005). Brazilian satintail (*Imperata brasiliensis* Trin.) is reported from Puerto Rico and five southern states (Louisiana, Mississippi, Alabama, Florida, and South Carolina; USDA Plants). The southern U.S. distribution of *I. brasiliensis* also co-occurs with cogongrass (*Imperata cylindrica*) presence in those states, also actively managing cogongrass infestations at this time. The native distribution of *I. brasiliensis* spans southern California in North America, to Brazil and Argentina in South America (Hubbard et al. 1944; Wiggins 1980; Barkworth et al. 2003). In 2010, it became a listed Federal Noxious Weed. However, it is also considered naturalized in Florida, where it is presumed to have been introduced via the Caribbean from South America (e.g., Brazil and Argentina) (Hall 1978, 1998; Bryson & Carter 1993; Welker & Longhi-Wagner 2012).

Though some distribution maps report a broad U.S. distribution for *I. brasiliensis*, populations identified with some degree of certainty by botanists and local experts are localized to south Florida (Hall 1998; Vergara et al. 2008; Keith Bradley, personal correspondence; Hall, personal correspondence). The stands of *I. brasiliensis* are generally characterized by lack of invasive characters, such as no evident spatial expansion over several years of observation, nor development of thick rhizomatous mats in monoculture (Welker & Longhi-Wagner 2012; Bradley, personal correspondence). The current and only paradigm, to identify and differentiate between *I. cylindrica* and *I. brasiliensis*, is a single morphological character: inflorescences that possess bi-staminate
flowers diagnose *I. cylindrica* whereas all other species within *Imperata* (including *I. brasiensis*) possess single staminate flowers (Hubbard et al. 1944; Gabel 1982; Welker & Longhi-Wagner 2012). Stamen number is the single non-overlapping diagnostic character available separating co-occurring congeneric *Imperata* species and therefore, relies on availability of intact flowers and flower parts among populations that can markedly differ in phenology (Burnell 2005; Howard 2005). The ability to morphologically differentiate between the two species in Florida is difficult and may be unreliable, considering Hall (1978) found populations that were both single and bi-staminate. Misidentification of both species likely occurs (Bryson & Carter 1993; Hall 1998). Recent studies have invoked interspecific hybridization between these two species in an effort to explain genetic patterns (i.e., Capo-chichi et al. 2008; Vergara et al. 2008). This may be due in part to evident phenotypic plasticity and morphological variability in *I. cylindrica*, further confounding physical differentiation between this species and congenerics worldwide (Al-Jaboory & Hassawy 1980; Cheng & Chou 1997; Bryson et al. 2010)

This portion of my research is specifically aimed at determining if genetic data provide supportive evidence for interspecific hybridization, defined as the co-mingling of genetic material between *I. cylindrica* and *I. brasiensis*. The expectation is that if interspecific hybridization has occurred, the supporting evidence will be molecularly detectable. This study examines genetic variation within and among sampled populations in FL, how that genetic variation is structured, and how these populations compare to one another on the population level in the aim of finding support, if any, for two separate species co-occurring and their genomes co-mingling. The highly reproducible, dominant
genetic markers known as amplified fragment length polymorphisms (AFLPs) are used, again, to explore genetic structure among populations sampled in Florida. AFLPs are arbitrarily amplified dominant markers and were selected for several reasons: no *a priori* sequence information was necessary, the entire genome can be simultaneously sampled, and the technique is highly reproducible and practical (in cost and data generation) (Bussell et al. 2005; Campbell et al. 2003; Meudt and Clarke 2007). AFLPs were utilized in a recent study of invasive *I. cylindrica* populations in Alabama, wherein the authors invoked interspecific hybridization with *I. brasiliensis* to explain some of patterns of genetic variation (Capo-chichi et al. 2008). This study utilizes these markers, since they are known to amplify and to adequately detect genetic variation. However, more selective primer pairs are applied in an effort to better detect the genetic signals of two different species or of interspecific hybridization, if it exists.

Populations were identified as cogongrass, *I. cylindrica*, or Brazilian satintail, *I. brasiliensis*, based on geographic location, growth pattern and patch size, and input from knowledgeable, local naturalists. All *I. brasiliensis* for this study were collected from populations located in Miami-Dade County, FL (Figure 3.1). This was to try to collect U.S. *I. brasiliensis* tissues from the most isolated and the most likely populations belonging to this species. Collections were targeted in this area due to information gleaned from previous studies (Hall 1978, 1998), and further confirmation was provided by on-site identification by a local expert. Populations considered *I. cylindrica* were collected from other counties throughout Florida. Naturalists at the Disney Wilderness Preserve (DWP, The Nature Conservancy) also identified sampled cogongrass populations on-site and provided management histories of populations.
The previous chapter included these Florida samples where genetic structuring or partitioning was observed at the statewide scale. This current analysis will focus on population-level analysis within Florida alone, to determine whether derived genetic data will suggest any partitioning of genetic variance among populations at a finer scale. The expectation is that if the populations considered *I. brasiliensis* from Miami are a different species; they will form a distinct genetic group separating it from populations considered *I. cylindrica*. To support interspecific hybridization, an intermediate cluster of individuals or populations may be detected that are a blend of populations considered to be *I. cylindrica* or *I. brasiliensis*, or possess a differing genetic signal all together due to hybridization.

Alternatively, these two species have co-occurred for almost 100 years. It is possible that interspecific hybridization occurred early in cogongrass invasion, swamping any detectable ‘pure’ *I. brasiliensis* genetic signal at this present time. Hybridization events may have genetically extirpated *I. brasiliensis* from the U.S., since populations sampled in Miami are isolated and under substantial canopy, reducing the probability of successful outcrossing. In this case, interspecific hybridization would be inferred to have been frequent and prevalent, such that all Florida populations (and potentially others throughout the U.S.) are ‘hybrid swarms’ (Howard 2005). Where no population structure is detected may be attributed to a complete lack of species-level partitioning between populations identified as *I. cylindrica* and *I. brasiliensis*. Of note, this study does not aim to delimit species, but intends to preliminarily explore if population genetic structure is present between populations identified as or presumed to be *I. cylindrica* or *I. brasiliensis*.
Materials and Methods

Sampling

Live leaf tissues were collected in the field from Imperata populations in the state of Florida during the summer of 2009. Each tiller was assumed representative of an “individual” or a ramet in the located patch or population (while acknowledging that individual patches may have arisen from only one to a few colonizing propagules resulting in potentially few genets comprising a population). Populations were identified as contiguous patches of cogongrass, often occurring as circular patches in open areas or as long, narrow patches along roadside rights-of-way. Tissues from individual leaves were collected from throughout sampled populations to obtain a representative sampling of population diversity. Aboveground leaf tissues were stored in individually labeled plastic bags in a cooler. Tissues then were dried in the lab by placing tissues in silica gel with color indicator.

Both I. cylindrica and I. brasiliensis are listed Federal Noxious Weeds, requiring permits to collect, transport, and store live tissue accessions. Imperata brasiliensis became a Federally Listed Noxious Weed in 2010. Sampling was conducted in 2009, so a permit was required only for I. cylindrica at the time. A permit was granted by the U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine to move live plant pests, noxious weeds, and soil for I. cylindrica ( Permit #: P526P-12-00211, P526-080721-005). In Osceola County, a collections agreement with The Nature Conservancy was obtained to conduct sampling from invasive I. cylindrica patches located within the Disney Wilderness Preserve. A permit to sample within municipal parks was applied for and approved by the Miami-Dade County Parks and Recreation
Department-Natural Areas Management (MDPR Permit #145). The Institute for Regional Conservation (Keith Bradley) assisted with locating and identifying *I. brasiliensis* populations located in Miami-Dade County, FL.

*Imperata cylindrica* tissues were obtained from Alachua (Gainesville), Osceola (Kissimmee), Sarasota, Indian River (Vero Beach), and Duval Counties (Jacksonville) in Florida, from eight populations (n = 66 individuals). Six *I. brasiliensis* populations were sampled, occurring in municipal parks in Miami-Dade County (n = 63 individuals). These populations of *Imperata* were sampled in Miami based on Hall (1978, 1998), geographic location, and habitat-type. These somewhat isolated populations of *I. brasiliensis* were without flower structures present during the time of collection. *Imperata brasiliensis* populations that were sampled in Miami have not been known to flower in the recent past. Patch location and on-site identification of *I. brasiliensis* was provided by Keith Bradley (Institute for Regional Conservation, Miami, FL). The sampled patches were small and non-expanding, occurring primarily on pine rockland habitats. These traits led Bradley to consider these populations as *I. brasiliensis* and, as such, they were not considered or managed as an invasive species (personal correspondence).

A total of 129 individuals were sampled from Florida (Figure 3.1). Along with leaf tissues, habitat type (e.g., right-of-way, forested, residential, commercial, etc.), location coordinates (global positioning system [GPS] coordinates, WGS 1984), and approximate patch size (area) were documented or collected.
**Tissue processing and molecular methods**

Extraction of DNA utilized a modified NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue, produced and published by Applied Biosystems (Life Technologies, Carlsbad, CA, USA). Approximately 1 cm² of *Imperata* leaf tissue was aseptically transferred into a 2-ml microcentrifuge tube. Samples were fully disrupted utilizing a Retsch mixer mill and then processed using the ABI NucPrep chemistry for DNA extraction (NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue, P/N 4333959). Purified DNA was transferred into sterile, individually labeled tubes and frozen (-20 °C) until analysis (-80 °C for long-term storage).

Amplified fragment length polymorphism (AFLPs) analysis utilized a modified protocol for capillary electrophoresis based on technique and methodology developed by Vos et al. (1995). Extracted, purified DNA underwent digestion by restriction enzymes, ligation of linking primers, followed by pre-selective amplification to generate fragments of interest, and then, a selective cycle to amplify fragments into a marker set to detect polymorphisms within the genome among DNA samples. Restriction digest of individual genomic DNA was achieved in 25μl reactions incubated at 37 °C for 2 hours in a thermal cycler, finalized by denaturing of enzymes by heating samples to 70 °C for 15 minutes. Restriction digest enzymes and reagents utilized per reaction were: 1 μl of EcoRI (20,000 U/ml, 5’-G^AATT, 3’CTTAA^G; New England BioLabs, Ipswich, MA, USA), 1 μl of MseI (10,000 U/ml, 5’-T^TAA, 3’-AAT^T; New England BioLabs, Ipswich, MA, USA), 2.5 μl of included 10X NEBuffer4, 0.25 μl of 100 μg/ml BSA, 10 μl of individually purified genomic DNA, and 10.25 μl of sterile water.
Eco AFLP linkers were annealed in a thermal cycler by heating to 95 °C for 5 minutes and cooled to 5 °C over 30 minutes using the following reagents: 1 μl of Eco Linker 1 (100 μM, 5’-CTC GTA GAC TGC CC), 1 μl of Eco Linker 2 (100 μM, 5’-AAT TGG TAC GCA GTG TAC), 90 μl of TE buffer (10 mM Tris & 1 mM EDTA), and 108 μl sterile water. Mse AFLP linkers were annealed in a thermal cycler by heating to 95 °C for 5 minutes and cooled to 5 °C over 30 minutes using the following reagents: 10 μl of Mse Linker 1 (100 μM, 5’-GAC GAT GAG TCC TGA G), 10 μl of Mse Linker 2 (100 μM, 5’-TAC TCA GGA CTC AT), 90 μl of TE buffer (10 mM Tris & 1 mM EDTA), and 90 μl sterile water. Annealed linkers are stored frozen at -20 °C until use for ligation of linkers.

Ligation of Eco and Mse linkers were conducted in 20 μl reactions comprised of: 1 μl each of Eco and Mse Linker (as annealed above), 1 μl T4 DNA Ligase enzyme and 2 μl of included 10X T4 DNA Ligase Reaction Buffer (New England BioLabs, Ipswich, MA, USA), 10 μl of individually digested DNA, and 5 μl sterile water. Ligation reactions were conducted in a thermal cycler at 16 °C overnight or at 37 °C for 3 hours. Individual ligated reactions were stored at -80 °C to prevent degradation.

Pre-selective 20 μl polymerase chain reaction (PCR)-conditions for amplification were: 1 μl each of Eco+A (10 μM, 5’-GAC TGC GTA CCA ATT CA) and Mse+C (10 μM, 5’-GAT GAG TCC TGA GTA AC) primers, 2.5 μl of dNTPs (0.2 mM dATP, dCTP, dGTP, and dTTP; Deoxynucleotide Solution Set, New England BioLabs, Ipswich, MA, USA), 4 μl 5X GoTaq™ FlexiBuffer (Promega Corp., Madison, WI, USA), 1.2 μl MgCl₂ (Promega Corp., Madison, WI, USA), 3 μl of individually ligated DNA, 7.1 μl sterile water, and 0.2 μl GoTaq™ DNA polymerase (5u/μl, Promega Corp., Madison, WI,
Pre-selective amplifications consisted of an initial denaturing step of 94 °C for 1 minute, 30-cycles of 30 seconds at 94 °C, 1 minute at 56 °C, and 1 minute at 72°C, and followed by final annealing for 2 minutes at 72 °C.

For selective amplification, pre-selective amplification products are individually diluted 1:20 with sterile water. Each combination of selective Mse and fluorescent Eco selective primers is a separate PCR amplification. Selective amplification for all individuals was achieved in 20 μl reactions consisting of: 1 μl each of Mse (5μM) and fluorescent Eco (1 μM) selective primer (see: Table 2.1), 2.5 μl of dNTPs (0.2 mM dATP, dCTP, dGTP, and dTTP; Deoxynucleotide Solution Set, New England BioLabs, Ipswich, MA, USA), 4 μl 5X GoTaq™ FlexiBuffer (Promega Corp., Madison, WI, USA), 3.7 μl MgCl₂ (Promega Corp., Madison, WI, USA), 3 μl of diluted pre-selective amplification product, 4.6 μl sterile water, and 0.2 μl GoTaq™ DNA polymerase (5u/μl, Promega Corp., Madison, WI, USA). Selective amplifications consisted of an initial denaturing step of 94 °C for 2 minutes, 10-cycles of 30 seconds at 94 °C, 30 seconds at 65 °C, and 1 minute at 72°C (reducing annealing temperature by 1 °C/cycle), 30-cycles of 30 seconds at 94 °C, 30 seconds at 56 °C, and 1 minute at 72°C, and finished with 30 seconds at 72 °C.

Six selective primer sets were applied to each individual in this study (Table 3.1). The selective primers are fluorescently tagged where products from multiple combinations of DNA primers can be analyzed at once. Three different fluorescently tagged products (1.5 μl of each) were combined per well with a single-stranded, fluorescent ROX-1000 size standard (0.25 μl; MapMarker (50-1000), BioVentures, Inc., Murfreesboro, TN, USA) and fixed with formamide (10 μl, Hi-Di™, Life Technologies,
Carslbad, CA, USA). Pooled fragment products were run on an ABI 3730 capillary sequencer at the Arizona State University DNA Lab (Tempe, AZ, USA) with positive and negative control sample replicates. Positive control replicates consisted of one individual randomly selected at the beginning of the study and followed each set and step of molecular analysis throughout to determine reproducibility. Negative control replicates consisted of reagents alone, and no genomic DNA to check for cross-contamination among individual samples. Standard error of positive control replicates suggests reproducibility of the AFLP methodology (SE = 0.004).

**Data management and analysis**

Fragment data were digitally visualized in GeneMarker® (SoftGenetics, LLC, State College, PA, USA), and data were exported into a general text format for input to Excel 2007 (Microsoft Corporation, Redmond, WA, USA.). Fragments were sorted based on migration size (basepairs) and auto-scored utilizing an independently developed procedure (Lucardi and Walker, unpublished methodology) that utilizes both Excel 2007 and PASW v.18.0 (SPSS, IBM Corporation, Armonk, NY, USA). This procedure sorted fragments, and differences were scored with a 0.3-basepair threshold. Polymorphisms were identified as scored (or ‘called’) alleles/bands that did not occur in all individuals. Scoring of polymorphisms sorted detected fragments and determined scored bands as polymorphic or not. Data matrices were created from scored fragment data and auto-populated over several steps in both software programs. Matrices were coded ‘0’ for absence and ‘1’ for presence. Detected polymorphic loci less than 200 basepairs in length were removed from statistical analyses in an effort to avoid potential effects of fragment-size homoplasy, due to disproportionate number of smaller fragments produced
by AFLPs (Koopman & Gort 2004; Bonin et al. 2007). Homoplasmy is the result of co-migrating bands during electrophoresis that are not of the same physical locus in the genome and therefore, lack homology. Homoplasious biases influence errors in allele frequency detection, generally toward overestimation, generating erroneous heterozygosity estimates and underrepresentation of genetic differentiation between subpopulations (Meudt & Clarke 2007; Caballero et al. 2008).

Data conversions of presence-absence matrices for input into population genetic software programs (such as, STRUCTURE and Arlequin) utilized the R-package, AFLPdat source script (Ehrich 2006). Genetic diversity, within populations, was assessed on the number of bands and private bands, the percentage of polymorphic loci, expected heterozygosity (biased ($H_e$) and unbiased ($UH_e$)) based on Hardy-Weinberg expectations (Nei 1978), and Shannon’s Information/Diversity Index ($I$), serving as a coefficient of similarity, for each of the 14 populations (GenAIEx 6.3, Peakall and Smouse 2006). Both Imperata species are able to reproduce asexually via belowground rhizomes. The number of unique multi-locus genotypes per population would therefore contribute toward more accurate assessments of genetic diversity within populations.

This study aims to determine if population structure of genetic variation support two species co-occurring and past interspecific hybridization. For the purpose of ascertaining genetic variation among populations, the genetic distance between populations utilized population pairwise $F_{ST}$ (Arlequin v.3.5). STRUCTURE v.2.3.3 (Pritchard et al. 2000; released in 2009; http://pritch.bsd.uchicago.edu/structure.html) was utilized to infer the number of clusters that best fit and are most appropriate for the data without apriori information applied to the number or structure of expected clusters within
the dataset. Since the most appropriate number of clusters was initially unknown, duplicate runs of $K=1-7$ were performed, where admixture ancestry model was applied with a burnin of 10,000 and 50,000 MCMC (Markov Chain Monte Carlo) (Pritchard et al. 2000). The expectation was that not more than three clusters would be detected in STRUCTURE analysis to support two differing species and/or a hybrid cluster; however, multiple iterations of $K=1-7$ were run to determine if that expectation was supported. To infer the most appropriate number of clusters ($K$), this study utilized the method developed by Evanno et al. (2005), to detect the second order rate of change in likelihood values, based on the difference between likelihood values generated with each $K$, from each STRUCTURE run. The difference between likelihood values and the second order rate of change was then plotted and allowed for a $\Delta K$ be determined suggesting the most likely number of clusters appropriate for the dataset. Additional analysis of population structure was assessed through principal coordinates analysis (PCA, GenAlEx v.6.3) of individual genetic covariance (with data standardization) among analyzed individuals. Analysis of molecular variation (AMOVA, Excoffier et al. 1992) was performed in Arlequin v.3.5 (Excoffier and Lischer 2010) based on species identification information gained during the collection phase to determine degree of genetic differentiation between the possibly two species.

**Results and Discussion**

**Genetic Diversity**

Eight populations of *I. cylindrica* were sampled (n=66) from five counties in Florida. Six populations of presumably *I. brasiliensis* were sampled (n=63) from Miami-Dade County, FL (Figure 3.1). The AFLP genome scan resulted in 568 polymorphic
AFLP markers. Number of polymorphic bands detected per sampled population ranged from 41 to 221. The number of private bands, which are polymorphic bands that are detected in only one population and no other, ranged from 0 to 95. Average percentage of polymorphic loci was 16% (SE ± 2%), with four *I. cylindrica* and two *I. brasiliensis* populations above that mean. Unbiased heterozygosity (UH<sub>e</sub>) ranged from 0.020 to 0.082, with an average value of 0.047 (SE ± 0.001). Shannon’s Information Index (I) ranged from 0.029 to 0.134, with an average value of 0.067 (SE ± 0.002). An *I. cylindrica* population located in Alachua County (Gainesville, FL) and an *I. brasiliensis* population located in Thompson Park (MDPR) both resulted in high genetic variation and are among the highest overall values for percentage of polymorphic loci, H<sub>e</sub> and UH<sub>e</sub>, and I. Summarized genetic diversity measures may be found in Table 3.2, for the sampled *I. cylindrica* and *I. brasiliensis* populations in this study.

Variability of genetic diversity indices was similar within and between *I. cylindrica* and *I. brasiliensis* populations in this study. Two *I. brasiliensis* populations, one from Thompson Park (39% polymorphic loci, H<sub>e</sub>/UH<sub>e</sub>=0.078/0.042, I=0.134) and one of the two populations sampled from Martinez Pineland (21% polymorphic loci, H<sub>e</sub>/UH<sub>e</sub>=0.046/0.051, I=0.079), resulted in above average diversity. More individual tillers were sampled (n=13) from Thompson Park than other populations (generally, n=10); thus, the greater amount of detected diversity may be attributed to greater sampling intensity. The population sampled from Ingram Pineland resulted in the overall lowest genetic diversity (7% polymorphic loci, H<sub>e</sub>/UH<sub>e</sub>=0.018/0.020, I=0.029). These data suggest that the populations considered *I. brasiliensis* in Miami-Dade contain substantial genetic diversity, but levels of diversity are variable among populations and
are comparable to observations for *I. cylindrica* in other states (Chapter II, Table 2.2). This pattern also was observed in sampled FL *I. cylindrica* populations. The population sampled from Jacksonville, FL, located in Duval County, resulted in the greatest number of detected polymorphic bands (221), but the population located in Alachua county, resulted in the highest genetic diversity over all sampled *I. cylindrica* populations (26% polymorphic loci, $H_e/UH_e=0.074/0.082$, $I=0.116$). *Imperata cylindrica* populations possessing lower levels of genetic diversity include one sampled from the Disney Wilderness Preserve (The Nature Conservancy) located in Osceola County (8% polymorphic loci, $H_e/UH_e=0.028/0.035$, $I=0.042$), and another, from a right-of-way in Indian River County (9% polymorphic loci, $H_e/UH_e=0.029/0.035$, $I=0.045$).

**Pairwise Population $F_{ST}$**

There was no clear pattern of genetic dissimilarity between sampled *I. cylindrica* and *I. brasiliensis* populations (Table 3.3). Almost equivalent genetic dissimilarity was found within species as there was between species. Within-species pairwise population $F_{ST}$ values ranged from 0 to 0.204 for *I. cylindrica*, and 0.012 to 0.325 for *I. brasiliensis*. Significant between species $F_{ST}$ values ranged from 0.059 to 0.292. Greater population genetic dissimilarity was observed within *I. brasiliensis* than within *I. cylindrica*, and these were greater than the dissimilarity observed between the two.

If Miami-Dade populations are assumed to be a separate species, the pattern of population pairwise genetic similarity would have resulted in the highest pairwise population $F_{ST}$ values between isolated populations of *I. cylindrica* and *I. brasiliensis* to have supported interspecific hybridization. Hybrid populations would show moderate or very low values, depending on source(s) of genetic material. Although statistically
significant genetic dissimilarity is observed between the two morphologically identified species, the observed values are low, providing poor support for genetic distinction between species. Furthermore, the observed $F_{ST}$ values are less than would be expected to exist between clearly separate plant species, even those that have hybridized (Wei et al. 2005).

Significant population structure is present and greater among *I. brasiliensis* populations than is among *I. cylindrica* populations and between presumed species, suggesting an alternative explanation to interspecific hybridization in Florida. All *I. cylindrica* populations (FL-1, 2, 3, 4, 5, 6, 13, and 14) are genetically similar ($F_{ST} < 0.2$) to FL-7, 8, and 12, which are grouped as *I. brasiliensis*. Of note is *I. brasiliensis* population FL-12, where no significant genetic differentiation was found between it and any other population, with the exception of a conspecific population, FL-9. This observed pattern, in conjunction with low, but significant pairwise $F_{ST}$ values, suggests that these two groups are not separate species. Populations located in Miami-Dade County are probably also *I. cylindrica*, but may be old populations, inhabiting very stressful environments such as pine rockland habitats, where these were sampled. These habitats are old, exposed limestone seabed with very little soil or accumulated organic matter. The resource-limited and stressful environments likely contributed to these populations’ lack of significant growth or spread, typically characterizing ‘invasive’ *I. cylindrica* populations found throughout the rest of the state.

The population genetic structure observed among *I. brasiliensis* suggests that populations in Miami-Dade County are more isolated and have experienced less genetic admixture within the county than with other populations in the state. Significant genetic
similarity between Miami-Dade populations and *I. cylindrica* populations (e.g. FL-13 and 14 to FL-10, $F_{ST} = 0.060, 0.059$) suggests that populations in South Florida may be sourcing genetic material to populations located on the eastern coast of the state, by propagules travelling northward. On the other hand, it is possible that populations sampled in Duval and Indian River counties are moving southward, introgressed into the Miami populations. Despite the lack of directionality in the data, I suggest that intraspecific hybridization, rather than interspecific hybridization, occurs among populations in Florida. This is likely due to anthropogenic transport of reproductively viable propagules between Miami and already established cogongrass stands throughout the state. The alternative may suggest that cogongrass populations in the north part of the state founded the populations in Miami some time in the past, and have genetically differentiated in isolation from each other. However, genetic diversity indices (Table 3.2) and strong genetic isolation (Table 3.3) among *I. brasiliensis* populations suggests substantial introduced diversity and/or lack of outcrossing with nearby stands.

**Population Structure**

Analyses of molecular variance (AMOVA) were conducted for 13 populations that were sampled in Florida. Population structure was tested specifically between the two groups: eight populations identified as *I. cylindrica* and six populations identified as *I. brasiliensis* (Table 3.4). This sought to detect genetic support for two different species, within the same genus, co-occurring in FL. This AMOVA resulted in a significant $F_{ST}$ value of 0.135 ($P < 0.001$). The degree of genetic differentiation between *I. cylindrica* and *I. brasiliensis* is less than that observed within *I. cylindrica* found in MS and AL ($F_{ST} = 0.499, P < 0.001$; Chapter II, Table 2.5.B). This AMOVA results should be a reliable
estimate of a low degree of genetic differentiation between the two groups (species) tested.

Analysis of the sampled populations from presumably two different species in FL was conducted in STRUCTURE, and the results were not consistent with expectations. Multiple iterations of each run (number of groups, $K=1-7$) failed to adequately support population structuring consistent with expectations that Miami-Dade populations would be clustered separately from all other sampled FL populations. When two populations are assumed ($K=2$), two clusters are detected showing one strong group with introgression by another genetic signal (Figure 3.2, likelihood value = -7898). When three populations are assumed ($K=3$), one main group was again primarily detected, with two genetic signals showing admixture intermittently across all populations (Figure 3.2, likelihood value = -6989). No distinct ‘clusters’ were observed to be consistent with the expectation that *I. brasiliensis* would separate out first from STRUCTURE analyses.

There is not enough evidence to reject the null hypothesis of one genetic group in Florida experiencing substantial introgression, possibly from two sources. These results fail to support the hypothesis that the sampled populations in FL are representative of two distinct species (*I. cylindrica* and *I. brasiliensis*).

**Principal Coordinates Analysis (PCA)**

A principal coordinates analysis (covariance with data standardization) was conducted on AFLP data specific to Florida populations (Figure 3.3). The first two axes account for 62.9% of the variation in this dataset. The bulk of the individuals tested form a broad cluster in the top and bottom-left quadrants of this PCA. The few individuals that did not cluster within the main cluster belong primarily to FL-7 (*I. brasiliensis*) and FL-1,
2, 4 (I. cylindrica), with only one or two individuals from FL-14 (I. cylindrica) and FL-8 (I. brasiliensis).

This pattern observed in this PCA suggests a single, somewhat heterogeneous genetic assemblage for the main cluster, consistent with STRUCTURE analysis (Figure 3.2). A few individuals from several populations appear to be differentiating from this source cluster, either due to significant recombination and/or isolation. This analysis of genetic data also does not support two genetically distinct groups forming hybridized offspring in these FL populations.

Conclusions

There appear no consistent patterns of genetic diversity or differentiation among sampled populations in Florida to determine the status of interspecific hybridization between I. cylindrica and I. brasiliensis. Genetic diversity is variable within and among both of the groups tested. STRUCTURE analysis failed to consistently support the presence of more than one genetic group among the sampled populations, and this was supported by analysis of molecular variance. Finally, tests of genetic dissimilarity among populations identified as I. cylindrica and I. brasiliensis resulted in low $F_{ST}$ ($F_{ST}=0.134$) from this survey of neutral markers. Other studies employing similar tests for the presence of hybridization (i.e., AFLP markers, pairwise FST, and AMOVA) generally detected stronger population structuring among populations, higher mean and pairwise $F_{ST}$ values, and stronger clustering between tested groups (e.g., Wei et al. 2005; Szczepaniak et al. 2007; Song et al. 2010). Although a statistically significant genetic dissimilarity ($F_{ST}=0.135$) was found between groups, the differences were relatively low, considering $F_{ST}$ values can range from panmixis ($F_{ST}=0$) to complete genetic isolation...
(F_{ST}=1) (Beaumont 2005). This result in conjunction with PCA suggests these samples probably originated from a single, heterogeneous (diverse) genetic assemblage and have recombined, likely through intraspecific hybridization as inferred in Chapter II.

Alternative interpretations of these data and observed patterns may be made. The lack of strong clustering in the data may be possibly due to shallow temporal detection of AFLPs, within a century or so, and the dominant nature of this type of marker, reducing resolution of the data. A hybridization event would be followed by hybrid offspring swiftly spreading both spatially and genetically, potentially swamping a genetic signal of two separate species in FL. Introggression of two new signals may indicate multiple introductions of cogongrass invasion, as it occurs broadly across the region, further reducing any signal of *I. brasiliensis* as a differing species. *Imperata cylindrica* and *I. brasiliensis* may have arisen from a single ancestral population and were then geographically isolated, restricting *I. cylindrica* to the Old World and *I. brasiliensis* to the New World. These may be two species solely due to geographic isolation, lacking both substantial genetic and morphological differentiation. There is a possibility that *I. cylindrica* and *I. brasiliensis* are not necessarily species upon secondary contact (depending on the species concept). This may have contributed to the little genomic differentiation detected in this study. Species delimitation between *I. cylindrica* and *I. brasiliensis* cannot be made from this molecular dataset, and especially not with this type of molecular marker or sampling. Nonetheless, population genetic analysis of these 14 Florida populations has not provided supportive evidence for coexistence of two differing species, or for hybridization generating ‘invasiveness’ in the Southeast. However, relationships and delineations among species within the genus *Imperata* are not
uniformly resolved and a global consensus has not yet been reached (Hubbard et al. 1944; Hall 1978; Gabel 1982, Welker & Longhi-Wagner 2012). The study of the phylogenetic relationship and genetic differentiation between *I. cylindrica* and *I. brasiliensis* has been attempted (see: Vergara et al. 2008); however, additional studies that incorporate global tissue samples will be more prescient, especially in light of intercontinental transport of propagules in this invasion.

Source propagules from both Mississippi and Alabama were purposefully introduced into Florida since the early-to-mid-20th century (Tabor 1949; MacDonald 2004). The known transport of propagules from MS and AL into FL suggest that if interspecific hybridization had occurred, backcrossing with *I. cylindrica* could have dominated, enabling ready interbreeding with cogongrass populations from other areas in the southeastern U.S. STRUCTURE analysis failed to reject one genetic group to best explain genetic data among these Florida samples, which possess substantial diversity. The substantial diversity of cogongrass populations in FL may influence the admixture of genetic material into other populations. The *Imperata* populations in Florida likely originated from Mississippi and Alabama, where populations have already experienced intraspecific hybridization.

Overall, these data do not suggest the presence of two separate species in FL; the populations sampled in Miami-Dade, presumed to be *I. brasiliensis*, are not sufficiently differentiated genetically to suggest differing species. Patterns also indicate that some of the populations in Miami-Dade have recombined with other FL populations. A potentially better approach would be to conduct a globally sampled phylogenetic analysis, for the purpose of examining larger temporal history of both *I. cylindrica* and *I.
brasiliensis, and especially to examine *I. cylindrica* tissues from alleged parent sources in Asia (e.g., Japan and the Philippines) and obtaining *I. brasiliensis* tissues from South America, primarily Brazil and Argentina. This would permit an analysis of genetic relationships between *I. brasiliensis*, from where it is considered native, and Miami-Dade populations, as well as characterizing phylogenetic relatedness between *I. cylindrica* and *I. brasiliensis* in their native and invaded range(s). Different studies, such as those including phylogenetic analysis using different markers and new technology may provide a broader temporal scale in which to understand the context of this particular invasion, its relationship to global specimens, and hopefully, provide additional data towards inferring relationships among species within *Imperata*. 
Table 3.1  Six AFLP selective amplification primer combinations utilized.

<table>
<thead>
<tr>
<th>AFLP Selective Amplification Primer Combinations</th>
<th>Fluorescent Dye-Labeled EcoRI Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MseI Primer</strong></td>
<td><strong>EcoRI Primer</strong></td>
</tr>
<tr>
<td>MseI-CAT</td>
<td>EcoRI-ACT-FAM</td>
</tr>
<tr>
<td>MseI-CTA</td>
<td>EcoRI-AGG-HEX</td>
</tr>
<tr>
<td>MseI-CTG</td>
<td>EcoRI-AGC-NED</td>
</tr>
<tr>
<td>MseI-CTT</td>
<td>EcoRI-ACT-FAM</td>
</tr>
<tr>
<td>MseI-CTC</td>
<td>EcoRI-AGG-HEX</td>
</tr>
<tr>
<td>MseI-CAC</td>
<td>EcoRI-AGC-NED</td>
</tr>
</tbody>
</table>

NOTE: Fluorescent dye-labeled selective primers are denoted by "EcoRI primer-[Axx]-[dye]. Each fluorescent dye is visualized as a different color for fragment analysis: FAM (blue), HEX (green), NED (yellow or black).
Table 3.2  Genetic Diversity Indices with Population Information (FL).

<table>
<thead>
<tr>
<th>Species</th>
<th>County</th>
<th>Other Information</th>
<th>Number of individuals (n)</th>
<th>Number of Bands Detected</th>
<th>Number of Private Bands</th>
<th>Percentage Polymorphic Loci</th>
<th>$H_e \pm SE$</th>
<th>$UH_e \pm SE$</th>
<th>Shannon’s Information Index (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. cylindrica</em></td>
<td>Alachua</td>
<td>ROW</td>
<td>10</td>
<td>151</td>
<td>48</td>
<td>7.63%</td>
<td>0.021±0.002</td>
<td>0.024±0.002</td>
<td>0.034±0.003</td>
</tr>
<tr>
<td>Osceola</td>
<td>Treated (IMP), Burned</td>
<td></td>
<td>10</td>
<td>112</td>
<td>34</td>
<td>6.17%</td>
<td>0.015±0.001</td>
<td>0.017±0.002</td>
<td>0.025±0.002</td>
</tr>
<tr>
<td>Osceola</td>
<td>Treated (GLY, IMP)</td>
<td></td>
<td>5</td>
<td>57</td>
<td>6</td>
<td>2.24%</td>
<td>0.008±0.001</td>
<td>0.010±0.001</td>
<td>0.012±0.002</td>
</tr>
<tr>
<td>Osceola</td>
<td>Logged, Treated (IMP), Burned</td>
<td></td>
<td>10</td>
<td>115</td>
<td>14</td>
<td>5.35%</td>
<td>0.015±0.002</td>
<td>0.017±0.002</td>
<td>0.024±0.002</td>
</tr>
<tr>
<td>Osceola</td>
<td>Treated (IMP), Burned</td>
<td></td>
<td>5</td>
<td>94</td>
<td>14</td>
<td>4.33%</td>
<td>0.016±0.002</td>
<td>0.020±0.002</td>
<td>0.024±0.002</td>
</tr>
<tr>
<td>Sarasota</td>
<td>ROW</td>
<td></td>
<td>10</td>
<td>84</td>
<td>2</td>
<td>3.74%</td>
<td>0.009±0.001</td>
<td>0.010±0.001</td>
<td>0.015±0.002</td>
</tr>
<tr>
<td>Indian River</td>
<td>ROW, Disturbed (construction)</td>
<td></td>
<td>6</td>
<td>65</td>
<td>17</td>
<td>2.82%</td>
<td>0.009±0.001</td>
<td>0.011±0.001</td>
<td>0.014±0.002</td>
</tr>
<tr>
<td><em>I. brasiliensis</em></td>
<td>Miami-Dade</td>
<td>Thompson Park</td>
<td>13</td>
<td>262</td>
<td>123</td>
<td>12.69%</td>
<td>0.025±0.002</td>
<td>0.027±0.002</td>
<td>0.042±0.003</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>Martinez Pineland</td>
<td></td>
<td>10</td>
<td>79</td>
<td>13</td>
<td>3.50%</td>
<td>0.008±0.001</td>
<td>0.009±0.001</td>
<td>0.013±0.002</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>Martinez Pineland</td>
<td></td>
<td>10</td>
<td>137</td>
<td>52</td>
<td>6.17%</td>
<td>0.013±0.001</td>
<td>0.015±0.001</td>
<td>0.023±0.002</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>Pine Shore Park</td>
<td></td>
<td>10</td>
<td>80</td>
<td>23</td>
<td>3.45%</td>
<td>0.009±0.001</td>
<td>0.010±0.001</td>
<td>0.014±0.002</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>Ingram Pineland</td>
<td></td>
<td>10</td>
<td>47</td>
<td>7</td>
<td>2.09%</td>
<td>0.006±0.001</td>
<td>0.006±0.001</td>
<td>0.009±0.001</td>
</tr>
<tr>
<td>Miami-Dade</td>
<td>Seminole Wayside Park</td>
<td></td>
<td>10</td>
<td>86</td>
<td>25</td>
<td>3.40%</td>
<td>0.008±0.001</td>
<td>0.009±0.001</td>
<td>0.013±0.002</td>
</tr>
</tbody>
</table>

NOTE: Species assignment, location, habitat information, band data, and genetic diversity indices ($H_e$ = expected heterozygosity, $UH_e$ = unbiased expected heterozygosity, $I$ = Shannon’s Information index, with ± standard error for 14 populations sampled. Overall totals and averages are in **bold**. Eight different I. cylindrica sites were sampled from five counties, and six different I. brasiliensis sites sampled, from Miami-Dade municipal parks. All I. cylindrica tissues sampled in Osceola Co., FL, were collected from the Disney Wilderness Preserve (The Nature Conservancy). Abbreviations: “ROW” right-of-way, “IMP” Imazapyr (herbicide), “GLY” glyphosate (herbicide).
Table 3.3  Pairwise population matrix of $F_{ST}$ values among 14 populations in Florida.

<table>
<thead>
<tr>
<th></th>
<th>FL-1</th>
<th>FL-2</th>
<th>FL-3</th>
<th>FL-4</th>
<th>FL-5</th>
<th>FL-6</th>
<th>FL-7</th>
<th>FL-8</th>
<th>FL-9</th>
<th>FL-10</th>
<th>FL-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>FL-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-2</td>
<td>0.144*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-3</td>
<td>0.173 0.000*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-4</td>
<td>0.051 0.029 0.051*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-5</td>
<td>0.016 0.045 0.000 0.051*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-6</td>
<td>0.137 0.052 0.077 0.000 0.065*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-7</td>
<td>0.204 0.107 0.080 0.114 0.134 0.092*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL-8</td>
<td>0.176 0.111 0.097 0.117 0.087 0.095 0.012*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Significant pairwise population $F_{ST}$ values are shaded in gray $(P<0.005)$.

Table 3.4  Results from analysis of molecular variation (AMOVA) using $F_{ST}$ between *I. cylindrica* and *I. brasiliensis* (“Groups”).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Percentage of variation</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Groups</td>
<td>1</td>
<td>114.30</td>
<td>8.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among populations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>within groups</td>
<td>12</td>
<td>279.61</td>
<td>5.37</td>
<td>0.003</td>
</tr>
<tr>
<td>Within populations</td>
<td>115</td>
<td>1710.66</td>
<td>86.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>128</td>
<td>2104.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F_{ST} = 0.135$ $(P<0.001)$, $F_{SC} = 0.058$ $(P<0.001)$, $F_{CT} = 0.081$ $(P<0.001)$
Figure 3.1  Map of population sites sampled in Florida of *I. cylindrica* and *I. brasiliensis*.

NOTE: *Imperata cylindrica* sites are represented by diamond symbols; *I. brasiliensis* sites are represented by circle symbols. Gray shaded areas represent National Forests acquisition boundaries.
Figure 3.2 Bar plots from STRUCTURE analysis in FL: two populations assumed ($K=2$, above), and three populations assumed ($K=3$, below).

**NOTE:** Individuals are represented by vertical bars and horizontally organized by population. Populations sampled from Miami-Dade (*I. brasiliensis*, n=63) occur in the middle of the bar plot, and are indicated by the grey area. All other individuals are cogongrass (*I. cylindrica*, n=66). Each bar represents an individual. Each cluster is represented by a different color. Different colors (red, green, and blue) represent the frequency of AFLP loci assigned to clusters, where some individuals are completely assigned to one cluster or another, while others show mixed ancestry.
Figure 3.3  Principal coordinates analysis (PCA) of genetic covariance (with data standardization) of *Imperata* samples in FL.

NOTE: A total of 129 individuals from 14 populations were analyzed. Population identifiers are provided in the legend, where “IB” denotes populations assigned as *Imperata brasiliensis*, and “IC” denotes populations assigned as *Imperata cylindrica*. The first two axes explain 62.90% of the variation in this dataset.
Literature Cited


Song, Ha-Yoon, Keun-Yong Kim, Moongeun Yoon, Yoon Kwon Nam, Dong Soo Kim and In-Chul Bang. 2010. Genetic variation of \textit{coreoleuciscus splendidus} populations (Teleostei; Cypriniformes) from four major river drainage systems in South Korea as assessed by AFLP markers. Genes and Genomics. 32:199-205.


CHAPTER IV
REGIONAL POPULATION GENETIC ANALYSIS OF SUCCESSFUL INVASION:
COGONGRASS (IMPERATA CYLINDRICA (L.) RAEUSCHEL)
INFESTATIONS IN THE SOUTHEASTERN
UNITED STATES

Abstract
This regional population genetic analysis of the invasive grass, cogongrass
(Imperata cylindrica (L.) Raeuschel), utilized amplified fragment length polymorphism
(AFLP) markers to assess genetic diversity within and among populations of this invasive
grass over most of the North American invaded range. A total of 676 cogongrass tillers
were collected from Alabama (AL), Florida (FL), Georgia (GA), Louisiana (LA),
Mississippi (MS), South Carolina (SC), and Texas (TX). The lowest genetic diversity
was detected in GA (2% of AFLP polymorphic fragments, \( H_e = 0.005 \pm 0.001 \)) and TX
(3% polymorphic, \( H_e = 0.008 \pm 0.001 \)). These results are attributed to less dense
cogongrass infestations present in those states. The highest genetic diversity was
detected in SC (56% polymorphic, \( H_e = 0.029 \pm 0.001 \)), despite fewer reported infestations
and a designation of “outlier” status for these populations at the current northeastern edge
of the invaded range. This pattern is inferred to have resulted from multiple introductions
of diverse genetic material from various established domestic locations in addition to
introduced intraspecific hybrid propagules from the epicenter of invasion. Cogongrass
has historically persisted in AL, FL, and MS for the longest period of time, and each of these states possesses substantial genetic diversity. Two genetic groups previously detected to exist in MS and AL were consistently observed in this study including the expansion of spatial scale, with all other states grouping within the AL-type, while the MS-type remains restricted to the central region of MS (\( K = 2; F_{ST} = 0.363, P<0.001 \)).

Data also indicate substantial admixture present in most states, which may be attributed to intraspecific hybridization occurring along the MS/AL coast, followed by hybridized propagules being introduced (multiple times) to other states. These results suggest that differential management and funding aimed at cogongrass eradication among these seven states may have contributed toward differential levels of genetic variation in each state.

**Introduction**

Cogongrass (*Imperata cylindrica* (L.) Raeuschel) is a warm-season, perennial, rhizomatous grass species considered invasive and problematic in the US, where it primarily affects the southeastern states (Bryson & Carter 1993; MacDonald 2004). For the seven states with significant infestations, the perceived negative economic and ecological impacts of this species are evident, including costly fire events in natural areas as well as its pyrogenic impact in managed forests and timber plantations. Cogongrass is generally transported anthropogenically, sometimes discovered in contaminated heavy machinery utilized in right-of-way maintenance and silviculture. Once introduced into a new area, invasive cogongrass propagules can establish especially if accompanied by soil disturbance (Bryson & Carter 1993; Holly 2008). Cogongrass often forms monodominant stands, essentially excluding other plant species (Bryson and Cater 1993; MacDonald 2004). In response to these sort of negative impacts in conserved natural or
carefully managed areas, organized regional cogongrass workshops are conducted across the southeast, and concerted management plans are available through regional and national Exotic Pest Plant Councils (EPPCs).

Records documenting cogongrass introduction into the Gulf Coast region of the U.S. suggest at least two introductions occurred in the early 20th century. Published records of cogongrass introduction point to inadvertent introduction of propagules originating from Japan, into Grand Bay, AL (near Mobile) in 1912; this was followed by purposeful introduction of cogongrass propagules from the Philippines into McNeil, MS in 1921 (Tabor 1949, 1952). The founding populations at these sites were able to establish and have now spread throughout the region. Secondary invasions were likely facilitated by humans interested in utilizing cogongrass as a forage crop or for erosion control (MacDonald 2004; Holly 2008). Cogongrass propagules were transported from the sites of introduction, along the Gulf Coast in MS and AL and into much of Florida (Tabor 1952; MacDonald 2004). Today, these three states experience the heaviest infestations of cogongrass (Figure 3.1). The spatial pattern of cogongrass infestations appears like a wave, where the density of reported infestations are most dense near sites of documented introduction, presumably with the longest period of time since establishment, and infestation intensity diminishes as geographic distance is increased. Infestations are reported by authorized state governmental agencies to the Southeast Exotic Pest Plant Council and to the Regional Cogongrass Conference, and compiled by the University of Georgia’s Center for Invasive Species and Ecosystem Health, in cooperation with the USDA-Forest Service (Miller 2007).
Cogongrass is actively managed in Alabama, Mississippi, Florida, Georgia, South Carolina, Louisiana, and Texas, by state and federal agencies, non-governmental organizations, and/or private enterprise. In 2008, the American Recovery and Reinvestment Act (ARRA) allocated funding (expiring 2012) specifically aimed at invasive plant remediation. Alabama ($6.3-mil, in US dollars) and Georgia ($1.8-mil) received ARRA funding aimed specifically at cogongrass eradication, whereas Mississippi ($1.2-mil) and South Carolina ($700-k) received funding for general invasive plant control or management. In addition to differential funding and aims across states, each state in the southeastern region has experienced a different cogongrass invasion history. Therefore, each state has approached the cogongrass problem a bit differently. For example: some states, like Georgia, have a long history of active control and eradication, whereas Texas only has one recorded population that has been actively managed for nearly a decade, but complete eradication of the population remains elusive. South Carolina is on the north and eastern edge of the spread and likely is experiencing the most recent invasions.

This study samples populations from seven states experiencing, and actively managing, cogongrass invasion as it occurs in the U.S. Two of these states presumably received direct introductions of cogongrass material from Asia (MS and AL). Cogongrass was introduced purposefully into much of Florida in the mid-20th century from both MS and AL (Tabor 1949). As such, Florida’s relationship with this invasive is within a few decades of primary introduction(s) of parent material. Cogongrass occurrence in the remaining states is likely the product of secondary invasion(s), otherwise known as the spatial spread/geographic range expansion of an invasive species
into new areas in the invaded range. Secondary invasions are subject to the same processes and barriers as the primary invasion, such as transport, introduction, establishment, subsequent spread and impact, cycling repeatedly. With each new cycle of secondary invasion, the potential success for establishment and subsequent impacts of new founding populations may be subject to the strength of propagule pressure.

Propagule pressure is a function of the number of founding individuals (or propagules) and the frequency of introductions into an area (Lockwood et al. 2005; Eppstein and Molofsky 2007). Therefore, propagule pressure can be a significant determinant and predictor of establishment and spread into new areas (Lonsdale 1999; Lockwood et al. 2005; Coulatti et al. 2006).

This study is meant to examine how genetic variation at the population level varies across an expanding range in an introduced species. The exploration of extant genetic variation and the partitioning of that variation within and among populations can provide information about how the genome of a species changes during the course of invasion. Specifically, what are the dynamics of genetic variation in populations following secondary invasions and geographic range expansion? By studying population genetic variation, detection of genetic-geographic population structure may point to populations or genetic groups that are sourcing secondary invasions. Degree of population structure may allow inferences to be made about the degree of genetic differentiation among populations, indicating differential introduction components of invasion, like propagule pressure, or differential conditions and environments due to environmental variation or due to variation in management and control applied to invasive populations. With genetic information, the role of propagule pressure, in terms
of multiple introductions of propagules and the amount of genetic variation introduced into new areas, may be incorporated into better understanding of the invasion process and may contribute to predicting the success of secondary invasion and biological invasions in general.

The population genetic analyses described here use highly reproducible, dominant genetic markers, known as amplified fragment length polymorphisms (AFLPs). AFLPs are arbitrarily amplified dominant markers and were selected because no \textit{a priori} sequence information was necessary, we were able to sample the entire genome simultaneously, and the technique is considered to be highly reproducible and practical (in cost and data generation) in studies such as this (Bussell et al. 2005; Campbell et al. 2003; Meudt and Clarke 2007). Polymorphisms are detected fragments (bands or loci) that are present in some but not in all individuals tested. A polymorphism is variation in the genome and its presence or absence is representative of genetic diversity.

From Chapter II, cogongrass populations in MS and AL formed two genetic groups or types, based on genetic similarity. The data supported a multiple introduction hypothesis and the documented introduction history. It is expected that the inclusion of the same MS and AL cogongrass populations will demonstrate the same pattern here; however, populations in the five other states sampled (FL, GA, LA, SC, and TX) are expected to be the result of secondary invasions. Genetic information should reflect the genetic relationships of these populations and potentially identify sources of secondary invasions that are already established in MS and AL. Each state sampled also actively manages cogongrass populations, but the degree of management and control efforts varies across states. In addition, differential funding (as stated above: ARRA) awarded to
each state, combined with variation in the degree of effort and matching-funds from state agencies, has contributed to genetic isolation and differentiation among cogongrass populations. Therefore, it is hypothesized that state-level population genetic structure will be detected due to differential propagule pressure and variation in management.

**Materials and Methods**

**Sampling**

To address regional population genetic patterns, leaf tissues were collected from seven U.S. states in the Southeast during 2008 and 2009, in Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, and Texas. 208 individuals were sampled from Alabama, 13 from Georgia, 62 individuals from Louisiana, 180 individuals in Mississippi, 10 individuals from Texas, 74 in South Carolina, and 129 from Florida. Six populations (n=63) in Florida should be noted, as they were collected under the assumption of being a morphologically different species, Brazilian satintail (*Imperata brasiliensis* Trin.) These individuals and populations were treated equally to all other samples in study sampling, molecular analysis, and data handling. There is no *a priori* assumption separating these populations. This distinction is treated explicitly in Chapter IV. The sampling from these seven states provides 676 total individuals for this study (Figure 3.2).

Leaf tissues were collected in the field from cogongrass populations in AL, FL, GA, LA, MS, SC, and TX. Each tiller was assumed representative of an “individual” or a ramet in the located patch or population (while acknowledging that individual patches may have arisen from only one to a few colonizing propagules resulting in few genets comprising a population). Populations were identified as contiguous patches of
cogongrass, often occurring as circular patches in open areas or as long, narrow patches along roadside rights-of-way. Minimum geographic distance between populations was 1-km, with the exception of paired populations each in AL (AL-2 and AL-3) and MS (MS-9 and MS-10), which were sampled from what appeared to be large contiguous patches in excess of 4-km². Another exception to the above minimum distance also occurred in FL, where two populations were taken from the same municipal park (Martinez Pineland). Distances between populations may be visualized in Figure 3.2. Tissues from individual leaves were collected systematically from the edges and the middle of sampled populations. Aboveground leaf tissues were stored in individually labeled plastic bags and stored in a cooler. Tissues then were dried in the lab by placing tissues in silica gel with color indicator.

Because cogongrass (*I. cylindrica*) is a listed Federal Noxious Weed, all sampling was conducted under approval by the U.S. Dept. of Agriculture, Animal and Plant Health Inspection Service, Plant Pest Quarantine (Permit #: P526P-12-00211, P526-080721-005). Additional permits were required for specific access to lands, including approval and permits from The Nature Conservancy (TNC) and Miami-Dade County Parks and Recreation (MDPR). Other sampling for this study was conducted on public land including National Forest holdings, interstate/highway rights-of-way, and private land with permission from state forestry agencies.

Additional data were collected during tissue collection. I documented habitat type (e.g., right-of-way, forested, residential, commercial, etc.), location coordinates (global positioning system [GPS] coordinates, WGS 1984), voucher specimens, and approximate patch size (area).
Tissue processing and molecular methods

Extraction of DNA utilized a modified NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue, produced and published by Applied Biosystems (Life Technologies, Carlsbad, CA, USA). Approximately 1 cm$^2$ of cogongrass tissue was aseptically transferred into a 2-ml microcentrifuge tube. Samples were fully disrupted utilizing a Retsch mixer mill and then processed using the ABI NucPrep chemistry for DNA extraction (NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue--P/N 4333959). Purified DNA were transferred into sterile, individually labeled tubes and frozen (-20 °C) until analysis (-80 °C for long-term storage).

Amplified fragment length polymorphism (AFLPs) analysis utilized a modified protocol for capillary electrophoresis based on technique and methodology developed by Vos et al. (1995). AFLPs are dominant, arbitrarily amplified DNA markers and a PCR-based methodology utilized to detect polymorphisms in the genome. This methodology scans the entire genome simultaneously, is considered highly reproducible and applicable for a wide array of investigations in population genetics, ecology and some evolutionary studies (Bonin et al. 2007; Meudt & Clarke 2007). It is considered ‘highly sensitive’ and is known to detect slight differences within species where little genomic study has previously occurred, and also, does not require sequence information (Campbell et al. 2003; Meudt & Clarke 2007).

Extracted and purified DNA underwent digestion by restriction enzymes, ligation of linking primers, followed by pre-selective amplification to generate fragments of interest, and then, a selective cycle to amplify fragments into a marker set to detect
polymorphisms within the genome among DNA samples. Restriction digest of individual genomic DNA was achieved in 25μl reactions incubated at 37 °C for 2 hours in a thermal cycler, finalized by denaturing of enzymes by heating samples to 70 °C for 15 minutes. Restriction digest enzymes and reagents utilized per reaction were: 1 μl of EcoRI (20,000 U/ml, 5’-G^AATT, 3’CTTAA^G; New England BioLabs, Ipswich, MA, USA), 1 μl of MseI (10,000 U/ml, 5’-T^TAA, 3’-AAT^T; New England BioLabs, Ipswich, MA, USA), 2.5 μl of included 10X NEBuffer4, 0.25 μl of 100 μg/ml BSA, 10 μl of individually purified genomic DNA, and 10.25 μl of sterile water.

Eco AFLP linkers were annealed in a thermal cycler by heating to 95 °C for 5 minutes and cooled to 5 °C over 30 minutes using the following reagents: 1 μl of Eco Linker 1 (100 μM, 5’-CTC GTA GAC TGC CC), 1 μl of Eco Linker 2 (100 μM, 5’-AAT TGG TAC GCA GTC TAC), 90 μl of TE buffer (10 mM Tris & 1 mM EDTA), and 108 μl sterile water. Mse AFLP linkers were annealed in a thermal cycler by heating to 95 °C for 5 minutes and cooled to 5 °C over 30 minutes using the following reagents: 10 μl of Mse Linker 1 (100 μM, 5’-GAC GAT GAG TCC TGA G), 10 μl of Mse Linker 2 (100 μM, 5’-TAC TCA GGA CTC AT), 90 μl of TE buffer (10 mM Tris & 1 mM EDTA), and 90 μl sterile water. Annealed linkers are stored frozen at -20 °C until use for ligation of linkers.

Ligation of Eco and Mse linkers were conducted in 20 μl reactions comprised of: 1 μl each of Eco and Mse Linker (as annealed above), 1 μl T4 DNA Ligase enzyme and 2 μl of included 10X T4 DNA Ligase Reaction Buffer (New England BioLabs, Ipswich, MA, USA), 10 μl of individually digested DNA, and 5 μl sterile water. Ligation
reactions were conducted in a thermal cycler at 16 °C overnight or at 37 °C for 3 hours. Individual ligated reactions were stored at -80 °C to prevent degradation.

Pre-selective 20 μl polymerase chain reaction (PCR)-conditions for amplification were: 1 μl each of Eco+A (10 μM, 5’-GAC TGC GTA CCA ATT CA) and Mse+C (10 μM, 5’-GAT GAG TCC TGA GTA AC) primers, 2.5 μl of dNTPs (0.2 mM dATP, dCTP, dGTP, and dTTP; Deoxynucleotide Solution Set, New England BioLabs, Ipswich, MA, USA), 4 μl 5X GoTaq™ FlexiBuffer (Promega Corp., Madison, WI, USA), 1.2 μl MgCl₂ (Promega Corp., Madison, WI, USA), 3 μl of individually ligated DNA, 7.1 μl sterile water, and 0.2 μl GoTaq™ DNA polymerase (5u/μl, Promega Corp., Madison, WI, USA). Pre-selective amplifications consisted of an initial denaturing step of 94 °C for 1 minute, 30-cycles of 30 seconds at 94 °C, 1 minute at 56 °C, and 1 minute at 72°C, and followed by final annealing for 2 minutes at 72 °C.

For selective amplification, pre-selective amplification products are individually diluted 1:20 with sterile water. Each combination of selective Mse and fluorescent Eco selective primers is a separate PCR amplification. Selective amplification for all individuals was achieved in 20 μl reactions consisting of: 1 μl each of Mse (5μM) and fluorescent Eco (1 μM) selective primer (see: Table 2.1), 2.5 μl of dNTPs (0.2 mM dATP, dCTP, dGTP, and dTTP; Deoxynucleotide Solution Set, New England BioLabs, Ipswich, MA, USA), 4 μl 5X GoTaq™ FlexiBuffer (Promega Corp., Madison, WI, USA), 3.7 μl MgCl₂ (Promega Corp., Madison, WI, USA), 3 μl of diluted pre-selective amplification product, 4.6 μl sterile water, and 0.2 μl GoTaq™ DNA polymerase (5u/μl, Promega Corp., Madison, WI, USA). Selective amplifications consisted of an initial denaturing step of 94 °C for 2 minutes, 10-cycles of 30 seconds at 94 °C, 30 seconds at
65 °C, and 1 minute at 72°C (reducing annealing temperature by 1 °C/cycle), 30-cycles of 30 seconds at 94 °C, 30 seconds at 56 °C, and 1 minute at 72°C, and finished with 30 seconds at 72 °C. Six selective primer sets were applied to PCR products in this study (Table 3.1).

The selective primers are fluorescently tagged such that product from multiple combinations of DNA primers can be analyzed at once. Three different fluorescently tagged, selectively amplified products (1.5 μl of each) were combined per well with a single-stranded, fluorescent ROX-1000 size standard (0.25 μl; MapMarker (50-1000), BioVentures, Inc., Murfreesboro, TN, USA) and fixed with formamide (10 μl, Hi-Di™, Life Technologies, Carlsbad, CA, USA). Pooled fragment products were run on an ABI 3730 capillary sequencer at the Arizona State University DNA Lab (Tempe, AZ, USA) with positive and negative control sample replicates. Positive control replicates consisted of one individual randomly selected at the beginning of the study and followed each set and step of molecular analysis throughout to determine reproducibility. Negative control replicates consisted of reagents alone, and no genomic DNA to check for cross-contamination among individual samples. Standard error of positive control replicates suggests reproducibility of the AFLP methodology (SE = 0.004).

Data management and analysis

Fragment data were digitally visualized in GeneMarker® (SoftGenetics, LLC, State College, PA, USA), and data were exported into a general text format for input to Excel 2007 (Microsoft Corporation, Redmond, WA, USA). Fragments were sorted based on migration size (basepairs) and auto-scored utilizing an independently developed procedure (Lucardi and Walker, unpublished methodology) that utilizes both Excel 2007
and PASW v.18.0 (SPSS, IBM Corporation, Armonk, NY, USA). This procedure sorted fragments, and bands were scored with a 0.3-basepair threshold. Polymorphisms were identified as scored (or ‘called’) alleles/bands that did not occur in all individuals. Scoring of polymorphisms sorted detected fragments and determined scored bands as polymorphic or not. Data matrices were created from scored fragment data and auto-populated over several steps with the use of both software programs. Matrices were coded ‘0’ for absence and ‘1’ for presence. Detected polymorphic loci less than 200-basepairs in length were removed from statistical analyses in an effort to avoid potential effects of fragment-size homoplasy, due to disproportionate number of smaller fragments produced by AFLPs (Koopman & Gort 2004; Bonin et al. 2007).

This dataset detected a large number of informative AFLP markers, from several selective primer combinations. Population genetic diversity metrics ($H_o$, $UH_e$, heterozygosity estimates, and $I$ Shannon’s Information Index), as presented here, estimate allele frequencies from dominant (presence-absence) data generated by AFLP analyses, and are subject to Hardy-Weinberg equilibrium assumptions. These assumptions can reduce accuracy in allele frequency estimations from dominant data, but reliable results for comparative study can be achieved with adequate population sampling and sufficient number of primer sets, which generate a large number of detected polymorphic loci (Mariette et al. 2002; Bonin et al. 2007; Meudt & Clarke 2007).

Data conversions of presence-absence matrices for input into population genetic software programs (such as STRUCTURE and Arlequin, below) utilized the R-package, $AFLPdat$ source script (Ehrich 2006) in R (R-project for statistical computing; www.r-project.org). Genetic diversity, within populations, was assessed on the number of bands
and private bands, the percentage of polymorphic loci, expected heterozygosity (biased \(H_e\) and unbiased \(UH_e\)) based on Hardy-Weinberg expectations (Nei 1978), and Shannon’s Information/Diversity Index (I), serving as a coefficient of similarity, for each of the 21 populations (GenAlEx 6.3, Peakall and Smouse 2006). Cogongrass is able to reproduce asexually via belowground rhizomes. The number of unique multi-locus genotypes per population would therefore contribute toward more accurate assessments of genetic diversity within populations. Determination of the number of different genotypes present in each population utilized the “Clones” function within AFLPdat, with a corresponding error parameter, which is the product of an error rate and the number of markers (Ehrich 2006). Standard error among positive control replicates served as the error rate for clonal diversity analysis. This function within AFLPdat estimates genotype diversity (Nei 1987), the effective number of genotypes (Parker 1979), and Nei’s gene diversity (1987). Discrepancies in sample size of individuals in each state can bias results and subsequent interpretations due to statistical dependence between variables. For normally distributed data, Pearson’s correlation value may be utilized; however, for data that does not conform to normal distribution, Spearman’s correlation may be used to determine the relationship between sample size and genetic diversity results. Since these data do not conform to a normal distribution, correlations and significance of relationships between sample size and genetic diversity values were tested using Spearman’s correlation method, “cor.s” in R v.2.15.1 (Roasted Marshmallows; www.r-project.org).

This intraspecific examination also evaluated among-population genetic variation and structuring. For the purpose of ascertaining genetic variation among populations, and
how much genetic distance exists between each population, this study utilized Nei’s pairwise unbiased genetic distances (Nei 1972, GenAlEx v.6.3) and population pairwise $F_{ST}$ (Arlequin v.3.5). STRUCTURE v.2.3.3 (Pritchard et al. 2000; released in 2009, http://pritch.bsd.uchicago.edu/structure.html) was utilized to infer the number of clusters that best fit and are most appropriate for the data without apriori information applied to the number or structure of expected clusters within the dataset. Since the most appropriate number of clusters was initially unknown, duplicate runs of $K=1$-$7$ were performed, where admixture ancestry model was applied with a burnin of 10,000 and 50,000 MCMC (Markov Chain Monte Carlo) (Pritchard et al. 2000). Based on non-genetic evidence, such as the introduction history of cogongrass, a $K=2$ is expected, however, multiple iterations of $K=1$-$7$ were run to determine if that expectation was supported. To infer the most appropriate number of clusters ($K$), this study utilized the method developed by Evanno et al. (2005), to detect the second order rate of change in likelihood values, based on the difference between likelihood values generated with each $K$, from each STRUCTURE run. The difference between likelihood values and the second order rate of change was then plotted and allowed for a $\Delta K$ be determined suggesting the most likely number of clusters appropriate for the dataset. Additional analysis of population structure was assessed through principal coordinates analysis (PCA, GenAlEx v.6.3) of genetic covariance (with data standardization) among all individuals molecularly analyzed. Analysis of molecular variation (AMOVA, Excoffier et al. 1992) was performed in Arlequin v.3.5 (Excoffier and Lischer 2010), between populations assigned to differing clusters from STRUCTURE analysis.
Results and Discussion

Genetic Diversity

Regional AFLP analysis of 676 individuals from seven US states resulted in 2057 detected polymorphic bands from six selective primer pair combinations. The benefit gained from the use of more selective primer sets is to reduce the number of polymorphisms captured per primer set, but to still generate a large number of polymorphic bands for accurate AFLP assessment of intraspecific genetic diversity. This reduces unnecessary introductions of error or bias through homoplasy, where greater the numbers of detected fragments over fewer selective primer combinations increases the potential strength of homoplasious errors/biases in the generated AFLP dataset (Koopman & Gort 2004). Homoplasy is the result of co-migrating bands during electrophoresis that are not of the same physical locus in the genome and lack homology. Homoplasious biases influence errors in allele frequency detection, generally toward overestimation, generating erroneous heterozygosity estimates and underrepresentation of genetic differentiation between subpopulations (Meudt & Clarke 2007; Caballero et al. 2008). Previous AFLP analyses performed by Capo-chichi, et al., (2008) on cogongrass populations from around one site of documented introduction in Mobile Bay, AL, utilized two selective primer sets. Cheng and Chou (1997) utilized a different dominant molecular marker, known as random amplified polymorphic DNA (RAPDs), on Taiwanese ecotypes of cogongrass.

In general, a recommended minimum of “500 AFLP loci” is suggested for intraspecific examination of an outcrossing species (Mariette et al. 2002; Bonin et al. 2007; Meudt & Clarke 2007). However, more than the recommended minimum number
of detected AFLP loci estimates diversity comparable to other molecular markers, such as microsatellites (Mariette et al. 2002). This is achieved in this regional study of cogongrass populations, where cogongrass is a known obligate outcrossing species with the capacity to reproduce clonally (Gabel 1982; Bryson & Carter 1993).

The average percentage of AFLP loci found to be polymorphic per state was 23% (SE ± 7%). The percentages of polymorphic loci were calculated from the number of polymorphic loci in a population (or in this case, state) by the total number of polymorphic loci detected and analyzed among all states. The number of polymorphisms found in a population represents the quantity of genetic diversity present. From the seven U.S. states with cogongrass invasion, percentages of polymorphic loci were found to range from 2% (in GA) to 56% (in SC). These values indicate that the highest genetic diversity was found in SC and the lowest genetic diversity found in GA (as reported above) and TX (3%); although, AL (30%), FL (32%), and MS (24%) are moderately diverse among the populations analyzed in this study. Mean heterozygosity ($H_e$ and $UH_e$) among all seven states was 0.016 (SE ± 0.001) and mean Shannon’s Information Index ($I$) was 0.030 (SE ± 0.001). Four states: AL, FL, MS, and SC, consistently resulted in values above the average in percentage of polymorphic loci, heterozygosity, and Shannon’s Index values (Table 4.2). Nei’s gene diversity for all states analyzed ranged from 0.006 in GA to 0.042 in SC (Table 4.3). It is observed that Nei’s gene diversity values are also similar among the states of AL (0.023), MS, (0.029) and FL (0.028).

Though genetic diversity, in numbers of polymorphisms, was high for most states, $H_e$ and $UH_e$ seem comparatively low for long-lived, perennial out-crossing plant species analyzed via dominant molecular markers (Nybom 2004). These values may have been
low due to the large number of polymorphic loci comprising this dataset. Low heterozygosity may also indicate that the populations analyzed here may be heterozygote deficient. Despite these seemingly low numbers, heterozygosity and Shannon’s Index values for GA and TX were 2-3 times lower than all other states analyzed and the values were highest in SC.

Since cogongrass can also reproduce clonally via belowground rhizomes, reduction in genetic diversity may be due to clonal reproduction during population expansion. The total number of genotypes detected from clonal analysis was reduced over 50%, from 676 total individuals sampled to 321 genotypes. Clonal analysis observed reductions in genotype diversity in each state, with some states possessing populations likely relying on clonal reproduction more than others (Table 4.3). For example, genotype diversity is lowest in GA at 0.154, reducing the effective number of genotypes to 1.166 from 13 sampled individuals; however, the states that received direct introductions of propagules from Asia (MS and AL) possess the highest, and most similar, genotypic diversity (>0.90) among sampled states. This pattern of genotypic diversity can support the previous finding that MS and AL have experienced cogongrass invasion for a longer period of time, and therefore, posses the greatest variation in the invaded U.S. range. This is likely due to the highest density of cogongrass infestations increasing the likelihood for successful outcrossing among populations to promote genetic variability in those states. Certain genotypes may affect the success of the overall invasion in a region, due to the possession of greater diversity or through the selection for more adaptable or invasive characteristics (Sakai et al. 2001; Vellend et al. 2010).
The values for genetic diversity (Table 4.2) consistently resulted in the lowest values in GA and TX, and were highest in SC. Low genetic diversity values may be potentially due to small sample sizes in TX (n=10) and GA (n=13) as compared to other states. Data for genetic diversity and sample size did not conform to a normal distribution, so non-parametric Spearman’s correlation coefficient (ρ) was used to test degree of statistical dependence between sample size and resulting genetic diversity values, including the number and percentage of polymorphic bands detected, the number of private bands, heterozygosity (H_e) and unbiased heterozygosity (UH_e), Shannon’s information index (I), and Nei’s gene diversity (Table 4.4). Tests for correlation between sample size and genetic diversity were conducted for all states, for the states with more than 50 individuals sampled (AL, FL, LA, MS and SC), and for TX and GA, alone. Positive correlations were found for all relationships between sample size and genetic diversity measures, with the exception of Nei’s gene diversity for states with greater individual sampling. However, in testing for significance, all relationships between sample size and genetic diversity were found to be insignificant, and therefore no significant relationships between sample size and genetic diversity could be found, suggesting small sample sizes in TX and GA are not skewing the data or interpretation.

The number of private bands detected in SC (682 of 1153) is remarkably high, comprising the majority of the genetic diversity detected. This may be due to treating each state as an individual population for state-level analysis. The effect of such a high number of private bands at low to very low frequencies may alternatively influence correlations between sample size and genetic diversity (as above). The frequencies of these private bands were not further investigated in this study. These private bands, if at
a very low frequency may be anomalous and thereby influencing inferences made about
cogongrass populations in SC. On the other hand, these private bands may be primarily
suggestive of an additional separate introduction(s) of foreign material. Possible direct
introduction of viable cogongrass propagules into Port of Charleston in SC are
anecdotally discussed, but without reliable documentation (R. Westbrooks, personal
communication). In addition, the location of cogongrass occurrence in SC appear to be
somewhat disjunct from the main invasion (Figure 4.1), further supporting an possible
separate introduction. An unknown introduction of completely different parent material
may potentially explain the high number of private bands found in SC relative to all other
states in the region.

The observed pattern of genetic diversity among states suggests a potential role of
management and management history, both in terms of time and funding, affecting the
quantity of extant genetic diversity in cogongrass populations. Management is
considered any effort toward controlling the growth and spatial spread of cogongrass, and
is accomplished through preventing introduction and spread of propagules, implementing
monitoring and detection programs, and control through physical disruption of soil,
herbicide application, and other approaches. Herbicide and disruption management in
efforts to kill and reduce patch area likely reduce genetic diversity by reducing the
number of fit individuals available for sexual reproduction to transmit their genes to the
next generation. Management of cogongrass is primarily funded and executed by state
agencies, sometimes supplemented by federal funding. For example, SC only recently
has begun to track and treat cogongrass patches in that state, potentially allowing
established patches to reproduce and spread during the intervening time since initial
introduction(s). The high amount of present genetic variation in SC supports this inferred pattern. Whereas in GA, treatment of cogongrass began nearly half a century ago, and their management strategy appears to be effective. The area and intensity of cogongrass infestations in GA are relatively low compared to neighboring states (Figure 4.1), and the pattern of low genetic diversity mirrors the reduced physical coverage in this state by cogongrass. These genetic data support a role of differential funding and management contributing to different levels of genetic diversity among states.

Regional Population Structure

Cluster analysis in STRUCTURE supported an inference of two distinct genetic groups ($K=2$) across the region (Figure 4.3). The most distinct division between clusters occurred within MS, indicating co-occurrence of two genetic groups in this state, also supported by data from the previous chapter. The MS-type is still inferred as one cluster and the AL-type as another. The addition of five more states in this analysis resulted in FL, GA, LA, SC, and TX grouping with the AL-type. Within the AL-type, substantial admixture with the MS-type appeared in AL, FL, and SC, whereas LA and TX have relatively low evidence of admixture, and very little to none was detected among GA samples. Spatial distribution of sample sites and genetic identity from STRUCTURE analysis is available in the sampling map, Figure 4.2.

Principal coordinate analysis of these populations also resulted in two clusters: one fairly organized cluster (MS-type/MS-central) and one very broad and scattered cluster (AL-type) (Figure 4.4). Individuals from MS occur in both clusters; MS-central constrained to the bottom right quadrant and MS-coast individuals group with AL, FL, and SC individuals. Individuals from AL are scattered throughout the cluster, indicating
the greatest variation among individuals from AL, followed by individuals from FL. Interestingly enough, TX individuals (all collected from a single population) did not form a tight cluster, but grouped with samples from AL, FL, LA, and SC. Georgia, however, forms a very tight cluster within the AL-type (bottom left quadrant), overlapping with a subset of the AL, LA, MS, and SC individuals, potentially indicating genetic relationships among cogongrass populations in these states.

Nei’s pairwise matrix of genetic distances among populations (Table 4.5.A) shows MS consistently distant (0.003-0.007) from all other states, supporting data from Chapter II of this work. The greatest genetic distance was found between TX and GA (0.009), with GA being the most distant from all other states analyzed (0.006-0.009), except for LA (0.001). This suggests the LA and GA populations are likely related genetically, with one state sourcing populations to the other, or originating from the same parent material. Anthropogenic activities, especially transport of propagules and associated genomes may have given rise to this unexpected pattern. Cogongrass management, especially by herbicide, can reduce genetic diversity by reducing the number of individuals able to reproduce, either sexually or asexually. Therefore, differential management from state to state compounded by stochastic anthropogenic activities likely generate unexpected patterns of genetic diversity and population structuring in invasive organisms.

Previous data suggested that MS-central populations are genetically distinct from MS-coast populations, and that distinction remains consistent in this study as expected. Population pairwise FST values (Table 4.5.B) support significant genetic dissimilarity between the two groups of MS populations (FST =0.330, P<0.05). Also of note, MS-
central was genetically dissimilar from all other groups tested ($F_{ST} > 0.3$), supporting that this group may have arisen from different parent material. AL and MS-coast populations were the least genetically differentiated ($F_{ST} = 0.094-0.217$), and this suggests these populations are the most related. Pairwise population $F_{ST}$ values were similar between SC and most other state-groupings ($F_{ST} < 0.2$), except for MS-central ($F_{ST} = 0.314$). If MS and AL populations are assumed to be the only primary introductions of cogongrass parent material and cogongrass presence in SC is more recent than in MS, AL, and FL, then these data suggest that SC populations may be the result of multiple introductions from other states already with established and intermixing populations. Data support the MS-central group as more spatially localized, which may be due to its location, possessing more remoteness from major interstates or less heavy equipment disturbance that are associated with cogongrass spread, than with other locations.

Pairwise population $F_{ST}$ values also provide genetic support for determining relatedness of cogongrass populations between states. For example, the pairwise $F_{ST}$ value between TX and AL ($F_{ST} = 0.090$) was very close to pairwise $F_{ST}$ values between TX and SC, and between TX and FL (both $F_{ST} = 0.083$). This suggests that the TX population is equivalently related to populations in those three states, and may have been founded by propagules from one or all those states. There was also evidence based on pairwise $F_{ST}$ that populations located in AL, the MS-coast, and FL, are most similar to each other and to outlier populations in LA, TX, and SC. This may suggest that these three locations are generalized areas of intraspecific hybridization event(s), and are sourcing propagules into other locations that expand cogongrass’ invaded range.
The greatest genetic dissimilarity existed between TX and GA ($F_{ST} = 0.553$). This substantial differentiation may be due to multiple factors including, but not limited to, geographic distance, differential invasion histories, continuous (or lack of) propagule pressure, and/or differential management strategies and histories. Only one population is present in TX and has been actively managed for nearly a decade (Texas Forest Service). Georgia was the first state to manage cogongrass, beginning in the late 1960’s (A. Miller, personal correspondence; Georgia Forestry Commission.) This state continues the most aggressive management of cogongrass, relative to density and distribution of cogongrass infestations throughout the region. Both TX and GA also possessed low genetic variation, suggestive of a genetic bottleneck, and may also have experienced genetic drift in isolation, thereby genetically differentiating these populations from one another.

Using inferred groups from STRUCTURE analysis, which were supported by patterns in pairwise population $F_{ST}$ values, an analysis of molecular variance (AMOVA) was conducted to test the degree of genetic dissimilarity between the MS-type (MS-central) vs. AL-type (all other groups) (Table 4.6). Significant genetic differentiation was found between the groups tested ($F_{ST} = 0.363$, $P<0.001$), greater than variation among populations within groups, which accounts for little of the molecular variation (9%). These results are consistent with data from preceding chapters, but indicate that inclusion of all regional data increases genetic similarity between the MS-type and the AL-type. This suggests regional secondary invasions may be the result of multiple introductions where some of the propagules introduced during secondary spread are offspring from intraspecific hybridization event(s) between these genetic groups.
Conclusions

The dynamics of secondary invasions are subject to the invasion process or cycle, where propagules must be successfully transported, introduced, and then become established. That success is dependent on primarily the strength of propagule pressure. For this study, propagule pressure can be considered as the amount of introduced genetic variability combined with the frequency of introductions. The geographic pattern of the partitioning of genetic variation on a state-by-state basis suggest that differential secondary invasion and propagule pressure, in conjunction management history, action plans, and funding, contribute toward reductions in population genetic diversity, consistently observed in analyses of collections from Georgia and Texas. Continued range expansion of cogongrass, in the U.S., will be the product of secondary invasion processes establishing in new areas, and are likely to become problematic and persistent.

Extant levels of genetic diversity were found to be highly variable among cogongrass populations located throughout seven states in the southeastern US. Detected levels of genetic diversity in each state mirrored the reported densities of cogongrass infestation found in Figure 4.1. The exception to this pattern was SC, which does not have a high relative density of reported infestations (Figure 4.1) but possesses the highest genetic variability across the board. Cogongrass populations in SC are considered ‘outlier’ populations (Miller 2007, 2011), and therefore are slated first for treatment and eradication according to coordinated management plans. Reduction of genetic variation in SC would likely benefit the eradication goals set out by the above management plans. The presence of substantial genetic variation in SC is suggestive of multiple introductions of cogongrass propagules over a decade or longer without active management. In
addition, its lack of clear genetic differentiation from other states, save MS-central, also suggests that propagules were sourced from multiple areas.

Other populations in states like TX, LA, and GA are also considered outlying as part of an advancing front of invasion. These states exhibited low genetic variability, consistent with infestation patterns and expectations. However, the lower genetic diversity indices in these areas were not correlated with genetic dissimilarity, which is contraindicative of an isolation-by-distance hypothesis (Figure 2.2, Chapter II; Capo-chichi et al. 2008). The patterns of genetic diversity in these states may possibly be attributed to a consequence of long-term management and eradication, thereby resulting in low infestation density and genetic diversity present in these areas. Lack of genetic dissimilarity also indicates that GA and LA are most similar among states analyzed and may be directly related. Both TX and GA consistently possess lowest genetic variability over all populations tested, and this can be a result of known, long-term historical management by herbicide. Herbicide management serves as a strong force of selection, which may have reduced genetic diversity in populations in these states, which also are most genetically dissimilar to one another. These results support genetic differentiation in isolation due to genetic drift, possibly combined with a lack of continued introduction of propagules. The phenomena of population genetic differentiation are not always necessarily a product of geographic distance, but can be the result of mechanisms like genetic drift and selection, altering allele frequencies within cogongrass populations (Nei & Chakraborty 1978). Both genetic drift and the force of selection can change an allele frequency directionally in a population, toward fixation (frequency = 1) or toward extinction of that allele (frequency = 0). These mechanisms drive genetic differentiation
between populations and may ultimately result in a speciation event over time and multiple generations. These principles are the basis for population genetic study and evolution.

The lack of genetic dissimilarity between most states may also be due to intraspecific hybridization event(s) recombining genomes of two detected genetic groups, consistently supporting data and conclusions from this and another chapter (Chapter II). Hybridized propagules have likely spread from the ‘epicenter’ of invasion, located in the coastal plains of Mississippi and Alabama (Figure 4.1; Miller 2007), and then secondarily invaded new states to establish closely related outlier populations located on the edge of the invaded range. The spatial pattern of cogongrass infestations across the seven states that were analyzed is supported by patterns of genetic identity and diversity as observed in this and other chapters.

The genetic data generated and inferences made from molecularly derived information are utilized to better understand the process of invasion limited primarily by propagule pressure. The quantity of extant genetic diversity and partitioning of this variation provides information to infer potential quantities of genetic information introduced, as well to determine frequency of separate, multiple introductions into a region from foreign sources contributing to the success of an invasion (Walker et al. 2003; Genton et al. 2005; Hughes et al. 2008). Secondary invasions during range expansion are the product of the number and frequency of propagules, from possibly different genetic sources, introduced to new, uninvaded areas (Lockwood et al. 2005). Genetic relatedness across the geographic range affected by cogongrass invasion suggests that secondary invasions are spreading from the hypothesized epicenter. The spread of
cogongrass from the Mississippi and Alabama coast into neighboring states was likely facilitated by human transport, with documented propagule transport and establishment into much of Florida by way of Georgia (Tabor 1952). Anthropogenic activities transporting propagules from the Mississippi and Alabama coastal plain to other areas are also implicated in present-day secondary invasions based on generated molecular data. Populations that are actively and aggressively managed are reported as less dense and, based on this study, possess less genetic variation. Populations that have not been under long-term management possess more genetic variability, and may be the product of multiple introductions of cogongrass propagules from not only the epicenter of invasion, but also from other secondarily established locations (Table 4.5).

The spatial distribution of cogongrass infestations can be explained by propagule movement, and supported by observed patterns from resulting molecular data, where Mississippi, Alabama, and Florida possess moderate genetic variation among all states analyzed. This may suggest that these three states may have experienced similar invasion histories in potentially the same temporal window, suggesting a longer amount of time experiencing cogongrass invasion than the other states. Documented introduction history, documentation of propagule transport is further supported by derived molecular data in this work. Genetic variation within the populations located in the area identified as the epicenter, located in areas along the Mississippi-Alabama border, suggest intraspecific hybridization between two different genetic groups and consistently support an initial two-introduction hypothesis (Chapter II).

Multiple introductions from previously isolated parent genetic material during invasions can increase the genetic information and variability introduced into an area
(Lee 2002; Genton et al. 2005). Increases in introduced genetic information can facilitate successful invasions by augmenting a founding population’s adaptability to biotic and abiotic conditions of the novel range (Lockwood et al. 2005). Hybridization is another vehicle capable of generating genetic diversity among populations of a single, invading species, and can therefore, promote establishment and spread into naïve areas (Ellstrand & Schierenbeck 2000; Ward et al. 2008). These phenomena contribute toward propagule pressure, which is hypothesized to be a significant and predictive component of biological invasions. Therefore, cogongrass invasion in the southeastern U.S. is primarily fueled by spread of propagules from populations proximal to sites of initial introduction from Asia (MS and AL). Ameliorating cogongrass invasion in the U.S. will require multi-state cooperatives, self-regulation by private entities, enforcement of decontamination protocols, and prevention of the transfer of cogongrass propagules and its inherent genome.

Catford et al.’s (2009) framework suggested that propagule pressure is the one component of invasion that is directly modifiable by anthropogenic activities. Therefore, transport and frequency of introductions of genetic material into invaded and non-invaded areas is a direct product of human behavior and action. Propagule pressure is a product of the number of introduced propagules and the frequency of introductions (Lockwood et al. 2005). In terms of genetic information, propagule pressure is the product of the quantity of genetic information introduced, increases in genetic information and variability, and the frequency of events increasing genetic variability and the frequency of introductions of new genetic material. Therefore, events increasing genetic information and variability strengthen propagule pressure; on the other hand,
genetic bottlenecks and isolation can reduce genetic variability and available genetic information during primary and secondary founding events of invasion (Lockwood et al. 2005; Coulatti et al. 2006; Dlugosch & Parker 2008). Using genetic diversity to estimate propagule pressure can be a powerful new tool in elucidating and predicting invasions. Integrating the genetic component in the study of invasions provides an additional perspective toward developing a more clear understanding of propagule pressure and its role in biological invasions.
Table 4.1  Six AFLP selective amplification primer combinations utilized.

<table>
<thead>
<tr>
<th>MseI Primer</th>
<th>Fluorescent Dye-Labeled EcoRI Primer</th>
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<tr>
<td>MseI-CAT</td>
<td>EcoRI-ACT-FAM</td>
</tr>
<tr>
<td>MseI-CTA</td>
<td>EcoRI-AGG-HEX</td>
</tr>
<tr>
<td>MseI-CTG</td>
<td>EcoRI-AGC-NED</td>
</tr>
<tr>
<td>MseI-CTT</td>
<td>EcoRI-ACT-FAM</td>
</tr>
<tr>
<td>MseI-CTC</td>
<td>EcoRI-AGG-HEX</td>
</tr>
<tr>
<td>MseI-CAC</td>
<td>EcoRI-AGC-NED</td>
</tr>
</tbody>
</table>

NOTE: Fluorescent dye-labeled selective primers are denoted by “EcoRI-primer-[Axx]-[dye]. Each fluorescent dye is visualized as a different color for fragment analysis: FAM (blue), HEX (green), NED (yellow or black).
Table 4.2  Genetic Diversity Indices with Population Location Information.

<table>
<thead>
<tr>
<th>State</th>
<th>Counties Sampled</th>
<th>Other Location Information</th>
<th>Number of individuals (n)</th>
<th>Number of Bands Detected</th>
<th>Number of Private Bands</th>
<th>Percentage Polymorphic Loci (%)</th>
<th>$H_e \pm$ SE</th>
<th>$UH_e \pm$ SE</th>
<th>Shannon’s Information Index (I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama (AL)</td>
<td>Baldwin, Hale, Lee, Mobile, Washington, Sumter</td>
<td>Talladega NF, Frank Boykin WMA</td>
<td>208</td>
<td>626</td>
<td>216</td>
<td>30.38%</td>
<td>0.017 ± 0.017</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Florida (FL)</td>
<td>Alachua, Duval, Indian River, Miami-Dade, Osceola, Sarasota</td>
<td>Disney Wilderness Preserve (TNC), Miami-Dade municipal parks</td>
<td>129</td>
<td>672</td>
<td>238</td>
<td>32.67%</td>
<td>0.016 ± 0.016</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Georgia (GA)</td>
<td>Baker, Crawford, Decatur, Mitchell, Thomas, Worth</td>
<td>Georgia Forestry Commission</td>
<td>13</td>
<td>45</td>
<td>-</td>
<td>2.04%</td>
<td>0.005 ± 0.006</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Louisiana (LA)</td>
<td>St. Tammany, Washington</td>
<td>Benscreek WMA</td>
<td>62</td>
<td>289</td>
<td>64</td>
<td>14.00%</td>
<td>0.012 ± 0.013</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Mississippi (MS)</td>
<td>Greene, Harrison, Jasper, Jones, Scott, Smith, Wayne</td>
<td>Desoto NF, Bienville NF</td>
<td>180</td>
<td>500</td>
<td>101</td>
<td>24.31%</td>
<td>0.022 ± 0.022</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>South Carolina (SC)</td>
<td>Berkeley, Greenwood, Saluda, Union</td>
<td>Frances Marion NF, Sumter NF</td>
<td>74</td>
<td>1153</td>
<td>682</td>
<td>56.05%</td>
<td>0.029 ± 0.029</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Texas (TX)</td>
<td>Tyler</td>
<td>Texas Forest Service</td>
<td>10</td>
<td>65</td>
<td>-</td>
<td>2.58%</td>
<td>0.008 ± 0.009</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Overall</td>
<td>676</td>
<td>7.20%</td>
<td>0.016 ± 0.016</td>
<td>0.001</td>
<td>0.001</td>
<td>0.030 ± 0.030</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

± standard error (SE)

NOTE: Seven states sampled for cogongrass ($I. cylindrica$): location information, band data, and genetic diversity indices ($H_e$=expected heterozygosity, $UH_e$=unbiased expected heterozygosity, I=Shannon’s Information index, with ± standard error (N=676). Overall totals and averages are in **bold**.
Table 4.3  Clonal Diversity Analysis based on AFLP Multilocus Data (Regional).

<table>
<thead>
<tr>
<th>Population Identifier</th>
<th>Number of individuals (n)</th>
<th>Number of Genotypes</th>
<th>Genotype Diversity</th>
<th>Effective Number of Genotypes</th>
<th>Nei’s Gene Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama (AL)</td>
<td>208</td>
<td>92</td>
<td>0.935</td>
<td>14.431</td>
<td>0.023</td>
</tr>
<tr>
<td>Florida (FL)</td>
<td>129</td>
<td>49</td>
<td>0.816</td>
<td>5.268</td>
<td>0.028</td>
</tr>
<tr>
<td>Georgia (GA)</td>
<td>13</td>
<td>2</td>
<td>0.154</td>
<td>1.166</td>
<td>0.006</td>
</tr>
<tr>
<td>Louisiana (LA)</td>
<td>62</td>
<td>14</td>
<td>0.640</td>
<td>2.699</td>
<td>0.029</td>
</tr>
<tr>
<td>Mississippi (MS)</td>
<td>180</td>
<td>117</td>
<td>0.940</td>
<td>15.429</td>
<td>0.023</td>
</tr>
<tr>
<td>South Carolina (SC)</td>
<td>74</td>
<td>41</td>
<td>0.888</td>
<td>8.077</td>
<td>0.042</td>
</tr>
<tr>
<td>Texas (TX)</td>
<td>10</td>
<td>6</td>
<td>0.778</td>
<td>3.333</td>
<td>0.011</td>
</tr>
<tr>
<td>Overall</td>
<td>676</td>
<td>321</td>
<td>0.940</td>
<td>15.429</td>
<td>0.023</td>
</tr>
</tbody>
</table>

NOTE: State populations, sample size, and resulting clonal diversity from multilocus AFLP data, including number of different genotypes detected, genotypic diversity, effective number of genotypes, and Nei’s gene diversity. Overall totals and averages are in bold.

Table 4.4  Non-parameteric Spearman’s correlation values between sample size and regional genetic diversity.

<table>
<thead>
<tr>
<th>Spearman's correlation coefficient (ρ), P-value set at 0.05</th>
<th>Number of Bands Detected</th>
<th>Number of Private Bands</th>
<th>Percentage Polymorphic Loci</th>
<th>Heterozygosity (H_e)</th>
<th>Unbiased Heterozygosity (UHe)</th>
<th>Shannon's Information Index (I)</th>
<th>Nei's Gene Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>All States</td>
<td>0.642</td>
<td>0.667</td>
<td>0.643</td>
<td>0.714</td>
<td>0.714</td>
<td>0.684</td>
<td>0.306</td>
</tr>
<tr>
<td>(P-value)</td>
<td>0.139</td>
<td>0.102</td>
<td>0.139</td>
<td>0.088</td>
<td>0.088</td>
<td>0.090</td>
<td>0.504</td>
</tr>
<tr>
<td>AL, FL, LA, MS, SC alone</td>
<td>0.100</td>
<td>0.100</td>
<td>0.100</td>
<td>0.300</td>
<td>0.300</td>
<td>0.205</td>
<td>-0.872</td>
</tr>
<tr>
<td>(P-value)</td>
<td>0.950</td>
<td>0.950</td>
<td>0.950</td>
<td>0.683</td>
<td>0.683</td>
<td>0.741</td>
<td>0.054</td>
</tr>
</tbody>
</table>

NOTE: Non-parametric Spearman’s correlation values (ρ) between sample size and genetic diversity with tests for significance (P<0.05). Correlations between sample size and genetic diversity grouped all states together (above), or excluded TX and GA to determine relationship of relatively small sample sizes (below). All Spearman’s correlation values were found to be insignificant, and genetic diversity values are not biased due to differing sample sizes among states.
Table 4.5  State by state pairwise population genetic distance (A) and differentiation (B).

A.  
**Pairwise Population Matrix of Nei Unbiased Genetic Distance**

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>AL</th>
<th>LA</th>
<th>SC</th>
<th>TX</th>
<th>GA</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>0.003</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>0.005</td>
<td>0.004</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.005</td>
<td>0.003</td>
<td>0.003</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>0.006</td>
<td>0.003</td>
<td>0.005</td>
<td>0.004</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX</td>
<td>0.007</td>
<td>0.007</td>
<td>0.001</td>
<td>0.006</td>
<td>0.009</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>0.004</td>
<td>0.002</td>
<td>0.003</td>
<td>0.002</td>
<td>0.002</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.385</td>
</tr>
</tbody>
</table>

B.  
**Pairwise Population Matrix of F<sub>ST</sub>**

<table>
<thead>
<tr>
<th></th>
<th>MS-Coast</th>
<th>AL</th>
<th>LA</th>
<th>SC</th>
<th>TX</th>
<th>GA</th>
<th>FL</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS-Coast</td>
<td>0.132</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AL</td>
<td>0.096</td>
<td>0.175</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.098</td>
<td>0.100</td>
<td>0.120</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>0.217</td>
<td>0.090</td>
<td>0.264</td>
<td>0.083</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TX</td>
<td>0.150</td>
<td>0.277</td>
<td>0.050</td>
<td>0.158</td>
<td>0.553</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>0.146</td>
<td>0.094</td>
<td>0.158</td>
<td>0.060</td>
<td>0.083</td>
<td>0.281</td>
<td>*</td>
</tr>
<tr>
<td>FL</td>
<td>0.330</td>
<td>0.320</td>
<td>0.444</td>
<td>0.314</td>
<td>0.348</td>
<td>0.478</td>
<td>0.385</td>
</tr>
</tbody>
</table>

NOTE: (A) Pairwise population matrix of Nei’s unbiased genetic distances, and (B) Pairwise population matrix of F<sub>ST</sub> values. All Pairwise F<sub>ST</sub> Values are Significant (P <0.05). 

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Table 4.6  Results from analysis of molecular variation (AMOVA) using $F_{ST}$ between groups inferred from genetic data.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Percentage of variation</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among Groups</td>
<td>1</td>
<td>1325.57</td>
<td>27.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>6</td>
<td>1283.18</td>
<td>8.88</td>
<td>0.11</td>
</tr>
<tr>
<td>Within populations</td>
<td>668</td>
<td>12151.01</td>
<td>63.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>675</td>
<td>14776.76</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$F_{ST} = 0.363$ ($P<0.001$), $F_{SC} = 0.122$ ($P<0.001$), $F_{CT} = 0.274$ ($P=0.11$)

NOTE: Group 1 is defined as cluster 1 (MS-type) and group 2 was defined as cluster 2 (AL-type) as inferred from STRUCTURE analysis ($K=2$; Figure 4.3).
Figure 4.1 Mapped densities of cogongrass infestations at the county level as is reported in the southeastern U.S.

NOTE: Darker shading represents heavier infestations. Pattern of spread appears as a wave with an evident advancing front. Outlying populations are geographically disjunct from primary and secondary invasions, evident in TX and SC. Map obtained from the Center for Invasive Species and Ecosystem Health, University of Georgia, in cooperation with reporting state agencies (www.cogongrass.org, via EDDmaps, the Bugwood Network, and Southeast Exotic Pest Plant Council).
Figure 4.2  Map of seven U.S. states sampled for cogongrass (*I. cylindrica*) populations.

NOTE: From west to east, one site sampled from TX, six sites from LA, 11 sites in MS, 10 sites in AL, 10 sites from GA, 14 sites from FL, and seven sites sampled in SC (N=676). Gray shaded areas represent the National Forest acquisition boundaries. Square symbols represent sampled populations that are genetically grouped within “MS-type”; circle symbols represent populations grouped within “AL-type”. Six sites sampled (63 individuals) from Miami-Dade County, FL, were collected under the assumption of potentially belonging to a different congener (*Imperata brasiliensis* Trin.) (see Chapter III).
Figure 4.3 Bar plot from STRUCTURE analysis with two populations assumed ($K=2$) for individuals sampled from seven US states.

NOTE: A total of 676 individual were analyzed in STRUCTURE for the seven states comprising the region. Individuals are represented by vertical bars and horizontally organized by population. State labels along bottom horizontal axis. Each bar represents an individual. Each cluster is represented by a different color. Different colors (black or white) represent the frequency of AFLP loci assigned to each cluster. Some individuals are completely assigned to one cluster or another, while others show mixed ancestry. Cluster 1 (“MS-type”) is predominantly white and cluster 2 (“AL-type”) is predominantly black, with admixture between the two groups. Two asterisks (**) represent the demarcation between two detected clusters, found to occur within population MS-6, which separates MS-central from MS-coast populations.
Figure 4.4 Principal coordinates analysis (PCA) of genetic covariance (with data standardization) of individuals sampled across seven US states

NOTE: A total of 676 individuals were sampled from seven U.S. states (MS, AL, LA, SC, TX, GA, and FL). Most individuals collected from MS form the cluster in the bottom right quadrant (MS-type), with some AL individuals. The other cluster forming the AL-type is less tight and more scattered. This cluster is comprised of most AL individuals, MS-Coast group, and individuals from all other states sampled. Individuals from AL are widely scattered. GA and LA form a tight cluster in the bottom left quadrant as part of the larger AL-type cluster. These first two PCA axes explained 60.76% of the variation in this dataset.


APPENDIX A

SAMPLED LOCATION DATA, DESCRIPTORS, AND HERBARIUM INFORMATION FOR *IMPERATA* COLLECTED

IN THE UNITED STATES
### Tables of Tissue Collections by State

#### Table A.1 Mississippi Collections.

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Location Description</th>
<th>GPS Coordinates</th>
<th>Date Collected</th>
<th>Population Identifier</th>
<th>Herbarium</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mississippi.</td>
<td>Jasper Co.:</td>
<td>Bienville NF, right-of-way Co. Rd. 16, Bienville NF, right-of-way bordering pasture</td>
<td>89.282864W 32.091628N</td>
<td>28 July 2008</td>
<td>MS-1</td>
<td>Dried leaf tissue available</td>
<td>RDL 001</td>
</tr>
<tr>
<td></td>
<td>Smith Co.:</td>
<td>on Co. Rd. 520 off of Co. Rd. 501, Bienville NF, Morton-Marathon Rd. right-of-way</td>
<td>89.398715W 32.211304N</td>
<td>28 July 2008</td>
<td>MS-2</td>
<td>Dried leaf tissue available</td>
<td>RDL 002</td>
</tr>
<tr>
<td></td>
<td>Scott Co.:</td>
<td>and ditch bordering pasture east of MS-HWY 35, Bienville NF, right-of-way and ditch</td>
<td>89.474334W 32.234789N</td>
<td>28 July 2008</td>
<td>MS-3</td>
<td>Dried leaf tissue available</td>
<td>RDL 003</td>
</tr>
<tr>
<td></td>
<td>Scott Co.:</td>
<td>bordering treeline on Highway 481 north of Strickland Road right-of-way, patch</td>
<td>89.604199W 32.284668N</td>
<td>28 July 2008</td>
<td>MS-4</td>
<td>Dried leaf tissue available</td>
<td>RDL 004</td>
</tr>
<tr>
<td></td>
<td>Jones Co.:</td>
<td>transitioning into new growth Pinus palustris forest stand, Desoto NF acquisition</td>
<td>88.797347W 31.566852N</td>
<td>04 August 2008</td>
<td>MS-5</td>
<td>Dried leaf tissue available</td>
<td>RDL 005</td>
</tr>
<tr>
<td></td>
<td>Greene Co.:</td>
<td>boundary, Off of Tom Jones Rd., Desoto NF, occuring with flagged gopher tortoise</td>
<td>88.792715W 31.498154N</td>
<td>04 August 2008</td>
<td>MS-6</td>
<td>Dried leaf tissue available</td>
<td>RDL 006</td>
</tr>
<tr>
<td></td>
<td>Wayne Co.:</td>
<td>Desoto NF, right-of-way of FR 205 intersected by Brown Cemetery Rd., EB I-10</td>
<td>88.694363W 31.354801N</td>
<td>04 August 2008</td>
<td>MS-7</td>
<td>Dried leaf tissue available</td>
<td>RDL 007</td>
</tr>
<tr>
<td>Mississippi.</td>
<td>Wayne Co.:</td>
<td>shoulder (ROW), approx 1 mi. west ofShorecrest Rd., near Gulfport, MS, Beneath</td>
<td>88.963143W 31.449731N</td>
<td>04 August 2008</td>
<td>MS-8</td>
<td>Dried leaf tissue available</td>
<td>RDL 008</td>
</tr>
<tr>
<td>Harisson Co.:</td>
<td>EB I-10 overpass at Cedar Lake Road (exit #44), Biloxi, MS, Beneath EB I-10</td>
<td>89.012256W 30.447631N</td>
<td>17 August 2009</td>
<td>MS-9</td>
<td>Dried leaf tissue available</td>
<td>RDL 009</td>
<td></td>
</tr>
<tr>
<td>Harisson Co.:</td>
<td>Beneath EB I-10 overpass at Cedar Lake Road (exit #44), Biloxi, MS,</td>
<td>88.934241W 30.449424N</td>
<td>17 August 2009</td>
<td>MS-10</td>
<td>Dried leaf tissue available</td>
<td>RDL 010</td>
<td></td>
</tr>
<tr>
<td>Harisson Co.:</td>
<td>Beneath EB I-10 overpass at Cedar Lake Road (exit #44), Biloxi, MS,</td>
<td>88.934241W 30.449424N</td>
<td>17 August 2009</td>
<td>MS-11</td>
<td>Dried leaf tissue available</td>
<td>RDL 011</td>
<td></td>
</tr>
</tbody>
</table>
Table A.2  Alabama Collections.

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Location Description</th>
<th>GPS Coordinates</th>
<th>Date Collected</th>
<th>Population Identifier</th>
<th>Herbarium</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama</td>
<td>Sumter Co.</td>
<td>Right-of-way Hwy AL-11, bordering open pasture, Large area (73 x 22m) with evidence of recent logging activity at crest of hill,</td>
<td>88.164537W 32.661376N</td>
<td>02 August 2008</td>
<td>AL-1</td>
<td>Dried leaf tissue available</td>
<td>RDL 012</td>
</tr>
<tr>
<td>Alabama</td>
<td>Hale Co.</td>
<td>Oakmulgee WMA, Talladega NF, Rights-of-way on both sides of new unimproved road leading to hillcrest, high disturbance area, Oakmulgee WMA, Talladega NF, SB Hwy 45 right-of-way and associated private drive leading into a wet area and pine plantation, Co. Rd. 35 right-of-way opening to large area containing abandoned logging deck, Frank Boykin WMA,</td>
<td>87.497343W 32.889016N</td>
<td>02 August 2008</td>
<td>AL-2</td>
<td>Dried leaf tissue available</td>
<td>RDL 013</td>
</tr>
<tr>
<td>Alabama</td>
<td>Hale Co.</td>
<td></td>
<td>87.4971W 32.8877N</td>
<td>02 August 2008</td>
<td>AL-3</td>
<td>Dried leaf tissue available</td>
<td>RDL 014</td>
</tr>
<tr>
<td>Alabama</td>
<td>Washington Co.</td>
<td></td>
<td>88.283885W 31.188116N</td>
<td>13 August 2008</td>
<td>AL-4</td>
<td>Dried leaf tissue available</td>
<td>RDL 015</td>
</tr>
<tr>
<td>Alabama</td>
<td>Washington Co.</td>
<td></td>
<td>88.156726W 31.22541N</td>
<td>13 August 2008</td>
<td>AL-5</td>
<td>(MISSA)</td>
<td>RDL 016</td>
</tr>
<tr>
<td>Alabama</td>
<td>Baldwin Co.</td>
<td>Large patch area off of AL Hwy-181</td>
<td>87.852504W 30.494266N</td>
<td>13 August 2008</td>
<td>AL-6</td>
<td>Dried leaf tissue available</td>
<td>RDL 017</td>
</tr>
<tr>
<td>Baldwin</td>
<td>Co.</td>
<td>Rights-of-way associated with intersecting Co. Rds. 55 &amp; 56, Disturbed, developed parking area in front of Tronox Corp. site off of AL Hwy-193, Smithtown Rd. right-of-way at Hwy-45 across road from Smithtown Baptist Church,</td>
<td>87.750333W 30.581782N</td>
<td>13 August 2008</td>
<td>AL-7</td>
<td>Dried leaf tissue available</td>
<td>RDL 018</td>
</tr>
<tr>
<td>Alabama</td>
<td>Mobile Co.</td>
<td></td>
<td>88.124076W 30.540986N</td>
<td>13 August 2008</td>
<td>AL-8</td>
<td>Dried leaf tissue available</td>
<td>RDL 019</td>
</tr>
<tr>
<td>Alabama</td>
<td>Mobile Co.</td>
<td></td>
<td>88.196509W 30.861242N</td>
<td>13 August 2008</td>
<td>AL-9</td>
<td>Dried leaf tissue available</td>
<td>RDL 020</td>
</tr>
<tr>
<td>Alabama</td>
<td>Lee Co.</td>
<td></td>
<td>85.50594W 32.56314N</td>
<td>13 August 2008</td>
<td>AL-10</td>
<td>Dried leaf tissue available</td>
<td>RDL 021</td>
</tr>
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</table>
### Table A.3  Florida Collections.

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Location Description</th>
<th>GPS Coordinates</th>
<th>Date Collected</th>
<th>Population Identifier</th>
<th>Herbarium</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Florida.</td>
<td>Alachua Co.:</td>
<td>Right-of-way of SB IH-75, exti #390, behind Harley-Davidson dealership in Gainesville, flowering at time of collection</td>
<td>82.450817W 29.692517N</td>
<td>28 June 2009</td>
<td>FL-1</td>
<td>(MISSA)</td>
<td>RDL 029</td>
</tr>
<tr>
<td>Florida.</td>
<td>Osceola Co.:</td>
<td>Heavily managed pineland within Disney Wilderness Preserve, heavy chemical management of persistent patch,</td>
<td>81.419159W 28.094404N</td>
<td>29 June 2009</td>
<td>FL-3</td>
<td>Dried leaf tissue available</td>
<td>RDL 031</td>
</tr>
<tr>
<td>Florida.</td>
<td>Osceola Co.:</td>
<td>Previously logged area of mixed pineland and pasture in the Disney Wilderness Preserve, grazing pressure and co-occurs with <em>Paspalum notatum</em>,</td>
<td>81.385234W 28.043943N</td>
<td>29 June 2009</td>
<td>FL-4</td>
<td>Dried leaf tissue available</td>
<td>RDL 032</td>
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<tr>
<td>Florida.</td>
<td>Osceola Co.:</td>
<td>Disturbed and burned airstrip in the Disney Wilderness Preserve with chemical treatment history,</td>
<td>81.404559W 28.05555N</td>
<td>29 June 2009</td>
<td>FL-5</td>
<td>(MISSA)</td>
<td>RDL 033</td>
</tr>
<tr>
<td>Florida.</td>
<td>Sarasota Co.:</td>
<td>Powerline right-of-way across Cranberry Blvd. and SB IH-75 in Northport</td>
<td>82.159612W 27.093905N</td>
<td>01 July 2009</td>
<td>FL-6</td>
<td>Dried leaf tissue available</td>
<td>RDL 034</td>
</tr>
<tr>
<td>Florida.</td>
<td>Miami-Dade Co.:</td>
<td>Pine rockland in Thompson Park (MDPR) Former marl prairie edged by pine rockland habitat within Martinez</td>
<td>80.395359W 25.598365N</td>
<td>01 July 2009</td>
<td>FL-7</td>
<td>Dried leaf tissue available</td>
<td>RDL 035</td>
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<tr>
<td>Florida.</td>
<td>Miami-Dade Co.:</td>
<td>Pine rockland in Martinez Pineland (MDPR), very little soil present at time of collection,</td>
<td>80.406411W 25.606809N</td>
<td>01 July 2009</td>
<td>FL-8</td>
<td>Dried leaf tissue available</td>
<td>RDL 036</td>
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<tr>
<td>Florida.</td>
<td>Miami-Dade Co.:</td>
<td>Pine rockland in Martinez Pineland (MDPR), very little soil present at time of collection,</td>
<td>80.409219W 25.606874N</td>
<td>01 July 2009</td>
<td>FL-9</td>
<td>Dried leaf tissue available</td>
<td>RDL 037</td>
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<tr>
<td>State</td>
<td>County</td>
<td>Location Description</td>
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<tr>
<td>Florida</td>
<td>Miami-Dade Co.:</td>
<td>Pine rockland in Pine Shore Park (MDPR), very little soil present at time of collection, Former pine rockland habitat logged through World War II, with complete canopy loss due to Hurricane Andrew and insect invasion in Ingram Pineland</td>
<td>80.374831W 25.650915N</td>
<td>01 July 2009</td>
<td>FL-10</td>
<td>Dried leaf tissue available</td>
<td>RDL 038</td>
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<tr>
<td>Florida</td>
<td>Miami-Dade Co.:</td>
<td>Maintained though disturbed walking path/trail within Seminole Wayside Park (MDPR),</td>
<td>80.461464W 25.497793N</td>
<td>02 July 2009</td>
<td>FL-11</td>
<td>(MISSA)</td>
<td>RDL 039</td>
</tr>
<tr>
<td>Florida</td>
<td>Miami-Dade Co.:</td>
<td>Construction zone on right-of-way of NB IH-95 near Atlantic Coastal City</td>
<td>80.549143W 27.711872N</td>
<td>04 July 2009</td>
<td>FL-13</td>
<td>Dried leaf tissue available</td>
<td>RDL 041</td>
</tr>
<tr>
<td>Florida</td>
<td>Duval Co.:</td>
<td>Right-of-way between several hotels and NB IH-95 in south Jacksonville, interstate and new construction present, old blooms present at time of collections,</td>
<td>81.588164W 30.246262N</td>
<td>04 July 2009</td>
<td>FL-14</td>
<td>Dried leaf tissue available</td>
<td>RDL 042</td>
</tr>
<tr>
<td>State</td>
<td>County</td>
<td>Location Description</td>
<td>GPS Coordinates</td>
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<tr>
<td>Louisiana.</td>
<td>St. Tammany</td>
<td>NB Hwy-59 right-of-way in Abita Springs, leaves appeared red at time of collection,</td>
<td>90.062855W 30.482416N</td>
<td>15 December</td>
<td>LA-1</td>
<td>MISSA</td>
<td>RDL 022</td>
</tr>
<tr>
<td></td>
<td>Pa.:</td>
<td>Large patch occurring on SB Hwy-21 right-of-way and two homesites along hwy. in Angie,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Benscreek WMA, right-of-way on LA Hwy 10, leaves appeared red at time of collection,</td>
<td>90.577213W 30.96874N</td>
<td>15 December</td>
<td>LA-2</td>
<td>MISSA</td>
<td>RDL 023</td>
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<tr>
<td></td>
<td></td>
<td>Chalky-clay outcrop with pines on EB Frontage Rd. right-of-way of IH-12 in Slidell,</td>
<td>90.687332W 30.834658N</td>
<td>15 December</td>
<td>LA-3</td>
<td>Dried leaf tissue available</td>
<td>RDL 024</td>
</tr>
<tr>
<td></td>
<td></td>
<td>leaves appeared red at time of collection,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Louisiana.</td>
<td>St. Tammany</td>
<td>Buffer zone between Oak Harbour residential subdivision and IH-10</td>
<td>90.583854W 30.234104N</td>
<td>16 December</td>
<td>LA-4</td>
<td>MISSA</td>
<td>RDL 025</td>
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<tr>
<td></td>
<td>Pa.:</td>
<td>Open field behind newly constructed office/medical offices in Oak</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Harbour residential sub-division</td>
<td>90.582946W 30.235558N</td>
<td>16 December</td>
<td>LA-5</td>
<td>MISSA</td>
<td>RDL 026</td>
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<tr>
<td>Texas.</td>
<td>Tyler Co.:</td>
<td>Pine Plantation about 1 mi N of Spurger off TX-FM 92</td>
<td>94.173405W 30.711444N</td>
<td>17 January</td>
<td>LA-6</td>
<td>MISSA</td>
<td>RDL 027</td>
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Table A.5  Georgia Collections.

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<tr>
<th>State</th>
<th>County</th>
<th>Location Description</th>
<th>GPS Coordinates</th>
<th>Date Collected</th>
<th>Population Identifier</th>
<th>Herbarium</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia</td>
<td>Crawford</td>
<td>Ca. 3.5 air mi. ENE center of Mosella; N of E Hopewell Road</td>
<td>83.97511W</td>
<td>26 May 2010</td>
<td>GA-1</td>
<td>(SWSL at MMNS)</td>
<td>Mark Raines</td>
</tr>
<tr>
<td>Georgia</td>
<td>Crawford</td>
<td>Ca. 3.5 air mi. ENE center of Mosella; N of E Hopewell Road</td>
<td>83.97511W</td>
<td>26 May 2010</td>
<td>GA-2</td>
<td>(SWSL at MMNS)</td>
<td>Mark Raines</td>
</tr>
<tr>
<td>Georgia</td>
<td>Worth</td>
<td>Ca. 16 air mi. NNW center of Sylvester, Warrior Creek Plantation</td>
<td>83.74837W</td>
<td>25 May 2010</td>
<td>GA-3</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Thomas</td>
<td>Ca. 7 air mi. NE center of Thomasville, Aucilla Plantation</td>
<td>83.86062W</td>
<td>25 May 2010</td>
<td>GA-4</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Baker</td>
<td>Ca. 7 air mi. SSW Leary, Island Pond Plantation</td>
<td>84.538024W</td>
<td>02 June 2010</td>
<td>GA-5</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Decatur</td>
<td>Ca. 4 air mi. S of Bainbridge, Aragon Farms</td>
<td>84.538024W</td>
<td>02 June 2010</td>
<td>GA-6</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Decatur</td>
<td>Ca. 2 air mi. NW Attapulgus, Sharber tract</td>
<td>84.524656W</td>
<td>02 June 2010</td>
<td>GA-7</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Decatur</td>
<td>Ca. 7 air mi. NE of Climax, Knight tract</td>
<td>84.381393W</td>
<td>02 June 2010</td>
<td>GA-8</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Decatur</td>
<td>Ca. 17 air mi. SW Bainbridge, Rozier tract</td>
<td>84.818857W</td>
<td>02 June 2010</td>
<td>GA-9</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Decatur</td>
<td>Ca. 10 air mi. SW Bainbridge, Silver Lake #6</td>
<td>84.71221W</td>
<td>02 June 2010</td>
<td>GA-10</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Decatur</td>
<td>Ca. 4 air mi. NW Whigham, Mobley tract</td>
<td>84.367878W</td>
<td>02 June 2010</td>
<td>GA-11</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Mitchell</td>
<td>Ca. 6 air mi. ENE of Camilla, Prichett tract</td>
<td>84.109888W</td>
<td>07 June 2010</td>
<td>GA-12</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
<tr>
<td>Georgia</td>
<td>Thomas</td>
<td>Ca. 7 air mi. NE center of Thomasville, Aucilla Plantation, this is the same area as GA-4</td>
<td>83.86062W</td>
<td>07 June 2010</td>
<td>GA-13</td>
<td>(SWSL at MMNS)</td>
<td>Mark McClure</td>
</tr>
</tbody>
</table>
Table A.6  South Carolina Collections.

<table>
<thead>
<tr>
<th>State</th>
<th>County</th>
<th>Location Description</th>
<th>GPS Coordinates</th>
<th>Date Collected</th>
<th>Population Identifier</th>
<th>Herbarium</th>
<th>Collector</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Carolina</td>
<td>Berkeley Co.:</td>
<td>Frances Marion NF, drainage ditch occurring from pines to the shoulder of south Hampton Rd., Frances Marion NF, drainage ditch occurring in longleaf pine savannah to shoulder of north Hampton Rd., N of Steed Rd.,</td>
<td>79.749397W 33.059822N</td>
<td>06 July 2009</td>
<td>SC-1</td>
<td>(MISSA)</td>
<td>RDL 043</td>
</tr>
<tr>
<td>South Carolina</td>
<td>Berkeley Co.:</td>
<td>Frances Marion NF, Schulknighter Rd., evidence of burn history, leaves appear red at tips at time of collection,</td>
<td>79.759493W 33.078458N</td>
<td>06 July 2009</td>
<td>SC-2</td>
<td>(MISSA)</td>
<td>RDL 044</td>
</tr>
<tr>
<td>South Carolina</td>
<td>Berkeley Co.:</td>
<td>Frances Marion NF, mixed forest area off FR 151</td>
<td>79.859367W 33.338664N</td>
<td>06 July 2009</td>
<td>SC-3</td>
<td>(MISSA)</td>
<td>RDL 045</td>
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<tr>
<td>South Carolina</td>
<td>Union Co.:</td>
<td>Sumter NF, right-of-way into mixed forest off of Santuc Rd., about 3 mi. W of Berry Farm Rd.,</td>
<td>81.512391W 34.582529N</td>
<td>07 July 2009</td>
<td>SC-5</td>
<td>(MISSA)</td>
<td>RDL 047</td>
</tr>
<tr>
<td>South Carolina</td>
<td>Saluda Co.:</td>
<td>Sumter NF, border of private land and a game management area on Ephriam Rd.</td>
<td>81.948178W 33.996761N</td>
<td>07 July 2009</td>
<td>SC-6</td>
<td>(MISSA)</td>
<td>RDL 048</td>
</tr>
<tr>
<td>South Carolina</td>
<td>Greenwood Co.:</td>
<td>Sumter NF, disturbed right-of-way on WB SC Hwy 178, leaves appear reddish at time of collection,</td>
<td>81.997397W 34.051149N</td>
<td>07 July 2009</td>
<td>SC-7</td>
<td>(MISSA)</td>
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