The performance of the baldcypress leafroller (Archips goyerana Kruse, Lepidoptera: Torticidae) in response to fertilization, thinning, and genetic variation in host baldcypress (Taxodium distichum L. Richard)

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THE PERFORMANCE OF THE BALDCYPRESS LEAFROLLER (*ARCHIPS GOYERANA* KRUSE, LEPIDOPTERA:TORTRICIDAE) IN RESPONSE TO FERTILIZATION, THINNING, AND GENETIC VARIATION IN HOST BALDCYPRESS (*TAXODIUM DISTICHUM* L. RICHARD)

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

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Crawford Wood Johnson
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ABSTRACT

Mississippi River diversions into coastal Louisiana wetlands aim to provide nutrient inputs and slow the impacts resulting from saltwater intrusion. Baldcypress (Taxodium distichum L. Richard) exhibits significant intraspecific tolerance to salinity and is being developed for restoration projects. Two studies were conducted using five half-sibling families of baldcypress planted at two locations in southeast Louisiana to investigate the effects of nutrient additions, thinning, and phenology on the growth of the baldcypress leafroller (Archips goyerana Kruse, BCLR). In the ‘Jeanfreau’ study, families were subjected to a control, low, and high level of fertilization simulating two-four years’ effects, respectively, from a Mississippi River diversion. In the ‘Delacroix’ study, an area impacted by a river diversion, these same families were subjected to control and thinned treatments.

BCLR larval bioassays were conducted in the laboratory to ascertain relative growth rate (RGR), development time, and pupal weights, a surrogate for potential fecundity. Tree growth and foliage nutrients, phenolics (Jeanfreau only), moisture, length and width were measured.

Fertilization did not consistently influence tree growth. Dry pupal weights and relative growth rates in most families were greater, and development times shorter, each year in the low fertilization treatment. Phenology differences among families were consistent across fertilization treatments and significantly affected BCLR growth. Pupal weights were lower on early-leafing families due to the decreasing suitability of the foliage at time of larval emergence. Thinning did not have a clear effect on BCLR development during the time of study. Phenological effects on larval growth and foliar nutrient samples were experimentally removed by allowing the foliage among families to reach comparable stages of growth in 2003. Females reared on family cb3 in the thinned treatment exhibited significantly heavier
pupae than the control, implying a potentially greater fecundity, but foliage analyses revealed total nutrients and moisture content were less concentrated in the thinned treatment. There were no other significant differences in larval performance.

Overall, this evidence suggests a more nutritious food resulting from limited fertilization inputs may lead to a BCLR population increase. Larval growth and performance may not be affected immediately by thinning. Phenological variation in budburst among families was found to be a significant factor affecting leafroller performance.
CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

History teaches us that North America’s swamps and marshes were seldom given much of a chance. Most early Americans associated these wetlands with mosquitoes and disease. Then, agriculture was the primary means of generating income; so many marshes and forested swamps were cleared and drained. Only now, in south Louisiana, are we realizing the value of wetlands and the multitude of benefits they provide.

Louisiana is home to 30% of the nation’s coastal marsh. It is estimated that there are 650,000 ha (1.6 million acres) of cypress-tupelo swamps (Taxodium distichum L. Richard and Nyssa aquatica L.) in the U.S., and 25% of this forest type occurs in coastal Louisiana (Allen, 1998). Together these ecotypes are responsible for providing both a monetary and an invaluable ecological wealth of resources to both the state of Louisiana and the nation. Nearly half of the residents of Louisiana live in the parishes delineated as the “coastal zone” for state and federal restoration projects (www.savelawetlands.org, 2003). Coastal wetlands provide jobs for many residents and provide income directly or indirectly to many local governments through the oil and natural gas industry, waterborne commerce, commercial fishing, recreational fishing and hunting, fur harvests from trapping, alligator harvests, and eco-tourism (www.savelawetlands.org, 2003). The oil and natural gas industry mines these resources from wells both onshore and offshore, and relies on coastal ports and waterways for access and support of offshore activities. The oil and natural gas industry provides 600 million dollars annually to the state budget through severance taxes, lease payments, and royalties.
(Coreil, 1997). Louisiana commercial ports annually account for 20% of the total shipping weight in the U.S. (www.savelawetlands.org, 2003). The annual “dock-side” value of commercial seafood landings totals near 300 million dollars and as of 1997 accounted for at least 30,000 jobs (Coreil, 1997). Recreational marine fishermen are estimated to create an annual economic effect totaling 944 million dollars (www.savelawetlands.org, 2003). The Louisiana Department of Wildlife and Fisheries recently reported that the coastal wetlands support a trapping industry which generates 1 million dollars annually (www.savelawetlands.org, 2003). The annual value of wild alligator eggs, hides, and meat are estimated between 25-30 million dollars annually (www.savelawetlands.org, 2003; Coreil, 1997). Tourists and residents who enjoy hiking, bird watching, photography, camping, and canoeing generate 220 million dollars annually (www.savelawetlands.org, 2003; Coreil, 1997). Though difficult to predict their value, the marshes and forested wetlands are known to decrease the destructive effects of hurricanes. It is estimated that for every mile of marsh, the storm surge associated with a hurricane is reduced by one vertical foot (Coreil, 1997). This could amount to billions saved in flood damages to metropolitan areas under severe circumstances.

The coastal marshes and forested wetlands are also vitally important from an ecological standpoint. Wetlands filter and absorb water pollutants and excess nutrients (Coreil, 1997). The forested wetlands and coastal marshes provide habitat for endangered and threatened species, including the bald eagle, brown pelican, and some black bear in southern forested wetlands (Coreil, 1997). It is estimated more than 93% of the bald eagle nests in Louisiana are found in baldcypress and water tupelo forests in southern Louisiana (Allen et al., 1998). It is estimated that 5 million migratory waterfowl overwinter each year in south Louisiana
Surveys of Louisiana’s coastal vegetation communities estimate 118 different species of plants (Chabreck, 1972). As of 1997, forested wetlands alone in south Louisiana were known to contain at least 17 orders of insects, comprised of 437 species within 157 families (Duerr, 1997). Freshwater, intermediate, brackish, and saline wetlands also serve as important nursery grounds for the juvenile stages of many freshwater and saltwater fish species, and habitat for a number of other aquatic invertebrates (Craig and Day, 1977).

In south Louisiana this ecosystem has been declared to be in a state of “system collapse” (Louisiana Dept. of Nat. Res., 1998). Much of the coastal marshes and forested wetlands in close proximity to the Gulf of Mexico are currently in a state of demise as a result of natural geological and climatic processes, fairly recent anthropogenic factors, and insect herbivory. The ultimate effect of these factors is the loss of land. It is estimated that Louisiana is losing 25-35 square miles annually, and even if current restoration efforts are included, Louisiana stands to lose roughly 500 square miles of land by the year 2050 (www.savelawetlands.org, 2003).

This loss of land is due largely to natural processes pre-dating any impact humans may have recently made. Much of coastal Louisiana was built by the massive deposits of the Mississippi River. The 1.25 million square mile Mississippi River drainage is estimated to carry an average sediment load of approximately 170 million tons per year (Harrison, 1951; Harmar and Priestnall). This massive annual sediment deposition at the terminus of the river has produced dynamic lobes of land projecting into the Gulf of Mexico. As vegetation takes root on newly-built delta, additional sediment is captured and the land area grows and advances into open water. The present active delta began forming approximately 700-1000 years ago,
but the river has changed its course several times, leaving remnants of once active deltas in the form of marshes and barrier islands (Louisiana Dept. Nat. Res., 1998; Conner and Brody, 1989).

When the river changes course upstream of the active delta, the flow of sediments immediately ceases and no new accretion of sediments can continue. Without additional sediment deposits, the land is lost to natural effects including subsidence, sea-level rise, and erosion from the Gulf of Mexico’s wave action and longshore currents (Louisiana Dept. Nat. Res., 1998). As freshwater inputs decrease, brackish water conditions become dominant, vegetation regimes change, and marine processes eventually erode the finer particles in the land masses and leave the heavier sand and shell, creating barrier islands and similar formations (Louisiana Dept. Nat. Res., 1998). The interior marshes are gradually lost to animal damage such as muskrat and nutria eat-outs, storm-event erosion, subsidence, and changes in global sea level. As barrier islands naturally subside and erode, the remaining interior marshes soon follow and seawater from the Gulf of Mexico advances landward (Louisiana Dept. Nat. Res., 1998).

Though all of the above mentioned forces are at work along the Louisiana coast and adjacent forested wetlands, subsidence and sea-level rise may be the most immediate and critical factors. The deltaic landmasses in south Louisiana are sinking at rapid rates due to the combined effects of natural subsidence and sea-level rise (Louisiana Dept. Nat. Res., 1998; Kuecher, 1995; Penland and Ramsey, 1990; and Delaune et al., 1987). Subsidence is due to the combined effects of geological movement along fault lines, the compaction of unconsolidated sediments, and to some degree the withdrawal of water from aquifers (Louisiana Dept. Nat. Res., 1998; Kuecher, 1995). Scientists estimate sea levels have been
rising for the last 20,000 years due to melting glaciers, expanding ocean waters as atmospheric air temperatures warm, tectonic motion of the earth’s crust and perhaps recently aided by the much-debated, greenhouse effect (Williams and Burkett, 2001). It is estimated that global sea level has risen 20 cm (8 in) in the last century (Douglas et al., 2001). The US Geological Survey projects a best estimate at a 50 cm (20 in) rise in global sea level by year 2100; however, this estimate could in specific areas be much higher due to more rapid rates of local subsidence (Hammar-Klose and Thieler, 2001). Land regions in the present Mississippi River delta near New Orleans are subsiding so quickly that relative sea level rise is averaging 10 mm each year (approximately 3 ft change by year 2100) (Louisiana Dept. Nat. Res., 1998; Hammar-Klose and Thieler, 2001).

The naturally occurring processes of sea-level rise and land subsidence have been exacerbated by human activities dating back as far as the mid 18th century (Moore, 1967; Mancil, 1972). The earliest French settlers erected levees to protect plantations and farmland from the annual Mississippi River flood waters (Moore, 1967). An extensive levee system now protects residents of Louisiana from potentially catastrophic floods, extending the length of the Mississippi as it winds its way through present coastal Louisiana, and ends at the head of the delta. This has effectively eliminated the greatest source of new sediment accretion in adjacent wetlands and marshes, leaving the effects of subsidence and sea-level rise largely unchecked (Louisiana Dept. Nat. Res., 1998). Most of the tremendous sediment load carried by the river is deposited beyond the continental shelf in deep Gulf waters (Louisiana Dept. Nat. Res., 1998). Though some sediment is deposited during storms as currents move and reshape landmasses, it is often not enough to offset the negative effects (Lou. Dept. Nat. Res., 1998).
The levee system not only blocks the flow of freshwater, which favors better wetland tree growth than stagnant water, and thus prevents new sediment accretion, but it also aids in saltwater intrusion, speeding marine processes in formerly freshwater-dominated, forested wetlands (Conner and Day, 1976; Lou. Dept. Nat. Res., 1998). Saltwater is lethal at relatively low levels to forested wetlands and the majority of understory vegetation. Heavier than freshwater, saltwater forms a “wedge” which migrates inland beneath the overlying layer of freshwater, in many cases penetrating deep into freshwater wetlands (Lou. Dept. Nat. Res., 1998; Coastal Environments, 1984; Goyer, 2002).

Saltwater has intruded inland through both natural and man-made waterways, depending on freshwater stream currents, tidal currents and wind direction. Channels for timber harvesting, the oil and gas industry, and commercial shipping vessels heavily lace Louisiana’s coastal marshes and forested wetlands. These channels have encouraged deeper and more prolonged flooding and proven efficient avenues for saltwater intrusion (Louisiana Dept. Nat. Res., 1998; Coastal Environments, 1984; Goyer, 2002; Allen et al., 1998).

The canals constructed for timber removal in the early 20th century were the earliest to alter hydrology patterns. The steam engine enabled cost-effective means of accessing and removing vast tracts of forested wetlands in coastal Louisiana (Mancil, 1972). Two methods of timber removal were primarily used that made a significant and lasting impact on the landscape (Mancil, 1972).

Access canals 30-50 feet wide and 6-8 feet deep were dug deep into interior baldcypress stands at systematic intervals. Spoil from the digging process was most often deposited on the canal edges, creating a damming effect. Barges equipped with a steam-driven winch system
dragged logs to the canal, creating deep ditches in the soft, waterlogged soils that can still be seen today in aerial photographs (Mancil, 1972). Experience dictated these pullboat crews start at the far reaches of canals and waterways and work backward, as the large amounts of sediment dragged into the channels impeded travel and had to be dredged at heavy expense (Mancil, 1972).

Railways also were systematically constructed through the swamps. Using the waste from sawmill operations and poles of less merchantable tree species, a stable pallet was laid down the length of the railway as conditions required, often 5-6 feet thick. These canals and railways significantly altered drainage patterns, resulting in higher and prolonged flooding, as well as allowing entrance through the canals for saline conditions.

Navigation channels for commercial shipping also have had tremendous effects on wetland ecology. The Mississippi River Gulf Outlet (“Mr. Go”) is perhaps the most often cited example of how destructive navigation channels can be. Intended for large, oceanic freight ships, it has severely affected wetlands in the far-east portion of coastal Louisiana. Preliminary stages of the channel opened in 1961, allowing commercial vessels the option to forego the longer passage down the river and travel a more direct path from the Gulf through the wetlands to port at New Orleans (Coastal Environments, 1984). The channel was completed in 1968, and has since been maintained at a depth of 36 feet and total width of 500 feet (Coastal Environments, 1984). For more than half of its length “Mr. Go” travels through the coastal wetlands of St. Bernard Parish. Salinity levels in the area prior to 1961 ranged from two to four parts per thousand (ppt) and supported some stands of forested wetlands, but soon after channel completion these areas ranged from 10-20 ppt (Coastal Environments,
Sea-water typically ranges from 20-30 ppt (Coastal Environments, 1984; Krauss et al., 1998).

Effects of salinity and increased flooding have far-ranging implications for future forest stand health and survival. Though existing, flood-tolerant tree species may persist in forested wetlands susceptible to the effects of saltwater intrusion and increased hydroperiod, these tree species require moist but unflooded soil conditions for seed germination and an extended period of time in unflooded conditions to reach heights affording survival of future flooding (Conner and Toliver, 1990). Baldcypress natural regeneration is generally considered to be poor in coastal forested wetlands of Louisiana due to higher water levels resulting from both anthropogenic alterations in landscape drainage patterns, relative sea level rise, saltwater intrusion, as well as mammalian herbivory from an exotic species, *Myocastor coypus* Kerr (nutria), and native species *Castor Canadensis* Kuhl (beaver) and the swamp rabbit *Sylvilagus aquaticus* (Bachman) (Souther, 2000; Conner, 1986; Conner and Toliver, 1990; United States Dept. Agriculture Forest Service, 1980).

The detrimental effects of these agents on forested wetlands in Louisiana are exacerbated by the effects of two insect herbivores. A defoliator of water tupelo, *Malacosoma disstria* Hubner (forest tent caterpillar) and a defoliator of baldcypress, *Archips goyerana* Kruse (baldcypress leafroller) in their larval stage have been known to annually defoliate hundreds of thousands of acres primarily in the coastal wetland zone (Goyer and Lenhard, 2002). Records indicate the forest tent caterpillar has periodically infested forested wetlands containing water tupelo in Louisiana at least as early as 1948 (Smith, 1983). The baldcypress leafroller, however, has only relatively recently emerged as a pest of baldcypress (Goyer and Lenhard, 1988). Early descriptions of the vast, primarily pure stands of virgin baldcypress
claim the species was mostly immune to serious insect and disease problems, and include no pest descriptions until the 1950s, after much of the virgin stands had been cut (Mancil, 1972; Brown and Montz, 1986; Conner and Day, 1976) The cypress looper, *Anacamptodes pergracilis* (Hulst), defoliated significant areas in Florida in the early 1980s, but has made little impact in Louisiana (Drooz et al., 1981). The bagworm *Thyridopteryx ephemaraeformis* (Haworth) defoliated an area of baldcypress covering approximately 6000 acres in the southern Atchafalaya Basin in 1994-1995 (Goyer et al., 2003), but it is not clear if this species will become a recurring pest. The most serious, consistent, economic insect pest reported to date is the baldcypress leafroller (*Archips goyerana* Kruse). The baldcypress leafroller was first reported in 1983 in the southern Atchafalaya Basin and the nearby watersheds east and south (Goyer, 2002). The larval stage of the leafroller feeds solely on baldcypress foliage and since first discovery, populations have spread eastward from the epicenter near Bayou Pigeon, Louisiana reaching the highest numbers primarily in the Atchafalaya River Basin south of Interstate 10, the nearby Lake Verret-Grassy Lake-Lake Palourde drainage system, and the Lake Maurepas-Pass Manchac-Lake Pontchartrain system (see figure 1.1) (Goyer, 2002).

It is unfortunate that much of Louisiana’s forested wetlands impacted by the natural and anthropogenic agents discussed above lies within the range of the baldcypress leafroller and forest tent caterpillar. Annual defoliation of both baldcypress and water tupelo have resulted in significant reductions in radial tree growth, as well as tree mortality to repeatedly defoliated baldcypress saplings (Goyer and Lenhard, 1988; Smith, 1983) The presence of multiple stress agents acting on these forests will only worsen growing conditions and speed the degeneration process in these fragile wetlands.
The degradation of Louisiana’s coastal wetlands has been recognized for decades, but serious, successful corrective action has not taken place until recently. Currently two public sources of funding are in place to combat these problems with restoration projects. The federal Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA) with a 25% state cost share provides 30 million dollars annually, and the state Louisiana Coastal Wetland Conservation, Restoration, and Management Act provides up to 25 million dollars annually from oil and natural gas severance tax, lease payment, and royalty revenues for coastal restoration projects (Coreil, 1997). Among the major methods of restoring coastal wetlands are Mississippi River water diversions into adjacent wetlands, as well as vegetative planting projects (Louisiana Dept. Nat. Res., 1998; Coreil, 1997). The Caernarvon diversion near New Orleans has proven very successful in building new land from sediment accretion, and slowing marine processes. Approximately 1500 acres of marshland have been positively affected since operation of the diversion began in 1990 (Goyer, 2002). The larger Davis Pond Miss. River diversion was opened upriver of New Orleans in 2002. Davis Pond is currently touted as the world’s largest diversion of its kind, and is expected to preserve 33,000 acres and benefit another 777,000 acres of marshes and bays in the Barataria Basin over the next 50 years (Hall et al., 2002). Studies have shown forested wetlands to be nutrient sinks, and diversions into adjacent wetlands would undoubtedly increase nitrate levels, as well as other nutrients (Louisiana Dept. Nat. Res., 1998; Lane et al., 2002). Analysis of the Lake Maurepas basin southeast of Baton Rouge, where a diversion is planned, indicates nitrogen levels are lower than that in the Mississippi River (Day Jr., 2002). A diversion would increase available nitrogen to these wetlands, and raises the likelihood of an insect herbivore response.
In addition to river diversions, restoration efforts also have included the use of nursery grown plants and trees to colonize newly restored areas and reestablish vegetation on degraded sites (Louisiana Dept. Nat. Res., 1998). Consequently, tree species occurring in coastal forested wetlands have been surveyed for their natural occurrence within freshwater swamp-brackish marsh interfaces and their response to increased flooding and salinity regimes. Native Quercus spp. (oaks) occur on the natural levees along streams which may be flooded for short durations, and research results support observations that these species are extremely sensitive to salinity. Seedlings did not survive past five months when flooded at two ppt salinity levels (Allen et al., 1998).

Baldcypress and water tupelo, tree species better adapted to extended periods of flooding, are relatively more resilient to low salinity levels (Allen et al., 1998). Baldcypress has been planted for experimental reasons and mitigation projects in areas scheduled to receive future inputs from diversions, and because of an apparent lack of natural baldcypress regeneration (Goyer, 2002; Conner and Toliver, 1990; Williams, 2003), nursery stock will likely be sought after in future planting projects. A high potential exists for saltwater intrusion and increased flooding stress at sites suitable for planting baldcypress. This has spurred research on the potential for identifying and testing salt-tolerant baldcypress genotypes for use in such situations (Krauss, 1999; Allen et al., 1996; Allen et al., 1997; Krauss et al., 2000; Krauss et al., 1998; Allen et al., 1994). Baldcypress in coastal Louisiana generally does not survive for long periods at salinities beyond 2-3 ppt, and most observations and research results predict mortality when seedlings are saturated for long periods of time at salinities beyond a 3 ppt threshold (Allen et al., 1998; Chabreck 1972; Wicker, 1981; Pezeshki et al., 1990). Remnant stands of more mature baldcypress, however, have been located within high salinity areas.
along the Gulf coast containing trees alive and producing viable seed at salinities as high as 6-8 ppt (Allen et al., 1998). Research has shown that offspring from brackish-water source trees are typically more tolerant to varying levels of salinity than offspring of freshwater source trees, though there is a degree of variation at high levels of salinity (Krauss, 1999, Allen et al., 1994, Allen et al., 1996; Allen et al., 1997).

The implications suggest that salt-tolerant baldcypress of several genetic lineages, thereby being more homogeneous than a natural stand, may be planted in plantation-like fashion on forested wetland restoration sites. These sites also are likely to receive nutrient inputs through river diversions. To achieve proper stocking densities and cover seedling mortality, higher numbers of seedlings may be planted initially, requiring an eventual stand density reduction as competition for resources increases with tree size. Tree response to these altered growing conditions and varying genotypes may have an impact on the baldcypress leafroller. The insect component should be addressed where possible as another threat to the health of these forests. The potential for tree defoliation, reducing tree growth or causing mortality, will put these restoration plantings under greater stress, as predicted salinities and/or prolonged flooding conditions arise. Thus, objectives of this study were to:

1. Determine the effects of fertilization on, and the importance of genotypic variation in *Taxodium distichum* L. Richard (baldcypress) and how the host-level response would affect the growth and development of an insect defoliator, *Archips goyerana* Kruse (Lepidoptera:Tortricidae), the baldcypress leafroller.
2. Determine the effects of thinning on, and the importance of genotypic variation in, baldcypress and how the host-level response also would affect the growth and development of an insect defoliator, the baldcypress leafroller.

The practical results of this study should shed light on the effects of host tree genetic variation, fertilization, and thinning on baldcypress leafroller populations and the potential implications for the health of baldcypress forests in restoration circumstances.

1.2 Literature Review

1.2.1 Taxonomy and Geographic Range

The baldcypress leafroller, *Archips goyerana* Kruse, is in the order Lepidoptera, superfamily Tortricoidea, family Tortricidae, and tribe Archipini (Kruse, 2000, Borror, 1989). It is confined to host tree *Taxodium distichum* L. Richard (baldcypress) for its entire life cycle. The baldcypress leafroller was originally identified as *Archips argyrospila* Walker, the fruittree leafroller, and that name was used in publications between 1988 and 2000 (Goyer, 2002). The fruittree leafroller occurs across much of the United States and southern Canada, and is known to feed on more than 24 host species (Weires and Reidl, 1991). A comparison of the baldcypress race of fruittree leafroller with another geographically separated population found sufficient differences to warrant classification of the baldcypress race as a separate species (Goyer et al., 1995). The “baldcypress” population exhibited significant genetic differences, a large degree of host specificity in feeding bioassays, extremely low male response to the non-baldcypress race pheromones, and subtle morphological differences from
Archips argyrospila Walker, and was subsequently described under the new species name Archips goyerana (Kruse, 2000).

The baldcypress leafroller is known to occur in south Louisiana and Mississippi, but has been most damaging in the extensive stands of baldcypress in south Louisiana (Kruse, 2000). The baldcypress leafroller was first reported in 1983 in the lower Atchafalaya Basin near Big Bayou Pigeon (Goyer and Lenhard, personal communication). Significant defoliation began in portions of Assumption, Iberia, Iberville, and St. Martin parishes, but the BCLR has since defoliated baldcypress stands as far north as Baton Rouge and eastward as far as New Orleans (Meeker, 1992). The baldcypress leafroller has affected as many as 242,000 ha (600,000 ac) in a single year (Goyer et al., 1990). Presently the Atchafalaya Basin (between Interstate 10 south to near hwy 90), the Lake Verret-Lake Palourde system, the upper Barataria Basin, and the upper Pontchartrain system are annually defoliated (R. Goyer, personal communication, 2003; See figure 1.1.)

1.2.2 Life History

The baldcypress leafroller is univoltine. Larvae hatch from egg masses in late February-early March concurrent with branchlet expansion. Egg masses contain approximately 54 individuals and emergence within and among egg masses may vary by as much as 10-16 days (Goyer and Chambers, 1997). Research results from studies on the closely related fruittree leafroller (Archips argyrospila Walker) suggest eggs hatch after completing an inherent developmental requirement and then eclose in response to environmental conditions, most importantly temperature (Judd et al., 1993). Emergence of larvae in a field setting during one year of the Judd et al. study (1993) spanned five weeks. Field collections of the baldcypress leafroller often find up to three instars feeding within the same tree (Braun, et al. 1990).
Figure 1.1. Range of host *Taxodium distichum* L. Rich. (baldcypress) and areas most heavily forested in baldcypress and annually defoliated by *Archips goyerana* Kruse (baldcypress leafroller). The map to the lower right also features the location of the two research plots used in the current study.
Upon hatching, first instar larvae move to expanding branchlet tips, where they spin silk to form a tight, protective shelter and feed from within these shelters. Later instars may web together two or more branchlets in a similar fashion (Meeker, 1992). Larvae also use silk to aid in dispersal and escape, generally dispersing with gravity and prevailing wind direction within and among trees (Goyer and Chambers, 1997). Larvae feed through five instars before pupating. First instars are a pale, cream color and not more than 2 mm long while fifth instars are characterized by black headcapsules during the instar, and are a bright, apple green color very similar to developing baldcypress foliage, reaching up to 2 cm in length (Goyer and Chambers, 1997). Larvae typically require 8-10 weeks to mature under natural field conditions. Larvae generally pupate near branch tips, and require 8-12 days to develop into adults (Goyer and Chambers, 1997). Pupae are characterized by a light-green coloration dorsally, often with black latitudinal stripes separating the abdominal segments, and developing wings on the ventral surface are a darker green overlaying the light-green colored ventral surface of the abdomen. The adult moths emerge in late April through early May and typically live for 14 days (Goyer and Chambers, 1997). Meeker (1992) reports adult moths range from 14 to 23 mm wide wing tip to wing tip. Forewings are mottled in a pale red-brown to dark brown with two white patches on each wing, while hind wings are colored light brown (Meeker, 1992).

1.2.3 Predators, Parasitoids, Pathogens of the Baldcypress Leafroller

A number of vertebrate predators, insect predators, insect parasitoids, and pathogens are known to affect population levels of the baldcypress leafroller. Since the early infestation years, two Coleopteran species in the family Carabidae as well as two Hymenopteran species of pupal parasitoids were recognized as potentially significant predators and parasitoids,
respectively (Goyer and Lenhard, 1988). Research results indicate the two arboreal carabid species, *Plochionus timidus* Haldeman and *Calleida viridipennis* (Say) were the most important insect predators (Braun et al., 1990). Both species are primarily spring breeders and adults commonly overwinter under the bark of baldcypress and black willow (*Salix nigra* Marshall) (Zhou et al., 1993). The immature stages of both species appear to depend significantly on the larval and pupal stage of the baldcypress leafroller in the spring, but are generalists and are also associated with the fall webworm *Hyphantria cunea* (Drury) where available, and others as the growing season progresses (Zhou et al., 1993). Besides requiring alternate hosts for the remainder of the year, evidence suggests that these predators require baldcypress trees at least 30 cm in diameter or black willow trees at least 15 cm in diameter at 40 cm above the water level, as these trees are presumed to provide adequate bark scale flaking for overwintering adults (Zhou, et.al., 1993). A collection of pupae from no less than three locations in 1985, the first year that significant predation by these species was observed, and again in 1988 revealed a range in predation rates by theses two species, from a low of 4% to a high of 32% between sites, and average predation rates of 18% and 15% respectively for the two years of study (Braun et al., 1990).

Research results have repeatedly indicated the baldcypress leafroller pupae to be the life stage most vulnerable to parasitism. Parasitism rates from all parasitoids recovered from each immature life cycle stage were consistently highest on pupae (Braun et al. 1990). The two parasitoids that have consistently emerged in the highest abundance from leafroller pupae collected across much of the leafroller’s range, spanning a time period from 1985 to 1995, were *Itoplectis conquisitor* (Say) (Hymenoptera: Ichneumonidae) and in much fewer numbers, *Brachymeria ovata* (Say) (Hymenoptera: Chalcidae) (Braun et al., 1990; Wei
Itoplectis conquisitor (Say) emerged most frequently in the Braun et al. (1990) study with a mean parasitism rate of 19%, followed by Brachymeria at 4% across all sites and the four years of study. Itoplectis again exhibited the highest parasitism rate in Wei’s (1996) two years of data, parasitizing 9% and 12% at each of two study sites and again followed by Brachymeria, which emerged from 1% and 3% of the total larvae collected from each site. A third pupal parasitoid, Cirrospilus sp. nr flavicinctus (Hymenoptera: Eulophidae) was reported in each study, and Hyphantrophaga sellersi (Sabrosky) (Diptera: Tachinidae) reported in Wei (1996), but both of these were present at very low levels and likely had minimal effects on populations during those periods (Braun et al., 1990, Wei 1996). Two species of hyperparasitoids, Cyclogastrella n. sp. (Hymenoptera: Pteromalidae) and Dimmockia incongrua (Ashmead) (Hymenoptera: Eulophidae) were found associated with Itoplectis in earlier work, however Wei (1996) recovered no individuals of Cyclogastrella. The effects of hyperparasitoids on the leafrollers and parasitoid-leafroller complex appeared to be minimal during the periods of study (Braun et al., 1990, Wei, 1996).

Larval parasitism has been relatively low throughout these studies and one reason may be the low level of diversity of parasitoids known to attack the larval stages of the leafroller. Apanteles polychrosidis (Viereck) (Hymenoptera: Braconidae) was recovered from the larval stage of the leafroller in earlier work, but the data suggested it had little impact as it was recovered sporadically and in low levels (Braun et al., 1990). The later survey of 1-3 instars from two field sites by (Wei, 1996) indicated parasitism rates near 2% each year due to Apanteles. Wei also recovered Bassus sp. (Hymenoptera: Braconidae), which was not previously found in leafroller populations. The Bassus sp.
was also present in low abundance, and appeared to parasitize 3, 4, and 5 instars at rates of 0.7-2.2 % depending on sites. *Hyphantrophaga sellersi* (Sabrosky) was also recovered from 4-5 instar larvae, but were found in very low numbers or not at all each year at the respective sites (Wei, 1996).

Several hundred egg masses with approximately 54 individual eggs within each were collected from multiple field sites during a period spanning from 1985 to 1995, and no egg parasitism was detected. Other studies of tortricid pests report mortalities as high as 85% due to egg parasitism (Wei, 1996). Braun et al. (1990) also listed three known vertebrate predators: a reptile, *Anolis caroliniensis* Voigt, and two birds, *Parus caroliniensis* (Audubon) and *Protonotaria citrea* (Bodaert). Other general insect predators observed feeding on the larval and pupal stages of the leafroller include species in the families Reduviidae, Pentatomidae, Formicidae, Libellulidae, and two species in Vespidae (Braun et al., 1990). The nests of *Polistes* spp. wasps (Hymenoptera: Vespidae) were observed in more than 50% of the baldcypress in infested stands and the wasps observed removing larvae from the webs in the branchlet tips (Braun et al., 1990). Various species of spider likely play a role in predation as well (Goyer and Lenhard, 1988).

The lack of egg parasitism, paucity of larval parasitoids, and overall lack of diversity of total parasitoids is thought primarily to be a product of the characteristics of the wetland forest composition and habitat condition (Goyer et al., 1990). The periodic and permanently flooded conditions restrict plant diversity and so restrict the diversity of the fauna as well. The parasitoid most frequently recovered in these wetlands was *Itoplectis*, which is known to parasitize as many as 81 lepidopteran species (Mills and Carl, 1991; Wei, 1996). *Itoplectis* has been observed in low levels in *Malacosoma disstria* Hubner (forest tent caterpillar)
populations, which co-occur with baldcypress leafroller populations (Smith, 1983).

*Brachymeria ovata* (Say), the second most abundant parasitoid, also has a diverse array of hosts (Wei, 1996). But these generalist predator species and parasitoids must find more prey and new hosts after the leafroller larvae are no longer available. The univoltine life cycle and local environment of the baldcypress leafroller may continue to limit the role to some extent of predation and parasitism.

Tortricids can be affected by multiple species of viruses, bacteria, fungi, protozoa, and nematode parasites (Zimmermann and Weiser, 1985; Poinar, 1985). Significant mortality appeared in the baldcypress leafroller population in 1990. A nuclear polyhedrosis virus and granulosis virus also appeared in 1990 and played a role in population mortality (Goyer et al., 1991). Frequent rainfall during that spring was thought to have aided in the virus transmission. Defoliation was not as severe in those local areas in 1990 or 1991 (Goyer et al., 1992). These viruses were found to infect populations again in 1994-1995 throughout most of the leafroller’s range at that time (Wei, 1996). Average infection rates for both years ranged from 3-17% depending on sites. Sites with higher leafroller population densities had the highest infection rates, which was attributed to higher rates of transmission.

1.2.4 *Archips goyerana* Kruse and Host *Taxodium distichum* L. Richard

Interactions

Mated female leafrollers lay egg masses which will remain dormant until the following spring on baldcypress twigs approximately 0.7 cm (0.27 in) in diameter, often in the upper to middle portions of the tree canopy (Goyer, 2002). However, the effect of gravity on larval dispersal within the crown is thought during low leafroller population conditions to cause the lower portions of the tree crown to become more heavily infested (Goyer and Lenhard, 1988).
Repeated observations have suggested relatively young, pole-sized and sapling baldcypress (30-45 cm diameter, 30 cm above butt-swell) growing along the stand edges are heavily preferred over older, larger, interior trees or smaller, understory trees (Goyer and Lenhard, 1988). Trees sampled at approximately 20 m intervals interior to the forest edge contained significantly fewer egg masses (Goyer and Lenhard, 1988). However during outbreak conditions, entire trees and entire forest stands were completely defoliated regardless of larval position within the canopy or tree position within the stand (Goyer and Lenhard, 1988). Gravity and wind aid in larval dispersal, and in conditions supporting high populations, have put understory saplings, less able to recover from repeated defoliation, at a higher risk of mortality (Goyer and Chambers, 1997). This is especially so in stands subject to the effects of stagnant, permanently flooded conditions (Goyer and Chambers, 1997). Larger trees have experienced significant reductions in radial growth, crown dieback, and likely reduced seed production (Goyer and Lenhard, 1988; Goyer and Chambers, 1997). Baldcypress will refoliate when severely defoliated, but at significant costs to the nutrient reserves in the tree bole cambium roots (Goyer et al., 1990). Radial growth was reduced by nearly 80% during subsequent years of heavy (>60% of tree canopy) defoliation (Goyer and Lenhard, 1988).

Baldcypress is unique in that it is one of the few deciduous conifers. It does not retain growth of any previous years (through the winter) as many other conifers do. Another unique character of baldcypress is the ability of some individuals to express an appressed branchlet morph which has a decidedly negative effect on the baldcypress leafroller and consistently escapes severe defoliation (Brown and Montz, 1986; Goyer and Meeker, 1993). The typical, “open,” branchlet morph is composed of photosynthetic needles which grow along a central stem in a flat, planar fashion. The needles radiate outward from the central stem at a greater
angle than the “appressed” branchlet morph (Meeker, 1993). Needles of appressed branchlets are generally shorter than the needles of open branchlets and grow closely to the central stem in a “scale-like” manner, and thus the entire branchlet has the appearance of a single needle similar to needles possessed by members of the Pinaceae family (Brown and Montz, 1986, Meeker, 1993). Trees expressing the appressed branchlet morphology are usually older, larger, and are most often the dominate trees in the tree canopy, which are exposed to more direct sunlight (Meeker and Goyer, 1993; Brown and Montz, 1986). A survey of these morphologies found trees possessing a range of these two branchlet morphs, from exclusively open, mixed open-appressed, to exclusively appressed (Goyer and Meeker, 1993). Those trees containing a mix of the foliage morphology always had appressed foliage in the crown tops, grading into open foliage in the lower levels of the crown. Furthermore, these branchlet morphs were manifested early in branchlet expansion and so were present during leafroller development (Meeker, 1993).

Field observations consistently found trees expressing the open morph branchlets were more heavily defoliated each year, regardless of the site (Meeker and Goyer, 1993). Four years of egg mass sampling results found no significant difference in egg masses on trees with the respective branchlet morphologies, suggesting no foliage quality differences that may have been detected by ovipositing females. Choice tests between the foliage types suggested first instar larvae consistently preferred the open foliage type, and feeding bioassays revealed that when reared on the appressed foliage type, early instar larvae had a substantially lower survival rate at the p < 0.07 level (Meeker, 1993). Foliage samples taken during initial foliage expansion indicated appressed foliage had nutrient concentrations similar to the open foliage, but appressed foliage appeared more tough.
Bioassays with 4-5 instar larvae reared on appressed foliage revealed that both males and females had significantly lower consumption rates and total consumption, and the appressed foliage type appeared slightly less digestible based on approximate digestibility values. Males seemed less affected by the foliage differences with respect to relative growth rates and dry pupal weights, while females reared on the appressed foliage type were characterized by significantly lower dry pupal weights and relative growth rates (Meeker, 1993). Meeker concluded that while the nutrient concentrations of the appressed foliage type and open foliage type were similar at both early and later sampling points, suitability differences were especially important during the early instar period and were manifested and by the physical structure and tougher, more fibrous nature of the appressed foliage. The results suggested characteristics appeared to prevent early instar larvae from webbing together the foliage and feeding, as well as maintaining these properties through the leafroller development period, which lowered the ability of the later instars of both male and female larvae to consume the appressed foliage type (Meeker, 1993).

Another significant product of Meeker’s research indicated that baldcypress as a species responded to insect defoliation in a manner similar to other tree species (Benz, 1985; Blais, 1985). A comparison of baldcypress trees expressing the repeatedly defoliated, open foliage morph trees with uninfested, open foliage morph trees revealed those trees that consistently received heavy defoliation produced smaller branchlets which grew at a slower rate between the early and late sampling points than trees receiving less or no defoliation (Meeker, 1993). The smaller branchlet size and slower branchlet growth rates were indicative of a loss of nutrients available to allocate to leaf production.
CHAPTER 2

EFFECTS OF FERTILIZATION AND GENETIC VARIATION IN BUDBREAK PHENOLOGY ON THE GROWTH AND DEVELOPMENT OF AN INSECT DEFOLIATOR, ARCHIPS GOYERANA KRUSE (BCLR) ON HOST TAXODIUM DISTICHUM L. RICHARD (BALDCYPRESS)

2.1 Introduction

Much of coastal Louisiana is rapidly eroding, leading to increased forested wetland loss and decline. It is estimated coastal Louisiana is losing land at rates between 65-91 square km per year, and may lose approximately 1036 square km of swamp, including forested wetlands, by year 2050 if corrective action is not taken (Louisiana Department of Natural Resources, 2003). These losses are due to both natural and anthropogenic factors. The US Geological Survey projects a 50 cm (20 in) rise in global sea level by year 2100; however sea levels could rise as much as three feet by year 2100 due to more rapid rates of local subsidence (Hammar-Klose and Thieler, 2001; Louisiana Dept. Nat. Res., 1998).

Man has exacerbated these problems by building the levee system along the lower Mississippi River to control flooding, and by constructing navigation channels through forested wetlands and coastal marshes for timber removal and oil and gas exploration (Louisiana Dept. Natural Resources, 2003; Hammar-Klose, 2001; Moore, 1967; Coastal Environments, 1984). The levee system prevents new sediment deposition which would offset land subsidence, nutrient additions, and the flushing effects the river floods once provided to adjacent wetlands (Louisiana Dept. Natural Resources, 2003; Lane et al. 2002; Conner and Day, 1976). Also, man-made impoundments have been created by levees and canal spoil
banks, wherein stagnant water persists, reducing or eliminating natural tree regeneration (Conner and Day, 1976). Man-made canals and ship channels allow saltwater to intrude into once protected interior wetlands, causing an inward movement of the brackish water-freshwater interface and increasing salinity levels above the physiological threshold for freshwater wetland vegetation (Coastal Environments, 1984; Louisiana Dept. Nat. Res., 1998). A combination of these factors is likely to result in the decline of an increasing percentage of the gulf states’ coastal forested wetlands over the next century.

The potential losses could mean severe consequences for the state of Louisiana and the nation. The oil and natural gas industry depends on resources from wells both inshore and offshore of Louisiana and other gulf states, and relies on coastal ports and waterways for access and support of offshore activities. The combined annual value associated with the economy in the coastal zone of Louisiana is well into the billions (Lou. Dept. Nat. Res., 2003; Coreil, 1997), and is affected by the oil and natural gas industry, waterborne commerce, commercial fishing, recreational fishing and hunting, fur harvests from trapping, alligator harvests, and eco-tourism. These wetlands are also important ecologically by providing water quality treatment functions, slowing and reducing the strength of hurricanes, and providing habitat for unique organisms and endangered wildlife (Allen et al., 1998; Smith, 1983; Chabreck, 1972; Coreil, 1997; Duerr, 1997; Craig and Day Jr., 1977).

The detrimental effects of saltwater intrusion on forested wetlands in Louisiana are exacerbated by the effects of an insect herbivore, *Archips goyerana* Kruse, the baldcypress leafroller (BCLR). The larval stage of the BCLR (Lepidoptera: Tortricidae) annually defoliates hundreds of thousands of acres, some of which occur in coastal forests subject to the effects described above (Goyer and Lenhard, 2002). The BCLR erupted as a pest of
baldcypress in 1983, and is currently expanding its range and has been recovered as far north as Jackson, Mississippi (Goyer and Lenhard 1988, Kruse, 2000). Annual defoliation of baldcypress has resulted in significant reductions in radial tree growth, as well as tree mortality to repeatedly defoliated baldcypress saplings (Goyer and Lenhard, 1988; Smith, 1983). The presence of multiple stress agents acting on these forests will only worsen growing conditions and speed the degeneration process in these fragile forested wetlands.

As these stands decline, federally and state sponsored programs have been implemented and more are proposed to prevent and delay the potential losses (Lou. Dept. Nat. Res., 1998). Currently two public sources of funding are in place to combat these problems with restoration projects. The federal Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA) with a 25% state cost share provides 30 million dollars annually, and the state Louisiana Coastal Wetland Conservation, Restoration, and Management Act provides up to 25 million dollars annually from oil and natural gas severance tax, lease payment, and royalty revenues for coastal restoration projects (Coreil, 1997). Among the methods currently proposed to restore forested wetlands are the use of Mississippi River diversions and vegetative planting projects (Lou. Dept. Nat. Res., 1998; Coreil, 1997).

River diversions for the purpose of coastal restoration and protection have been emplaced at various points along the course of the Mississippi River between Baton Rouge and the river delta and more are planned (La. Dept. Nat. Res., 1998) These diversions mimic the effects of the annual spring floods the river once provided prior to the construction of the levee system. “Caernarvon” is one such diversion that was opened in 1990 and since has become a showcase for the potential of river diversions to improve and protect wetlands (La. Dept. Nat. Res., 1998). It is estimated that 607 ha of marshland have been built since 1990, and 6, 475 ha
are expected to be preserved over the next 40 years (La. Dept. Nat. Res., 2003; US Army COE, 2003; Goyer 2002). The larger Davis Pond Miss. River diversion was opened upriver of New Orleans in 2002, and is currently touted as the world’s largest diversion of its kind. Davis Pond is expected to preserve 13,355 ha and benefit another 314,442 ha of forested swamps, marshes and bays in the Barataria Basin over the next 50 years (Hall et al., 2002).

The Lake Maurepas Basin located southeast of Baton Rouge contains extensive stands of baldcypress, is within the range of the baldcypress leafroller, and is one of many sites for a planned river diversion (Louisiana Dept. Nat. Res., 1998). Baseline analyses indicate nutrient levels in the water column (nitrogen particularly) are lower than that in the Mississippi River (Day et al., 2002). Forested wetlands are known to function as nutrient sinks, and diversions into adjacent wetlands would increase nutrient levels available for soil adsorption and subsequent plant uptake (Louisiana Dept. Nat. Res., 1998; Lane et al., 2002; Ewel and Odum, 1979; Richardson and Nichols, 1985; Coreil, 1997). A wide range of literature has examined herbivore responses to increased nutrients available to host plants. Nutrients present in high levels in the Mississippi River water column, most notably nitrogen, are required by both plants and insect herbivores, and if limited, can result in lowered fitness for producers and consumers (White, 1984; Mattson, 1980; Mattson and Scriber 1987). Nutrient additions to wetland systems have the potential to increase growth of baldcypress, thus potentially increasing foliage suitability to the baldcypress leafroller as host growth processes are favored over differentiation (Herms and Mattson 1992; Bryant et al., 1983; Lorio, 1986).

Restoration objectives also include the use of vegetative planting projects. Projects incorporating vegetative plantings completed to date are composed primarily of marshgrass plantings (La. Dept. Nat. Res., 1998). However demand is growing for tree plantings in
forested wetland areas impacted by encroaching salinity and increased flooding. Baldcypress regeneration is generally considered to be lacking in coastal forested wetlands of Louisiana due to the hydrological conditions described above and mammalian herbivory (Souther and Shaffer, 2000; Conner, 1986; Conner and Toliver, 1990; Williston et al., 1980). Such conditions have spurred research on the potential for using more salt-tolerant vegetation. Baldcypress (*Taxodium distichum* L. Richard), has exhibited significant intraspecific variation in its ability to grow under saline conditions (Krauss et al., 1999; Krauss et al., 1998; Krauss et al., 2000; Allen et al., 1998; Allen et al., 1997; Allen et al., 1996). Intraspecific genetic variation in host plants also has been shown to have substantial effects on associated herbivores (Allen 1994, Lindroth and Hwang 1996; Herms 1999; Osier and Lindroth 2001, Lindroth et al. 2002; Mutikainen et al. 2000). One such expression of genetic variation in baldcypress is the occurrence of trees within the population which produce an appressed foliage morph, characterized by needles which lay tightly pressed against the central rachis (Brown and Montz, 1986). Trees expressing this foliage morph receive virtually no defoliation damage, whereas trees expressing the “open” morph, on which the needles spread outward from the rachis in a planar fashion, are annually defoliated by the baldcypress leafroller (Meeker and Goyer, 1993). Phenological differences in budbreak among hosts also is an important factor affecting the quality of foliage available to herbivores. Studies have shown with other spring feeding lepidopteran species that asynchrony between the host foliage and insect emergence have significant bearing on survival and growth (Hough and Pimentel, 1978; Feeny, 1970; Parry et al., 1998; Watt and McFarlane, 1991). The ten half-sibling families of baldcypress used in this study (cultured by Krauss, 1996) exhibit considerable variation in budbreak phenology. These families of baldcypress saplings were
screened for tolerance to salinity and planted in 1996 in a predominantly freshwater environment located east-southeast of New Orleans, LA as part of a larger study evaluating the growth of these families in more saline environments nearby. Currently, efforts are underway to produce offspring from these salt-tolerant baldcypress in state owned nurseries, which can then be used in a coastal restoration context (R. Goyer, personal communication). The performance of the baldcypress leafroller in response to intraspecific variation among these half-sibling families under increased nutrient conditions has not been investigated.

Earlier results suggest that salt-tolerant baldcypress of distinct genetic lineage, possibly being more homogeneous than a natural stand, may be planted in plantation-like fashion on formerly forested wetland restoration sites scheduled to receive increased nutrient loads from a river diversion. Intraspecific characteristics in salt-tolerant families (Allen et al., 1994; Allen et al., 1996; Allen et al., 1997; Krauss et al., 2000) and increased nutrient uptake may predispose some trees to an increase in herbivore pressure. The potential for increased herbivore populations may result in increased tree defoliation, reduced tree growth, and/or tree mortality, and may subject these restoration plantings to even greater stress as predicted salinities and/or prolonged flooding conditions arise. This herbivore component should be addressed where possible as another threat to the health of these future forests. Thus, objectives of this study were to:

1. Determine the effects of fertilization on baldcypress and how the host-level response in foliar quality also would affect the growth and development the BCLR.

2. To evaluate the performance of the BCLR in response to genetic variation in five half-sibling families of baldcypress subjected to varying nutrient regimes.
2.2 Materials and Methods

2.2.1 Site Characteristics, Stand History, and Current Study Design and Methods

2.2.1.1 Site History and Purpose

The ‘Jeanfreau’ site used in this study, established by Krauss et al. 2000, is located near New Orleans, Louisiana on the north side of Highway 46 in St. Bernard Parish, at 29.52.818 north latitude and 89.48.575 west longitude. The objective of the Krauss et al. study (2000) was to evaluate the growth and survival of ten, half-sibling genetic families of baldcypress in an area with residual soil salinity resulting from past exposure to saltwater intrusion. The site is located near the Mississippi River Gulf Outlet, and numerous dead baldcypress still stand in the area, evidence of the saltwater intrusion that occurred prior to the construction of the hurricane protection levee in that portion of St. Bernard Parish in the early 1970s (US Army Corps of Engineers, personal communication, 2003c). Soil-water salinity measurements taken during this study indicate salinity levels ranged from 0.3 to 1.7 g L⁻¹. This is within the physiologically tolerable range (up to 3 g L⁻¹) in areas where baldcypress naturally occurs (Allen et al., 1998; Chabreck, 1972; Wicker, 1981), however these measurements do not negate the possibility that residual salinities have impacted growth of some trees. The site is relatively low-lying and was at times during this study known to have been inundated to a depth of approximately 6 inches by heavy rainfall, the only water input into the area since the protection levee was completed in the 1970s. It was, however, mostly free of standing water during the study, though the soil remained saturated throughout most of the study. Krauss et al. (2000) reported the Jeanfreau site soil type was classified as the Barbery series (thermic Typic Hydraquents), that were poorly drained and contained a relatively high mineral content.
In preparation for establishing the Jeanfreau site, seeds were collected by Krauss et al. (2000) from eight brackish-water baldcypress stands and two freshwater baldcypress stands. Four hundred baldcypress seedlings were grown in a greenhouse for nine months and subsequently planted on the Jeanfreau site in January of 1996 (Krauss et al. 2000). Of the five families used for insect feeding assays, four were from brackish-water source stands and one was from a freshwater source stand. Family cb3 was collected from a survivor in brackish-water in the Mobile Bay area, Alabama. Family fa7 was collected from a brackish-water source in the Falgout Canal area near Houma, LA in Terrebonne Parish. Family sg2 was collected from a brackish-water source near the St. Gore Pumping Station, near the junction of HWY 39 and 46 in St. Bernard Parish. Family sw2 was collected from a freshwater source in the Sherburne Wildlife Management Area, in the swamp adjacent to the Atchafalaya River in Iberville Parish. Family ve2 was collected from a brackish-water source in the present Mississippi River Delta near the mouth of the river in Plaquemines Parish.

The seedlings were planted according to a complete randomized block design consisting of ten blocks, each containing four replicate trees of the ten families. The site was thinned in 1999 by removing every other diagonal row, and with the exception of scattered mortality, two trees each of ten families remained in each of ten blocks, totaling approximately 200 trees.

2.2.1.2 2001 Plot Design and Treatment Allocation Rationale

In the winter of 2001, all trees at the Jeanfreau site were measured for diameter at one meter above ground-level using a diameter tape and total height using a Lasertech® Impulse laser altimeter. Diameter and total height means and tree mortality were used to determine the location and extent of tree growth differences between blocks. All the trees in one block were
excluded based on obviously lower height and diameter means and higher mortality predating the current study. The remaining nine blocks were evenly divided and assigned to two fertilization treatment blocks and one control. The blocks were fertilized on February 8, 2002, and again in early February 2003 with Scott’s Controlled Release Osmocote® 18-6-12 fertilizer. The fertilization rates were chosen in an attempt to mimic the result of one and two years’ worth of nutrient deposition at 8000 cubic feet per second, the maximum flow rate of the nearby Caernarvon diversion operated by the LA Dept. of Natural Resources (Day Jr., 2002; La. Dept. Nat. Res. 1998). The low fertilization treatment was fertilized at a rate of 236 g/m² and the high fertilization treatment received double, at 472 g/m², or the annual equivalent of a 16,000 cfs diversion. A two meter buffer was established to separate the treatment blocks, and trees within the buffer zones were not used for insect rearing or analyzed for tree growth measurements (Figures 2.1; 2.2). Trees along the periphery were excluded from measurement and feeding as well, as they had been exposed to more light than those within the interior.

Each trees’ diameter one meter above ground and total height was measured at the end of the 2002 growing season following the methods previously described, and in September of 2003. One source reports ‘mature’ baldcypress trees measured near New Orleans having completed radial growth during that study by mid-Summer, however height growth was not measured (Eggler, 1955). Another source indicates a plantation of 20 year old baldcypress growing in Georgia ceased diameter growth in mid-September (Jackson 1952). Height growth in many trees is known to occur over a shorter period of time than diameter growth (Kozlowski 1971). However, it is known that the growth period within a year of individual trees within the same species is variable and the growth period between years is also variable (Kozlowski 1971). Though some trees may have continued to grow beyond the 2003 final
measurements, one might expect the majority of the growth to have taken place earlier in the
growing season.

All insect data for the Jeanfreau site is based on three replicate trees of the five families
mentioned above, (cb3, fa7, sg2, sw2, and ve2) within each of the three fertilization
treatments. One tree of family sg2 was missing in the control fertilization treatment and one
tree of family sw2 was missing in the high fertilization treatment, for a total of 43 trees.

2.2.1.3 Soil Samples

Soil samples were obtained at the close of the 2002 growing season prior to the second
fertilization application using a Nasco stainless steel, one-inch diameter soil corer. Cores were
taken to a depth of six inches (Wilde et al., 1964). Multiple cores were taken systematically
in each fertilization block to obtain two composite samples per fertilization block. The
samples were air dried at approximately 50°C then ground with mortar and pestle. Analyses
were conducted by the Louisiana State University Dept. of Agricultural Chemistry
Laboratory. Total Kjeldahl nitrogen, phosphorous, potassium, chloride, and pH was obtained.
Total N was determined by colorimetric semi-automated block digestion procedure (AOAC,
1995a). Soil P and K were determined using microwave assisted digestion and elemental
analysis by inductively coupled plasma-atomic emission spectroscopy (AOAC 1995b).

2.2.2 Foliage Collection and Analysis Methods and Larval Bioassay Setup 2002-2003

2.2.2.1 2002-2003 General Larval Setup and Foliage Sampling Methods

There was an important difference between 2002 and 2003 in the methodology used to
begin larval feeding assays and used in collecting foliage for chemical analyses. In 2002, the
trees used for feeding assays were allowed to develop sufficient foliage to support larval
feeding assays, then all trees were sampled and the leaves transported to the laboratory.
**Figure 2.1.** Jeanfreau plot map. The fertilization treatments are labeled and the broken borders within fertilization treatment blocks mark buffer rows (not used for analyses). Cells contain tag number and family ID. Feeding assay trees are bolded. Letter “c” denotes coppiced Krauss (1996). Bottom of page is entry point to site from drainage ditch levee.
Figure 2.2. Jeanfreau site, spring 2003. Note differences in leaf emergence among trees in foreground, left.
Foliage samples in 2002 for nitrogen, phosphorous, potassium, and total phenolics were collected on April 30 from all 43 larval assay trees, when BCLR in the field were primarily in 4-5th instars and all those in the laboratory had nearly completed larval development.

In 2003, I began the feeding study by collecting foliage by families in the order of budbreak, in essence lessening the differences in foliage age. There was a gap of nearly two weeks between starting dates separating the earliest-leafing families and the latest leafing family. Because the phenotypic variation in the time the families began leafing out relative to each other was strong, very homogeneous within families, and consistent, this design was utilized to ascertain the extent of the these effects in phenological foliage characteristics on larval growth and performance. Thus, when the two early-leafing families fa7 and sg2 had leafed out sufficiently across the three fertilization treatments, foliage from those families was collected (on March 25, 2003) and returned to the laboratory to begin larval bioassays on those families only. On April 1, 2003 the late-leafing families cb3 and ve2 were collected and the larvae assigned to foliage in the laboratory, and the latest-leafing family sw2 was collected across fertilization treatments on April 4, 2003 and larvae started on those trees. Foliage for chemical analyses was collected in a manner analogous to that of the feeding assay setup in 2003. The larvae completed development approximately in the order in which they were begun on the foliage. The foliage for chemical analyses was collected when the larvae in the respective families were in 4th-5th instars. Families fa7 and sg2 were sampled across fertilization treatments for chemical analyses on April 16, 2003; families cb3 and ve2 on April 23, 2003; and family sw2 on April 30, 2003.

Each year, foliage for all parameter measurements was sampled (from each of the 43 total replicate trees in the family and fertilization treatments used for feeding assays) between
10:00 am and 2:00 pm, at random from the crown from all aspects, but primarily from the mid- to lower-crown. Trees were chosen randomly and sampled across fertilization treatments to minimize potential changes due to temperature fluctuations during the four hour sampling period.

### 2.2.2.2. Foliage Chemical Analyses-2002-2003

Foliage for chemical analyses (nitrogen, phosphorous, potassium, and total phenolics) each year required approximately 5% (60 g fresh weight) of each trees' foliage. The samples were placed in plastic bags, and transported on ice to the laboratory, and promptly stored in a deep freeze at -80° C until needed. Approximately two-thirds of these samples were allocated to NPK analysis, and one-third to total phenolics analysis.

Approximately 40 g wet weight (8 g dry wt of needles only, 20-30 branchlets) was obtained for foliar NPK analyses for each tree. The foliage was dried at approximately 57°C for 48 hours. Needles were separated from the central rachis and the rachis excluded from chemical analyses. Dried needles were ground to a fine powder using a mortar and pestle. Foliar NPK analyses were conducted by the Louisiana State University Dept. of Agricultural Chemistry. Total Kedhal N was determined by colorimetric semi-automated block digestion procedure (AOAC, 1995a). Foliar P and K were determined using microwave assisted digestion and elemental analysis by inductively coupled plasma-atomic emission spectroscopy (AOAC 1995b). Foliar N, P, and K concentrations were expressed as a percent dry weight.

Total phenolics were quantified following the Folin-Ciocalteau procedure (Stout et al., 1998; as adapted from Waterman and Mole, 1994 ). Approximately 20 g (beginning fresh weight, approx. 10-20 branchlets) of foliage per tree was used to determine foliar phenolics. This quantity was taken from the 60 g fresh weight composite sample collected for both NPK
and total phenolics samples. Foliage was dried in an ATR Inc. 3.0 freeze-drier, the central rachis removed, and the remaining needles ground with a Wiley mill using a size 40-mesh screen, yielding approximately 4 g dry weight of beginning material. From this, two 100 mg aliquots per tree each year were extracted for two weeks in 10 ml of 50:50 HPLC-grade methanol and distilled water at room temperature. (+)- catechin was used to generate a standard curve. (+)- Catechin is a major flavan-3-ol present in *Taxodium distichum*, and this compound is known to link with other monomers to form condensed tannins, among others (Stafford and Lester 1986, Waterman and Mole 1994). The standard curve was generated by adding 14.5 mg of the (+)- catechin to a 50:50 water : HPLC methanol solution, using 25 ml quantities of each. A range of increasing (+)- catechin solution from 0 to 200 µl was added to a decreasing volume of pure water, maintaining a total volume of 2.75 ml in each test tube. The Folin-Ciocalteau reagent (Sigma) and a 20% solution of sodium bicarbonate were added in 500 µl quantities to each test tube. The volume was vortexed and allowed to stand for 1.5 hours. Total phenolics were determined using a Shimadzu UV-1601 UV-Visible Spectrophotometer and reading absorbance at 720 nm. The absorbance values were used to generate a standard curve and absorbance values from foliar samples used to determine concentration in (+) - catechin reagent equivalent. Total foliar phenolics are reported as a percent of the leaf dry weight. For foliage sample phenolic determinations, 25 µl of each 100 mg aliquot solution was used and the same procedures used in mixing the appropriate chemicals and reading absorbance described above were followed.

**2.2.2.3 Foliage Samples for Physical Characteristics and Moisture Levels-2002**

Branchlets were sampled randomly within each tree primarily from the mid-crown. Two separate samples of 15 branchlets from all 43 larval assay trees were collected for length and
width measurements on April 18, 2002 and again on April 25, 2002. Branchlet length was measured to the nearest millimeter, from the petiole base to the terminal needle tip. Branchlet width was measured at the widest point of each branchlet, typically at the middle of the branchlet, from needle tip on one side of the rachis (central stem on which needles are attached) to the needle tip directly opposite. Needles typically project from the central rachis at similar angles along the length of the rachis, and needles were maintained in their natural state for width measurements. Two separate samples of foliage for moisture were also obtained simultaneously across all 43 feeding assay trees in 2002, the first collected on April 12, and the second on April 25. Foliage samples for moisture analysis were taken from mid-crown at all crown aspects, sealed in plastic bags, stored on ice and transported to the laboratory. Immediately upon reaching the laboratory, one subsample of 30 branchlets per feeding assay tree was selected from foliage collected for larval feeding, weighed to the nearest tenth of a milligram to obtain the fresh weight, and then dried at approximately 57°C until a stable dry weight was obtained (ca: 48 hrs). The dry foliage was then removed from the plastic bag and weighed again to the nearest tenth of a milligram.

2.2.2.4 Foliage Samples for Physical Characteristics and Moisture Levels-2003

Branchlet length and width samples in 2003 were collected at one sampling date on April 16. The foliage was sampled primarily from the lower- to mid-crown. In the context of the larval feeding assay, the majority of the larvae set up on the early-leafing foliage had pupated by April 16. Foliar moisture samples were collected by two sampling methods. On April 16, each of the 43 trees used in the feeding assay were sampled “simultaneously,” or across all treatments on the same day. Two subsamples of approximately 2 g fresh weight (approx. 10 branchlets) for each tree of fresh foliage was immediately weighed upon returning to the
laboratory and then oven-dried at 57°C for 48 hours. Cup weights were subtracted to determine percent foliar moisture.

The trees also were sampled according to budbreak, or “staggered” in the order in which larvae were started on the respective families for moisture analysis. The values recorded for the early-leafing families fa7 and sg2 in the simultaneous method above also were used for these two families when comparing to the staggered sampling method. One week following the collection of the two early-leafing families, ve2 and cb3 were collected on April 23 and the identical procedures followed for preparing and drying the subsamples. Family sw2 was sampled one week later and the same methods followed. Each family was sampled across fertilization treatments at the three sampling intervals.

2.2.2.5 Larval Collection and Feeding

Field-collected larvae were used for all feeding bioassays. Larvae were collected from field populations near Gramercy, LA and Norco, LA on HWY 61. Larvae were collected by clipping infested branches and transporting back to the laboratory, where larvae were collected from within the webs and as they left the foliage. In 2002, thirty second-instar larvae were randomly assigned to each tree in the family-fertilization treatments that were equally represented with three trees each, and in the two cases where a tree was missing from that treatment combination, 45 larvae were assigned, totaling 1,350 initial larvae.

In 2003, 30 larvae were assigned to the early-leafing families fa7 and sg2, however these target numbers were reduced to only 20 larvae for the remaining three families for logistical reasons (a concurrent study with the Delacroix research plot described hereafter). Larvae were randomly assigned to individual replicate trees across fertilization and family treatments to minimize variation in foliage quality and to avoid using larvae that may have remained in
collection materials longer than others. Foliage used in rearing was collected weekly from the Jeanfreau plot trees and transported to the lab on ice, where it was refrigerated until no longer needed. Foliage was collected on one side of the tree each collecting trip, and the side sampled was rotated each trip so that all cardinal directions were sampled. At each collection, foliage was collected from lower to upper crown, and along the inner and outer segments of lateral branches. Larvae were reared in 28 g diet cups. The foliage was cut with scissors and placed into cups and replaced as needed or every three to four days. The larvae were reared at 23° C under 12:12 light: dark photoperiod. Beginning weight, fresh pupal weight, development time in days, dry pupal weight, and sex were recorded. Fresh pupal weights were obtained immediately upon pupation. Pupae were then oven-dried at approximately 57° C. Mortality was recorded and parasitism occurrence noted.

2.2.3 Statistical Analysis

2.2.3.1 Insect Performance

The data were analyzed using the SAS mixed procedure as an unbalanced, complete randomized design, 2 x 2 x 5 x 3 factorial ANOVA (SAS Institute, Inc., 2001). Year was considered a fixed effect with two levels in this case due to the altered methodology in 2003. Sex was entered as a treatment with two levels, family as a treatment with five levels, and fertilization as a treatment with three rates. Insects were considered subsamples within the replicates (trees). The error term used to test the main effects of years, fertilization, family, and the interaction consisted of the replicates nested in the year by fertilization by family by sex interaction, and was used in the random statement of the mixed procedure. Relative growth rates, dry pupal weights, and development time were analyzed. Mortality was not
analyzed, as it was likely confounded by factors predating the setup (collection and transport, storage, etc.).

2.2.3.2 Foliage Parameters

Foliage samples for nitrogen, phosphorous, and potassium were also analyzed as an unbalanced CRD, 2 x 3 x 5 ANOVA with two years, three fertilization levels and five families. Again the error term consisted of the replicate nested in the year by fertilization by family interaction. A comparison of leaf length, width, and moisture between two foliage samples (early and late) collected simultaneously across fertilization treatments and all families was made in 2002. These data were analyzed similarly with the exception that the two sampling dates were considered a treatment ‘time’ with two levels, early and late, and so analyzed as a 2 x 3 x 5 ANOVA. The error term used to test treatments here was the replicate nested in the time by fertilization by family interaction.

In 2003, no early vs. late samples within the same year were compared as in 2002. With the exception of foliar moisture analysis, foliage samples were collected at a single date. Foliar moisture analysis in 2003 differed in that both a simultaneous sampling method was used as well as a budbreak sampling method. The 2003 moisture data collected simultaneously (across all treatments on the same day) were analyzed alone within 2003 as an 3 x 5 factorial ANOVA with three fertilization levels and five families. A contrast of the two early-leafing families versus the three late-leafing families was also used at an alpha level of 0.05. The 2003 moisture data collected by the budbreak ‘staggered’ method were also analyzed alone within 2003 in the same manner, including family as a treatment, and the same contrast constructed comparing the early-leafing families vs. late-leafing families. These foliar moisture samples obtained in 2003 consisted of two subsamples per tree, and were entered in
the model as such. The error term used to test the main effects fertilization and family however, remained the replicate nested in the fertilization by family interaction.

To compare foliage samples across years, the ‘late in year’ samples of foliar moisture, length and width measurements sampled simultaneously across treatments in 2002 were very near the same calendar date (within same week) of collection in 2003, and so the data were analyzed as such, with year included in the model as a treatment to detect any possible differences between years. The foliar moisture methods however differed slightly between years, such that in 2002, only one subsample was obtained per tree, and in 2003 two subsamples were obtained per tree. So to compare the ‘late in year’ simultaneous moisture samples between the two years, the two subsamples in the ‘late in year’ 2003 moisture sample were averaged and the data analyzed as a 3 x 5 factorial with three fertilization treatments and five families, such that each year was based on one observation per tree. For branchlet length and width analysis, fifteen branchlets (subsamples) were used per tree each year. For each of these analyses, the error term consisted of the replicate nested in the interaction between year by fertilization by family, and was used in the random statement of the mixed procedure.

2.2.3.3 Tree Growth

For tree growth response, all trees of each family (non-feeding assay and feeding assay trees) falling within predetermined treatment blocks were included to increase the total experimental units, resulting in a ‘full model’ 3 x 7 x 2 factorial ANOVA containing the fertilization treatments, the family effect, and the two years. Trees used in feeding assays were included in overall tree growth measurements despite the leaf removal. There was insufficient replication in the interaction to consider defoliation as a treatment. For height growth, only seven of ten families were used due to storm damage and an inability to sight tree tip through
the stand canopy at the September measurement in 2003. Families sw2, sw1, and sg3 were not included due to insufficient replication across years or fertilization treatments.

Because by excluding these families a substantial loss of sample size was incurred, the family effect was removed from the model and the tree height growth analyzed irrespective of the genetic effect. This overlooks possible interactions between fertilization treatments and families, as well as the propensity of some families for greater inherent growth rates than others, but this reduced model results in 18 additional trees and balances out the three fertilization treatments.

Diameter growth was analyzed similarly. First a full model was fitted including year as a treatment, family, and fertilization in a 2 x 8 x 3 factorial ANOVA. Families sg2 and sg3 were removed due to insufficient replication across years and among treatments due to storm damage or suspected measurement problems. Again because the removal of these families results in a loss of sample size, a reduced model with the family treatment removed was run using all available trees. This reduced model again requires the assumption that there were no family by fertilization interactions and no family differences in growth, which may well be a false assumption, but is likely a safe assumption that all trees regardless of genetic quality will respond similarly to increased nutrients.

Finally, both diameter and height growth of the 43 trees used in feeding assays were analyzed alone to elucidate possible differences obscured by the full model described above. Both height growth and diameter growth were analyzed for these trees. The data were analyzed as a 2 x 5 x 3 factorial ANOVA.

Diameter and height growth were calculated as the difference between initial diameter and height and the final diameter and height each year (Dec. 2002, Sept. 2003). When needed,
variables were log-transformed. The design of this analysis makes the assumption that after the block of trees were excluded based on preliminary measurements, the remainder of the site is relatively homogeneous with respect to tree growth and indirect effects on larval growth. In each analysis, the error term used to test the main effects consisted of the replicate nested in the interaction of the main effects and was listed in the random statement of the mixed procedure.

All hypothesis tests were conducted at the 0.05 alpha level. The least squares means were calculated for all datasets and separated using the Tukey-Kramer method for unbalanced designs, the Satterthwaite option to calculate degrees of freedom for unequal sample sizes, and type III sums of squares option used to test hypotheses. The Shapiro-Wilkes test was used to test the assumption of normality. Observations with legitimate reasons (listed in appendix) were excluded prior to analysis and are listed in the (Appendix A, Tables A3-A5). Upon analysis, variables were log-transformed to meet the assumptions of normality and the homogeneity of variance. In a very few cases additional outliers were excluded following transformation based on the stem and leaf plot in the univariate procedure in SAS, which denotes extreme residual values (observations) with an asterisk. Extreme values in this sense are defined as those values located beyond the 25th or 75th quartile by a distance of 3 times the interquartile range, which are beyond the 99th percentile of the data range (SAS Ver. 8, Technical Support). A list of these observations is located in the (Appendix A, Tables A6-A7). Deletion of observations following this procedure was followed through only once per dataset, and additional observations identified as asterisks were not removed.

In some cases the Shapiro-Wilkes values still indicated the assumption of normality had been violated in the insect datasets, however the violation was largely due to the extension of
the tails in each case, and insect sample sizes were quite large. With males and females combined, each insect variable measured totals not less than 500 individuals. This large sample size is very powerful, and examination of stem and leaf plots support this decision. Also, the distribution of the means of these individuals were more normally distributed than the individuals alone. A plot of the residuals on the predicted values as well as a regression of the variance on the means was used to evaluate the assumption of homogeneous variance. Many analyses were conducted on log-transformed variables, but are reported here as the original means and accompanied by their standard errors.

2.3 Results

2.3.1. Larval Growth and Performance Parameters

2.3.1.1 Order of Presentation and Differences between Sexes in Growth Response

Three indices of larval performance are presented in the following: dry pupal weights, RGRs, and developmental time. There was no significant interaction between larval sex with year, fertilization rate, or family. This result indicated males and females performed similarly in response to treatment effects.

2.3.1.2 Dry Pupal Weight

There was no significant three-way interaction between year, fertilization level, and family in analysis of dry pupal weight. Dry pupal weights did not differ significantly between years of study, and trends in means among fertilization rates were similar (F_{1,105}=2.62, p=0.1082). When pooled across years, dry pupal weights of both sexes were greatest in the fertilization treatments (Table 2.1). Females were, as expected, significantly heavier than males in all treatments. Females were significantly heavier in the low fertilization rate than those in the control, but there was no significant difference from those in the high fertilization treatment,
nor did the high and control treatments differ significantly from each other (Table 2.1). Males were significantly heavier in the low fertilization treatment than in both the control treatments, but, again, the high and control treatments did not differ significantly (Table 2.1).

Results indicated performance among families differed significantly across years with a family-year interaction ($F_{4,105}=8.64$, $p<0.0001$; Figure 2.3). Performance of males did not differ significantly among families either year. Female mean dry pupal weight in 2002 was significantly lower in early-leafing family fa7 than late-leafing families cb3, ve2 and sw2 (Figure 2.3). Female mean dry pupal weight in the early-leafing family sg2 in 2002 was significantly lower from that in the late-leafers ve2 and sw2 but not cb3. With the exception of family sg2, the means in each respective family were generally lower than 2002 means.

2.3.1.3 Larval Relative Growth Rate (RGR)

For RGR it is perhaps most prudent to accept and consider the relevance of an interaction between fertilization treatments, family, and year (with sexes combined), at an alpha level of 0.10. Again, no interaction involving sex and the other treatments was significant. When sexes are combined, results indicate a marginally significant interaction between years, fertilization treatments, and families ($F_{8,111}=1.96$, $p=0.0582$). Accepting this result as significant is supported by the detection of significant interactions between year and fertilization rate ($F_{2,111}=5.26$, $p=0.0066$), year and family ($F_{4,111}=4.51$, $p=0.0020$), and fertilization rate and family ($F_{8,111}=2.62$, $p=0.0117$).

In 2002, RGRs were generally greater in late-leafing families cb3, sw2, and ve2 than early-leafing families fa7 and sg2, and this was most readily seen in the control treatment.
Table 2.1. Male and female mean dry pupal weight (mg) by fertilization rate. Sample sizes ranged from 278-330 females and 302-364 males on 14-15 trees per fertilization treatment (years combined). Means ± 1 SE marked with different letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

<table>
<thead>
<tr>
<th>Fertilization Rate</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.26 ± 0.15 b</td>
<td>9.84 ± 0.08 d</td>
</tr>
<tr>
<td>Low</td>
<td>19.19 ± 0.12 a</td>
<td>10.44 ± 0.06 c</td>
</tr>
<tr>
<td>High</td>
<td>18.76 ± 0.13 ab</td>
<td>10.26 ± 0.06 c</td>
</tr>
</tbody>
</table>

Figure 2.3. Female mean dry pupal weight by year and family. Sample size ranged from 103-115 larvae in 2002 and 54-104 larvae in 2003 on 8-9 trees per bar each year. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.
Figure 2.4). In the low and high fertilization treatments, a similar trend in means was evident, however RGRs did not differ significantly among early or late-leafing families.

In 2003, when the larvae were assigned to foliage in the order of budbreak, the effects of fertilization were more readily visible. There were no significant differences within the same fertilization treatment between the early and late-leafing families. Only larvae reared on late-leafing family cb3 grew at a significantly greater rate than late-leafing family ve2 in the high fertilization treatment. Late-leafing family sw2 grew at a significantly greater rate in the fertilized treatments compared to the control, and late-leafing family ve2 grew at a significantly greater rate in the low fertilization treatment than either the control or high fertilization treatment, which were very similar to each other. Growth rates in general were greater in 2002 than in 2003. Late-leafing families in sw2 and ve2 in 2002 grew at significantly greater rates in the control treatments than in 2003.

2.3.1.4 Developmental Time

Results indicated a significant interaction ($F_{8,113}=2.11$, $p=0.0402$) between family, fertilization rates, and years (both sexes combined). In 2002, development times were generally longer among the early-leafing families fa7 and sg2, and this was especially obvious in the control treatment. Development time was significantly longer in the control treatment on the early-leafing family fa7 than the late-leafing family ve2 (Figure 2.5). A similar trend among early- and late-leafing families was evident in the fertilized treatments, however families did not differ significantly across the same fertilization rate. In general development time means were lowest on the fertilized treatments, particularly the low; however, family ve2 in 2002 had the shortest mean development time in the control treatment. In 2003, there were no significant differences among family-fertilization treatment combinations.
Figure 2.4. Mean RGR by year, fertilization rate, and family (sexes combined). Sample size ranged from 59-80 larvae in 2002 and 34-84 larvae in 2003 on 2-3 trees per bar each year. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.
Figure 2.5. Mean development time by year, fertilization rate, and family (sexes combined). Sample size ranged from 59-80 larvae in 2002 and 34-84 larvae in 2003 on 2-3 trees per bar each year. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.
Development times in 2003 also were longest in the control treatments in families cb3, sg2, sw2, and ve2, and differences between the control and the fertilization treatments within an individual family in 2003 were generally greater than in 2002. Development time was significantly longer in 2003 in the control treatment on families ve2 and sw2 than in 2002. In 2003 the shortest development time was more consistently found in the low fertilization level.

2.3.1.5 Larval Mortality

Though analyses were not performed on larval mortality due to potentially confounding factors involved in the cause of death, the percent mortality by year of study overall are presented in Table 2.2, and by fertilization each year in Table 2.3. Of ancillary interest is the repeated higher percent mortality in the control than the fertilized treatments each year. Early instar parasitism was very low each year (Table 2.2). Percent Mortality by family each year is included in Appendix A (table A-2).

<table>
<thead>
<tr>
<th>Year</th>
<th>Handler</th>
<th>Parasitism</th>
<th>Unknown</th>
<th>Total</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>0.42</td>
<td>0.21</td>
<td>21.07</td>
<td>21.70</td>
<td>1433</td>
</tr>
<tr>
<td>2003</td>
<td>0.19</td>
<td>1.71</td>
<td>16.44</td>
<td>18.34</td>
<td>1052</td>
</tr>
</tbody>
</table>

*a* Handler = died as result of handling injury.

*b* Unknown = cause of mortality unknown.

Table 2.2. Percent larval mortality by year.

<table>
<thead>
<tr>
<th>Fertilization</th>
<th>Handler</th>
<th>Parasitism</th>
<th>Unknown</th>
<th>Total</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.21</td>
<td>0.62</td>
<td>27.16</td>
<td>27.99</td>
<td>486</td>
</tr>
<tr>
<td>Low</td>
<td>0.62</td>
<td>0.00</td>
<td>19.34</td>
<td>19.96</td>
<td>486</td>
</tr>
<tr>
<td>High</td>
<td>0.43</td>
<td>0.00</td>
<td>16.49</td>
<td>16.92</td>
<td>461</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>2.36</td>
<td>24.78</td>
<td>27.14</td>
<td>339</td>
</tr>
<tr>
<td>Low</td>
<td>0.00</td>
<td>1.65</td>
<td>10.99</td>
<td>12.64</td>
<td>364</td>
</tr>
<tr>
<td>High</td>
<td>0.57</td>
<td>1.15</td>
<td>14.04</td>
<td>15.76</td>
<td>349</td>
</tr>
</tbody>
</table>

*a* Handler = died as result of handling injury.

*b* Unknown = cause of mortality unknown.

Table 2.3. Percent larval mortality by fertilization rate.
2.3.2 Foliage Chemical Parameters

2.3.2.1. Total Phenolics

There was no evidence of a significant interaction between family, fertilization treatment, or these two treatments in combination, with year, suggesting that the alternate sampling method in 2003 (order of budbreak) did not significantly change the relative concentrations of total phenolics among the respective families in the fertilization treatments between years. Total phenolic concentration pooled across all treatments was significantly higher in 2002 when sampled simultaneously than when sampled by budbreak in 2003 (t_{43}=3.17, p=0.0024). There was evidence, however, of a significant fertilization-family interaction (p=0.0347). The trend in means within the fertilization and family treatment groups was consistent each year. Visual analysis of the fertilization by family graph (Figure 2.6) indicated that overall, the early-leafing families sg2 and fa7 were consistently, and in several cases significantly, higher in total phenolic levels than the later leafing families cb3, sw2, and ve2 in the low fertilization treatment. Late-leafing families cb3 and ve2 differed in that the high fertilization treatment contained slightly greater amounts of total phenolics than did the control and low treatments. In some instances trees within the same family differed more across fertilization levels than others, likely due to low sample size. The Tukey-Kramer post-anova comparison of means indicated no significant differences within any family across fertilization levels.

2.3.2.2 Nitrogen

Analysis of foliar nitrogen narrowly missed a significant fertilization by family interaction (F_{8,56}=1.97, p=0.0679). Foliar nitrogen followed a significant negative correlation with total phenolics each year, supporting the possibility of differential growth among fertilization
Figure 2.6. Foliar total phenolic concentrations. Sample sizes ranged from 2-3 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05. Treatments depending on the family (Figure 2.7). Foliar nitrogen concentrations, in general, were significantly greater in 2003 than in 2002 ($t_{56}=3.02$, $p=0.04$). While this trend was consistent across fertilization treatments, only the low fertilization treatment in 2003 was significantly greater than low fertilization treatment in 2002 (Figure 2.8). Foliage collected from the low fertilization treatment contained significantly greater nitrogen concentration than the control each year. The high fertilization treatment did not differ significantly from either the control or low fertilization treatment, but each year concentrations were intermediate to
Figure 2.7. Regression analysis of foliar nitrogen on total phenolics. 2002 analysis is based on foliage collected simultaneously among all treatment combinations on April 30 and 2003 is based on samples obtained in order families began leafing out beginning when larvae reached 4th-5th instar.

the theses treatments. Each year the early-leafing families generally contained significantly less foliar nitrogen than the late-leafing families. In 2002, when foliage was sampled simultaneously, the differences among early and late-leafing families were more pronounced than in 2003 (Figure 2.9). Late-leafing families cb3 and ve2 contained significantly higher concentrations of nitrogen than early-leafing families fa7 and sg2, while late-leafing family sw2 fell intermediate and did not differ significantly from the other families. In 2003 late-leafing families cb3 and ve2 maintained significantly greater foliar N concentration than
Figure 2.8. Foliar nitrogen content by fertilization treatment. Samples obtained from 14-15 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

Figure 2.9. Foliar nitrogen by family and year of study. Sample sizes ranged from 8-9 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.
early-leafing family fa7, however early-leafing family sg2 did not differ from any late-leafing families.

2.3.2.3. Potassium

Foliar potassium concentration also was significantly greater in 2003 than in 2002 ($t_{56}=3.26$, $p=0.0019$), and followed patterns of slightly higher K content in each fertilization treatment in 2003. When viewed within years, K concentration was generally greater in the low fertilization treatment than either the control or high fertilization treatment each year, however differences were not significant (Table 2.4).

Differences in foliar potassium among families were more pronounced in 2002 than 2003, and generally each year the late-leafing families contained greater K concentration than the early-leafing families (fa7 and sg2; figure 2.10). Mean foliar potassium was significantly higher in the late-leafing family cb3 than the early-leafing family sg2 in 2002, but there were no significant differences among early and/or late-leafing families in 2003.

Table 2.4. Mean K ± 1 S.E. concentration by fertilization treatment. Means followed by differing letters were considered significantly different at an alpha level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002</td>
<td>0.511 ± 0.03</td>
<td>0.592 ± 0.03</td>
<td>0.488 ± 0.03</td>
<td>0.532 ± 0.19b</td>
</tr>
<tr>
<td>2003</td>
<td>0.605 ± 0.04</td>
<td>0.631 ± 0.02</td>
<td>0.569 ± 0.02</td>
<td>0.602 ± 0.18a</td>
</tr>
</tbody>
</table>

2.3.2.4. Phosphorous

Foliar phosphorous also was significantly higher in 2003 than in 2002 ($t_{56}=7.19$, $p<0.0001$).

While foliar P levels did not differ significantly among fertilization levels within separate
Figure 2.10. Foliar potassium levels by family and year of study. Sample sizes ranged from 8-9 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

years, levels were significantly higher between years in all fertilization treatments (Figure 2.11). Foliar phosphorous varied least across fertilization levels each year when compared to analyzed regardless of treatment combination. When compared within years, foliar P levels did not differ significantly among families. Relative differences among families were more strongly expressed in 2002 and relatively flat across the family effect in 2003 (Figure 2.12). Early-leafing families in 2002 were generally lower in percent P than the late-leafing families cb3, sw2, and ve2, and this difference was less clear in 2003, as only late-leafing families cb3 and ve2 maintained their status of the greatest percent phosphorous content.
Figure 2.11. Foliar phosphorous levels by fertilization rate and year of study. Sample sizes ranged from 14-15 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

Figure 2.12. Foliar phosphorous levels by family and year of study. Sample sizes ranged from 8-9 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.
2.3.2.5. Moisture

Foliar moisture sampled simultaneously from each treatment combination at comparable dates (4/12/02 and 4/16/03) indicated no significant difference between years (F_{1.56}=1.06, p=0.3076). When pooled over years, foliar moisture in the low fertilization treatment was significantly greater than the high and control (Figure 2.13). The high fertilization treatment contained greater mean percent moisture content but did not differ significantly from the control. The analysis of early season moisture vs. late season moisture levels (two weeks separation) found no significant difference between sampling dates (F_{1.56}=0.30, 0.5868), however when pooled across sampling dates, the low fertilization treatment again contained the greatest moisture content. In 2003 foliage samples were collected for moisture analysis by sampling foliage simultaneously across all families and fertilization treatments as well as by budbreak (fa7 and sg2; cb3 and ve2; sw2). When sampled simultaneously, contrast analyses indicated that foliar moisture was significantly higher in the low fertilization treatment than in the combined means of the control and high fertilization treatments (F_{1.27}=6.17, p=0.0195). When sampled by budbreak (families) across fertilization treatments in 2003, the low fertilization treatment again had significantly higher moisture content than the high fertilization treatment (t_{26}=3.8, p=0.0022) and near significantly different from the control (t_{26}=2.41, p=0.0586). Foliar moisture levels were consistently higher in the late-leafing families than the early-leafing families. A comparison of foliage samples collected at similar dates in 2002 and 2003 (within four days of each other) when leafrollers were nearing 4^{th}-5^{th} instars indicated no significant differences in moisture levels between years. When pooled across years, late-leafing families cb3 and sw2 contained significantly more foliar moisture than early-leafing families fa7 and sg2 (Fig 2.14). A comparison of moisture levels among
**Figure 2.13.** Foliar moisture by fertilization treatments. Sample sizes ranged from 14-15 trees per bar. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

**Figure 2.14.** Foliar moisture levels by family across the two year period of study, when sampled simultaneously across treatments. Sample size ranged from 8-9 trees per bar each year. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.
families sampled twice within the 2002 growing season with an interval of 13 days suggested no significant differences in moisture levels between sampling dates, however when pooled over the two sampling dates, foliar moisture levels were significantly greater in the late-leafing families sw2 and cb3 than the early-leafing families fa7 and sg2.

A comparison of the simultaneous sampling method to the budbreak sampling method (by family) in 2003 revealed that the two methodologies may have affected foliar moisture. When foliar moisture levels were collected by budbreak (fa7 and sg2 first, cb3 and ve2, then sw2) mean moisture levels were nearly equal among families ($F_{4,26}=0.12$, $p=0.9756$). An analysis of foliar moisture levels among all families sampled simultaneously on April 16, 2003 at the beginning of the budbreak (by family) sampling sequence resulted in a significant family effect ($F_{4,27}=3.34$, $p=0.0240$), however the Tukey-Kramer tests detected no significant differences among individual families. This result was followed with a contrast analysis consisting of the two early-leafing families (fa7 and sg2) vs. the three late-leafing families (cb3, sw2, and ve2), indicating that the early-leafing families had significantly less foliar moisture than the late-leafing families ($F_{1,27}=12.74$, $p=0.0014$).

2.3.3. Physical Characteristics

2.3.3.1. Branchlet Length

When foliage samples were obtained from comparable dates each year (two days separation), and compared, leaf length did not differ significantly between 2002 and 2003. Branchlets collected from families in the low fertilization level were significantly longer than the control ($t_{55}=2.96$, $p=0.0123$) while the high fertilization treatment fell intermediate and did not differ significantly from either extreme. A comparison of two samples collected across all families and fertilization treatments separated by one week in 2002 indicated that, while
leaves were on average longer at the second sampling date, perhaps the sampling interval was not long enough, or the sample sizes inadequate, to detect a significant difference. When pooled across sampling intervals, the branchlets in the low fertilization treatment were on average longer than those in the control and high treatment, but did not differ significantly. This analysis supported the importance of the family effect.

Family effects explained a significant portion of the variation in leaf length. Foliage sampled at comparable dates each year (April 18, 2002 and April 16, 2003) did not differ significantly in length between years, however when pooled over both years, the early-leafing families (fa7 and sg2) were generally longer than the late-leafing families. Early-leafer fa7 was significantly longer than late-leafers ve2, sw2, and cb3. Early-leafing family sg2 was considerably longer than the late-leafers, but did not differ significantly from fa7 or the other families (Figure 2.15). The analysis of leaf length comparing the two sampling dates in 2002 also supported the importance of the family effect. When pooled over the two sampling dates, again the results indicated family fa7 was significantly longer than family ve2, while families cb3, sg2, and sw2 did not differ significantly from fa7 or ve2.

### 2.3.3.2 Branchlet Width

When width was compared across years using the comparable sampling dates, interestingly leaves were found to be significantly wider in 2003 ($t_{1.55} = 4.53$, $p < 0.0001$), but generally followed trends similar to leaf length among fertilization treatments. When compared by fertilization treatments within years, leaves were generally wider in the low fertilization level than those in the control and high fertilization treatment each year, however they did not differ significantly among fertilization treatments (Table 2.5).
**Figure 2.15.** Mean branchlet length by family. Sample size ranged from 8-9 trees per bar (15 branchlets per tree) each year. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

**Table 2.5.** Mean branchlet width (mm) by fertilization treatment and family. Mean ± 1 SE are presented. Sample size ranges from 14-15 trees for fertilization rate means and 8-9 trees for family means (15 branchlets per tree). Samples were collected same day on comparable dates each year (4/18/02 and 4/16/02).

<table>
<thead>
<tr>
<th>Fertilization Rate</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16.1 ± 0.30</td>
<td>18.4 ± 0.33</td>
</tr>
<tr>
<td>Low</td>
<td>18.5 ± 0.27</td>
<td>21.3 ± 0.43</td>
</tr>
<tr>
<td>High</td>
<td>17.2 ± 0.31</td>
<td>20.5 ± 0.33</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Family</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td>cb3</td>
<td>16.7 ± 0.34</td>
<td>19.6 ± 0.46</td>
</tr>
<tr>
<td>fa7</td>
<td>17.3 ± 0.40</td>
<td>19.3 ± 0.45</td>
</tr>
<tr>
<td>sg2</td>
<td>16.3 ± 0.33</td>
<td>18.5 ± 0.39</td>
</tr>
<tr>
<td>sw2</td>
<td>18.2 ± 0.48</td>
<td>21.2 ± 0.49</td>
</tr>
<tr>
<td>ve2</td>
<td>18.2 ± 0.35</td>
<td>21.9 ± 0.56</td>
</tr>
</tbody>
</table>
When branchlet width was compared among families, width did not differ significantly among families either year. Of ancillary interest, however is the observation that though total branchlet length is a good indicator of branchlet age, branchlet width is not. Mean branchlet width was greater in the late-leafing families sw2 and ve2, while the early-leafing families fa7

2.3.4 Tree Growth Parameters and Soil Properties

2.3.4.1 Height Growth

The analysis of height growth by year, fertilization rate, and family was based on a total of 61 trees, or approximately 20 trees in each fertilization level. A lack of replication across all fertilization rates in families sg3, sw2, and sw1 limited analyses to the seven remaining families. There were no significant differences among these remaining families, nor were there significant interactions between family, year and fertilization rate. A table of the height growth by family and fertilization means is included (Appendix A, Table A-1). To strengthen the investigation of the effect of the fertilization treatments within and between the two years of study, family was removed as an effect and all available trees used in a reduced model containing only year and fertilization as treatments. The reduced model (excluding family) (Figure 2.16) contained 18 more trees than did the full model and replication was more equally balanced between fertilization levels (n=28, 26, 25).

When family was excluded, the trends in mean height growth among fertilization treatments did not change from that of the model including the family effect, though the interaction between fertilization rates and years of study was nearly significant (F_{2, 38.1}=3.19, p<0.0523). Mean height growth was greater in the low fertilization treatment than height growth in the control and high fertilization treatment in 2002, but mean height growth did not differ significantly within years (Figure 2.16). In 2003 there were no significant differences in
Figure 2.16. Mean height growth in the reduced model, year and fertilization level but family excluded. Sample size in years 2002 and 2003 was Control, n=28, n=28; Low n=26, n=26; and High n=25, n=25. Means marked with differing letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

Height growth among fertilization treatments. The trees in the control had the greatest mean height growth, and those in the low fertilization treatment the least. Height growth was not significantly different between years at the two fertilization treatments, however trees in the control treatment grew significantly more in 2003 than did trees in the control in 2002.

2.3.4.2 Diameter Growth

Analysis of diameter growth by year, fertilization, and family was based on 77 total trees in 8 of the 10 families (families sg2 and sg3 dropped due to insufficient replication). Initial
ANOVA results indicated a marginally significant interaction between family, fertilization rate, and year in diameter growth ($F_{14, 88.6}=1.80, p<0.0508$). Mean diameter growth tended to be lower in 2003 than 2002 in nearly all families. Families at the low fertilization rate grew the least in diameter in 2002 but grew more than the control and high rate in many cases in 2003 (Table 2.6). Family fa8 in the control treatment grew significantly more in diameter in 2002 than did several families in both the control, low and high fertilization treatments in 2003, however the remaining pairwise comparisons found no significant differences within years and within individual families across the fertilization treatments (Table 2.6).

A significant interaction between families and the two years of study was detected when pooled across fertilization treatments ($F_{7, 90.5}=2.28, p<0.0349$, figure 2.17). Families fa7, sw2, and ve2 did differ significantly between years, however each of the other families grew significantly less in diameter in 2003 than in 2002 (Figure 2.17). Family fa2 exhibited the greatest mean diameter growth each year, and the ‘fa’ families generally did well in comparison to the other families each year. The pairwise comparisons indicated no significant differences among families within the same year (Figure 2.17).

Of the five families used for the larval feeding bioassays (cb3, fa7, sg2, sw2, ve2), mean height growth was higher for the low fertilization rate in 2002; however in 2003, trees exhibited the lowest mean height growth in the low fertilization treatment (Figure 2.18). There were no significant differences among these families in height growth.

2.3.4.3 Soil Properties and Environmental Conditions

Soil analysis based on samples taken eight months following the first fertilization treatment indicate differences in N, P, K, and Cl ions, as well as pH were not consistent across fertilization areas (Table 2.7). The control fertilization treatment unexpectedly contained more
Table 2.6. Mean diameter growth (cm) in full model year by fertilization level by family interaction. Sample size for both years ranged from 2-5 trees among year by fertilization by family interaction cell means.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>cb3</td>
<td>1.40 ± 0.3</td>
<td>1.13 ± 0.2</td>
<td>1.26 ± 0.2</td>
<td>0.53 ± 0.02</td>
<td>0.58 ± 0.2</td>
<td>0.80 ± 0.3b</td>
<td>0.98 ± 0.11</td>
</tr>
<tr>
<td>fa1</td>
<td>1.73 ± 0.2</td>
<td>1.30 ± 0.3</td>
<td>1.63 ± 0.1</td>
<td>0.50 ± 0.2b</td>
<td>1.23 ± 0.2</td>
<td>0.85 ± 0.3</td>
<td>1.19 ± 0.13</td>
</tr>
<tr>
<td>fa2</td>
<td>1.8 ± 0.3</td>
<td>1.04 ± 0.1</td>
<td>2.10 ± 0.3</td>
<td>1.01 ± 0.2</td>
<td>1.10 ± 0.3b</td>
<td>0.73 ± 0.2</td>
<td>1.26 ± 0.13</td>
</tr>
<tr>
<td>fa7</td>
<td>1.43 ± 0.2</td>
<td>0.95 ± 0.2</td>
<td>1.43 ± 0.4</td>
<td>0.88 ± 0.04</td>
<td>0.80 ± 0.3b</td>
<td>0.98 ± 0.2</td>
<td>1.05 ± 0.11</td>
</tr>
<tr>
<td>fa8</td>
<td>1.70 ± 0.3a</td>
<td>1.10 ± 0.3</td>
<td>1.20 ± 0.3</td>
<td>0.7 ± 0.2b</td>
<td>0.78 ± 0.2</td>
<td>0.35 ± 0.3</td>
<td>1.03 ± 0.15</td>
</tr>
<tr>
<td>sw1</td>
<td>1.17 ± 0.3</td>
<td>1.25 ± 0.3</td>
<td>1.13 ± 0.5</td>
<td>0.27 ± 0.1</td>
<td>0.30 ± 0.1</td>
<td>0.50 ± 0.3b</td>
<td>0.76 ± 0.15</td>
</tr>
<tr>
<td>sw2</td>
<td>1.40 ± 0.2</td>
<td>1.57 ± 0.4</td>
<td>0.95 ± 0.1</td>
<td>0.75 ± 0.3</td>
<td>0.85 ± 0.4</td>
<td>0.93 ± 0.1</td>
<td>1.10 ± 0.13</td>
</tr>
<tr>
<td>ve2</td>
<td>0.45 ± 0.5</td>
<td>1.03 ± 0.4</td>
<td>1.03 ± 0.4</td>
<td>1.25 ± 0.1</td>
<td>0.41 ± 0.3</td>
<td>0.70 ± 0.4</td>
<td>0.80 ± 0.15</td>
</tr>
<tr>
<td>Fert. Means</td>
<td>1.47 ± 0.1</td>
<td>1.14 ± 0.1</td>
<td>1.37 ± 0.1</td>
<td>0.71 ± 0.1</td>
<td>0.76 ± 0.1</td>
<td>0.76 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.17. Mean diameter growth by year and family. Sample sizes in each family by year combination ranged from 8-12 trees. Bars bearing differing letters are considered significantly different at the 0.05 alpha level using the Tukey-Kramer test.
Figure 2.18. Mean height growth in families by fertilization rate used for larval feeding bioassays.

a: Storm damaged one of two replicate trees in family sw2 in June 2003
total nitrogen, however it contained generally less P and K than did the fertilized areas. The low fertilization block had the lowest pH. Chloride ions also were higher in the control, followed by the low and high fertilization treatments (Table 2.7).

Rainfall and temperatures were typical of long-term trends over the two-year duration of this study. The site characteristics were such that moisture was likely not a limiting factor on tree growth. Though standing water was not constant throughout the study, the soil was saturated for much of the time.

Table 2.7. Soil analysis. Mean of two composite samples per treatment block. Samples collected December 2002 following one season of fertilizer application.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>pH</th>
<th>P</th>
<th>K</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.15</td>
<td>4.275</td>
<td>0.077</td>
<td>0.164</td>
<td>0.185</td>
</tr>
<tr>
<td>Low</td>
<td>1.07</td>
<td>4.175</td>
<td>0.081</td>
<td>0.184</td>
<td>0.170</td>
</tr>
<tr>
<td>High</td>
<td>1.09</td>
<td>4.275</td>
<td>0.074</td>
<td>0.184</td>
<td>0.165</td>
</tr>
</tbody>
</table>

2.4 Discussion

2.4.1. Insect Growth and Corresponding Foliage and Tree Characteristics

The quality of the foliage available to eclosing baldcypress leafroller (BCLR) larvae depends on the growth status of the host tree as it is affected by environmental and site conditions, the synchronicity of the egg mass hatch to foliage budbreak, and intraspecific host differences, such as foliage morphology. In the present study, a combination of these effects were found to influence larval performance, however, variation in the study methodology magnified either one effect or the other each year. The methodology used in 2002 accentuated the effects of an asynchronous larvae-foliage emergence, by exposing larvae to differences in budbreak phenology expressed among families. In 2003, family differences in budbreak were
experimentally reduced, resulting in a more accentuated expression of differences in foliage quality attributable to the fertilization effect.

Each year, both insect sexes responded to treatments with growth in similar directions, however, female growth and development was seemingly more sensitive to foliage differences than were males. This has been observed in studies with other lepidopteran species. Raupp et al. (1988) found male gypsy moth (Lepidoptera: Lymatriidae) pupal weights were less dependent on foliage quality than females when offered differing host species. Similarly, Redak and Cates (1984) found foliar soluble N content was less predictive of male pupal weight than female spruce budworm (Lepidoptera: Tortricidae), pupal weight, however, males were better predicted by differing foliar monoterpene profiles than those predicting female pupal weights.

In 2002, all families had leafed out across fertilization treatments before feeding assays began, and greater differences in larval growth (especially females) could be attributed to differences in family budbreak than fertilization. In association with the lower nutrient and moisture content, and generally higher total phenolics, larvae reared on the early-leafing families fa7 and sg2 required a longer development period, during which time they grew at a reduced rate and attained a smaller dry pupal mass. That was not to say the nutrient availability and growth status of the tree did not affect foliage and larval growth, but those effects were seemingly less strong than family differences affecting foliage age. An example of this is seen in RGRs in 2002, which were less variable among fertilization treatments, suggesting if the foliage emerged later and was of better quality at the time larval bioassays were begun relative to early-leafing families, the growth status of the family among fertilization treatments had less of an effect on larval growth than it would have had the
foliage been older. Tree height growth, which would have expectedly been favored over
diameter growth under the given conditions of lower light availability (Kozlowski, 1971) was
not significantly different among families, however, mean height growth in 2002 was
generally greatest in the low or high fertilization treatment for all families except ve2. Family
ve2 had a lower mean height growth among all fertilization treatments than most families, but
because it was a late-leafing family, RGRs and dry pupal weights were as high as or higher
among all fertilization treatments than the other families, which in many cases appeared to be
growing more in height than family ve2. BCLR larvae did generally exhibit higher initial
survival, greater relative growth rates, and attain greater dry pupal mass in a shorter time
period in the two fertilization treatments, rather than the control in 2002, but these differences
among fertilization treatments were more strongly expressed in the early-leafing families,
which had older foliage, and RGRs showed a greater increase from the control to fertilized
treatments.

In 2003, dry pupal weights and RGRs varied more among fertilization treatments than
families. The significant interaction between the fertilization level, family, and year effects in
RGR and development time showed that in 2003 the pattern differed from 2002 in that RGRs
were pronouncedly greater in the low fertilization treatment in three of five families, and
RGRs on the remaining two families together were greater in the fertilized treatments than the
control. Results in 2003 also showed that the differences in larval growth among fertilization
treatments were stronger in late-leafing families than in 2002, suggesting that with the loss of
the advantage of younger foliage, the differences among fertilization treatments in foliage
quality may have been more valuable to developing larvae.
Though these results may have in some ways been affected by the treatments in the previous year or other varying conditions between years, one potential explanation for the results observed in 2003 is that the foliage age differences were lessened by the sampling technique, and differences due to the growth status of the tree were strengthened. The foliage nutrient content averaged across families again was higher in the low fertilization treatment than either the control or high fertilization treatment. RGRs and dry pupal weights varied less among the early- and late-leafing families, and foliar analyses showed that when samples were obtained according to budbreak, nutrient, moisture, and total phenolic levels were comparable across early- and late-leafing families. Simultaneously obtained moisture samples revealed the same pattern in foliage characteristics between early- and late-leafing families observed in 2002. Interestingly, foliage quality in 2003 did not follow tree growth patterns in 2003, but rather the physical and chemical characteristics of the foliage in 2003 were better predicted by the tree height growth observed in 2002. This seasonal pattern in tree growth and foliage characteristics has been observed in other studies. The quality of emerging foliage in trees often depends more on the ability of the previous years’ foliage to provision the developing buds for the following year, and that ability is determined by environmental conditions and resource availability to trees in the previous year (Haukioja and Honkanen, 1997; Kozlowski, 1971). Examples of this are found in studies and reviews of the effects of severe defoliation on the foliage quality (of deciduous trees particularly) in following years, which have found foliage to be smaller, contain less nutrients and moisture, and contain more defensive compounds such as tannins, as a result of removing nutrients and growth hormones prior to the development of the leaf primordia (Quiring and McKinnon, 1999; Ruohomaki and Chapin, 1996; Herms and Mattson, 1992; Tuomi et al., 1988; Kozlowski, 1971).
Prior to this study and one other ongoing study with the baldcypress leafroller at Louisiana State University, the total phenolics procedure used here had not been applied to baldcypress foliage. Total phenolic levels observed here followed the widely shown pattern of increasing levels as the foliage becomes older and ceases growth (higher in early-leafing families), and greater levels in the presence of lower nutrient content and less tissue growth (control foliage) (Herms and Mattson, 1992; Bryant et al., 1983; Haukioja and Neuvonen, 1985; Ruohomaki et al. 1996). It is difficult to say to what degree the values obtained from this study affected the BCLR, because as foliar phenolic levels increased, insect larval growth rates and dry pupal weights decreased, but foliar N, moisture content, and other necessary nutrients also decreased. Total phenolics were strongly negatively correlated with foliar N content in 2002 ($r^2=-0.70$), and this correlation was again significant in 2003 ($r^2=-0.54$).

There was a significant interaction in total phenolics between the fertilization treatment and family effect ($p=0.0347$), and given the tight correlation between total phenolics and foliar N, it is not surprising the interaction between fertilization treatments and family in foliar N was nearly significant ($p=0.0679$). The pattern in these means among fertilization treatments and families was strongly similar each year despite sampling technique differences between years. Though tree height growth was variable and not always correlated with foliar nutrients as in other studies (Zhang et al. 1997, Bigras et al. 1996, Bigras and Colombo, 2001, Ruohomaki et al. 1996), one possible cause for these results may have been a combination of the tree growth status and the foliage age as it was determined by the date of budbreak.

The date in any given year budbreak begins in a tree is closely dependent on warming temperatures, but relative to surrounding members of the same species, it is often consistently different due to genetic control (Bigras and Colombo, 2001; Chen et al., 2003; Kozlowski,
The date of budbreak has been shown to be advanced by abundant nutrient and moisture reserves (fertilization) allocated to developing buds in the previous growing season (Kozlowski, 1971; Quiring and McKinnon, 1999; Lerdau et al. 1995), and to be delayed following severe defoliation, especially late in the season (Quiring and McKinnon, 1999; Dumerle, 1988), as a result of removing the nutrients that would otherwise have been invested in bud production for the following years’ growth. Phenotypic variation in budbreak has been shown to have significant effects on forest lepidopteran species and other herbivores’ success (Dumerle, 1988; Chen et al., 2003; Clancy, 2002; Larsson, 2002; Barbosa and Baltensweiler, 1987; Ivashov et al., 2002; Lawrence et. al, 1997; Watt and McFarlane, 1991; Watt, 1987; Hunter et al., 1991; Hunter, 1992; Onstad and Reissig, 1986). Clancy (2002) includes phenological asynchrony between herbivore and host in a list of ten mechanisms of resistance of trees to defoliators. Watt and McFarlane (1991) found higher larval survival and greater growth in two consecutive years when the hatch of the winter moth (Lepidoptera: Geometridae) was closely synchronized with its host, sitka spruce, however in the three following years survival and growth were decreased, in part due to a loss in hatch and budbreak synchrony.

Actual dates of foliage emergence were not recorded in the present study, and branchlet length was used as a surrogate for branchlet age. The assertion that the foliage of the early-leafing families (fa7 and sg2) was older is supported by the observations that the branchlets were longer than late-leafing families, were lower in nutrient and moisture content than those families, and contained higher levels of total phenolics, which were indicative of slowing tissue growth (aging foliage) (Herms and Mattson, 1992; Bryant et al., 1983). The foliage from the two fertilization treatments also was generally longer than the control, but it is
difficult to say if budbreak occurred earlier than conspecifics in the presence of greater nutrient supply or not, given that this foliage also contained a greater percent nutrient and moisture content and lower total phenolics content than did the shorter foliage of the control treatment. Other than total phenolics, and possibly foliar N, the tests of the interaction in foliage length and many other variables between the fertilization treatments were not significant, supporting the strong genetic component controlling this trait. This pattern in budbreak was again observed in 2003. But the fact that larval growth rates were at times greater in the low fertilization treatment for a family and at other times greater in the high fertilization treatment, and the observed interaction in total phenolics. Tree height growth variability between families and fertilization treatments in 2002 suggests this was a possibility.

2.4.2. Tree Growth and Soils Analyses

Trees grew an overall average of approximately 75 cm in the control treatment in 2002, and 92 cm in overall total height each year. Average diameter growth across treatments was 1.1 cm (at one meter high on stem) per year for the period 2001-2003 at the Jeanfreau site. Krauss (2000) reported a height growth increment of 38.5 cm and diameter growth increment of 1.7 cm per year in the first two seasons following planting at this site. Other sources recorded annual height growth of four-year old baldcypress at 51 cm per year and average diameter growth at age 21 of 0.81-1.34 cm per year (Krinard and Johnson, 1976). Baldcypress saplings planted in a weeded, non-flooded but well-watered situation grew an average of 123 cm in height per year over a three-year period (Williston et al. 1980).

Krauss et al. (2000) reported that height growth increment after two growing seasons (1996-1997) of these families planted at three separate sites including Jeanfreau was greatest in
families sw1 and sw2. Mean initial tree height prior to the fertilization treatments in this study in 2001 indicated families sg3, sg2, and fa8 were the three tallest families and families sw1 and sw2 were among the shortest. Interestingly, families sg3, sg2, and fa8 were from brackish-water swamp seed-sources and families sw1 and sw2 were from freshwater seed sources. Also, the only four trees at the Jeanfreau site to produce enough cones for seed collection in the fall of 2003 were from the “sg” and “fa” family groups (Appendix C, Table C2).

Diameter growth in the Krauss (2000) study across the three sites was greatest in families sg2, fa7, and sw2. Interestingly, families sw1 and sw2 were again among the best performing families in diameter growth in the Krauss et al. (2000) study. Initial diameter measurement in 2001 indicated families sg3, fa2, and fa8 were the three largest trees, and families cb3, sw2, and sw1, the three smallest (in descending order).

Given that stem radial growth is more susceptible to the effects of overcrowding due to competition for light, fertilization might be expected to increase terminal shoot growth more readily than diameter growth (Kozlowski 1971). This may explain the observed lower diameter growth in the low fertilization treatment in comparison to the control and high fertilization treatments in 2002. Mean height growth was on average greatest in the low fertilization level in 2002. The control and high fertilization treatments exhibited reduced height growth in 2002. The reduction in height growth at the high fertilization rate could be due to inherent site conditions, competition, or that the high fertilization levels may have had a cumulative neutralizing effect on shoot growth, acting as salts and creating an imbalance between other necessary nutrients (Kozlowski 1971).
Soils analysis following the 2002 growing season (August) indicated the pH was lower, P- and K-concentrations greater, and chloride ions lower in the low fertilization treatment. Though not markedly so, the control treatment contained a higher mean N concentration than the fertilization treatments. The high fertilization treatment contained a lower mean Cl and P concentration and higher pH than the low fertilization treatment but comparable N and K concentrations. Initial diameter and height indicated growth (over the five-year interval leading up to the point the stand was fertilized in the present study) in some families from brackish sources may have outperformed the families from freshwater sources (sw1, sw2); and perhaps the interstitial soil water-salinity levels affected tree growth and larval growth. Family sw2 was a larval bioassay tree, however the late-leafing status of this tree and the given methodology showed that larval growth was as good as presumably less affected salt-tolerant families (cb3, fa7, sg2, ve2).

Patterns in height growth in 2003 were not consistent with an earlier fertilization response. Trees in the control treatment grew greatest in height in 2003. The decrease in height growth in the low fertilization level in 2003 could have been due to a real decrease in growth. However, in early summer of 2003, Tropical Storm Bill struck the coast of Louisiana and 15 of the taller trees in the two fertilized treatments were wind-damaged, whereas trees in the control were downwind, somewhat protected, and suffered very little damage. When height measurements were taken in 2003, many of the trees in the fertilized treatments had to be physically righted to allow for comparable measurements, but tipping could have damaged feeder roots, resulting in less moisture and nutrient uptake.

The differences in diameter growth between years could be attributed to many things separately or in combination with each other, including a delayed change in growth,
photosynthesize allocation within trees in response to fertilization, competition between trees as growing space became limited, differentiation in the measurement time (December 2001, 2002; September 2003), rainfall and other abiotic factors, or root disturbance due to foot traffic during foliage collection and tree measurement.

2.4.3 Limitations

There were conditions in this study which limit conclusions. The three fertilization blocks were assumed to be fairly homogeneous. The results of the soils analysis indicated some variation was present. The plot size also limited family replication. It is very likely that given a larger sample size and/or longer time frame, greater differences in tree growth among families could be detected, and likely differences in larval growth due to these and other inherent differences.

The larvae were reared in a growth chamber at a constant temperature, which maintained growing conditions for the BCLR at a near-optimum level, and so development in the field would likely have been longer and subject to greater variation in foliage changes than were provided in the rough three-week period provided here.

The foliage was collected from the same trees in the early spring, however the trees were not noticeably defoliated. Most deciduous trees are less affected by defoliation than evergreens due to greater below ground storage of carbohydrate reserves, and early spring defoliation tends to be less severe because trees may refoliate and have the remainder of the growing season to produce photosynthesize to allocate to the production of leaf primordial and growing meristems (Haukioja, and Honkanen, 1997; Kozlowski, 1971; Herms and Mattson, 1992). Timing is important however, and as foliage can begin producing photosynthesize before the entire leaf has ceased expansion, the removal of source leaves even in the early
spring may in some cases alter the following years’ foliage quality (Ruohomaki and Chapin 1996; Kozlowski, 1971). Though it is possible the foliage removal had an effect on the tree growth and foliage quality in 2003, it was unclear what role this may have played in this study. Foliage samples for chemical analyses were obtained earlier in the year and contained generally greater nutrient content among all treatments, including the control. Foliage length, usually smaller/shorter in years following severe defoliation, was not significantly different in 2003. Tree diameter growth was reduced in 2003, however it was also reduced in trees not used in larval feeding assays.

Foliage constituents other than those analyzed here are important to larval growth and performance. Larval growth is maximized in the presence of optimal ratios of macronutrients, moisture content, and necessary trace elements to secondary compounds and tissue toughness (Mattson and Haack, 1987; Clancy, 1992; Mattson and Scriber, 1987; Feeny, 1970; Wagner and Tinus, 1984; Redak and Cates, 1984). Foliar carbohydrates have also been found in some instances to stimulate Lepidoptera: Tortricidae larval feeding (Albert and Bauce, 1994; Guertin and Albert, 1992). How much and in what ratios to each other these constituents were present in the foliage available to these larvae is unknown.

2.4.4 Conclusions

Initial family mean tree diameter and height at this site suggests that in the absence of a freshwater diversion, a site impacted by salinity may have cumulative, stressing effects on more halophytic individuals over time, and as per the plant stress hypothesis, may potentially predispose those individuals to herbivory (White, 1984). In these situations, the performance of individuals better adapted to saline conditions would be much more important than was illustrated here. It is well documented that genetic provenances of trees can vary significantly
in rates of growth (Bigras and Colombo, 2001), and the propensity of some families to out-compete others in stressful situations could have important implications from the herbivore-management standpoint.

This study also showed tree and larval growth were generally reduced at the high fertilization rate. These results suggest that should a diversion enter an existing baldcypress stand, the loading-rate of these additions, in conjunction with soil and other site conditions present may combine to determine the timing and direction of the response, and the long-term health of the stand. One of the effects of a diversion this study could not duplicated was the removal of stagnant water and the flushing action of interstitial soil water, which here showed residual salinity levels existing from past hydrological events. The removal or dilution of harmful levels of toxic ions via flowing water is known to improve baldcypress growth (Louisiana Dept. Nat. Res., 1998; Lane and Day, 1999; Conner and Day, 1976; Day et al., 1988).

If tree vigor is increased by the long-term effects of a river diversion, host foliage suitability may increase as well. Because pupal mass is often positively correlated with measures of adult fitness such as fecundity, male flight capability and fertilization success, there is the potential for BCLR population increase (Awmack and Leather, 2002; Haukioja and Neuvonen, 1985; Brewer et al., 1985; Hennebary and Kishaba, 1966; Clancy, 1992; Hunter and Docherty, 1991; Redak and Cates, 1984). Conversely, because vigorously growing trees are often more associated with lower levels of soluble nitrogen, increased N content may not necessarily favor an outbreak (White, 1984). And if an outbreak did occur, the more vigorous trees may be better suited to tolerate or compensate for defoliation, with greater growth rates,
greater nutrient and carbohydrate reserves, and greater total foliage production, than stressed conspecifics.

In either case, this study showed the importance of the effects of host phenology on herbivore growth, and this and other genetic components also should be taken into consideration when planning coastal restoration plantings using baldcypress or other trees/plants with potential insect pests. The leafroller is known to span a period of 10-16 days in larval emergence from the same eggmass, which is also roughly the range in area baldcypress budbreak, which implies that use of tree differences in budbreak would require employing trees at either extreme of the phenological window to be effective. If baldcypress is obtained from seed source in the northern or southern limits of its range, these extremes may produce such phenology differences among individuals. The genetic control (and relative consistency over time) of budbreak supports the potential for planting the early-leafing, salt-tolerant families fa7 and sg2 in a coastal restoration context, which may allow some degree of escape from the effects of defoliation.

From this study I conclude that differences in host phenology and other genetic traits and insect phenology are important to the fitness of each, and host genetic differences may be used to offset the effects of herbivory as well as saltwater intrusion. Further, I would argue strongly against planting monocultures of salt-tolerant baldcypress genotypes, but rather several with widely varying dates in budbreak and other inherent differences to reduce the potential for BCLR population growth. Additional study is warranted to explore the possibilities of increased host palatability to the BCLR in the event a river diversion is allowed into a host stand, and how differing families will perform with respect to phenological differences in budbreak and other inherent differences.
CHAPTER 3

EFFECTS OF THINNING AND GENETIC VARIATION ON THE GROWTH AND DEVELOPMENT OF AN INSECT DEFOLIATOR, *ARCHIPS GOYERANA KRUSE* (THE BALDCYPRESS LEAFROLLER) ON HOST *TAXODIUM DISTICHUM* L. RICHARD (BALDCYPRESS)

3.1 Introduction

Much of coastal Louisiana is rapidly eroding, leading to increased forested wetland loss and decline. It is estimated that coastal Louisiana is losing land at rates between 25-35 square miles per year, and may lose approximately 400 square miles of swamp, including forested wetlands, by year 2050 if corrective action is not taken (Lou. Dept. Nat. Res., 2003). It is likely that much more of the Gulf states’ coastal forested wetlands will decline over the next century, as natural processes such as land subsidence, sea level rise, and coastal erosion couple with anthropogenic factors including hydrological changes (river levees) and canal construction, and lead to increased and prolonged flooding and saltwater exposure (Lou. Dept. Nat. Res., 2003; Hammar-Klose, 2001; Moore, 1967; Coastal Environments, 1984).

The potential losses could mean severe consequences for the state of Louisiana, other states along the Gulf of Mexico, and the nation. The oil and natural gas industry depends on resources from wells both inshore and offshore Louisiana and other gulf states, and relies on coastal ports and waterways for access and support of offshore activities. The combined annual value associated with the economy in the coastal zone of Louisiana is well into the billions, and is affected by the oil and natural gas industry, waterborne commerce, commercial fishing, recreational fishing and hunting, fur harvests from trapping, alligator harvests, and eco-tourism (Lou. Dept. Nat. Res., 2003). These wetlands also are important
ecologically by providing water quality treatment functions, slowing and reducing the force of hurricanes, and providing habitat for unique organisms and endangered wildlife (Allen et al., 1998; Smith, 1983; Chabreck, 1972; Coreil, 1997; Duerr, 1997; Craig and Day Jr., 1977).

The detrimental effects of saltwater intrusion on forested wetlands in Louisiana are exacerbated by the effects of an insect herbivore, *Archips goyerana* Kruse, the baldcypress leafroller (BCLR). The BCLR in the larval stage has been shown to annually defoliate hundreds of thousands of acres, many of which occur in the coastal wetland zone (Goyer Lenhard, 2002). The BCLR emerged in 1984 as a pest of baldcypress, and is currently expanding its range, being recovered as far north as Jackson, Mississippi (Goyer and Lenhard 1988, Kruse, 2000). Annual defoliation of baldcypress has resulted in significant reductions in radial tree growth, as well as tree mortality to repeatedly defoliated baldcypress saplings (Goyer and Lenhard 1988; Goyer and Chambers, 1997). The presence of multiple stress agents acting on these forests will only worsen growing conditions and speed the degeneration process in these fragile forested wetlands.

In response to declining swamplands, federally and state sponsored programs have been implemented and more are proposed to prevent and delay the potential losses (Lou. Dept. Nat. Res., 1998). Currently two public sources of funding are in place to combat these problems with restoration projects. The federal Coastal Wetlands Planning, Protection, and Restoration Act (CWPPRA) with a 25% state cost share provides 30 million dollars annually, and the state Louisiana Coastal Wetland Conservation, Restoration, and Management Act provides up to 25 million dollars annually from oil and natural gas severance tax, lease payment, and royalty revenues for coastal restoration projects (Coreil, 1997). Among the methods currently
proposed to restore forested wetlands are vegetative planting projects (Lou. Dept. Nat. Res., 1998; Coreil, 1997).

Successful baldcypress regeneration is generally considered to be lacking in coastal forested wetlands of Louisiana due to the hydrological conditions described above and mammalian herbivory (Souther and Shaffer, 2000; Conner, 1986; Conner and Toliver, 1990; Williston et al., 1980). Such conditions have spurred research on the potential for using more salt-tolerant vegetation. Baldcypress (\textit{Taxodium distichum} L. Richard), has exhibited significant intraspecific variation in its ability to grow under saline conditions (Krauss, 1996; Krauss et al., 1998; Krauss et al., 1999; Krauss et al., 2000, Allen et al., 1998; Allen et al., 1997; Allen et al., 1996). The present study enlists the use of half-sibling families of baldcypress saplings screened for tolerance to salinity and planted in 1996 in a predominantly freshwater environment located east-southeast of New Orleans, LA as part of a larger study evaluating the growth of these families in more saline environments nearby (Krauss 1996). Currently efforts are underway to produce offspring from these salt-tolerant baldcypress in state owned nurseries, which can then be used in a coastal restoration context (R. Goyer, personal communication).

Protocols for artificial regeneration might employ baldcypress of several genetic lineages, thereby being more homogeneous than a natural stand, in plantation utilizing suitable formerly-forested sites. To achieve proper stocking densities and cover seedling mortality, higher numbers of seedlings may be planted initially, requiring an eventual stand density reduction as competition for resources increases with tree size. Tree response to these altered growing conditions and varying genotypes (salt tolerant families) may alter susceptibility to the BCLR. The potential for defoliation, reducing tree growth and/or causing mortality, will
put these restoration plantings under greater stress, as predicted salinities and/or prolonged flooding conditions arise. The insect component should be addressed where possible as another threat to the health of these future forests.

Repeated observations suggest that relatively young, pole-sized and sapling baldcypress (30-45 cm diameter, 30 cm above butt-swell) growing along the stand edges are heavily preferred over older, larger, interior trees or smaller, understory trees (Goyer and Chambers, 1997; Meeker and Goyer, 1993; Goyer and Lenhard, 1988). Trees sampled at approximately 20 m intervals interior to the forest edge were found to contain significantly fewer egg masses than those on the stand edge (Goyer and Lenhard, 1988). It is likely that a combination of many factors are responsible for the occurrence of BCLR on these trees, but these trees likely receive more light than interior studies.

There are no reported studies of differential response among baldcypress half-sibling families to thinning. It is expected that the stand as a whole will respond to increased growing space and greater light radiation similarly to other studies with baldcypress, and generally all forest trees, with increased crown growth and increased radial growth (Prenger, 1985; Dicke and Tolliver, 1988; McGarity, 1979; Kozlowski, 1971; Goelz, 1999; Miller, 1999). Foliage-level responses to thinning, which allows more light into the tree crown, generally include greater total photosynthetic rates, leading to greater C availability and increased structural integrity, which is often expressed in terms of greater toughness, and in some cases greater secondary compounds (Mitchell et al., 1992; Kolb, et al., 1998; Larsson et al., 1986, Schoonhoven et al., 1998; Waterman and Mole, 1989). Another possible response to higher light intensity is the occurrence of the appressed morphology. Baldcypress is known to possess two foliage morphs, an open and appressed form (Brown and Montz, 1986). These
two morphs are sometimes found within the same trees, and the appressed morph is often expressed in the upper portions of the crown (Meeker and Goyer, 1993). Trees expressing the appressed foliage type repeatedly and completely escape defoliation from the BCLR, and though nutrient content is similar, the appressed morph tends to be more tough (Meeker and Goyer, 1993; Meeker, 1992). The appressed leaf morphology is characterized by very narrow-angled needles, which lie closely to the rachis (mid-rib) of the branchlet, while the “open” morphology needles grow at wide angles to the central midrib and are more exposed to solar radiation, particularly at midday (Meeker, 1992). The closed leaf morphology is largely expressed in more mature trees than those used in the current study, however it is a phenotypic expression and so is, in part, under genetic control (Meeker, 1992). Other plants have been found to produce leaves with lower surface area and angled surfaces in parts of the plant receiving high amounts of solar radiation, and these leaf characteristics are thought to be expressions aimed at conserving foliar moisture, allowing more light in the canopy, and are resistant to some foliage consuming herbivores (Mitchell et al., 1992). Whether or not any of the salt-tolerant families present in this study are capable of expressing the appressed foliage morphology in increased light conditions at this early age have not been evaluated and could prove useful in restoration context should they exhibit this ability.

Thinning also will reduce the degree of competition among these trees, and allow for remaining individuals to eventually gain access to greater resources. Based on proposed plant growth-differentiation curves, these conditions may favor improved tree growth as well as greater carbon assimilation, depending on nutrient availability status, which may (or may not) necessarily lead to lower foliage suitability (Lorio, 1986; Herms and Mattson, 1992). The dual increase in resources resulting from thinning should favor tree growth over differentiation.
The differential performance of the BCLR reared on five half-sibling families of salt-tolerant baldcypress in response to these altered growing conditions is unknown and sets the stage for the research presented herein. The practical results of this study should shed light on the potential effects of host tree genetic variation and thinning on BCLR populations, elucidate possible families innately better adapted to withstanding the BCLR, and the potential implications for the health of baldcypress forests in restoration circumstances. Thus, the objective of this study was to:

Determine the effects of thinning on five half-sibling families of *Taxodium distichum* L. Richard (baldcypress) and how the host-level response also would affect the growth and development of an insect defoliator, *Archips goyerana* Kruse (the baldcypress leafroller).

### 3.2 Materials and Methods

#### 3.2.1 Site Characteristics, Stand History, and Current Study Design and Methods

##### 3.2.1.1 Site History and Purpose

The “Delacroix” site chosen by Krauss et al. 2000 is located near New Orleans, Louisiana on the east side of Highway 39 in Scarsdale, Plaquemines Parish, at N 29°49.9', and W 89°57.5'. The soil at the site is of the Clovelly muck association (Krauss, 1996) and is located on the unprotected side of the hurricane protection levee. However the area is dominated by primarily freshwater conditions due in part to a nearby flood-control pumping station. Soil salinity measurements indicated a mean of 0.1 g/l (0.1 ppt) at Delacroix during the Krauss (1996) study, and measurements taken here indicated a mean salinity level of 0.6 g/l (0.6 ppt). The objective of the Krauss study (1996) was to evaluate the growth and survival of ten, half-sibling genetic families of baldcypress selected for salt-tolerance in a field situation. The
Delacroix site was the freshwater habitat site of three total plots. The site was periodically inundated at times to a depth of up to 0.75 m. In preparation for establishing the Delacroix site, seeds were collected by (Krauss et al., 2000) from eight brackish-water baldcypress stands and two freshwater baldcypress stands. Four hundred baldcypress seedlings were grown in a greenhouse for nine months and subsequently planted on the site in January of 1996 (Krauss, 1996).

Of the five families used for insect feeding assays, four were from brackish-water source stands and one was from a freshwater source stand. Family cb3 was collected from a brackish-water source stand in the Mobile Bay area, Alabama. Family fa7 was collected from a brackish-water source in the Falgout Canal area near Houma, LA in Terrebonne Parish. Family sg2 was collected from a brackish-water source near the E.J. Gore Pumping Station, near the junction of hwy 39 and 46 in St. Bernard Parish. Family sw2 was collected from a freshwater source in the Sherburne Wildlife Management Area, in bottomland swamp adjacent to the Atchafalaya River in Iberville Parish. Family ve2 was collected from a brackish-water source in the present Mississippi River Delta near Venice, LA in Plaquemines Parish. Previous work under greenhouse conditions had determined that of these five families, cb3 exhibited the greatest tolerance to salinity, and family sw2 was the least tolerant (Allen et al. 1994). Results of the Krauss study indicated families fa7, sg2, and even sw2 performed well in a field environment among the ten families used (Krauss, 1996).

The seedlings were planted according to a complete randomized block design consisting of ten blocks, each containing four replicate trees of the ten families. The site was thinned in 1999 by removing every other diagonal row, and with the exception of scattered mortality,
two trees each of ten families remained in each of ten blocks, totaling approximately 200 trees.

### 3.2.1.2 2001 Plot Design and Treatment Allocation Rationale

In the winter of 2001, all trees at the Delacroix site were measured for diameter at one meter above ground-level using a diameter tape and total height using a Lasertech® Impulse laser altimeter. Diameter and total height means and tree mortality were used to determine the location and extent of tree growth differences between blocks. Half of the plot was thinned by removing every other diagonal row, and the other half maintained as the control (Figure 3.1). Trees along the periphery as well as one block with noticeably smaller trees were excluded from measurement and feeding. All insect data was based on four replicate trees each of families cb3, fa7, sg2, sw2, and ve2 within each of the treatments for a total of 40 trees. See figure 3.1 for a map of the experimental plot and figure 3.2 for a photograph of the Delacroix site.

Each tree’s diameter one meter above ground and total height was measured at the end of the 2002 growing season following the methods previously described, and again in September of 2003. Though the trees may have continued to grow beyond the September 2003 measurement, comparisons at the given level of precision should still be valid, as one might expect the majority of the growth to have taken place earlier in the growing season.

### 3.2.1.3 Soil Samples

Soil samples were obtained at the close of the 2002 growing season application using a Nasco stainless steel, one-inch diameter soil corer. Cores were taken to a depth of six inches (Wilde et al., 1964). Multiple cores were taken from each of Krauss’ ten original blocks (shown in detail in results-Figure 3.13) and combined into one composite sample per block,
totaling 5 composite samples each in the control and thinned treatments. Samples were air
dried at approximately 50° C then ground with mortar and pestle. Analyses were conducted
by the L.S.U. Department of Agricultural Chemistry Laboratory using methods for total
Kjeldahl nitrogen described in the AOAC 16th Edn (1995a) and phosphorous and potassium
by methods described in the AOAC 16th Edn (1995b).

3.2.2 Foliage Collection and Analysis Methods and Larval Bioassay Setup Procedure

3.2.2.1 General Larval Setup and Foliage Sampling Method

Foliage for feeding assays was collected from families in the order of budbreak. Field
observation indicated families fa7 and sg2 began leafing out the last week of February. On
March 3, 2003, the foliage on families fa7 and sg2 was noticeably longer than families cb3
and ve2, and many of the trees in family sw2 had not begun leafing out. The first leaf
collection was made from families fa7 and sg2 across thinning treatments on March 25, 2003
when second instars were becoming available in the field. Family ve2 was collected one week
later on April 1, 2003. Lastly, families cb3 and sw2 were collected on April 4, 2003. There
was a gap of nearly two weeks between starting dates separating the earliest-leafing families
and the latest leafing family. Foliage for chemical analyses was collected in a manner
analogous to that of the feeding assay setup. The foliage for chemical analyses was collected
when the larvae in the respective families were in 4th-5th instars, which was in the order in
which the families were sampled (families fa7 and sg2 were sampled across fertilization
treatments for chemical analyses on April 16, 2003; families cb3 and ve2 on April 23, 2003;
and family sw2 on April 30, 2003).

Foliage for all parameter measurements was sampled between 10:00 am and 2:00 pm, at
random from the crown from all aspects, but primarily from the mid-crown. Trees were
Figure 3.1. Delacroix 2001-2003. The control is located on the left half, the thinned treatment on the right half. Cells denote tree location, contain tag numbers, family name. “c” denotes trees coppiced in 1996 (Krauss 1996). Broken lines denote buffer strip. Families embolded are larval bioassay trees. The bottom of page parallels the levee; top of page parallels marsh.
Figure 3.2. Delacroix site, spring 2003. Center of photograph is looking down a thinned row from the thinned side of the plot. Background is looking toward rear of plot, away from levee.

Photo by Jerry Lenhard.
randomly chosen and sampled across treatments to minimize potential changes due to temperature fluctuations during the four hour sampling period. Approximately 40 g fresh weight (8 g dry weight) of each trees’ foliage in families cb3, fa7, and ve2 was collected for nitrogen, phosphorous, and potassium. Only three of five families were sampled due to a lack of significant differences obtained in the feeding assays. These families contained the earliest-leafer, fa7, and two late-leafers, cb3 and ve2. The samples were stored in plastic bags, placed on ice, and transported to the laboratory, and promptly stored in an ultra-freezer at -80° C until needed. The foliage was dried at approximately 57°C for 48 hours. Needles were separated from the central rachis and the rachis excluded from chemical analyses. Dried needles were ground to a fine powder using a mortar and pestle. The foliar NPK analyses were conducted by the Louisiana State University Dept. of Agricultural Chemistry using methods described in the 16th Edn AOAC (1995a) for foliar N, and methods described in the 16th Edn AOAC (1995b) for foliar P and K concentrations were expressed as a percent dry weight.

3.2.2.2 Foliage Samples for Physical Characteristics and Moisture Levels

Samples were taken from the trees between 10:00 am and 2:00 pm. Trees were randomly chosen and sampled across fertilization treatments to minimize potential changes due to temperature fluctuations during the four hour sampling period. Branchlets were randomly sampled within each tree primarily from the lower- to mid-crown. One sample of 15 branchlets from all 40 larval assay trees was collected simultaneously for length and width measurements, on March 10 2003. Branchlet length was measured to the nearest millimeter, from the petiole base to the terminal needle tip. Branchlet width was measured at the widest point of each branchlet, typically at the middle of the branchlet, from needle tip on one side of
the rachis (central stem on which needles are attached) to the needle tip directly opposite. Needles typically project from the central rachis at similar angles along the length of the rachis, and needles were maintained in their natural state for width measurements. One sample of foliage was collected from each of the feeding trees used to measure the angle of needle appression on May 30, 2003 at the close of the feeding study. Samples for foliar moisture analysis also were collected using both a simultaneous method, whereby all families were sampled on the same day and the effects of differential dates of leaf emergence would be most strongly expressed, and families were also sampled by budbreak, in the order in which larvae were started. These two methods give an estimate of the foliar value differences on given day as well as the moisture values available to larvae if there was a reduction in budbreak variation among host trees. The date of the first collection for the early-leafing families in the sample by budbreak scheme was the same day that all families were sampled (across the control and thinning treatment) for the simultaneous sample, on April 16, 2003. The next two later-leafing families, ve2 and cb3, were sampled one week later on April 24, 2003 and the final family sw2 sampled on May 2, 2003. Foliage for moisture measurement was collected between 10:00 am and 2:00 pm at each sampling date, sealed in plastic bags, stored on ice and transported to the laboratory. Two subsamples of approximately 2 g foliage fresh weight (approx. 10 branchlets each) for each tree was immediately weighed upon returning to the laboratory and then oven-dried at approximately 57°C for 48 hours. Cup weights were subtracted when calculating percent foliar moisture.

3.2.2.3 Larval Collection and Feeding

Field-collected larvae were used for all feeding bioassays. Larvae were collected from field populations near Gramercy, LA and Norco, LA near HWY 61. Larvae were collected by
clipping infested branches and transporting to the laboratory, where larvae were collected from within the webs and as they left the foliage. Fifteen second-instar larvae were randomly assigned to each tree in the family-thinning treatments, totaling 600 larvae. Larvae were randomly assigned to individual replicate trees across thinning and family treatments to minimize variation in foliage quality and to avoid using larvae that may have remained in collection materials and starved longer than others. Foliage used in rearing was collected weekly and transported to the lab on ice, where it was refrigerated until no longer needed. Foliage was collected on one side of the tree each collecting trip, and the side used rotated each trip so that all cardinal directions were sampled. At each collection, foliage was collected from lower to upper crown, and along the inner and outer segments of lateral branches. Larvae were reared in clear plastic diet cups (29.5 ml). The foliage was cut with scissors and placed into cups and replaced as needed or every three days. The larvae were reared at 21°C under 12:12 light: dark photoperiod. Beginning weight, fresh pupal weight, development time in days, dry pupal weight, and sex was recorded. Relative growth rates were calculated as the fresh pupal weight – beginning weight/ development time (days). Fresh pupal weights were obtained daily upon pupation. Pupae were then oven-dried at approximately 57°C.

3.2.3 Statistical Analysis

3.2.3.1 Insect Data Analysis

The insect data were analyzed using the SAS mixed procedure (SAS Institute, Inc., 2001) as an unbalanced, complete randomized design, 2 x 2 x 5 factorial ANOVA. Sex was entered as a treatment with two levels, thinning with two levels, and five half-sibling families were used. Each factor was considered fixed for all variables analyzed. Larvae were considered
subsamples within the replicates (trees). The error term used to test the main effects thinning, family, and the interaction consisted of the replicates nested in the thinning by family by sex interaction, and was used in the random statement of the mixed procedure.

Larval mortality was included as tabular data in the results; it was not analyzed due to confounding factors not controlled for while setting up. The time larvae a single larvae collection bag was used was minimized to ensure vigorous larvae were selected, however some larvae were replaced early in the bioassay and it is suspected some larvae may have been in collection bags long enough to influence second instar mortality. Mortality was classified according to unknown factors, parasitism, and mishandling injury.

3.2.3.2 Foliage Parameter Analysis

Foliage samples for nitrogen, phosphorous, and potassium also were analyzed as an unbalanced CRD, 2 x 3 ANOVA with two thinning levels and three families. Again the error term consisted of the replicate nested in the thinning by family interaction, but sex (insects) was removed from the model. Foliage samples for branchlet length, width, angle of appression, and moisture were analyzed similarly with the exception that all five families used in insect bioassays were used in the model, so that it was a 2 x 5 ANOVA. For foliar moisture analysis, two subsamples were used within each tree. For branchlet length and width analysis, fifteen branchlets (subsamples) were used per tree. For needle angle of appression, ten branchlets (subsamples) were used per tree. For each of these analyses, the error term consisted of the replicate nested in the interaction between thinning and family, and was used in the random statement of the mixed procedure.
3.2.3.3 Tree Growth Parameter Analysis and Other General Procedures

For tree growth response, all trees of the ten total families in the plot falling within predetermined treatment blocks and containing sufficient replication were included to increase the total experimental units. Trees used in feeding assays were included in overall tree growth measurements despite the leaf removal. There was insufficient replication in the interaction to consider defoliation a treatment. Both diameter and height growth were calculated as the difference between initial diameter and height and the final diameter and height each year (Dec. 2002, Sept. 2003). The data were analyzed precisely as described above, with the exception that year and thinning were included as a treatments, and four other families added, so that the model was an unbalanced 2 x 2 x 9. Only nine families were used due to no replication of family sg3 in the thinned treatment. Where needed, variables were log-transformed. The design of this analysis makes the assumption that after the block of trees were excluded based on preliminary measurements, the remainder of the site is relatively homogeneous with respect to tree growth and indirect effects on larval growth.

All hypothesis tests were conducted at the 0.05 alpha level. The least squares means were separated using the Tukey-Kramer method for unbalanced designs, Satterthwaite option used to calculate degrees of freedom for unequal sample sizes, and type III sums of squares option used to test hypotheses. The Shapiro-Wilkes test was used to test the assumption of normality. Upon analysis, variables were log-transformed to meet the assumptions of normality and the homogeneity of variance. In a very few cases additional outliers were excluded based on the stem and leaf plot in the univariate procedure in SAS, which denotes extreme residual values (observations) with an asterisk. Extreme values in that sense are defined as those values located beyond the 25th or 75th quartile by a distance of three times the interquartile range, or
beyond the 99th percentile of the data range. A list of these observations is located in Appendix B (Table B1). Deletion of observations following this procedure was followed once per dataset, and additional observations identified with asterisks were not removed.

In some cases the Shapiro-Wilkes values indicated the assumption of normality had been violated in the insect datasets after the above procedures were applied. However, the violation was largely due to the extension of the tails in each case, and insect sample sizes were quite large. With males and females combined, each insect variable measured totals not less than 500 individuals. This large sample size is very powerful, and examination of stem and leaf plots support this decision. Also, the distribution of the means of these individuals were more normally distributed than the individuals alone. A plot of the residuals on the predicted values as well as a regression of the variance on the means was used to evaluate the assumption of homogeneous variance. Analyses were conducted on transformed variables where necessary, but reported here are the original means accompanied by their standard errors.

3.3 Results

3.3.1. Larval Growth and Performance Parameters

3.3.1.1 Dry Pupal Weight

There was no significant interaction in dry pupal weights between sex and either of the thinning and/or family treatment combinations. When sexes were combined, a significant interaction in dry pupal weights revealed that families cb3 and fa7 in the thinned treatment were heavier than in the control, while pupal weights in families ve2 and sw2 were heavier in the control than the thinned treatments \(F_{4,59.9}=5.85, p=0.0005\). Subsequent mean comparisons indicated female dry pupal weights in family cb3 were significantly heavier in the thinned treatment than in the control \(F_{67.8}=3.75, p=0.0446\) (Figure 3.3). The females and
Figure 3.3. Female mean (± 1 SE) dry pupal weight by family and thinning treatment. Means marked with different letters were considered significantly different at the 0.05 alpha-level using the Tukey-Kramer test. Sample sizes ranged from 15-32 pupae (4 trees) per bar.

males in family cb3 exhibited the greatest difference between the thinned treatment and control. Females were significantly larger in the thinned treatment than those in the control, which is somewhat unexpected given the lower foliar moisture in the thinned treatment. The trend in means does not support any differences that may have been expected based on the order of leaf expansion among families.

3.3.1.2 Relative Growth Rate

The analysis of larval relative growth rate (RGR) indicated that the interaction between thinning and families was not significant (F$_{4,64}$=2.09, p=0.0925). Results, however, suggested that family was a significant factor affecting growth rate (F$_{4,64}$=6.55, p=0.0002), as females grew at a significantly greater rate in late-leafing family sw2 than did those on family cb3,
which is unexpected given that cb3 is also a late-leafing family. The two early-leafing families fa7 and sg2 did not differ significantly from the other families, but were lower in rates of growth than the late-leafing families sw2 and ve2 (Figure 3.4). A plot of the means in the thinning by family treatment combinations is included as well (Figure 3.5). Females grew at a higher rate in the thinned treatment than did those in the control, however the difference was not significant at the given sample size. Family sw2 exhibited the highest RGR in each treatment. Families ve2, fa7, and sg2 were fairly static across treatments.

3.3.1.3 Development Time

The analysis of larval development time suggested a significant interaction between family and the thinning treatment ($F_{4,62.7}=2.62, p=0.0430$), as larvae on families cb3 and fa7 exhibited lower shorter development times in the thinned treatment, which was in agreement with the higher relative growth rates and dry pupal weights in the thinned treatment. However, families sw2 and ve2 exhibited longer development times in the thinned treatment. The post-anova pairwise comparisons failed to find significant differences between the treatments within the same family, but female mean development times are presented below (Figure 3.6). Larvae generally had shorter development times on the late-leafing families sw2 and ve2 than the early-leafing families fa7 and sg2.

3.3.1.4 Larval Mortality

Larval mortality was monitored and are presented below. Of 618 larvae reared for the Delacroix study, 15.4 % died due to unknown factors, parasitism, and mishandling injuries. See Table 3.1 for mortality rates within treatments.
Figure 3.4. Female mean (± 1 SE) RGR by family. Means marked with different letters were considered significantly different at the 0.05 alpha-level using the Tukey-Kramer test. Sample sizes ranged from 37-54 larvae (within 8 trees) per bar.

Figure 3.5. Female mean RGR (± 1 SE) in thinning by family treatment interaction. Means marked with different letters were considered significantly different at the 0.05 alpha-level using the Tukey-Kramer test. Sample sizes ranged from 15-32 larvae (4 trees) per bar.
**Figure 3.6.** Female mean (± 1 SE) development time (days) in the thinning by family interaction. Means marked with different letters were considered significantly different at the 0.05 alpha-level using the Tukey-Kramer test. Sample sizes ranged from 15-32 (4 trees) per bar.

**Table 3.1.** Percent mortality due to unknown factors (left) and percent mortality due to factors listed in Overall column (right). Total larvae (n=618)

<table>
<thead>
<tr>
<th>Family</th>
<th>Control</th>
<th>Thinned</th>
<th>Overall</th>
<th>% (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cb3</td>
<td>9.8</td>
<td>21.5</td>
<td></td>
<td>15.9</td>
</tr>
<tr>
<td>fa7</td>
<td>8.2</td>
<td>13.3</td>
<td></td>
<td>10.7</td>
</tr>
<tr>
<td>sg2</td>
<td>11.7</td>
<td>8.1</td>
<td></td>
<td>9.8</td>
</tr>
<tr>
<td>sw2</td>
<td>18.0</td>
<td>14.5</td>
<td></td>
<td>16.3</td>
</tr>
<tr>
<td>ve2</td>
<td>16.7</td>
<td>8.3</td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>Treatment</td>
<td>12.9</td>
<td>13.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.3.2. Chemical Foliar Parameters

3.3.2.1 Chemical Parameters- Nitrogen, Phosphorous, Potassium

Analyses of foliar nitrogen (N), phosphorous (P), and potassium (K) indicated generally
greater concentrations of these nutrients in the control treatment at the onset of the 2003
growing season. There was no significant difference between the control and thinning
treatment in N, P, or K when analyzed individually. However, when these elements were
combined, total percent NPK ions were significantly higher in the control than the thinned
treatment ($F_{1,18}=5.75$, $p=0.0277$; Figure 3.7). There were no significant differences among
families in N, P, or K when analyzed separately or when combined, nor was there a
significant interaction between thinning and family in these variables. Family cb3 had nearly
equal mean N concentrations in the thinned treatment and the control at the time of sampling,
whereas the other families exhibited greater foliar N in the control (Table 3.2). Total NPK
was higher across all families in the control treatment (Table 3.2). Family cb3 had a lower
mean foliar P and K in the thinned treatment than the control, however differences were not
significant.

3.3.2.2 Foliar Moisture Content

Analysis of foliar moisture levels ascertained that the control treatment had significantly
greater foliar moisture levels than the thinned treatment using both the simultaneous sampling
method as well as the staggered sampling method (when foliage was sampled by budbreak).
When sampled simultaneously across families and the thinning treatment, the foliage in the
control treatment had a significantly higher moisture content at 76.5 %, while the mean
moisture content in the thinned treatment was 72.8 % ($F_{1,30}=29.95$, $p<0.0001$). The families
Figure 3.7. Mean (±1 SE) foliar NPK combined by thinning treatments. Means marked with different letters were considered significantly different at the 0.05 alpha level using the Tukey-Kramer test. Sample sizes were composed of 12 trees per bar from families cb3, fa7, and ve2.

Table 3.2. Mean (±1 SE) foliar nutrient content by family. Foliage was collected by order of budbreak when larvae in each family were primarily in 4th-5th instars. Means are presented as percent of dry weight per sample. Sample size: n=4 trees, 20-30 branchlets per tree.

<table>
<thead>
<tr>
<th></th>
<th>Total N</th>
<th>Total NPK</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cb3</td>
<td>fa7</td>
</tr>
<tr>
<td>Thin</td>
<td>2.00±0.13</td>
<td>1.77±0.05</td>
</tr>
<tr>
<td>Control</td>
<td>1.99±0.10</td>
<td>1.91±0.09</td>
</tr>
</tbody>
</table>
also varied significantly in foliar moisture content ($F_{4,30}=6.31, p<0.0008$), as the late-leafing families sw2 and ve2 exhibited significantly higher foliar moisture content than the early-leafing family sg2. The late-leafing family sw2 had significantly higher moisture content than the early-leafing family fa7 as well. The late-leafing family cb3 did not differ significantly from the other families (Figure 3.8). There was not a significant interaction between thinning and families.

The significant thinning effect on foliar moisture levels remained strong when the families were sampled by budbreak ($F_{1,30.1}=40.75, p<0.0001$), with greater foliar moisture in the control treatment. However the differences among families pooled across the thinning treatments were no longer significant ($F_{4,30.1}=0.79, p=0.5429$). The range in foliar moisture between the two extremes was approximately 1.4% (Figure 3.9), whereas when sampled simultaneously, the range was greater than 4%.

3.3.3 Physical Foliage Parameters

3.3.3.1 Branchlet Length

There was no significant effect of thinning on angle of appression, foliage length, or foliage width. However, the early-leafing families fa7 and sg2 exhibited significantly longer branchlets at the date of collection than did the late-leafing family sw2 ($F_{4,30}=5.18, p=0.0027$). The late-leafing families ve2 and cb3 did not differ significantly from the other families, but were generally shorter than the early-leafing families fa7 and sg2 (Figure 3.10). There was no significant interaction in leaf length between thinning and family.

3.3.3.2 Branchlet Width

Baldcypress was shown to exhibit significant difference in foliage morphology from tree to tree and, also, to some extent within the same tree. The branchlets grew in width somewhat
**Figure 3.8.** Mean (± 1 SE) foliar moisture content by family using the simultaneous sampling method. Means based on 8 trees per family (thinning treatment combined). Means marked with different letters significantly different at the 0.05 alpha level using Tukey-Kramer test.

**Figure 3.9.** Mean (± 1 SE) foliar moisture by family using the staggered sampling method (by budbreak. Means based on 8 total trees per family (thinning treatment combined). Means marked with different letters significantly different at the 0.05 alpha level using the Tukey-Kramer test.
Figure 3.10. Mean (± 1 SE) branchlet length by family. Means based on 15 branchlets per tree, and 8 total trees per family (thinning treatment combined). Samples obtained simultaneously. Means marked with different letters were considered significantly different at the 0.05 alpha level using the Tukey-Kramer test.

independently of when the majority of each trees’ foliage began emerging.

The late-leafing family sw2 had branchlets nearly as wide as the early-leafing family fa7, despite the fact that the late-leafing family sw2 had significantly shorter branchlets at the time of sampling (Table 3.3).

3.3.3.3. Angle of Appression

Analysis of the angle of needle appression along the central rachis suggested thinning did not affect this trait (F_{1,20}=0.09, p=0.7679), nor was there a significant interaction between family and thinning (F_{4,20}=0.95, p=0.4564). The family effect, though not significant, did suggest some genetic variation in needle appression (F_{4,20}=2.23, p=0.1024). Family cb3
displayed the foliage with the highest degree of needle appression in both the thinning treatment and the control, followed by families fa7 and then sg2. The lowest mean angle of appression was exhibited by family cb3, at 41° (Table 3.3).

**Table 3.3.** Mean (± 1 SE) branchlet width (mm) and angle of needle appression (degrees) by family. Width means are based on 15 branchlets per tree, and means for angle of appression are based on ten branchlets per tree. Smaller values (degrees) of needle appression denote greater appression. Means consisted of eight total trees per family. E denotes early-leafing families, L denotes late-leafing families.

<table>
<thead>
<tr>
<th>Family</th>
<th>Branchlet Width</th>
<th>Needle Appression</th>
</tr>
</thead>
<tbody>
<tr>
<td>cb3</td>
<td>17.3 ± 0.38</td>
<td>41.8 ± 1.00</td>
</tr>
<tr>
<td>fa7</td>
<td>18.4 ± 0.36</td>
<td>45.2 ± 0.95</td>
</tr>
<tr>
<td>sg2</td>
<td>18.1 ± 0.39</td>
<td>47.3 ± 0.84</td>
</tr>
<tr>
<td>sw2</td>
<td>18.4 ± 0.43</td>
<td>49.9 ± 1.40</td>
</tr>
<tr>
<td>ve2</td>
<td>17.8 ± 0.35</td>
<td>50.5 ± 1.22</td>
</tr>
</tbody>
</table>

3.3.4. Tree Growth and Soil Characteristics

3.3.4.1 Height Growth

Trees in the thinned treatment exhibited significantly lower height growth than those of control trees in the first year, but by the second year of study, this response was less pronounced (F1, 5.74=32.64, p=0.0014). The trees within the control treatment did not vary in height growth between years. The interaction between years and thinning effects narrowly misses significance at the 0.05 alpha level (F1, 5.74=4.53, p=0.0794). The mean height growth in the control treatment in 2002 was 1.04 m and in 2003 was 0.93 meters. Height growth was generally lower in the thinned treatment than the control both years, and in 2002 was significantly lower than the control (Figure 3.11). There were no significant differences among families in height growth. Of the five families used for larval feeding analysis, family sw2 consistently ranked near the top in height growth in both the control and thinned
treatment. Family cb3 consistently ranked lower in mean height growth than most of the other four families in both the control and thinned treatments (Table 3.4).

### 3.3.4.2 Tree Diameter Growth

There was a significant year by thinning interaction in diameter growth ($F_{1,130}=23.53$, $p<0.0001$, Figure 3.12). Mean diameter growth in 2002 was very similar across the thinned and control treatments, at 1.96 cm in the control, and 1.95 cm in the thinned treatment. In 2003, however, mean diameter growth was significantly lower in both the control and thinned treatments than 2002 growth, and in 2003 the trees in the control treatment grew significantly less in diameter than trees in the thinned treatment (see Figure 3.12). As with height growth, results indicated no interaction between families and year or families and thinning, and no significant advantages in diameter growth among families at the given sample size.

Of those families used in the feeding analysis, diameter growth differences among families and treatments were not significant within the same year, but sg2 exhibited the greatest, most consistent diameter growth each year in both the control and thinned treatment (Table 3.5). In 2003, when larval feeding assays were conducted, family sw2 grew half as much in diameter in the control and thinned treatment than the remaining four families.

**Table 3.4.** Mean (± 1 SE) height growth (m) of feeding assay trees only. Means are presented in meters. Sample size was 4 trees in the interaction.

<table>
<thead>
<tr>
<th>Family</th>
<th>2002 Control</th>
<th>2003</th>
<th>2002 Thinned</th>
<th>2003 Thinned</th>
</tr>
</thead>
<tbody>
<tr>
<td>cb3</td>
<td>1.21 ± 0.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fa7</td>
<td>1.08 ± 0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sg2</td>
<td>0.90 ± 0.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sw2</td>
<td>1.26 ± 0.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ve2</td>
<td>1.49 ± 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.11. Mean (± 1 SE) height growth in the thinned and control treatments for years 2002 and 2003. Sample sizes were equal across years in the respective treatments-control: n=33; thinned: n=47. Means marked with different letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

Families cb3, fa7, sg2, and ve2 performed comparably in the control in 2003, but in the thinned treatment family sg2 grew the greatest amount, followed by ve2, cb3, fa7 and finally sw2.

3.3.4.3 Soils Analysis

Soils analyses indicated the upper soil horizons generally decreased in nitrogen and phosphorous content as distance increased from the levee. Potassium levels were generally more variable among blocks. Also, soil N, P, and K concentrations were generally greater in the thinned portion of the plot than in the control (Figure 3.13).
Figure 3.12. Mean (± 1 SE) diameter growth in the thinned and control treatments for years 2002 and 2003. Sample sizes in the respective treatments were control: n=44; thinned: n=39. Means marked with different letters were considered significantly different using Tukey-Kramer at an alpha level of 0.05.

Table 3.5. Mean (± 1 SE) diameter growth by family and thinning treatment of feeding bioassay trees only. Means presented in centimeters. Sample size consisted of 4 trees in the interaction cell (family x thinning).
Figure 3.13. Total Kjeldhal nitrogen (N) is expressed as percent; Phosphrous (P) and Potassium (K) as parts per million. Each block represents one composite sample of four core samples. Elevation decreases as one moves away from the levee (upward in figure).

3.4 Discussion

3.4.1 Larval Response and Corresponding Foliage and Tree Growth

Females responded more sharply to treatment effects than did males, an effect which has been observed in other studies. Raupp et al. (1988) found male gypsy moth (Lepidoptera: Lymatriidae) less responsive to foliage quality when larvae were reared on differing host species than females with respect to pupal weights. Redak and Cates (1984) found foliar soluble N content was less predictive of pupal weight in males than females. Female pupal mass of many Lepidopteran forest defoliators has been shown to be strongly correlated with realized fecundity in females and potential population growth (Awmack and Leather, 2002; Haukioja and Neuvonen, 1985; Brewer et al., 1985; Hennebary and Kishaba, 1966; Clancy,
Female BCLR dry pupal weights on family cb3 were significantly heavier in the thinned treatment than the control, but larval growth among other families was not significantly affected by the thinning treatment. Though not significant, female BCLRs also required less time to develop and exhibited higher relative growth rates (RGR) on family cb3 in the thinned treatment. On average larval performance was similar on family fa7; while in families sw2 and ve2, dry pupal weights and RGRs were greater and development times shorter in the control treatment.

Greater BCLR performance on family cb3 in the thinned treatment was unexpected given foliar moisture in all families was lower in the thinned treatment. Larval growth is known to be impeded by foliar toughness (Larrson and Ohmart, 1988; Feeny, 1970; Meeker, 1992). One explanation for lower foliar moisture in the thinned treatment may be the increased exposure to light. If nutrients and water are not limiting, thinning a stand increases exposure to light and will lead to a greater production of photosynthate, and because sugars important to the construction of cell components are in adequate levels, a tree’s foliage receiving ample light is often lower in foliar moisture and tougher than foliage in shaded and crowded conditions (Mitchell et al., 1992; Kolb et al., 1998; Sucoff and Hong, 1974; Dudt and Shure, 1994).

Foliar N content in family cb3 was equal in the thinned and control treatments (2.00 % vs. 1.99 %), but K and P content was greater in the control treatment. Families fa7 and ve2 contained greater total NPK content in the control treatment than the thinned treatment. The greater moisture content among all families and total NPK in families cb3, fa7, and ve2 in the control than the thinned treatment could also have been due to tree growth differences between the treatments. Overall diameter growth was similar among treatments in 2002,
however it was significantly greater in the thinned treatment among all families in 2003, a
similar result obtained in other thinning studies (Dicke and Toliver, 1988; Goelz, 1999;
Kozlowski, 1971; McGarity, 1979; Miller 1999). Overall height growth was significantly
greater in the control treatment than the thinned treatment in 2002, a pattern shared by
families cb3 and fa7. Thus, greater nutrient and moisture content observed in the foliage in the
control treatment may have been an artifact of the greater height growth in the control
treatment in 2002, as the content of new, expanding foliage of larger/older plants is often
more closely correlated with the previous years’ growth (Kozlowski, 1971). Examples of this
effect are found in studies and reviews of the effects of heavy defoliation on the foliage
quality in following years, which have found foliage to be smaller, contain less nutrients and
moisture, and contain more defensive compounds such as tannins as a result of removing
nutrients and growth hormones prior to the development of the leaf primordia (Quiring and
McKinnon, 1999; Ruohomaki and Chapin, 1996; Herms and Mattson, 1992; Tuomi et al.,

These observed foliage and tree growth differences all would suggest better BCLR
performance on trees within the control treatment, as the growth-differentiation hypothesis
predicts more foliar nutrients is an indication of more photosynthate allocated to growth rather
than C-based secondary compounds and other cell differentiation. One possible explanation for
better larval growth in family cb3 in the thinned treatment may in part be explained by the
observation that needles on branchlets from trees in the control treatment exhibited a greater
degree of needle appression and narrower branchlet width than those in the thinned treatment.
The mean needle angle of attachment to the rachis in family cb3 measured here (41°), was
considerably larger than that recorded by Meeker (1992) on the typical appressed morphology
trees (20°), and so this foliage was not truly representative of the appressed foliage type. However the cb3 branchlets in the control treatment also were narrower than foliage in the thinned treatment, and may have shared some similarities with the appressed morph. Trees in natural baldcypress stands exhibiting the appressed foliage morph were shown to receive significantly less defoliation by the BCLR, and laboratory assays indicated this foliage type was more fibrous than the open morphology (Meeker and Goyer, 1994; Meeker, 1992). Larval consumption rate and total consumption was significantly lower on the appressed foliage type than on the open branchlet morphology despite comparable nutrient and tannin levels (Meeker and Goyer, 1994; Meeker, 1992). Thus, foliage from cb3 in the control treatment may have been tougher in the control treatment, despite the observed higher moisture content, than foliage in family cb3 in the thinned treatment. From a tree physiological viewpoint, the possibility that foliage from family cb3 should be more appressed in the control treatment, which was exposed to less light and contained more total NPK content than the thinned treatment, is unexpected. Studies of the effects of crown position (light exposure) on foliage size and angle of exposure in baldcypress and other trees have found sun leaves are smaller and often angled differently than shade leaves, and are often located in the upper portion of the crown, such that tissues receive less direct radiation during the middle portion of the day and can conserve water (Kozlowski, 1971; Brown et al., 1981; Neufeld, 1983; Mitchell et al., 1992).

Differences in budbreak lead to differences in foliage age. Phenological variation in budbreak has been shown in chapter 2 of the present thesis and other work to have significant biological effects on larval growth and success (Lawrence et al., 1997; Chen et al., 2003; and Quiring and McKinnon, 1999; Dumerle, 1988; Mattson et al., 1991). Variation in budbreak
phenology did not have an obvious effect on the observed larval growth differences in the present study. Larval bioassays were begun on the individual families as sufficient foliage became available across thinning treatments, and foliage collections for moisture and nutrient analyses were collected in the same manner, such that budbreak phenology differences were lessened. Females reared on the early-leafing family fa7 in the thinned treatment attained the largest dry pupal weight among all families. Measurements of branchlet length and moisture content from samples collected simultaneously from all trees indicated the same sequence in budbreak among these families as was found in the Jeanfreau study (Ch. two). Early-leafing families fa7 and sg2 had significantly longer branchlets than the late-leafing family sw2 and on average longer branchlets than late-leafing families cb3 and ve2. Foliar moisture content in samples collected simultaneously were also significantly lower in the early-leafing families fa7 and sg2 than in the late-leafing family sw2 and on average, moisture content was lower than the late-leafing families ve2 and cb3. Foliar moisture content when sampled by budbreak indicated foliar moisture was equal among families when the age effect was removed. The lack of a significant interaction between the thinning effect and these response variables suggested thinning did not affect the relative sequence in the date of budbreak among these families. Branchlet length did not differ between the control and thinning treatment, suggesting no difference in budbreak as a result of the thinning treatment.

The time “budbreak” begins in a tree is known to be under strong genetic control and is therefore consistent relative to other individuals in the population (Bigras and Colombo, 2001; Chen et al. 2003, Quiring and McKinnon, 1999). However, budbreak is a phenotypic expression and is therefore affected by environmental factors such as temperature, nutrients, and light resources (Bigras and Colombo, 2001; Kozlowski, 1971). Though the date of
budbreak among families in each of the thinned treatments in this study was shown to be consistent by foliage length, the possibility that thinning a stand could alter the date of budbreak should not be ruled out. Actual measurements at the time of budbreak, including the calendar date, would be more thorough and provide a more reliable result than the branchlet length measurement discussed here. McGee (1975) found when oak seedlings were grown in pots under a forest canopy versus those in open and unprotected conditions, budbreak occurred up to 4 days earlier in the shaded seedlings. He attributed these differences to thinner bud scales which were more responsive to temperature changes, and to a lesser degree, increasing photoperiod in the spring. Conversely, fertilization and irrigation in resource limited plants improves growth and allocation to leaf primordial developing for the following year, and may advance budbreak by a few days relative to resource limited trees (Kozlowski, 1971; Quiring and McKinnon, 1999; Lerdau et al. 1995). Whether advances or delays such as these would affect BCLR populations is questionable; the leafroller varies in emergence within an egg mass by as many as 16 days (Goyer and Chambers 1997).

3.4.2. Tree Growth and Soils

Of ancillary interest, soils analyses indicated slightly higher nutrient content in the thinned treatment, which was unexpected given greater tree height growth in the control. It is known that the physical characteristics of the soil are important, as well as other properties such as the cation exchange capacity and the pH (Kozlowski, 1971). Soil water salinity and salinity levels when the plot was flooded indicated a mean salinity of 0.6 g L⁻¹, which was comparable to Krauss’ (2000) measurement at 0.5 g L⁻¹. Krauss et al. (2000) reported the first two years’ height growth increment on the Delacroix site was 54 cm per year (though this mean was based on all surviving individuals of the original 400 seedlings) whereas in the current study
annual mean height growth averaged 96.5 cm in the control and 62.0 cm in the thinned treatment. Of the five families used for larval feeding analysis, family sw2 consistently ranked near the top in height growth in both the control and thinned treatment, which is consistent with results from Krauss et al. (2000). Family cb3, as in Krauss et al. (2000), consistently ranked lower in mean height growth than most of the other four families in both the control and thinned treatments. Cones were collected on October 16, 2003 and were in greatest abundance on family groups ‘fa’ and ‘sg.’ Most notably, family sg2 produced the heaviest cone crop at the site. Cone production was closely associated with greater light availability. All cone bearing trees were located either in the thinned treatment or in the control treatment in an outside row. Other families producing cones included two trees of family fa8, two trees of family cb3, and one tree of family fa7. Tree tag numbers are listed in Appendix C.

The Krauss et al. (2000) study reported an annual diameter growth increment at the close of the second growing season of 2.1 cm, which was similar to what was measured in the present study in 2002, at 1.96 cm and 1.95 cm in the control and thinned treatments. However this growth increment decreased in 2003 to 0.78 cm in the thinned treatment and 0.31 cm in the control. Of those families used in the feeding analysis, family sg2 exhibited the greatest, most consistent diameter growth each year in both the control and thinned treatment, which was consistent with Krauss et al. (2000). The overall decrease in the rate of diameter growth between 2002 and 2003 was unexpected. This may be the result of measurement error, as the trees were measured at one meter above ground at each sampling interval, but I did not place permanent tags (measurement points) on trees until 2002. The magnitude of growth being measured is small; so variation in measurement points could considerably alter accuracy of growth measurement. The diameter measurement for 2003, however, was deemed to be more
accurate and therefore the differences observed between 2002 and 2003 are deemed more reliable.

3.4.3 Limitations

There were conditions in this study which limit conclusions. The plot was of insufficient size to thin randomly within the plot, and so the site was assumed to be fairly homogeneous. The results of the soils analysis indicated that as elevation decreased from the levee end of the plot toward the rear of the plot, nutrient content decreased as well. However the plot was thinned perpendicular to this gradient, such that each thinning treatment was subjected to comparable flooding and/or soil qualities. The plot size also limited family replication. It is very likely that given a larger sample size, differences in tree growth among families could be detected, and likely differences in larval growth due to these and other inherent differences.

Thinning a stand will undoubtedly lead to less competition for resources among the remaining trees, and should lead to increased tree growth and foliage differences which may have had a more noticeable impact on larval growth given the proper time period or greater replication. The larvae were reared in a growth chamber at a constant temperature, which maintained growing conditions for the BCLR at a near-optimum level, and so development in the field would likely have been longer and subject to greater variation in foliage changes than were provided in the rough three-week period provided here.

Foliage constituents other than those analyzed here are important to larval growth and performance. Larval growth is maximized in the presence of optimal ratios of macronutrients, moisture content, and necessary trace elements to secondary compounds and tissue toughness. Mattson and Haack, (1987) and Clancy (1992) found the balance in foliar constituents in artificial diet was a better predictor of larval growth than individual element concentrations;
as sugars were increased at a constant N content in artificial diet, spruce budworm performance declined. Other work with a related tortricid (spruce budworm) indicated late instars were stimulated to feed by nonstructural carbohydrates, and the combination of carbohydrates and amino acids produced an additive effect on larval feeding (Albert and Bauce, 1994; Guertin and Albert, 1992).

Secondary compounds have also been found to deter feeding and/or reduce digestibility of host foliage. Foliar terpenes in artificial diet decrease growth of a related tortricid, the spruce budworm (Choristoneura sp.) (Wagner and Tinus, 1984; Redak and Cates, 1984). The growth-differentiation and C:N balance hypothesis predicts, and studies have found, greater concentrations of C-based secondary compounds in high-light intensity situations (nutrients held constant), which may possibly result in higher concentrations of carbon-based defensive compounds (Bryant et al., 1983; Lorio, 1986; Herms and Mattson, 1992; Schoonhoven et al., 1998). In this study, however, females attained a significantly greater pupal weight in the thinned treatment on family cb3, suggesting either that carbon was not in excess for secondary metabolism, or if it was, growing conditions were adequate and C was not shunted into the production of secondary compounds but into growth processes (Lorio, 1986; Herms and Mattson, 1992; Bryant, 1983; Tuomi et al., 1988).

**3.4.4 Conclusions**

Within the limitations presented above, thinning does not appear to have had a strong effect across all families on the performance of the BCLR within the given time period and at the given sample size. Some differences in larval performance were attributed to the genetic component (families), but additional study is warranted to evaluate the cause of these differences.
The differences in budbreak, shown by foliage length and moisture content among these families, mirrored the pattern at Jeanfreau, and supports the strong genetic component controlling this event. The foliage of families fa7 and sg2 were consistently longer in both the control and thinned treatment at the day of sample collection, and the moisture analysis indicated lower foliar moisture in these families within the respective treatments when collected at one sampling date. Larval growth results showed, however, that when budbreak differences were removed, growth generally was comparable among families.

Given that thinning a stand of baldcypress will eventually lead to greater tree vigor as a result of increased resources, tree growth response, and larval response to foliage changes, may be expected to be greater after a longer time frame than was tested here. Other forest pests such as the Nantucket Pine Tip Moth *[Rhyacionia frustrana* (Comstock); Lepidoptera: Tortricidae] have been known to favor open grown, young and rapidly growing hosts over more mature hosts (Otvos, 1991). Other studies have found more vigorously growing stands to be less susceptible to insect attack (Price, 1991; Lorio, 1986) and stressed stands more susceptible as a result of higher levels of soluble N (White, 1984). The BCLR has in years past inflicted significant reductions in radial growth on continuously flooded, smaller sapling and pole-sized baldcypress (30-45 cm in diameter at breast height), and mortality on the stressed, understory saplings in flooded situations (Goyer and Chambers, 1997; Meeker and Goyer, 1993; Goyer and Lenhard, 1988). These patterns suggest trees growing less vigorously are less able to maintain fitness under the combinations of multiple stresses. Though the largest mean female dry pupal weight observed here was produced from a family in the thinned treatment, suggesting a greater propensity of the BCLR to reproduce, the growth of the trees given adequate resources could be expected to better withstand defoliation than
stressed individuals. Stands of baldcypress planted for coastal restoration purposes should be maintained at stocking densities that allow sufficient growing room to maintain vigor.
CHAPTER 4

SUMMARY AND CONCLUSIONS

4.1 Background and Justification for Study

Coastal forested wetlands are becoming increasingly susceptible to decline due to increased and prolonged flooding, increased exposure to salinity, and to some extent, herbivory (Conner and Day, 1976; Goyer and Lenhard 2002; Hammar-Klose and Thieler, 2001). Coastal restoration projects include the use of salt-tolerant vegetation and the diversion of nutrient-rich Mississippi River into vulnerable areas (La. Dept. Nat. Res. 1998). There is potential for the use of salt-tolerant *Taxodium distichum* L. Richard (baldcypress) in areas that may be exposed to alternating periods of fresh, nutrient-rich water from river diversions, and brackish to highly saline conditions (Allen et al., 1994; Krauss et al., 2000). Baldcypress constitutes a major percentage of the forested wetland ecotype in southern Louisiana, and at times extensive areas have been defoliated each spring by *Archips goyerana* Kruse (baldcypress leafroller-BCLR) (Goyer and Chambers, 1997; Goyer and Lenhard, 2002). If genetically related baldcypress are planted en masse in areas subject to the alternating effects of fertilization (diversions) and saltwater intrusion, plans for restoration projects using baldcypress should consider the herbivore component to prevent or reduce the damage from another potential stress agent. Large plantings of baldcypress will require planting at initially high densities to ensure proper stocking levels, and will require a future stand thinning.

Thus, the objectives of this study were to determine the effects of fertilization and thinning on five half-sibling families of *Taxodium distichum* L. Richard (baldcypress), and how these cultural treatments, as well as inherent genetic traits such as differing dates of budbreak,
would affect the suitability of these families to the baldcypress leafroller, *Archips goyerana* Kruse (Lepidoptera:Tortricidae). These objectives were evaluated in two separate studies on separate sites: one evaluating fertilization (Jeanfreau) and the other thinning (Delacroix); and in each study the respective treatment was compared utilizing the same five half-sibling families. The sites were established in 1996 to evaluate the field performance of previously laboratory-screened half-sibling families (Krauss et al., 2000). The two sites were once subjected to saline conditions above the physiological threshold for baldcypress, however the Jeanfreau site has been protected from intruding saltwater since the 1970s and the Delacroix site since the early 1990s (USACE, 2003c; Lane and Day, 1999).

### 4.2 Delacroix: The Effects of Thinning (Chapter III)

The Delacroix site was divided in halves and one side thinned by 50% of the stand density in January, 2001. One full growing season after the thinning treatment was administered, the effects were evaluated by collecting foliage and beginning larval bioassays in the sequence the five families (cb3, fa7, sg2, sw2, and ve2) began leafing out, which would emphasize thinning effects and minimize the effects of budbreak phenology. Larvae were reared in the laboratory under constant 12:12 light:dark conditions at 21°C.

Female BCLR mean dry pupal weight was significantly greater in the thinned treatment, and female mean relative growth rate was on average greater and development time shorter in the thinned treatment in family cb3, however there were no other clear and consistent differences between the thinning and control treatment in the other families. All families in the thinned treatment responded in 2003 with increased diameter growth. However, larval growth did not respond as expected. Foliage from the various families possessed comparable foliar N between treatments, but lower P and K content and significantly lower foliar moisture in the
thinned treatment. One possible explanation was that family cb3 in the control treatment possessed a greater degree of needle appression and had a narrower branchlet width than foliage in the thinned treatment, and though not the truly appressed foliage morph described in Meeker and Goyer (1993) and Brown and Montz (1986), this foliage was more appressed than the other families at the plot. Meeker (1992) found the appressed foliage to be tougher than the typical open branchlet foliage morph of less needle appression. Otherwise, foliar nutrient levels across all families were higher in the control than the thinned treatment and foliar moisture content significantly lower in the thinned treatment. Tree height growth was significantly lower in the thinned treatment in 2002, the year the plot was thinned, but diameter growth was significantly greater in the thinned treatment in 2003.

These results suggested that the trees in the thinned treatment were beginning to respond to the thinning treatment by increasing diameter growth in 2003. This confirms the assumption that baldcypress under the proper site conditions will respond to increased resources, and if stocking densities become too great to sustain growth, baldcypress families will become more susceptible to stress components. Family was not a significant source of variation in height or diameter growth at the given sample size and within the time frame of study, however it is likely that some families may be on average faster growers than others, as certain provenances of trees have been proven to possess inherently greater growth rates than others (Bigras and Colombo, 2001). It also is likely that given a longer time frame, greater differences between the thinning treatments in foliage quality and BCLR performance would develop.

4.3 Jeanfreau: Fertilization Effects (Chapter III)

To evaluate the effects of fertilization on the host suitability to the BCLR, the Jeanfreau experimental plot (Krauss et al. 2000) was divided into thirds and subjected to a control, a low
fertilization rate receiving the approximate equivalent of one years’ worth of nutrient additions (236 g/m²) from an 8000 cubic foot per second Mississippi River Diversion (Caernarvon diversion, maximum capacity, see Lane and Day, 1999) and a high fertilization rate, equivalent to two years’ worth of nutrient additions (472 g/m²). Trees were measured for height and diameter growth one meter above ground level. Foliage was collected from the trees and transported to the laboratory for larval bioassays and chemical analyses of nitrogen, phosphorous, potassium, total phenolics, and moisture content, and physical characteristics including length and width.

Larvae were reared in the laboratory from second instar through pupation and relative growth rates, development times, and dry pupal weights recorded. In 2002, larvae were assigned to the families when all trees had sufficient foliage to begin feeding assays, a manner similar to the way the majority of the second instars in field populations would have naturally encountered the foliage differences exhibited by the families among fertilization treatments. This methodology led to data that strongly depicted the differences among early-leafing families fa7 and sg2 and late leafing families cb3, sw2, and ve2 in foliage. These results were in agreement with other studies on the importance of synchronicity in larval eclosion and host budbreak on herbivore performance (Aide and Londono, 1989; Dumerle, 1988; Chen et al., 2003; Clancy, 2002; Larsson, 2002; Barbosa and Baltensweiler, 1987; Ivashov et al., 2002; Lawrence et. al, 1997; Watt and McFarlane, 1991; Watt, 1987; Hunter et al., 1991; Hunter, 1992; Onstad and Reissig, 1986.). Larvae performed well on the young, newly expanded and more nutritious foliage of the late-leafing families, attaining greater pupal weights at a greater rate of growth and shorter time period than early-leafing families, regardless of the fertilization treatments. RGRs suggested the differences in foliage quality among fertilization
treatments were of greater importance in the older foliage of the early-leafing families. On late-leafing families the foliage quality was more than adequate for larval growth irrespective the fertilization treatment.

In 2003 larvae were experimentally assigned to families in the sequence of budbreak. The methodology employed in 2003 somewhat masked the family differences and strengthened differences attributable to the growth status of the tree. Dry pupal weights and relative growth rates were again primarily greatest among families growing in the low fertilization treatment. Foliage was longer and wider in the low fertilization treatment, and generally contained greater foliar N and moisture content than did families in the control and high fertilization treatment. Dry pupal weights did not differ significantly among early- and late-leafing families, and RGRs also were less different among families. Foliage nutrient and moisture content varied less among early- and late-leafing families when sampled by budbreak in 2003.

Significant interactions among fertilization and family treatments in foliar total phenolics, relative growth rates, and development times also were detected. In some cases larval growth was greater and total phenolics levels were lower among families in the control treatment than the high fertilization treatment; at other times it was the just the opposite. These differences might be explained by a lack of uniformity across the fertilization blocks, resulting in greater growth for some individuals in a treatment. The observed results also may have been due to the high fertilization treatment acting as a salt and in toxic levels; some families may have been better adapted to exclude those ions, preventing them from interfering with photosynthesis. This mechanism would be similar to how it is suspected salt-tolerant trees and plants differ in their ability to exclude Cl⁻ ions, while others cannot (Pezeshki, 1990).
Height growth was generally greatest in the low fertilization treatment in 2002, and foliage suitability was generally greatest in the low fertilization treatment in 2002 and 2003. Tree growth in 2003 was less predictive of the physical and chemical content of the foliage in 2003, but this pattern has been observed in other trees (Kozlowski, 1971; Haukioja and Honkanen, 1997; Quiring and McKinnon, 1999). Family differences in tree growth were not significant, however initial tree height and diameter prior to the first fertilizer application was greater in families from brackish-water source parent trees in the ‘far’ and ‘sg2’ group and lower in freshwater source parent trees in the ‘saw’ group. These data suggest that the site conditions present were, over time, affecting the growth potential of these trees.

Results of my study suggest that areas impacted by saltwater intrusion in the absence of a freshwater diversion may predispose non salt-tolerant baldcypress stands to stress. Mississippi River diversions may prevent these situations; however nutrient-rich diversions also may create a fertilizer effect and increase stand growth and foliage suitability to the BCLR. Larval growth and pupal weight was shown here to depend on the foliage quality as it is affected by the growth status of the tree as well as phenology differences among families. Female pupal mass is often positively correlated with fecundity, and the larger pupal weights in the late-leafing families and the trees growing more rapidly in the low fertilization treatment suggest there is the potential for a BCLR population increase (Awmack and Leather, 2002; Haukioja and Neuvonen, 1985; Brewer et al., 1985; Hennebary and Kishaba, 1966; Clancy, 1992; Hunter and Docherty, 1991; Redak and Cates, 1984). Larval development times also were longer in the control treatment and early-leafing families, which has been shown to increase larval susceptibility to predation or other natural mortality factors (Parry et al., 1998).
Though trees may respond positively to increased resources, in some instances more vigorously growing trees have been shown to better withstand the effects of defoliation (Clancy, 2002). It is not known whether a diversion will result in such a situation. If baldcypress leafroller populations respond positively to increased foliar nutrient and moisture levels, the strong genetic differences among families in budbreak and other inherent, unique qualities should be utilized to offset the potential effects of future herbivory and salinity-induced stress. Though there is variation in larval emergence from egg masses (up to 16 days) which matches at least the approximate known range in baldcypress budbreak among the five families studied here, the genetic factors controlling budbreak are strong, and (Bigras and Colombo, 2001; Chen et. al., 2003) may provide trees leafing out at either extreme in the larval emergence period with a means of temporal escape from herbivory. It is noteworthy that the sequence in budbreak among the five families of study was consistent at the Delacroix site as well. The tendency of families fa7 and sg2 to leaf out earlier, exhibit greater growth on the Jeanfreau site, which has been exposed to some residual salinity, and develop cones at a relatively early age (seven years) suggests this family would be a good candidate for a coastal restoration project in need of both a salt-tolerant and herbivore resistant family. These results also show the importance of mixing genotypes or half-sibs to avoid a monoculture of the same or similar individuals, which could increase herbivore pressure through budbreak and larval emergence synchrony.

From this study I concluded that there was a combination of factors which affected BCLR growth. This study illustrated that the nutrient status of the hosts’ foliage as well as differences in host phenology and other genetic traits affecting the foliage can have significant bearing on leafroller populations. A relative lack of parasitoids and predators associated with
the BCLR due to low availability of alternate hosts and prey in the wetland habitat baldcypress inhabits (Goyer et al., 1990) makes variation in host suitability even more important. If host genetic differences are planned for use areas within the BCLR range in a coastal restoration context, these differences should also incorporate tree characteristics which confer an advantage over potential defoliators. Phenological asynchrony as exhibited by families fa7 and sg2 may be used to offset the effects of herbivory as well as saltwater intrusion. And though not shown conclusively in my research, it is likely that (at a higher level of replication) inherently faster growing families may better compensate for defoliation than other families, as has been shown in studies of other tree-herbivore interactions. This may be another mode of dealing with herbivore damage, and an increasingly important mode in the face of the expected increased flooding and salinity levels. Lastly, I would argue strongly against planting monocultures of salt-tolerant baldcypress genotypes, but rather families with widely varying dates in budbreak and other foliar differences that might tend to reduce the potential for BCLR population growth.
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Harrison, R. W. 1951. Levee Districts and Levee Building in Mississippi: A study of State and Local Efforts to control Mississippi River Floods. Delta Council, The Board of Mississippi Levee Commissioners, The Board of Levee Commissioners for the Yazoo-Mississippi Delta, and Mississippi Agricultural Experiment Station cooperating with USDA Bureau of Agricultural Economics. October.


APPENDIX A: CHAPTER 2-EXCLUDED DATAPoints

Table A-1. Jeanfreau 2002-2003 mean height growth by family and fertilization rate.

<table>
<thead>
<tr>
<th>Family</th>
<th>2002</th>
<th>2003</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low</td>
</tr>
<tr>
<td>cb3</td>
<td>0.67 ± 0.28</td>
<td>1.35 ± 0.24</td>
</tr>
<tr>
<td>fa2</td>
<td>1.15 ± 0.52</td>
<td>1.00 ± 0.15</td>
</tr>
<tr>
<td>fa7</td>
<td>0.78 ± 0.04</td>
<td>1.19 ± 0.18</td>
</tr>
<tr>
<td>fa8</td>
<td>0.78 ± 0.13</td>
<td>1.04 ± 0.14</td>
</tr>
<tr>
<td>sg2</td>
<td>0.99 ± 0.14</td>
<td>1.30 ± 0.21</td>
</tr>
<tr>
<td>sw2</td>
<td>0.72 ± 0.06</td>
<td>0.60 ± 0.35</td>
</tr>
<tr>
<td>ve2</td>
<td>0.77 ± 0.28</td>
<td>0.53 ± 0.16</td>
</tr>
</tbody>
</table>

Table A-2. Percent larval mortality by family for Jeanfreau Study, 2002-2003

<table>
<thead>
<tr>
<th>Family</th>
<th>Handler(^a)</th>
<th>Parasitism</th>
<th>Unknown(^b)</th>
<th>Total</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>cb3</td>
<td>0.34</td>
<td>0.34</td>
<td>25.08</td>
<td>25.76</td>
<td>295</td>
</tr>
<tr>
<td>fa7</td>
<td>0.00</td>
<td>0.70</td>
<td>16.55</td>
<td>17.25</td>
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<tr>
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<td>18.28</td>
<td>279</td>
</tr>
<tr>
<td>sw2</td>
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<td>0.00</td>
<td>21.45</td>
<td>21.80</td>
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</tr>
<tr>
<td>ve2</td>
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<td>0.00</td>
<td>23.78</td>
<td>25.18</td>
<td>286</td>
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2003

<table>
<thead>
<tr>
<th>Family</th>
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<th>Parasitism</th>
<th>Unknown(^b)</th>
<th>Total</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>cb3</td>
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<td>16.30</td>
<td>16.84</td>
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<td>2.56</td>
<td>17.95</td>
<td>20.88</td>
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<td>10.92</td>
<td>247</td>
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<td>1.23</td>
<td>23.46</td>
<td>24.69</td>
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<td>ve2</td>
<td>0.00</td>
<td>2.69</td>
<td>17.74</td>
<td>20.43</td>
<td>186</td>
</tr>
</tbody>
</table>

\(^a\) Handler= died as result of handling injury.

\(^b\) Unknown= cause of mortality unknown.
Table A.3. Jeanfreau: Not counted because replaced more than seven days after original larvae set up.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fertilization Level</th>
<th>Family</th>
<th>Rep</th>
<th>Larvae #</th>
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<tr>
<td>2002</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
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<td>3</td>
<td>1</td>
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<td>24</td>
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<td>9</td>
</tr>
<tr>
<td>2002</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2002</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>8</td>
</tr>
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Table A.4. Jeanfreau. Larvae not counted for reasons listed below.

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<th>Larvae #</th>
<th>Reason</th>
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<tbody>
<tr>
<td>2003</td>
<td>3</td>
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<td>2</td>
<td>2</td>
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</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>5</td>
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</tr>
<tr>
<td>2003</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>small, sickly-white</td>
</tr>
<tr>
<td>2003</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td>small, sickly-white</td>
</tr>
<tr>
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<td>30</td>
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</tr>
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<td>3</td>
<td>4</td>
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<tr>
<td>2003</td>
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<td>4</td>
<td>3</td>
<td>4</td>
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<tr>
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<td>5</td>
<td>1</td>
<td>15</td>
<td>small, sickly-white</td>
</tr>
<tr>
<td>2003</td>
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<td>2</td>
<td>1</td>
<td>7</td>
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</tr>
<tr>
<td>2003</td>
<td>3</td>
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<td>2</td>
<td>15</td>
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</table>

Table A.5. Jeanfreau. Removed from fry pupal weight analysis alone or from both RGR and dry pupal weight analysis because of handler injuries.

<table>
<thead>
<tr>
<th>Year</th>
<th>Fertilization Level</th>
<th>Family</th>
<th>Rep</th>
<th>Larvae #</th>
<th>Reason</th>
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<tbody>
<tr>
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<td>26</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>handler injury</td>
</tr>
<tr>
<td>2003</td>
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<td>4</td>
<td>1</td>
<td>1</td>
<td>rem. from dry pup. wt analysis only-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>handler injury</td>
</tr>
<tr>
<td>2003</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>6</td>
<td>rem. from dry pup. wt analysis only-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>handler injury</td>
</tr>
<tr>
<td>2003</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>rem. from rgr and dry pup. wt anal.-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>handler injury</td>
</tr>
<tr>
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<td>3</td>
<td>2</td>
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<td></td>
<td>handler injury</td>
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<td>14</td>
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<td></td>
<td></td>
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<td></td>
<td>handler injury</td>
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<td>4</td>
<td>1</td>
<td>14</td>
<td>rem. from rgr and dry pup. wt anal.-</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td>handler injury</td>
</tr>
<tr>
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<td>4</td>
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<td>13</td>
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</tr>
<tr>
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<td></td>
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<td></td>
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Table A.6. Jeanfreau dry pupal weight, relative growth rate, and development time (asterisks).

<table>
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<tr>
<th>Year</th>
<th>Fertilization Level</th>
<th>Family</th>
<th>Rep</th>
<th>Larvae</th>
</tr>
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<td>2002</td>
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B. RGR

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<th>Family</th>
<th>Rep</th>
<th>Larvae</th>
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C. Development Time

<table>
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<th>Fertilization Level</th>
<th>Family</th>
<th>Rep</th>
<th>Larvae</th>
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<tr>
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Table A.7. Foliage Parameters and Tree Growth (asterisks).

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B. Moisture-Staggered Analysis 2003

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<tr>
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C. Moisture-Simultaneous Analysis 2003

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D. Tree Height Growth Year, fertilization, family in model

<table>
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## APPENDIX B-CHAPTER 3-EXCLUDED DATAPPOINTS

Table B.1. Delacroix outliers (asterisks)-Insect dry pupal weights and development time.

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<th>Larvae</th>
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<td>7</td>
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<table>
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<th>Treatment</th>
<th>Family</th>
<th>Rep</th>
<th>Larvae</th>
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* No asterisks detected for other analyses.
APPENDIX C-CONE PRODUCTION AT DELACROIX AND JEANFREAU SITES, 2003

Table C1. Delacroix Cone Production-10/16/2003.

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<thead>
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<td>fa8</td>
<td>thinned treatment-interior</td>
</tr>
<tr>
<td>210</td>
<td>sg2</td>
<td>control-edge</td>
</tr>
<tr>
<td>211</td>
<td>sg2</td>
<td>control-edge</td>
</tr>
<tr>
<td>237</td>
<td>sg2</td>
<td>control-edge</td>
</tr>
<tr>
<td>695</td>
<td>sg2</td>
<td>thinned treatment-interior</td>
</tr>
<tr>
<td>312</td>
<td>fa7</td>
<td>thinned treatment-interior</td>
</tr>
<tr>
<td>315</td>
<td>fa8</td>
<td>thinned treatment-edge</td>
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<tr>
<td>248</td>
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Table C2. Jeanfreau Cone Production-10/16/2003

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</tr>
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<td>sg2</td>
<td>edge</td>
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<tr>
<td>12</td>
<td>sg2</td>
<td>edge</td>
</tr>
<tr>
<td>73</td>
<td>sg3</td>
<td>edge</td>
</tr>
</tbody>
</table>
VITA

Wood Johnson was born in Selma, Alabama, on October 28, 1976, and is the son of Earl C. and Anne W. Johnson. He completed his Bachelor of Science degree in forest management at Louisiana State University in May of 2001. He enrolled in the Louisiana State University, Department of Entomology in the fall of 2001 in pursuit of a Master of Science degree in entomology. Under the guidance of Dr. Richard Goyer, he completed the requirements of this degree in December of 2003 and is now a candidate for a Master of Science degree in entomology, which will be awarded in May of 2004.