AN INVESTIGATION INTO THE ROLE OF GENETICS IN THE TOLERANCE OF TEXAS LIVE OAKS TO *Ceratocystis fagacearum*

A Dissertation

by

MYRON CROWLEY GRAY

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2007

Major Subject: Plant Pathology

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Approved by:

Chair of Committee,	David A. Appel
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ABSTRACT

An Investigation into the Role of Genetics in the Tolerance
of Texas Live Oaks to *Ceratocystis fagacearum.* (May 2007)
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The fungus *Ceratocystis fagacearum* (Bretz) Hunt causes the vascular disease of oak wilt and has been decimating live oaks (*Quercus virginiana* Mill. and *Quercus fusiformis* Small.) and red oaks (*Quercus texana* Small and *Quercus marilandica* Muenchh.) in Texas. The purpose of this research was to test the hypotheses that live oaks have heritable tolerance to oak wilt, and that allozyme markers are associated with this tolerance. One-year-old half-sib and two-year-old clonal progeny of live oaks (*Q. fusiformis*) were grown from acorns and ramets from a disease center and then challenged with *C. fagacearum*.

Allozyme analyses were used to compare the pre- and post-epidemic populations in two natural disease centers to search for alleles associated with survivability and decreased crown loss. Half-sib and clonal challenge tests supported the hypothesis that heritable tolerance to the pathogen occurs in live oaks. The progeny tolerances seen in half-sib and clonal groups did not correlate with parental tree performance. This finding suggests that the tolerance of one-year-old seedlings in the greenhouse setting is not a good predictor of how mature trees will do in a natural setting. Seedlings may not be a good model for testing tolerance to a pathogen. The ability to survive this vascular pathogen is containment, and seedlings may be too small to test this type of tolerance. The clonal groups from post-epidemic trees performed better than the seedlings. They may have an increased resistance because they are mature or they may have a post-disease immunity. No significant allele frequencies between pre- and post-epidemic trees were consistent among sites or with previous research. The different disease sites had remarkably similar allele frequencies which indicate high levels of gene flow among

sites. Both sites were found to contain significant numbers of clones, but the Izoro site had significantly larger clonal groups.

Sites were in Hardy-Weinberg equilibrium which indicates substantial sexual reproduction and not just clonal reproduction is taking place. Several cases of linkage disequilibrium occurred at the Izoro site, but population structure was responsible in all but one case.

DEDICATION

I owe a great debt to Margaret Mossman and Mary Burger for being mentors throughout my life. I am very lucky to have known such inspiring souls.

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CHAPTER I

INTRODUCTION

Oak wilt is a vascular disease of oak trees that is caused by the fungal pathogen *Ceratocystis fagacearum* (Bretz) Hunt. *Ceratocystis fagacearum* is a heterothallic ascomycete with two mating types (A and B) that colonizes the xylem of oaks and causes wilting. This pathogen infects oaks and other trees in the family Fagaceae. Within the Genus *Quercus* those oaks in the red oak subgenus (*Eyrthrobalanus*) have no tolerance for the pathogen and die within 3 to 6 weeks of showing symptoms (12). In the white oak subgenus (*Leucobalanus*) only the semi-evergreen live oaks are severely affected. The live oaks (*Quercus virginiana* Mill and *Q. fusiformis* Small) show a differential tolerance to the pathogen with a survival rate of about 10 to 25 %. Surviving trees are left with 0-99 % crown damage. This fungus can spread locally underground through common root systems with adjacent trees. Long distance overland spread is by insects.

It was initially feared that this fungal tree disease could be as devastating to red and live oaks throughout the United States as were the Dutch elm disease and chestnut blight epidemics (47). This has not happened because oak wilt in contrast lacks an efficient vector for long distance spread (63).

In Texas, oak wilt is presently devastating stands of live oak and red oaks, primarily Spanish oak (*Q. texana* Small*)* and black jack oak (*Q. marilandica* Muenchh.) (6). To date, there have been hundreds of thousands of Texas oak wilt centers created (personal communication, Dr. David Appel, Dept. Plant Pathology and Microbiology, TAMU, Sept. 20, 2006). Oak wilt in Texas has been a devastating disease. Live oaks and red oaks are extremely important in both urban and rural environments as sources of shade and beauty for property owners as well as food and shelter for ranch animals and wildlife.

This dissertation follows the style of Phytopathology.

In some areas, such as those where the golden cheeked warbler is prevalent, oak wilt may be responsible for the destruction of endangered species habitat. Several measures have been taken in Texas to control and eradicate oak wilt. To date, they have included active research on the disease process, public assistance, and education. Ongoing management efforts include: 1. elimination of sources of inoculum by destroying diseased red oaks to prevent fungal mat production, 2. reducing available infection courts by wound treatment and by not pruning during wet, cool months, and, 3. protection of healthy trees by creating barriers to root grafts, and 4. by prophylactic treatments with fungicide injections (11, 12, 13, and 25). These measures have reduced the danger of the epidemic in Texas, but the disease still persists and is hard to treat in urban areas. While trenching to create barriers, albeit expensive, works in pure live oaks stands in rural areas and fungicide injections can save many already diseased trees and high risk trees, there are limitations to these methods. It is not affordable and sometimes it is impossible to trench around oak wilt sites in many urban areas because of preexisting structures (13). Fungicide injections can prevent a tree from dying but cannot prevent spread of the spores through its root system to other trees (25).

An important addition to the program for controlling oak wilt would be the ability to find sources of tolerance that are heritable and could therefore be used to propagate resistant trees. Two previous projects at Round Rock, TX. have found evidence that resistance is present, under selection, and associated with molecular markers (19, 72). Greene and Appel (43) demonstrated in live oak seedlings that the difference in response to artificial inoculation of *C. fagacearum* among live oaks is a heritable trait. Using isozymes, Bellamy (19) and McDonald et al. (72) identified a difference in the frequency of an allele for trees that had the least crown loss from oak wilt. They also identified significant allele frequency differences between a post-epidemic live oak population and an adjacent pre-epidemic population. McDonald et al. (72) hypothesized that selection is taking place at the Round Rock disease site, and a biotrophic pathogen is driving evolution in the host plant for genotypes that express resistant phenotypes.

The purpose of this project is to further test the findings that the survival of live oak challenged by *C. fagacearum* can be attributed to heritable, genetically determined resistance. The objectives of this study are to:

i. compare allozyme frequencies between pre- and post-epidemic oak wilt sites and among survivor trees as markers correlated with survivorship or resistance,
ii. test heritabilities of tolerance or resistance of half-sib live oak seedling families grown from pre-epidemic and post-epidemic trees and challenged with *C. fagacearum,*
iii. use allozyme profiles taken from a sample of live oak seedlings to test for correlations of alleles with survivorship,
iv. test for heritability of tolerance to *C. fagacearum* by comparing tolerance of clones from different diseased live oaks that have different levels of crown loss.

CHAPTER II

LITERATURE REVIEW

Prior Research

Live oak taxonomy: The semi-evergreen live oaks are classified into the subgenus *Leucobalanus* (86). *Leucobalanus* (white oaks) and *Erythrobalanus* (red oaks) are subgenera in the genus *Quercus*, family Fagaceae, and order Fagales. The Fagaceae family contains approximately 1000 species (69), and the genus *Quercus* consists of approximately 500 species (69). There are about 50 species of oaks in the US (69). There are no species of oak reported to be completely resistant to inoculation with *C. fagacearum*.

Quercus virginiana is the most common species of live oak in the US. This species was originally named *Quercus sempivirens* by Catesby in 1731, but *Q. sempivirens* was reduced to a varietal status of *Quercus phellos*, a red oak, by Linnaeus in 1753 (86). In 1768 Miller renamed *Q. sempivirens* as *Q. virginiana* (86). In 1838 Loudon was the first to recognize the live oaks as a group and named them as Series Virentes as one of the ten sections of *Quercus* (67). The live oak was renamed *Quercus virens* Aiton in 1783 until 1890 when Sargent recognized the original name and renamed *Q. sempivirens* as *Q. virginiana* (86).

From 1897-1933 Small split *Q. virginiana* into several additional species (e.g. *Q. minima* (Sargent) Small, and *Q. fusiformis*). However, Sargent disagreed with Small and reduced *Q. fusiformis* to a varietal rank under Q. virginiana (86). In 1961 Muller (79) supported species recognition for *Q. fusiformis*. Muller reported acorn morphology as the major separation between *Q. virginiana* and Q. *fusiformis*. Muller (79) also reported morphological integration between *Q. virginiana* and *Q. fusiformis*.

Nixon (86) reported that populations of *Q. virginiana* were limited to south and east Texas, whereas *Q. fusiformis* populations were generally located in the central area of Texas from the coastal plain to the Edward's Plateau. He further reported introgression between *Q. fusiformis* and *Q. virginiana* on the Edward's Plateau that

resulted in populations that consist of complex mixes of hybridization between the two species. "The populations of live oak that occur in the area bounded by Columbus, Austin, and San Antonio, Texas can all be considered morphologically intermediate between the populations of *Quercus fusiformis* that occur on the Edward's Plateau and *Quercus virginiana* of coastal southeastern Texas" (86). Previous research using hybridization studies between *Q. virginiana* and *Q. fusiformis* was conducted by Ness (84) and by Hardin (45). They both concluded that *Q. virginiana* and *Q. fusiformis* were one species that adopted different growth patterns in different environments. Presently *Q. fusiformis* and *Q. virginiana* are treated as two separate species with the ability to freely hybridize (86).

Disease history, impact, and control: The causal agent of oak wilt was first shown to be *Chalara quercina* by Henry et al. (47) in Wisconsin based on finding only the asexual stage of the fungus. The sexual stage of this fungus was later reported by Bretz (22) and the fungus was renamed *Endoconidiophora fagacearum* Bretz. The fungus was finally renamed *Ceratocystis fagacearum* (Bretz) Hunt by Hunt (53). Bretz (22) discovered that the fungus was heterothallic, and Hepting et al. (48) showed that the conidia from either mating type could function as spermatia to fertilize the thalli of the opposite mating type, which then produces the sexual stage with the resulting formation of the ascomycetous fruiting bodies (perithecia).

Prior to Henry's discovery, there were reports of oak disease across the country that all had the same characteristics (including disease centers in Texas). Some of these were reported as early as the 1930's and now they are thought to be oak wilt (113). Oak wilt may have been active in the early 1900's in Wisconsin and Minnesota (38). The first definitive report of oak wilt caused by *C. fagacearum* in Texas was made by Dooling (30) in the Dallas area in 1961. Oak wilt is presently reported in 23 states and has been found in 60 counties in Texas with hundreds of thousands of individual disease centers reported (Personal communication, Dr. David Appel, Department of Plant Pathology and Microbiology, TAMU, September 20, 2006).

Shortly after the initial confirmation of oak wilt as one disease found in numerous active disease centers across the United States, there was speculation that this epidemic would become as devastating to oaks in America as Dutch elm disease and chestnut

blight had been to elms and chestnuts (47). These early fears were not realized, and although fungal control within established disease centers is often not achieved, the discovery of new disease centers has slowed considerably. However, oak wilt is still occurring at epidemic rates in parts of Texas. It was also thought that this disease would not be found in states as far south as Texas because the hot summer climate was expected to limit the southern spread of the disease (92, 115, and 116). This belief was based on experiments that showed this fungus to be inhibited and even killed by high temperatures that can occur during Texas summers (35, 92, 111, and 115). In fact, the pathogen already existed in Texas and the disease now shows the fastest rates of annual growth within Texas disease centers when compared to other regions. The hot summers are limiting to pathogen spread but the numerous months of mild weather are ideal for growth of the pathogen (115). *Ceratocystis fagacearum* can survive the hotter months in the root system and lower boles of oak trees. (64, 65) The optimum temperature for growth of this fungus is 24-26º C and this temperature range corresponds to ambient temperature during the milder months in Texas (7, 8, and 12).

It is unknown if oak wilt is the result of a recent introduction of *C. fagacearum* into the United States, as was the situation with Dutch elm disease and chestnut blight (64, 65, and 115). The lack of diversity detected within this pathogen suggests either a relatively recent introduction or a host jump of this biotrophic pathogen from another host (60). Unlike chestnut blight and Dutch elm disease, oak wilt is not known to occur outside of the United States. The extent of the disease range in Texas prior to its confirmation in Dallas (30) is unknown, but the homogeneous distribution of both fungal mating types suggests that this disease is not new to the state (8, 60).

The history of settlement and land transformation in Texas has made conditions more conducive to the spread of oak wilt (7). Prior to settlement most of central Texas (where the disease is concentrated) was an open grass prairie with oak forests that were smaller, more distant from one another, and mostly limited to river valleys and hills. The extent of the forested area was limited by a cycle of prairie grass fires which destroy new seedlings and by the health of the native prairie grasses. Settlement resulted in both overgrazing which resulted in loss of healthy soil for prairie grasses as well as fire suppression which allowed seedlings and clonal ramets from live oaks and red oak

species to proliferate into more closely spaced and dense oak tree forests (8). These changes were helpful, and possibly necessary, to allow a pathogen to become epidemic that is inefficiently vectored over large distances but spreads well within tight clusters of root-connected trees (8). The influence of civilization in the form of fire suppression and alteration of native forests also created conditions conducive to the spread of this fungus in red oaks in Wisconsin (39).

In Texas, existing oak stands are very important in both urban and rural settings. The trees that are seriously damaged and killed by oak wilt in Texas are the red oaks and the live oaks (7). In urban settings live oaks are considered high value trees because of their beauty and shade and thereby are important to property values. In rural Texas settings, both red oaks and live oaks are important for the aforementioned reasons and for creating mast and shade for wild and domesticated animals in the hot summers.

Because of the value of oaks in the United States and Texas, there has been a tremendous amount of research into understanding the epidemiology of this disease and finding ways to halt the epidemic. In Texas, the Texas Forest Service created the first programs to study and fight oak wilt: the 1982 "Texas Oak Wilt Demonstration Project" which led to the 1988 "Texas Oak Wilt Suppression Project" (25, 122). Another program created by the Texas Forest Service has been the Urban Tree Improvement Program (UTIP), which "is working to genetically improve the species (live oaks) by identifying and propagating superior individuals" (43). Efforts to control this epidemic in rural Texas focus on: preventing localized root-to-root spread (by trenching); lowering available inoculum for long distance spread by limiting mat formation; and preventing the availability of infection courts in unaffected sites (11). The localized, root-to-root spread has been limited with good success where the expense of isolating root systems with heavy machinery is justified.

The prevention of spore inoculum for long distance transmission includes cutting, burning, and girdling infected red oaks to prevent fungal spore mat prevention (11). The prevention of infection courts for long distance spread is done mostly through public education on when to prune, how to prune, and the importance of treating fresh wounds in susceptible oaks (25).

Prevention in urban areas is more challenging because of man made structures and utilities that often make trenching to prevent local spread impossible. For high value properties it is highly desirable to prevent infected trees from dying or being ruined. Therefore, the treatment of high risk urban trees with injection of an effective fungicide was developed for therapy and maintenance when economically justified, but fungicide treatment of one tree cannot contain the spread of the fungal spores into adjacent root grafted trees.

The oak wilt control programs in Texas have been quite successful at slowing but not stopping the spread of oak wilt. Presently more research is being done to improve methods of control and to find sources of resistance in oaks and to make resistant trees available to the public.

Epidemiology: In order to understand the epidemiology of oak wilt in Texas it is necessary to address the taxonomic distinctions between the red oak subgenus (Erythrobalanus), the white oak subgenus (Leucobalanus), and the relationship of the semi-evergreen live oaks to these subgenera. Oaks are divided into these two subgenera based mainly on differences in reproduction, hybridization, wood structure, and tyloses density and morphology (86, 114). White oaks have an annual reproductive cycle; they hybridize with other white oaks; they are ring porous with xylem summerwood vessels that are small, angular, and thin walled; and they form dense, thick walled tyloses in xylem vessels (19, 79, and 114). In contrast, red oaks have a biannual reproductive cycle; they do not hybridize with white oaks; they have a ring porous xylem with vessels that are larger, rounder, and thicker walled than white oaks; and they have sparse formation of thin walled tyloses (79, 114). In response to stress, white oaks form tyloses more quickly than red oaks (7).

Live oaks have been classified in the white oak subgenus based on their annual reproductive habit and their ability to hybridize with other white oaks (45, 79, and 114). They have a wood structure that is most similar to red oaks, however production of tyloses in live oaks is somewhere in between those levels produced in deciduous red and white oaks. Live oaks have a semi-diffuse porous ring structure which is unique to this semi-evergreen group, are very susceptible to oak wilt caused by *Ceratocystis fagacearum,* and die within weeks after showing symptoms of infection (38, 39). In

contrast, white oaks have a high tolerance to oak wilt, showing limited symptoms and then recovering with little damage (9, 12, 80, and 81). Live oaks show a tolerance to oak wilt intermediate to deciduous white and red oaks. Approximately 80 to 90 % of live oaks die within 3 to 6 months (12). Trees that survive have varying levels of permanent crown loss (12). The xylem morphology of live oaks may partially explain why tolerance in live oaks is intermediate to the deciduous red and white oaks (12). When *C. fagacearum* colonizes the vascular system of a susceptible oak, the vessel elements and tracheids of the xylem eventually become plugged by fungal hyphae, conidia, and high molecular weight breakdown products (gummosis) (34). This plugging results in the wilting and subsequent death of the entire crown in red oaks and some live oaks, and partial death of the crown in live oaks (107, 108). It has also been suggested that toxins contribute to the induction of oak wilt, because toxic compounds that induce symptoms that are consistent with oak wilt have been found in lab grown colonies of *C. fagacearum* (50). However, Parmeter et al. (89) reported that "cells of the cambial region adjacent to the infected area remained alive and active" in bur oaks infected with *C. fagacearum*. Darker and more defined staining of parenchyma cells adjacent to colonized vessels may contribute to tolerance in white oaks (94). In northern pin oaks, it was observed that the cambium was still alive and functioning after severe wilting (107, 108), and they concluded that there was no "direct" toxic action from fungal metabolites. The faster and more prolific production of tyloses in white oaks may play a role in preventing lateral spread around the trunk to prevent complete loss of xylem to the crown (81, 89, 120, and 121). Because live oaks show concentrations of tyloses that are intermediate between the red and white oaks, this is possibly associated with the intermediate response to this disease by live oaks. It is the lateral spread of the fungus around the trunk of trees that determines if the tree survives with partial crown loss or suffers total crown loss and dies (97, 108). If the fungus completely girdles the trunk, the tree will die. The fungus moves laterally around the trunk by hyphal penetration of the cell walls and by penetrating pit membranes (55, 89, 97) and by penetrating parenchyma cells (108). White oaks have a lower number of vessel interconnections than red oaks, and this may be responsible for containing the colonization of the fungus to a more limited arc of the circumference, thus preventing death of the whole crown (89). White oaks also respond to infection by

producing a double band of xylem which may aid in localization of the fungus (61). In pin oaks, *C. fagacearum* "grew outward by penetrating the bordered pits to adjacent ray and xylem parenchyma cells."(108). Introduction of dye into the xylem of pin oak trees (a red oak) resulted in an ever widening arc of dyed xylem ascending up the trunk, but dye introduction into the xylem of bur oaks (a white oak), resulted in a decreasing arc of dyed xylem upward from the point of introduction (89). In red oaks the lateral spread around the circumference of the crown is rapid and completed within a few weeks, whereas in white oaks, the lateral growth is contained to a small arc (89). In addition, diseased white oaks have been found to produce an atypical, extra band of xylem in response to infection, which may limit loss of conduction due to vascular plugging and help contain spread of the fungus to the current growth season (61, 97). It was demonstrated that mechanical girdling of three-quarters of the crown of pin and bur oaks produced no visible effects on the crown (89). Several environmental factors may explain variability in the survival and extent of lateral spread of *C. fagacearum* within individual trees including: temperature and time of year of inoculation (80, 81, 110, 111, and 112), amount of inoculum (89), available nitrogen that may vary due to competing saprophytes such as *Hypoxylon truncatum* (63, 110), and variation in the number of simultaneous points of inoculation around the crown (89).

Ceratocystis fagacearum is a heterothallic, bisexual, xylem colonizing pathogen (5) that reproduces asexually via endoconidia and sexually when the two different mating strains come together to form ascospores in perithicia (22). The hyphae are fertilized by endoconidia of the opposite mating type that function as spermatia (48). *Ceratocystis fagacearum* most commonly infects healthy oak trees either by local spread through common root systems (from root grafts or common roots of clones) (57, 58, 59). Long distance transmission is by spores produced on fungal mats forming on red oaks and vectored to fresh wounds on healthy oaks by insects (56). *Ceratocystis fagacearum* will form fungal mats in dying red oaks during the proper environmental conditions of cool, wet weather (12, 28). Mat formation is the result of the fungus entering a saprophytic growth phase to grow through the inner cambium and form parenchymatous tissue underneath the outer bark (26). This tissue matures into spore forming mats that have pressure pads. The pads swell to crack open the bark and expose the sweet smelling

fungal sporulating mat to insect spore vectors. Colonization of the fungal mats by saprobic fungi may hinder mat viability and function (93). Both conidia and ascospores (if present) can then be vectored to infection courts in healthy oaks.

The spread of oak wilt into new stands of healthy oaks requires insect vectors that will frequent both sporulating mats and then deposit spores into fresh wounds on healthy oaks. There are several species of insects that visit the mats to feed and are capable of carrying spores to other mats to complete the sexual cycle of this fungus (16, 92, and 93). Only the mat feeding nitidulid beetles (Coleoptera: Nitidulidae) are known to vector the *C. fagacearum* spores into fresh wounds on healthy oak trees (56). An oak bark beetle that may spread this fungus in some areas (92), but this insect feeds on a variety of tree species and prefers to feed in the upper tree branches (75), where survival of *C. fagacearum* is short because of the hot climate (12, 51, 56). In Texas, only the two red oak species *Quercus texana* Small (Spanish oak) and *Quercus marilandica* Muenchh (blackjack oak) are reported to form *C. fagacearum* fungal mats (12). Therefore, all deciduous white oak and semi-evergreen live oak species in Texas are dead ends for long distance, insect vectored transmission to new sites.

On a local scale, disease centers in oak stands expand by transmission of conidia through common root systems (12, 78). These centers spread from the point of infection in more or less circular patterns in pure live oak stands. Spread may be more varied in mixed red and live oak stands (when red oaks are also infected) and can create new "satellite" foci via spread of spores from fungal mats to fresh wounds in trees that are outside of the initial disease center (12). In the thin soil and hot dry climate of central Texas live oaks have root systems that are shallow, extensive, and commonly grafted to other trees (12). The extensive grafting allows the spores of *C. fagacearum* to spread to trees tens of meters apart and at rates measured up to 40 meters per year (10). The root-to-shoot ratio may be as high as ten to one in Texas oaks, which offers the fungus the cool environment to survive where ambient summer temps were originally speculated to be too hot for this fungus to grow and even survive (92, 116). It has been shown that the temperature in the lower boles of oaks as well as in root systems is cool enough for survival of this fungus (65). A further reason for a faster spread of this fungus in live

oaks in Texas than seen in other states is the long, temperate growing season for this semi-evergreen species (10, 115).

Following the initial discovery of oak wilt in 1944, there were several new disease sites discovered, but symptoms consistent with oak wilt were reported in the latter 19th century (12, 65). It is unknown how old the sites are that were found in the last 50 years, or how fast they were being initiated in the past. New sites are still being discovered in Texas, and some are in areas known to have been free of any reported symptoms until recently. The finding of low levels of genetic variation (60) in the pathogen suggests a relatively recent host jump or recent introduction (60). The even distribution of the two mating types throughout Texas supports findings that the disease has been in the state for a considerable time (7). It is known that after an initial high rate of discoveries of new disease centers in several states, including Texas, the rate of finding new sites has diminished dramatically (7). The findings suggest that the scope of oak wilt epidemic that was feared isn't happening, and this epidemic will not be as severe as the chestnut blight or Dutch elm disease epidemics. Present knowledge of the disease cycle suggests why initial fears have not been realized. It is thought that the weak link in the long distance spread of oak wilt is due to an inefficient vectoring of the spores (75).

Root grafting has been reported to occur at low levels between red and white oaks in mixed stands in other states (33). Of those red oaks that have oak wilt only a fraction will develop mats because they can only do so during a short time before dying. This has to happen when the weather is cool and wet (8, 28). A further limit on the availability of spores for dispersal is that a fungal mat will only remain viable for a short time (32). The mats dry out quickly once exposed to the environment and they become exposed to invasion of antagonistic, aggressive saprophytic fungi that can be vectored in by the many species of insects that visit the mats (12, 110). In Texas, the saprophytic fungus *Hypoxylon atropunctatum* is known to grow into these wounds (12). The vector most implicated as having the capability to spread inoculum to fresh wounds (the Nitidulid beetle) is not a specialist on oak trees and will visit wounds on other available species of trees where it can loss its spore load of *C. fagacearum* and pick up spores from saprophytic fungi (75). It has been shown that inoculation of saprophytic fungal spores with *C. fagacearum* spores into fresh oak wounds can prevent the establishment of the

disease (89, 93). Furthermore, wounds on oak trees are only receptive to infection by *C. fagacearum* spores for short periods, up to eight days in red oaks and as little as 24 hours in some white oaks (89). Several of these factors make the long distance vectoring of *C. fagacearum* spores into healthy live oak centers an inefficient process, and explain why this epidemic has not been as devastating as Dutch elm disease or chestnut blight (75).

Present Research

Allozymes: Allozymes are used in population genetics to detect such population characteristics as genetic drift, bottleneck events, migration, and selection (14). The analysis of allozyme data has been used to estimate genetic variation in plant populations since the 1960's and is still frequently used by population biologists to estimate genetic parameters in trees. Allozyme analysis is based on the separation and visualization of enzymatic proteins that are alleles of the same locus (103, 104). This is achieved by using gel electrophoresis to separate enzymatic proteins based on size, electrical charge, and shape. These enzymatic proteins may function as a single polypeptide chain (monomer), or as two connected chains (dimer), or as four connected chains (tetramer). The banding patterns of allozymes not only can be used to show the different enzymes created by polymorphic alleles but can also detect the presence of both alleles in heterozygotes. Because either of the alleles in a heterozygote can contribute to the connected chain and in any combination, the heterozygotes of dimeric enzymes have three gel bands and those of tetrameric enzymes have five gel bands. This codominant expression is needed to directly determine population allele frequencies and genotype frequencies for use in population genetics research (14). Several loci may contain enzymes that catalyze the same reaction and these loci are known as isozymes (104). Different isozyme loci that catalyze the same reaction can be created by gene duplication followed by divergence and by genotypic convergence (104). Nuclear allozymes are inherited according to Mendel's Laws and most are codominantly expressed. Mitochondrial allozymes do not show Mendelian inheritance and have only one allele per locus (104).

The most useful allozyme systems for population genetics are those enzymes that are expressed constitutively. Allozymes only detect approximately one third of the genetic changes because most of the mutations do not alter the size, shape, or electrical charge of the resulting enzyme (14). Therefore, allozymes do underestimate diversity. However, starch gel electrophoresis of enzymatic proteins is a commonly used method of population genetics analysis, primarily because allozyme alleles are codominantly expressed (14).

Prior to the discovery of allozymes the use of individual genetic variation within populations was limited to only a few traits controlled by a few well understood genes with clear phenotypic expression of polymorphic alleles (54, 70). It was the discovery of allozymes that gave scientists the ability to track the expression of individual gene loci to individual enzymes. It was the further discovery that the individual allozymes often had more than one allele that allowed this process to be useful in population genetics (14).

Smithies (100) developed the starch gel electrophoresis technique that set the stage for allozyme analysis. The subsequent development of histochemical stains (54, 70) made it possible to determine that several forms of enzymes exist. The combination of starch gel electrophoresis and histochemical staining make it possible to detect the presence of several different allozymes by migrational separation (54).

Allozymes have been used to compare genetic diversity in live oak populations after an oak wilt epidemic and to establish relationships between vitality of oak trees and genetic structure in oak stands (19, 44, and 49). They have been used to compare genetic divergence among populations of several species of red oaks (69). Allozymes have also been used to study enzyme variation in populations of numerous other tree species such as Eurasian pine (*Pinus nigra* Arnold), Douglas fir (*Pseudotsuga menziesii* Mirb), Gambel oak (*Quercus gambelli* Nutt.), and bur oak **(***Quercus macrocarpa* Michx.) (96, 98, and 126). Allozymes have been used to study population structure and clonality in several oak species: turkey oak (*Quercus laevis* Walt.) (21), sand post oak (*Q. margaretta* Ashe) (21), and shin oak (*Quercus havardii* Rydb.) (71). Allozymes have been used to detect small scale population structure and heterozygote deficiency in mixed stand of two oak species by Bacilieri (15). Allozymes were used to differentiate two contested white

oak species: English oak (*Quercus robor* L.), and sessile oak (*Quercus petraea* (Matt.) Leibl) by Gomory (40).

In tomato (*Lycopersicon esculentum* L.) and clover (*Trifolium repens* L.) allozymes were linked to pathogen resistance (17, 73). In sugar beets (*Beta vulgaris* L.) an allozyme marker for resistance to root-knot nematode was discovered by Yu (127). Allozyme polymorphism has also been associated with higher resistance in date palms (20). In jack pine (*Pinus banksiana* Lamb.) allozymes were found to be associated with differences in soil nutrients by Xie (124). Using allozymes, Allard (3) reported genetic changes associated with evolution in wild and domesticated plants. In holm oak (*Quercus ilexs* L.) allozyme analysis showed that genetic variation increased with geographic discontinuity of populations (76).

CHAPTER III

SEEDLING AND CLONE EXPERIMENTS

The experiments described in this chapter were designed to increase understanding of the importance of genetics in the tolerance of some live oaks to oak wilt. Understanding the role of live oak genetics will contribute to the process of managing oak wilt in Texas by contributing to the effort for developing disease resistant selections.

Previous research has reported that genetic variation and heritability for resistance to oak wilt exists in naturally occurring stands of live oaks (19, 72). Artificially inoculated seedlings grown from post-epidemic live oaks survived better than seedlings from pre-epidemic trees.

The goal of this research was to test live oak progeny from Texas oak wilt sites to identify sources of tolerance to *C. fagacearum*.
The specific objectives were to:

i. Test seedlings from pre- and post-epidemic live oaks from oak wilt sites to determine if post-epidemic trees produce offspring with higher tolerance,
ii. Compare half-sib seedlings from post-epidemic trees to test the hypothesis that less damaged trees produce more tolerant seedlings because of superior genetics,
iii. Test half-sib groups from different live oaks to identify trees with the highest genetic tolerance for future breeding stock,
iv. Determine if significant differences in latent period can be confirmed between seedlings groups from pre- and post-epidemic parents and among half-sib groups,
v. Create clonal lineages from post-epidemic live oak trees that have varying levels of damage and compare the tolerance of the clones to previous parental crown loss when challenged with *C. fagacearum.*

Materials and Methods

Acorn collection: Acorns from live oak trees were collected at three oak wilt infection sites during two seasons (1998 and 1999). Each year's seedling crop was used in independent screening trials. Seedlings were grown for approximately one year in a greenhouse, inoculated, and subsequently monitored for disease response. The largest acorn collection came from Williamson County approximately ten kilometers west of the town of Round Rock on State Highway 620. This site was diagnosed with oak wilt in 1982 and was previously used to study genetics of oak survival (13, 19, and 72). Each acorn crop was collected from two groups of trees at this site: 1) "pre-epidemic" trees located just outside the disease center and therefore unchallenged with infection by *C. fagacearum*, and 2) "post-epidemic" trees within the epidemic area that had survived the epidemic. The two areas were easily distinguishable because a trench had been dug around the active disease site to isolate the epidemic, and the fungus had not spread to trees beyond the trench. Trees within the post-epidemic area were rated as to the percentage of their crowns lost due to disease. In the post-epidemic area, trees were chosen for acorn collection with the goal of propagating seedlings from parental trees with a wide variability of crown loss. In the pre-epidemic area, within approximately two kilometers of the post-epidemic area, acorns were collected from live oak trees. Pre-epidemic trees were selected based on large numbers of acorns and with sufficient distances between trees to limit the chances of picking clones. The number of collected acorns was maximized for every tree up to 150 acorns when possible. There were many trees of interest that produced too few acorns to be of use in this study. Small samples of acorns were also collected from two additional oak wilt sites. The Izoro disease center is approximately fifteen hectares in size and is located five kilometers west from the town of Izoro in Lampasas County (Figure 1). The GPS coordinates for the Izoro site are shown in Tables F-3 and F-4. The Austin disease center is approximately ten hectares in size and is located in the Balcones Canyon Lands Preserve next to Highway 360 on the west side of Austin in Travis County (Figure 2). The GPS coordinates for the Austin site are shown in Tables F-1 and F-2.

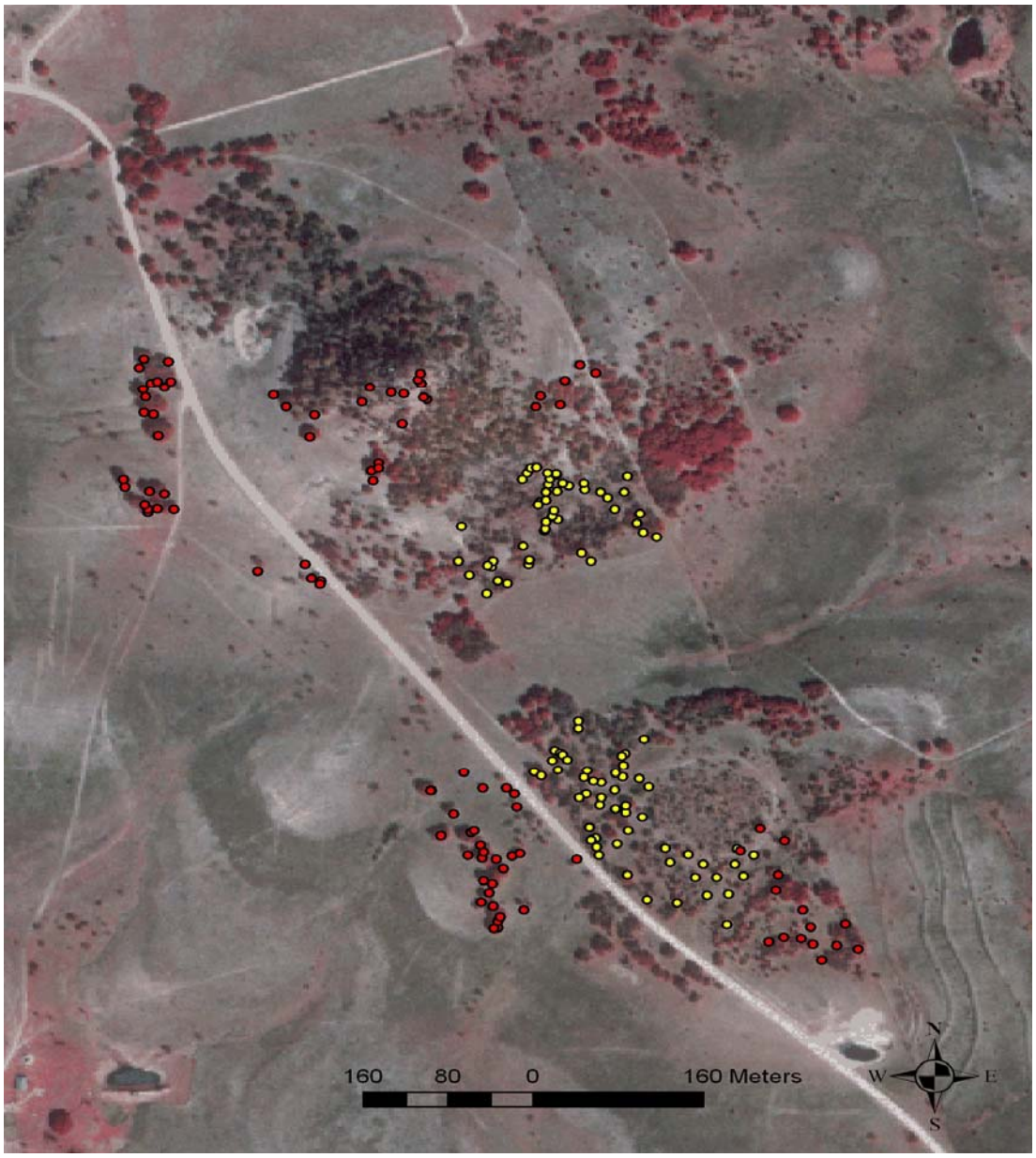

FIGURE 1 Aerial Photograph of Izoro Disease Center. Pre-epidemic trees are represented by yellow points and post-epidemic trees are represented by red points. Only those trees with GPS data are shown. Trees with mapped locations, but no GPS coordinates, are excluded from map.

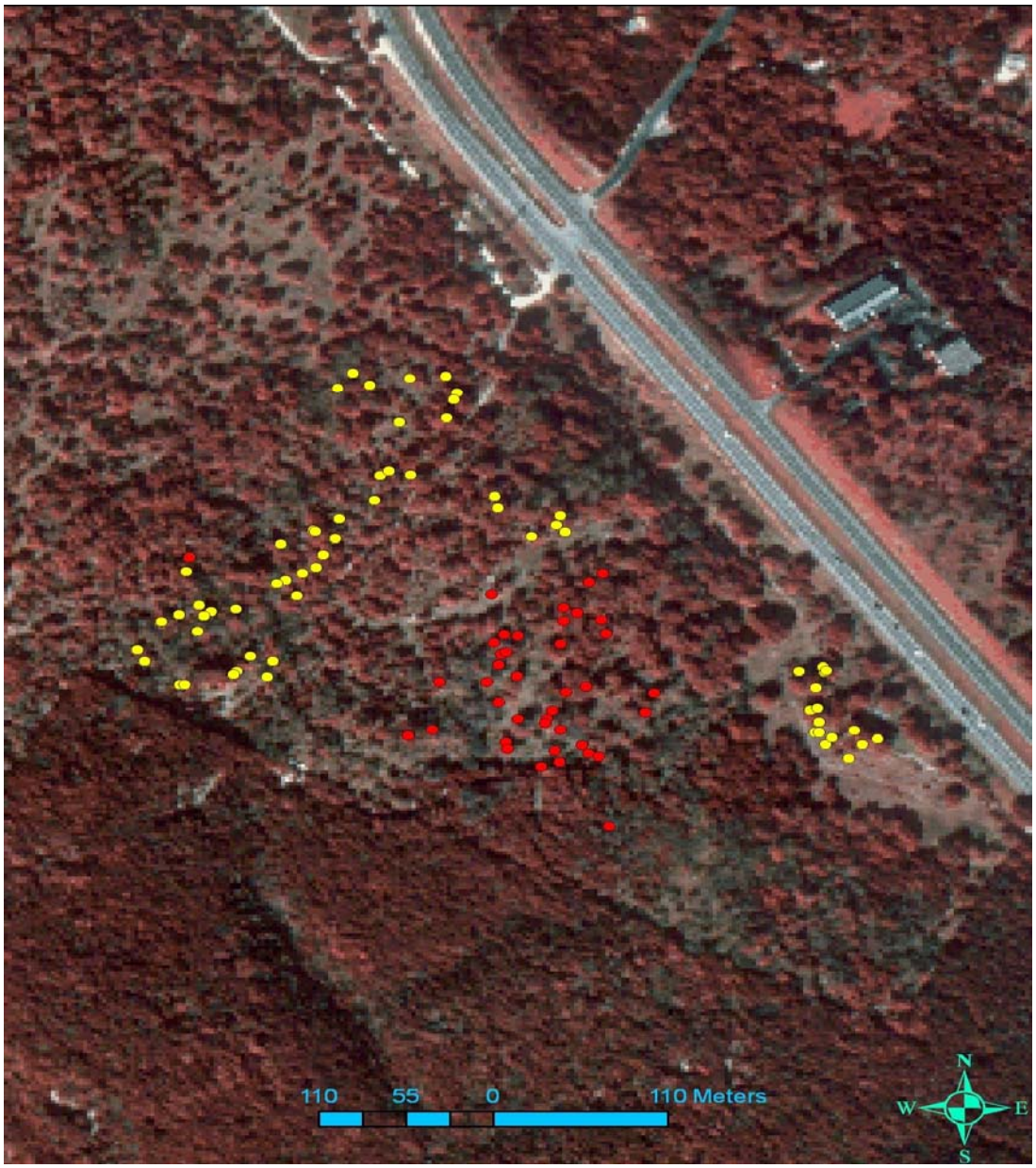

FIGURE 2 Aerial Photograph of Austin Disease Center. Pre-epidemic trees are represented by yellow points and post-epidemic trees are represented by red points. Only those trees with GPS data are shown. Trees with mapped locations, but no GPS coordinates, are excluded from map.

Growth of seedlings: Acorns from the 1998 seedling crop were germinated in damp paper towels and then transferred to 0.7 liter pots containing a 4:1 sand to bark potting mixture. The 1998 seedlings were transferred to one gallon pots after three months of growth. Because of problems with arrested germination and damping-off for the 1998 seedlings, the 1999 seedling crop was germinated in flat containers that contained only sand. After emergence above the sand, the 1999 seedlings were transferred to 0.7 liter pots containing a 4:1 sand to bark mixture. Seedlings for the 1998 and 1999 crops were watered daily for the first two months then watered every other day as needed to prevent root desiccation. Nutricote 20-7-10 (Plantco Inc. Brampton, Ontario, Canada) slow release, granular fertilizer was added to soil mixtures as needed.

Inoculation procedure: *Ceratocystis fagacearum*, strain ML-971-A, was grown in Petri plates on malt-extract agar using refrigerated spores that had been recently isolated from a live oak tree. This strain was chosen through previous trials that used 5 different strains to find the most virulent strain of the group (data not shown). All fungal strains were provided from the collection of Dr. David Appel located in the Plant Pathology Department at Texas A&M University. Conidia were collected by washing two week old cultures of *C. fagacearum* with sterile water, filtering the solution through cheesecloth, and diluting the solution to1 x 10^6 spores/mL. Seedlings were inoculated through a 1 cm long by 1 cm deep vertical wound made with a razor blade that was flame sterilized. Each seedling was laid horizontally and pierced 3 cm above the soil surface. One drop of spore suspension was placed over each wound, and the razor blade was reinserted through the solution into the wound to break the surface tension.

Half-sib analysis: Both the 1998 and 1999 acorn half-sib seedlings groups were kept in a greenhouse until their subsequent inoculations with the pathogen after approximately one year's growth (May 15, 1999 and June 10, 2000). However, the first year's crop (1998) was moved outside to the Texas A&M University Nursery / Floral Field Laboratory seven days after inoculation and was kept under 59% light exclusion. The 1998 crop was moved back into the green house in September and re-inoculated on November 15, 1999. This move was necessary because the summer temperatures caused the fungus to go dormant. Two subsets of the 1998 acorn crop were inoculated separately and moved to growth chambers after inoculation. The first subset of 50 seedlings was

inoculated on February 3, 1999 with 5 strains of *C. fagacearum* for the purpose of comparing the virulence of the different strains of the fungus. This group of seedlings was then transferred into the growth chamber at 21º C and a photoperiod of 13 hours light to 11 hours of darkness. The second subset of 150 seedlings was inoculated on July 10, 1999 with strain ml-971-A and placed into the growth chamber under the same conditions. These two small subsets of the 1998 crop were later transferred to the same greenhouse as the main group of seedlings. The second year's seedling crop (1999) was maintained in the greenhouse during all phases of the experiment.

Evaluation of post-inoculation seedlings: Interest was centered on disease progress comparisons as well as final disease ratings for the seedlings. After inoculation each group was screened for disease on a weekly schedule. The complete death of one leaf on a seedling was marked as the beginning of disease symptoms. Seedlings were rated for crown loss (which was estimated by the percentage of the main stem with dead leaves). Because the seedling growth pattern consisted of dominant main stems and minimal branching, the monitoring of main stem leaf death was considered accurate for assessing disease progress.

Half-sib group comparisons: For the 1998 and 1999 crops, half-sib groups of seedlings were kept together from germination until inoculation. After inoculation, seedlings were placed into randomized incomplete blocks. Seedlings that belonged to the same half-sib group were put into separate blocks. Seedlings chosen for each block were then randomly placed within the block. The goal of having at least 30 half-sib seedlings from each tree was not achieved in either year because of low yields for many trees of interest and poor germination results.

Clonal group comparisons: Clones were created from live oak ramets. Ramets are root sprouts. They were collected as propagative material using a regimen reported by Wang and Rouse (117). Ramets were collected as available during several months in 1999 from post-epidemic trees from the Round Rock disease site. Two large clonal groups were also collected from two live oak trees on the Texas A&M University campus in College Station. More than one clone per tree was created when possible. Post-epidemic trees with a wide variation in disease injury were selected as parental material. Ramets that were growing close to trees of interest were located and confirmed to be physically part

of those trees via root connections. The ramets were pulled from the soil until the root nodes were exposed and were severed below the nodes. Nodal tissue is necessary for successful root regeneration. Ramets with above ground stems from eight to fourteen inches long were collected. Ramets were placed into a foil lined cooler and kept chilled, dark, and moist while being transported to the laboratory. Ramet preparation for cloning included: 1) dipping the nodes into 14 ppm indole-3-butyric acid (Sigma, St. Louis, MO.) solution for one minute, 2) placing the ramets into pots containing a mixture of three parts perlite to one part sphagnum moss, 3) placing into a shaded, mist chamber and 4) spraying ramets with mist for fifteen seconds per five minutes during daylight hours (117). Ramets were pulled from the pots weekly to check for root formation. Ramets that developed numerous rootlets were transferred to gallon pots in a 4:1 sand to bark soil mixture, and then returned to the mist chamber for one week before placement into the greenhouse environment.

Clonal trees were grown for a minimum of two years before inoculation with *C. fagacearum*. The clones were inoculated and monitored for symptom responses in the same manner as previously described for the half-sib seedling groups. Although the ramets that were made into clones were collected individually over a one year period, ramets of live oak trees are mature tissue (117). Several clones produced flowers and acorns within a few months of clone generation, which confirmed that they were mature plants. Furthermore, age variation in mature live oak trees is not believed to be a factor in survivability.

Comparative analyses performed: The following analyses were performed to assess and compare genetic resistance components in live oak disease centers by assessing and comparing resistance among half-sib seedling groups, clones, and between pre- and post-epidemic seedlings:

1) Comparisons were made among half-sib seedling groups for percentage of successes (with success defined as less than 25 percent crown loss one year after inoculation).

2) Comparisons were made among half-sib seedling groups for percentage of survivors one year after inoculation.

3) Two comparison were done between inoculated pre- and post-epidemic groups of seedlings for percentage of successes where success is:

a. less than 25 percent crown loss (first trial),

b. survivability (second trial).

4) Comparisons were made of the average latent period between inoculation and initial appearance of symptoms between inoculated pre- and post-epidemic seedlings.

5) Correlation and regression analysis was carried out between inoculated post-epidemic half-sib seedling groups and their parent tree for:

a. parental crown losses compared to percent survivors in half-sib seedling groups,

b. parental crown losses compared to percentage of seedlings in half-sib groups having less than 25 percent stem death.

6) Correlation and regression analysis was carried out between post-epidemic trees and their clonal progeny for:

a. parental percent crown loss versus crown loss of individual clones,

b. parental percent crown loss versus average crown loss of clonal groups (clonal group defined as all the clones from one tree).

7) Crown loss profiles were made and compared for among the clonal groups.

a. clonal groups from post-epidemic trees were compared to each other,

b. clones from two campus trees were compared to post-epidemic clones.

8) Comparisons were made for a group of seedlings where one-half of the group was root inoculated and the other half was stem inoculated for:

a. percent of group survival after one year,

b. percent of group with less than 25 percent stem death.

9) A comparison was made between a group of seedlings that produced ramets in response to inoculation and a group of non-ramet producing seedlings for:

a. average latency period until disease symptom expression

10) Comparisons were made of average heights of half-sib seedling groups (after one year's growth and prior to inoculation).

Statistical methods: All half-sib group crown loss comparisons were done using nonparametric statistical methods because the data had a bimodal distribution.

Comparisons among groups were done using confidence intervals to estimate significant differences in both crown loss and survivability among half-sib live oak groups. The binomial formula is normally used for large group bimodal comparisons where either category of the bimodal is not extremely low. Because several group sample sizes were variable and small, and because several groups had low or no seedlings in one of the two outcomes, a variation of the binomial formula was used (85). This method is modeled to approximate results of the Agresti Coull method (1, 2) which does not depend on large sample sizes or intermediate levels of success (p) to give significant confidence interval estimations for binomial confidence intervals. The two-tail p-value uses the calculation from Agresti (2). This binomial method has been amended to work in excel © MSM by following the directions given by Nist/Sematech (85).

Comparison of crown heights among half-sib groups was done using the t-test to create confidence intervals for the mean. Comparison of the average latency times between ramet producing and non-ramet producing seedling groups were performed by using t-statistics to do a two sample comparison of means.

Simple linear regression and Pearson's correlation analysis were used to determine the relationship and strength of the relationship between 1) clonal crown loss response to *C. fagacearum* versus post-epidemic parental crown loss, 2) half-sib group percentage survivors versus parental crown loss, and 3) half-sib group survival percentage versus post-epidemic parental crown loss.

Results

Comparison among live oak half-sib groups for tolerance to oak wilt: Comparison of percentages for "success" among inoculated 1998 half-sib groups (where each seedling's success was defined as ≤ 25 percent stem death) resulted in no significant differences among 31 groups, when using 90 percent confidence intervals (Figure 3). When the same comparison was used for the 1999 crop of half-sib seedlings, the best performing half-sib group of the 31 half-sib seedling groups (no. 20) had a significantly higher percentage of successful seedlings than the eight poorest half-sib groups, when using 95% confidence intervals (Figure 4). And, the second best performing half-sib group (no. 24) had a significantly higher percentage of successful seedlings than the four poorest groups.

Comparisons among 21 first year (1998) half-sib groups for survival (with survival defined as a seedling living for one year after inoculation) resulted in five groups (no.'s 1, 2, 3, 12, and 13) that had significantly higher percentages of surviving seedlings than the five poorest groups (no.'s 6, 7, 8, 10, and 15), when using 95 percent confidence intervals (Figure 5). The best performing seedling group (no. 12) had a significantly higher percentage of successful seedlings than the poorest 15 groups. In a comparison of 32 second year (1999) half-sib seedling groups for survival, one group (no. 13) had a significantly higher percentage of surviving seedlings than the 11 groups with the fewest survivors, when using 95 percent confidence intervals (Figure 6).

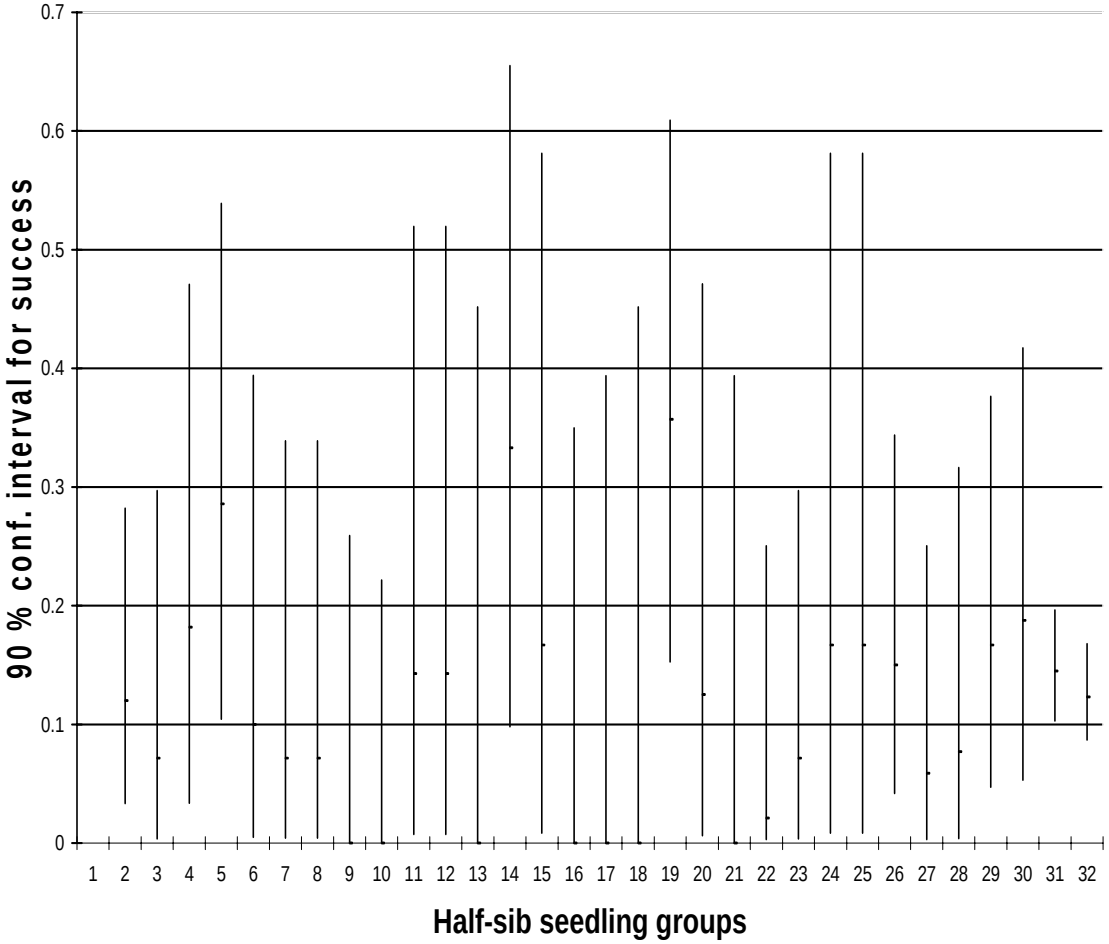

FIGURE 3 Comparison of Seedling Groups from 1998 for Percentage Crown Loss. Thirty groups of live oak, half-sib seedling groups from the 1998 crop were assessed and compared for the percentage of seedlings with ≤ 25 % stem death as a result of inoculation with *Ceratocystis fagacearum.* This graph has a 90% confidence interval for percentage of seedlings with ≤ 25 % stem death. (The last two groups on the right are the confidence intervals for all pre-epidemic and post-epidemic seedlings, respectively).

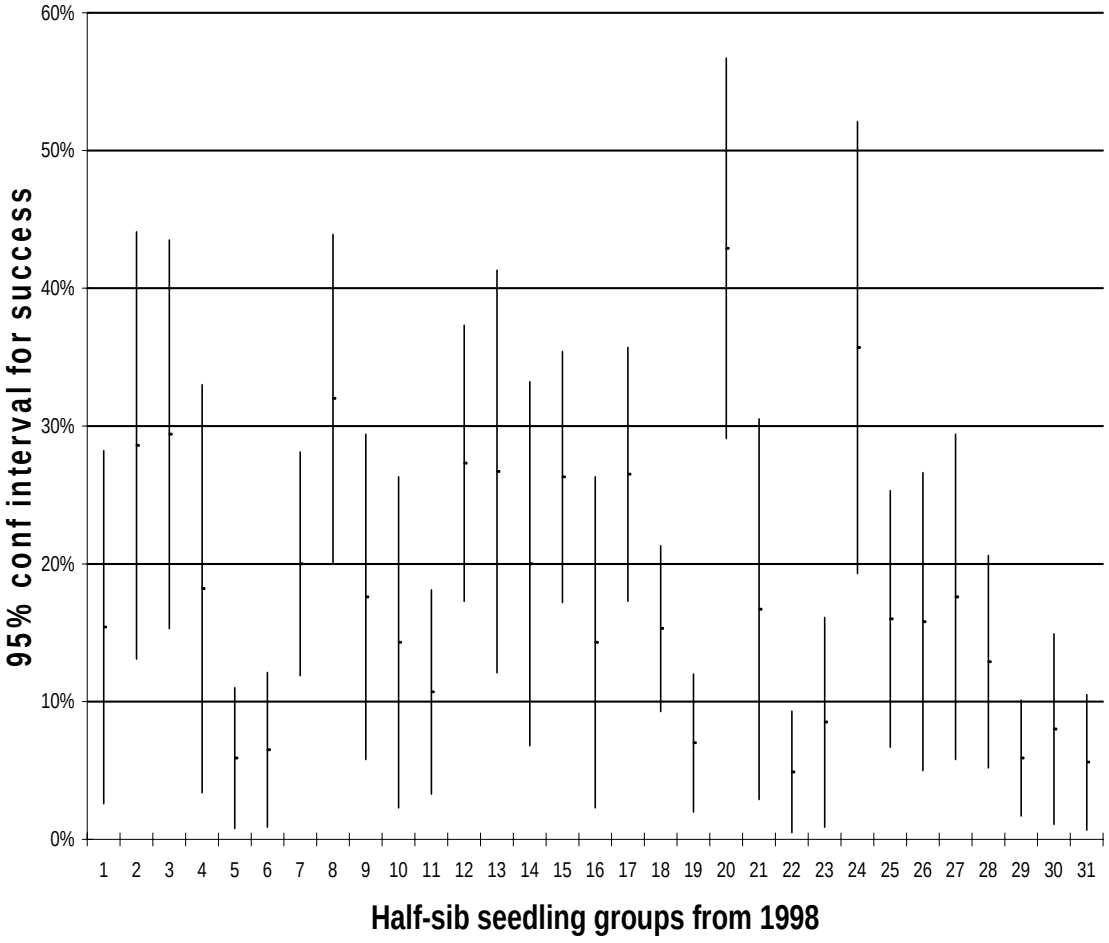

FIGURE 4 Comparison of Seedling Groups from 1999 for Percentage Crown Loss. Thirty-one groups of live oak, half-sib seedling groups from the 1999 crop were assessed and compared for the percentage of seedlings surviving with ≤ 25% crown loss for one year after inoculation with *Ceratocystis fagacearum.* This graph has a 95% confidence interval for the percentage of seedlings in each group that were successes.

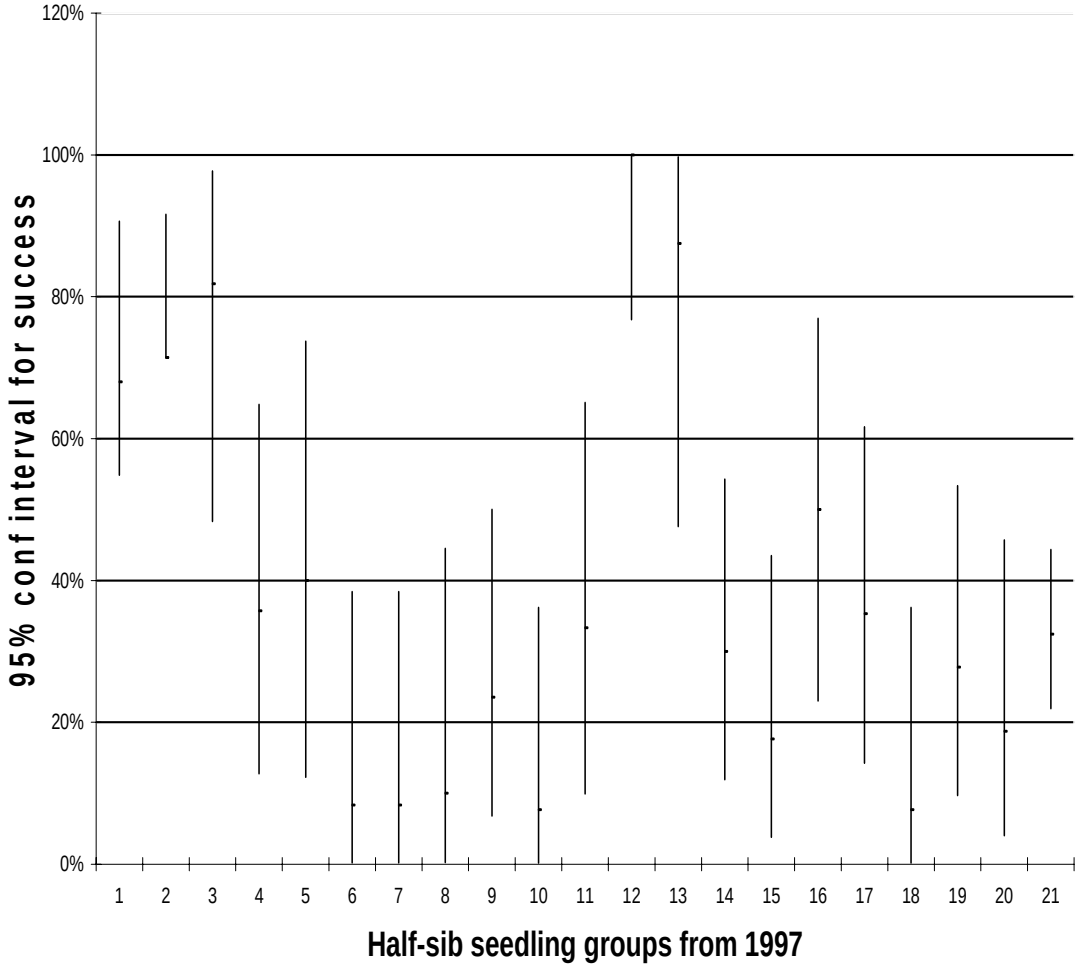

FIGURE 5 Comparison of Seedling Groups from 1998 for Survival Percentage. Twenty-one groups of live oak, half-sib seedling groups from the 1998 crop were assessed and compared for the percentage of seedlings that survived for one year after inoculation with *Ceratocystis fagacearum.* This graph has a 95% confidence interval for the percentage of seedlings that survived.

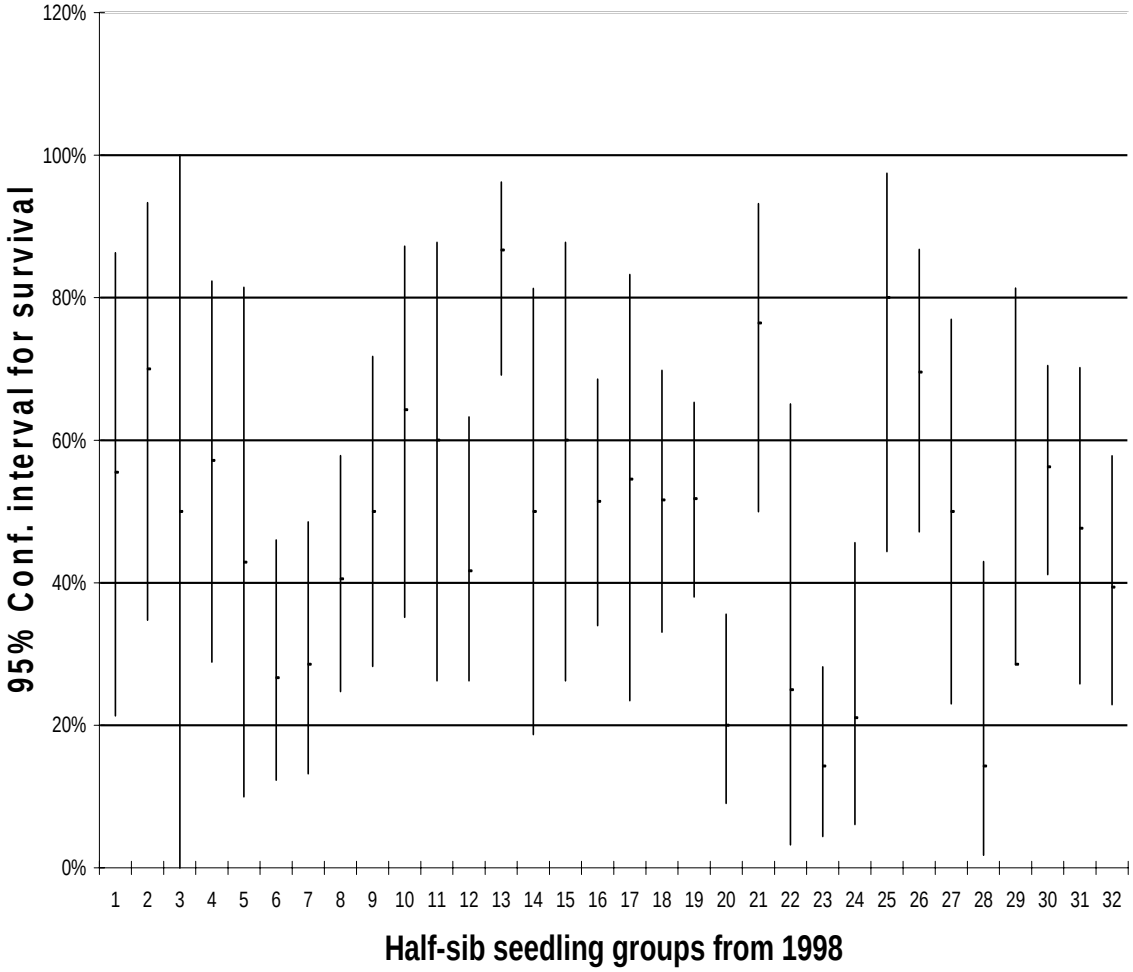

FIGURE 6 Comparison of Seedling Groups from 1999 for Survival Percentage. Thirty-two groups of live oak, half-sib seedling groups from the 1999 crop were assessed and compared for the percentage of seedlings that survived for one year after inoculation with *Ceratocystis fagacearum.* This graph has a 95% confidence interval for the percentage of seedlings that survived.

Comparison of live oak parents and half-sib progeny for tolerance to oak wilt: The disease responses of inoculated, half-sib seedling groups were compared to that of their respective post-epidemic, maternal parents. Because most inoculated seedlings either had little damage or severe damage, the disease response of each seedling was estimated as a binomial. In the first comparison, the tolerance of each half-sib "group" was estimated by using the percentage of seedlings within the group having ≤ 25 percent crown loss. The parental tolerance for each tree was estimated by using the percentage of crown loss resulting from the oak wilt epidemic. In the estimation of half-sib group tolerance by the fraction of the group with ≤ 25 crown loss, there was a significant negative correlation between estimated group tolerance and parental crown loss for the 1998 crop (Figure 7) (Table 1), but not for the 1999 crop (Figure 8) (Table 2). In a second type of comparison, the average tolerance of inoculated half-sib seedling groups was estimated by using the percentage of surviving seedlings within a group. Using this method, the estimated tolerances of half-sib groups were compared to parental tolerance (with parental tolerance again estimated by using the percentage of crown loss). In this comparison, there was no significant correlation between parental tolerance and half-sib progeny tolerance for the 1998 seedling crop (Figure 9) (Table 3), but there was a significant positive correlation for the 1999 crop (Figure 10) (Table 4).

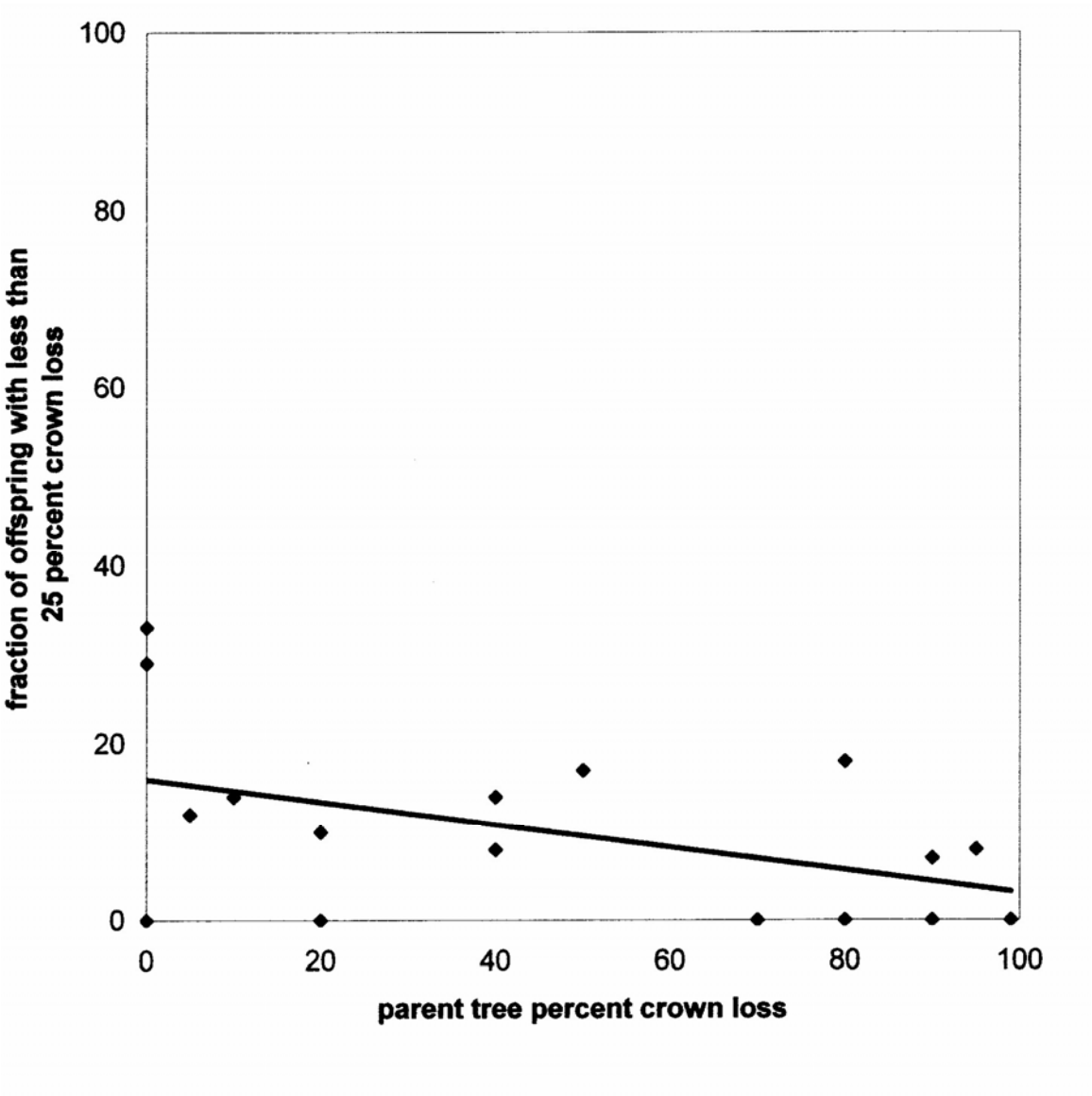

FIGURE 7 Comparison of Crown Losses of Seedling Groups from 1998 to Parental Crown Losses. The percentage of seedlings in half-sib seedling groups having ≤ 25% crown loss were compared against the percentage crown losses of their post-epidemic maternal parent trees for the 1998 seedling crop. The line is the least-squared line for regression. The value of R^2 is .228. The 95% confidence interval for the slope of the line is minus -.260 to .000.

TABLE 1 Summary Output and ANOVA for Figure 7.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.477549
R Square	0.228053
Adjusted R Square	0.176589
Standard Error	9.231635
Observations	17

ANOVA

	df	SS	MS	F	Significance F
Regression	1	377.6551	377.6551	4.4313	0.05255
Residual	15	1278.345	85.2230		
Total	16	1656.000			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	15.9821	3.6178	4.4176	0.000	8.2709	23.6934
X Variable	-0.1290	0.0612	-2.1051	0.053	-0.2594	0.0001

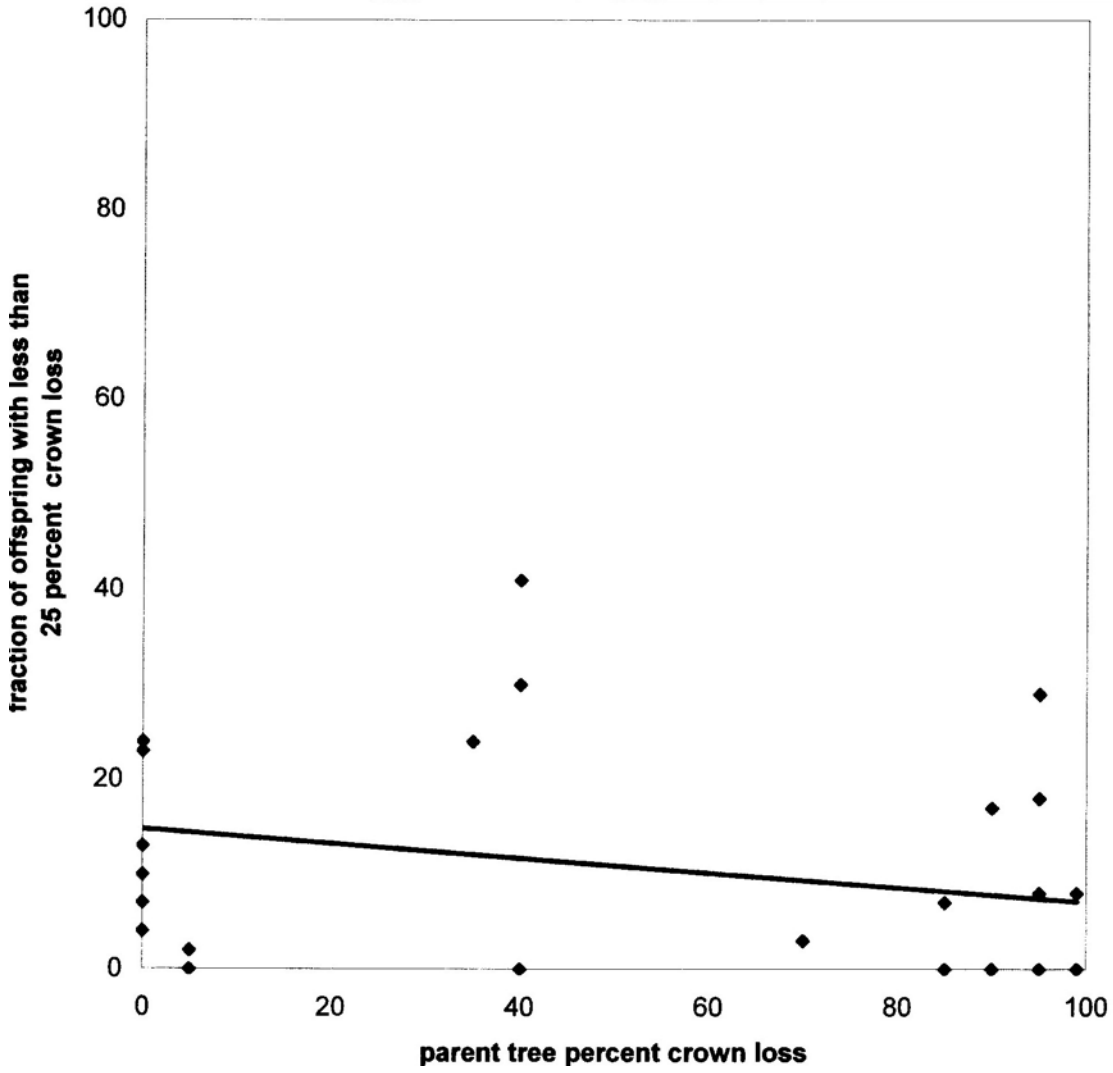

FIGURE 8 Comparison of Crown Losses of Seedling Groups from 1999 to Parental Crown Losses. The percentage of seedlings in half-sib seedling groups having ≤ 25 % crown loss were compared against the percentage crown losses of their post-epidemic maternal parent trees for the 1999 seedling crop. The line is the least-squared line for regression. The value of R^2 is .226. The 95% confidence interval for the slope of the line is minus -.190 to .035.

TABLE 2 Summary Output and ANOVA for Figure 8.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.266399
R Square	0.070968
Adjusted R Square	0.035236
Standard Error	11.607772
Observations	28

ANOVA

	df	SS	MS	F	Significance F
Regression	1	267.6112	267.6112	1.9861	0.170596
Residual	26	3503.246	134.7402		
Total	27	3770.857			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	14.7474	3.7687	3.9131	0.000	7.0006	22.4941
X Variable	-0.0771	0.0547	-1.4093	0.171	-0.1896	0.0353

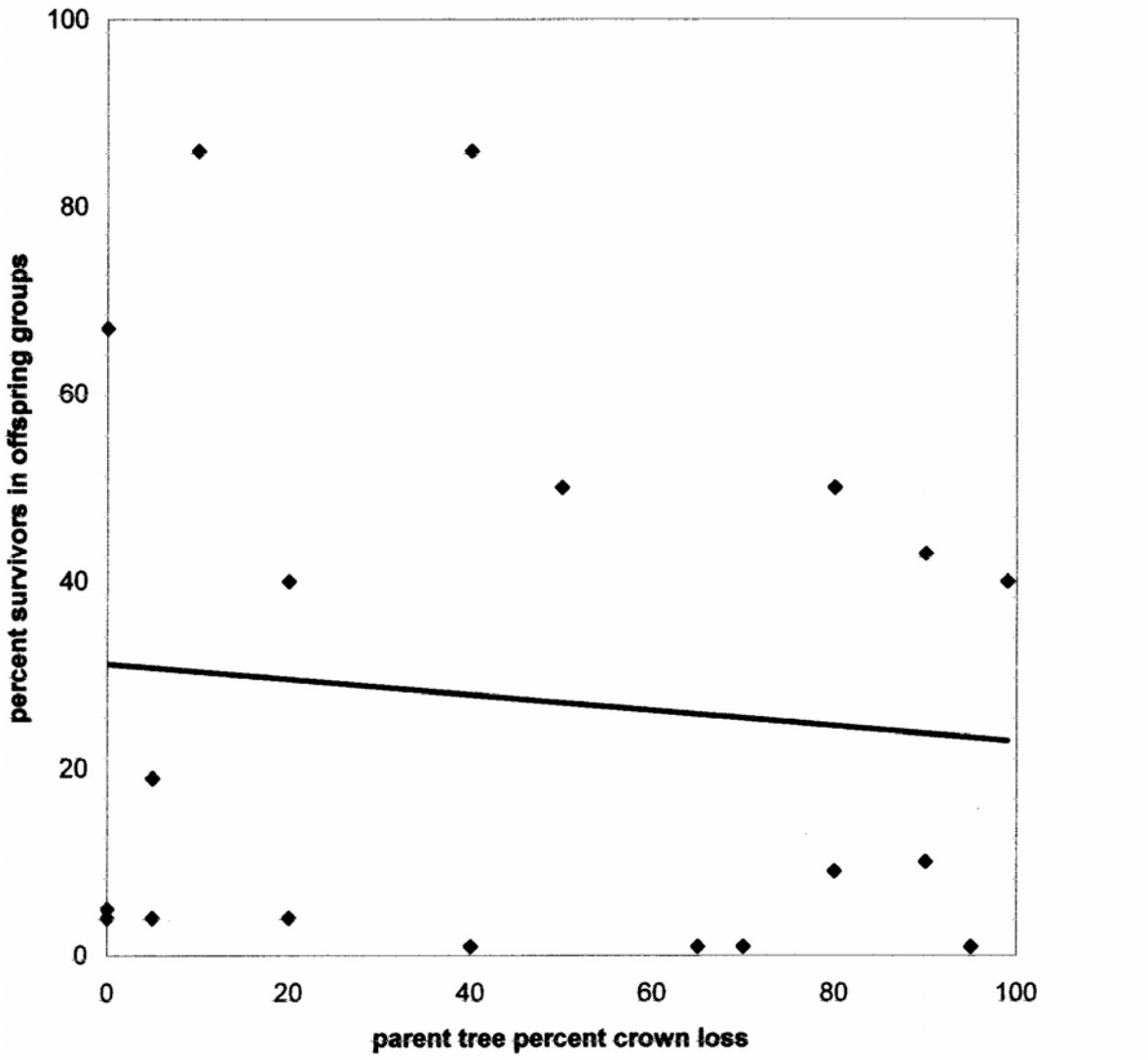

FIGURE 9 Comparison of Survivability of Seedling Groups from 1998 to Parental Crown Losses. The percentage of survivors in half-sib seedling groups from the 1998 seedling crop were compared against the crown loss percentages of their post-epidemic maternal live oak parent trees The line is the least-squared line for regression. The value of R^2 is .011. The 95% confidence interval for the slope of the line is minus -.487 to .234.

TABLE 3 Summary Output and ANOVA for Figure 9.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.103564
R Square	0.010726
Adjusted R Square	-0.047467
Standard Error	30.19022
Observations	28

ANOVA

	df	SS	MS	F	Significance F
Regression	1	167.9905	167.9905	0.1843	0.67309
Residual	17	15494.64	911.4495		
Total	18	15662.63			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	31.1446	11.0994	2.8060	0.012	7.7268	54.5634
X Variable	-0.0824	0.1918	-0.4293	0.673	-0.4871	0.3239

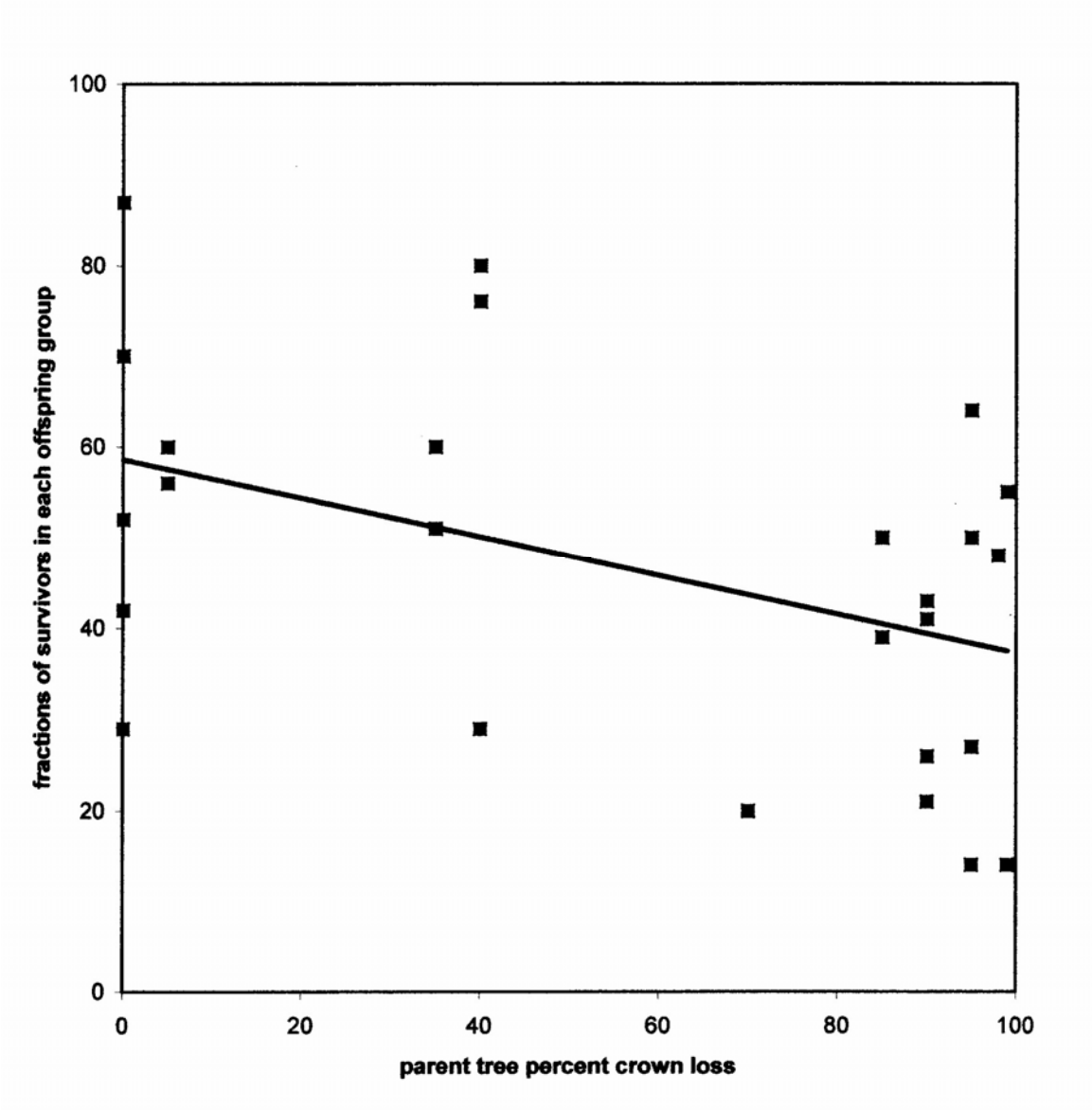

FIGURE 10 Comparison of Survivability of Seedling Groups from 1999 to Parental Crown Losses. The percentage of survivors in half-sib seedling groups from the 1999 seedling crop were compared against the crown loss percentages of their post-epidemic maternal live oak parent trees. The line is the least-squared line for regression. The value of R^2 is .203. The 95% confidence interval for the slope of the line is -.384 to -.483.

TABLE 4 Summary Output and ANOVA for Figure 10.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.450504
R Square	0.202954
Adjusted R Square	0.172299
Standard Error	17.626034
Observations	28

ANOVA

	df	SS	MS	F	Significance F
Regression	1	2056.825	2056.825	6.6205	0.016138
Residual	26	8077.603	310.6771		
Total	27	10134.436			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	58.6067	5.7198	10.2464	0.000	46.8496	70.3638
X Variable	-0.2132	0.0829	-2.5730	0.016	-0.3836	-0.0429

Comparison between pre- and post-epidemic live oak seedling tolerances to oak wilt: Inoculated pre- and post-epidemic 1998 crop seedling groups were compared for relative tolerance using two estimates: 1) percentage of a group with ≤ 25 percent crown loss, and 2) percentage of a group that survived. The percentage of seedlings with ≤ 25 percent crown loss in the pre- and post-epidemic seedling groups were 5/99 (9.3 %) and 23/246 (5.0 %) respectively. The post-epidemic population had a higher percentage of seedlings with ≤ 25% crown loss, but the difference was not significant (χ^2 = .2). The percentages of surviving seedlings in the pre- and post-epidemic groups were 77/97 (79.4 %) and 185/246 (75.2 %) respectively. There was no statistical difference between the populations of survivors (χ^2 = .413).

Comparison of stem inoculation versus root inoculation of live oak seedlings: When a population of 20 stem inoculated live oak seedlings from the 1998 crop was compared to a population of 20 root inoculated seedlings, there was no difference in the percentage of survivors (10 survivors each group) and no difference in the percentage of seedlings with ≤ 25 percent crown loss in each group (2 seedlings per group).

Comparison of stem height among live oak half-sib seedling groups: There was a wide diversity among half-sib groups from the 1999 seedling crop for mean stem height, and several half-sib groups had means that were significantly different when using the t-test to estimate 95% confidence intervals (Figure 11). There were no significant differences in seedling height between the different, randomized, mixed blocks for the 1999 seedling crop (Figure 12).

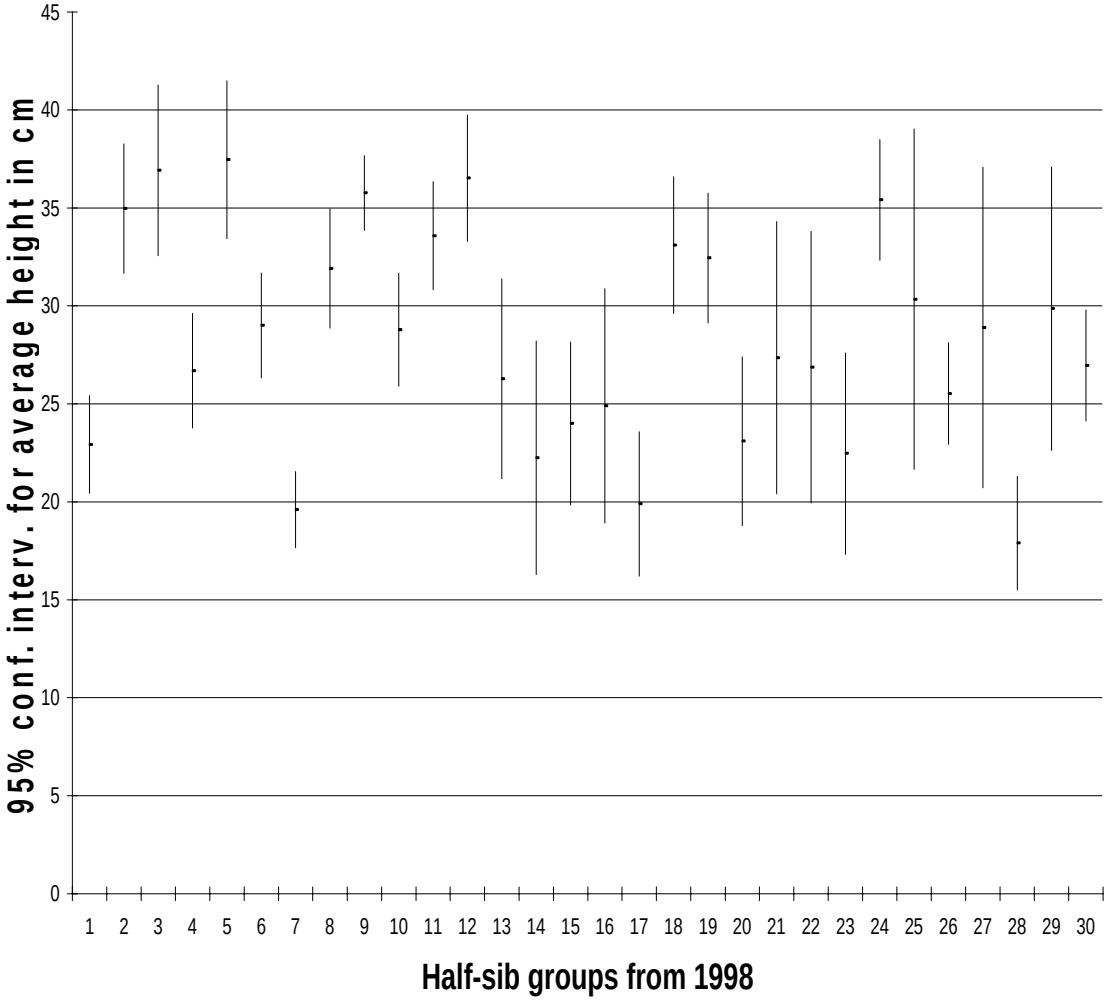

FIGURE 11 Stem Height Comparisons for Half-sib Groups of Seedlings. Average main stem heights were compared among half-sib groups of live oak seedlings from the 1999 crop. The vertical lines represent 95% confidence intervals.

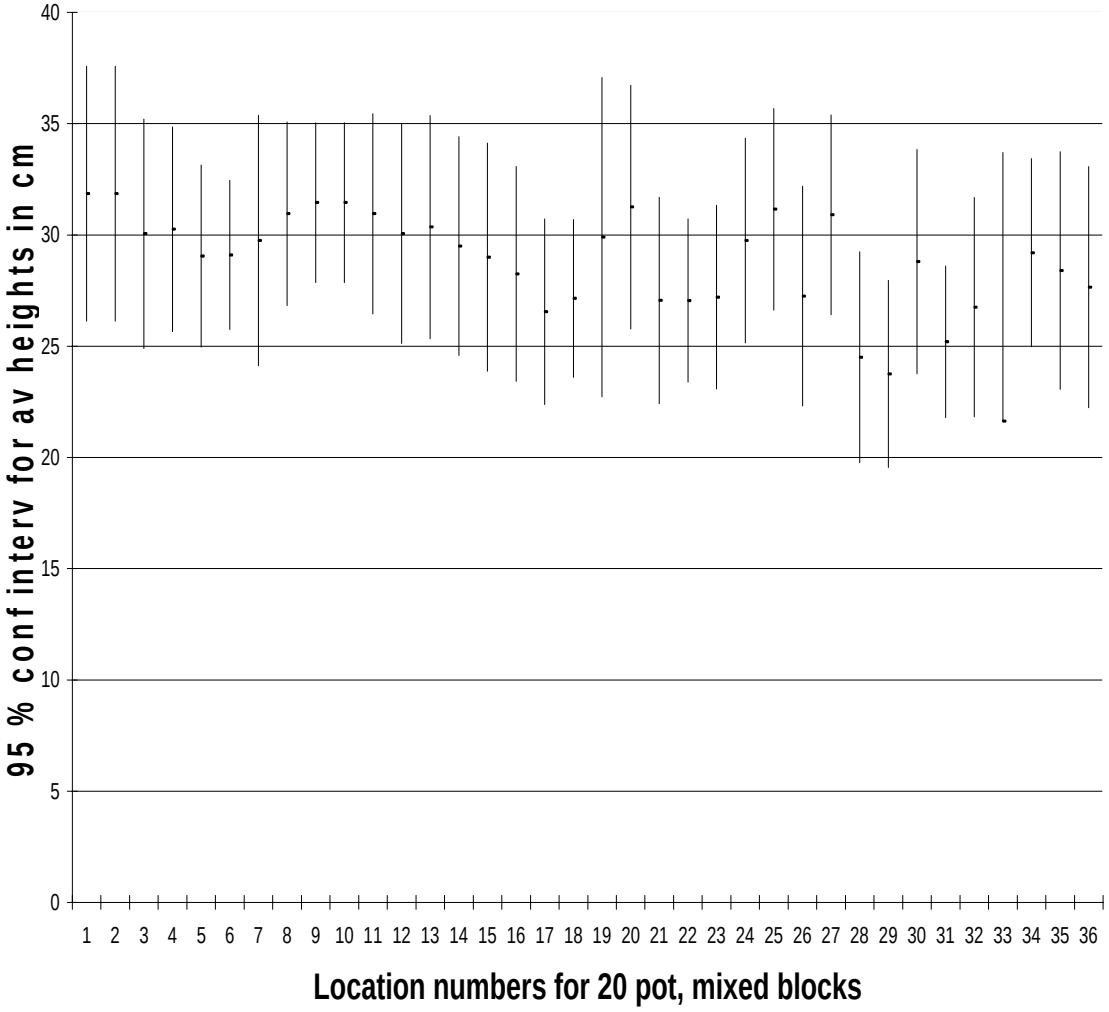

FIGURE 12 Stem Height Comparisons for Randomized Blocks of Seedlings. Average main stem heights were compared among randomized blocks of live oak seedlings from the 1999 crop. The vertical lines represent 95% confidence intervals.

Comparison of clonal resistance to parental resistance of live oaks: The creation of clones from pre- and post-epidemic live oak tree ramets had a limited success rate of approximately 25 percent. Ninety-four clonal trees were grown from thirty-three trees, and the clonal groups ranged in size from one to nine clones per parental tree. Fifty-eight individual clones from post-epidemic parent trees were inoculated with *C. fagacearum* and their resulting crown losses were compared to parental crown losses of the 28 parental trees. There was no significant correlation between previous parent tree performance and the crown loss of their individual clone (Figure 13) (Table 5). In a second correlation, the average crown losses of clonal groups from the 28 parental trees were compared to the clonal trees and also resulted in no correlation (Figure 14) (Table 6).

The clonal group sizes were too small for conclusive statistical comparisons of tolerance to oak wilt among groups. However, in comparisons of nine clone groups that had at least three members, one group was consistently tolerant and two groups were consistently susceptible to oak wilt, as estimated by crown loss averages (Figure 15)**.**

Two large clonal groups from campus live oak trees were originally used solely to perfect the propagation procedure of Wang and Rouse (117). Because we were able to generate so many clones from these two trees (more than 30 per tree), we inoculated these clones to see how much variation we would see in large groups of clones. Both of these groups had less than five survivors out of 30 challenged clones, and only one clone in each group had less than 99 % crown loss. These clones were tested as an addition to our experiment and they were grow in smaller pots than the campus clones and in some cases two clones shared a pot.

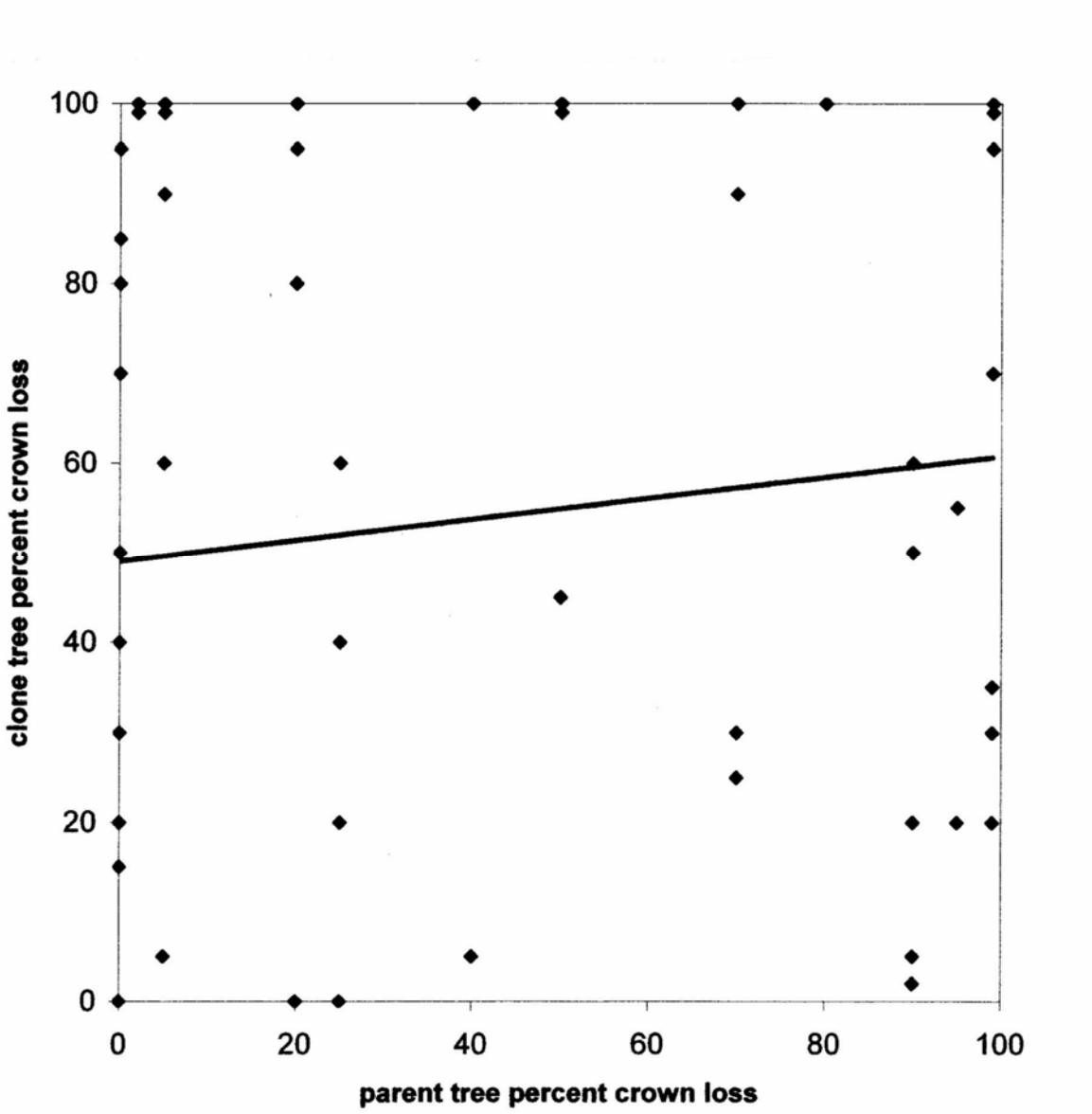

FIGURE 13 Comparison of Crown Losses of Clones to their Parent Trees. The percentage crown losses of parent trees were compared to the percentage crown losses of all individual clones. The number of clones from each of the twenty-eight parent trees varies from one to five. The line is the least-squared line for regression. The value of R^2 is .016. The 95% confidence interval for the slope of the line is -.126 to .361.

TABLE 5 Summary Output and ANOVA for Figure 13.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.126950
R Square	0.016116
Adjusted R Square	-0.0011 44
Standard Error	41.196480
Observations	59

ANOVA

	df	SS	MS	F	Significance F
Regression	1	1357.395	1357.395	0.9337	0.3380
Residual	57	82867.55	1453.817		
Total	58	84224.95			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	48.99067	7.3181	6.6945	0.000	63.6449	34.3364
X Variable	0.11749	0.1216	0.9663	0.3380	-0.1264	0.3610

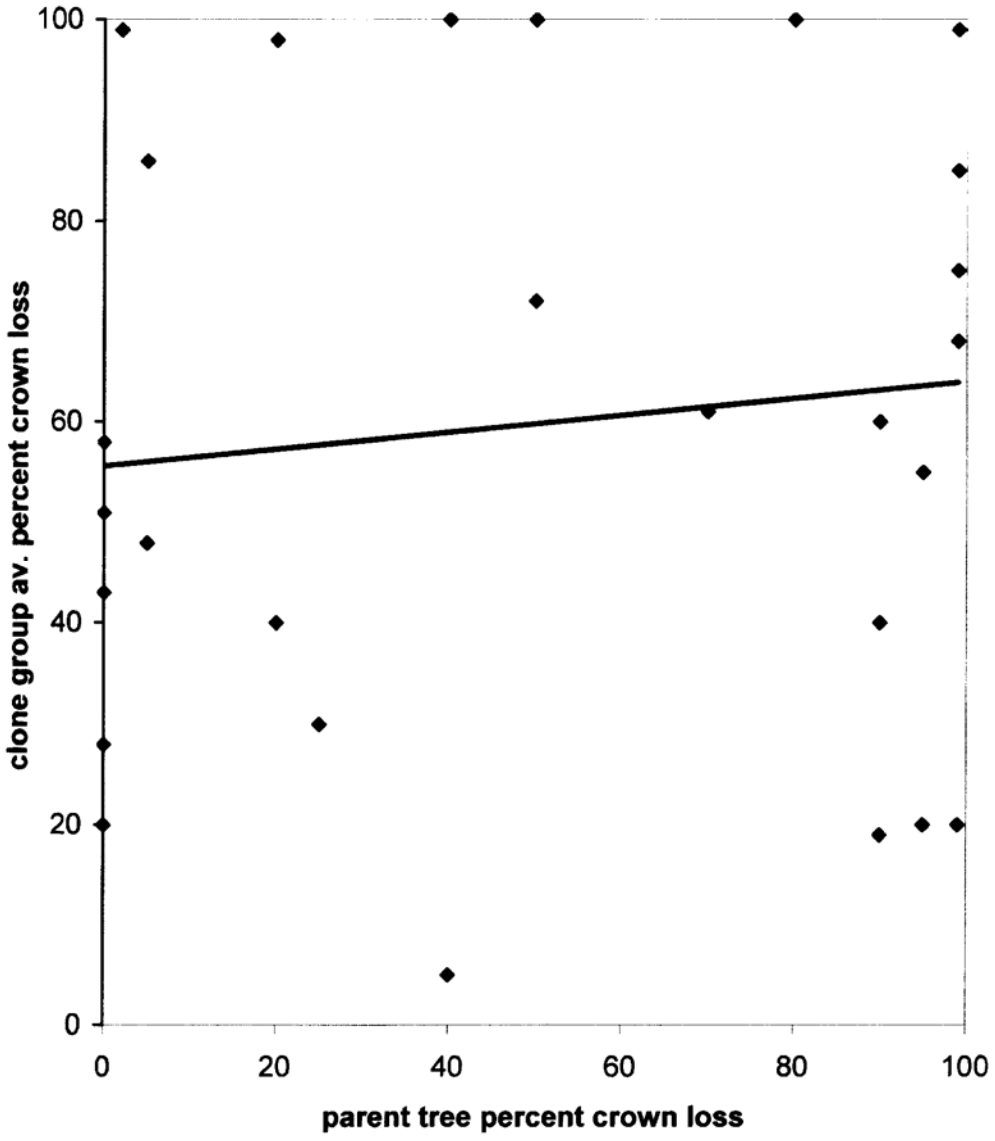

FIGURE 14 Comparison of Average Crown Losses of Clonal Groups to their Parent Trees. The percentage crown losses of parent trees were compared against the average percentage of crown loss of their two-year-old clonal groups. The line is the least-squared line for regression. The value of R^2 is .013. The 95% confidence interval for the slope of the line is -.214 to .381.

TABLE 6 Summary Output and ANOVA for Figure 14.

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.112331
R Square	0.012618
Adjusted R Square	-0.025358
Standard Error	31.00591
Observations	28

ANOVA

	df	SS	MS	F	Significance F
Regression	1	319.432	319.432	0.3322	0.569283
Residual	26	24995.53	961.367		
Total	27	25314.96			

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	55.6115	9.5580	5.8183	0.000	35.9646	75.2584
X Variable	0.0834	0.1447	0.5764	0.569	-0.2141	0.3809

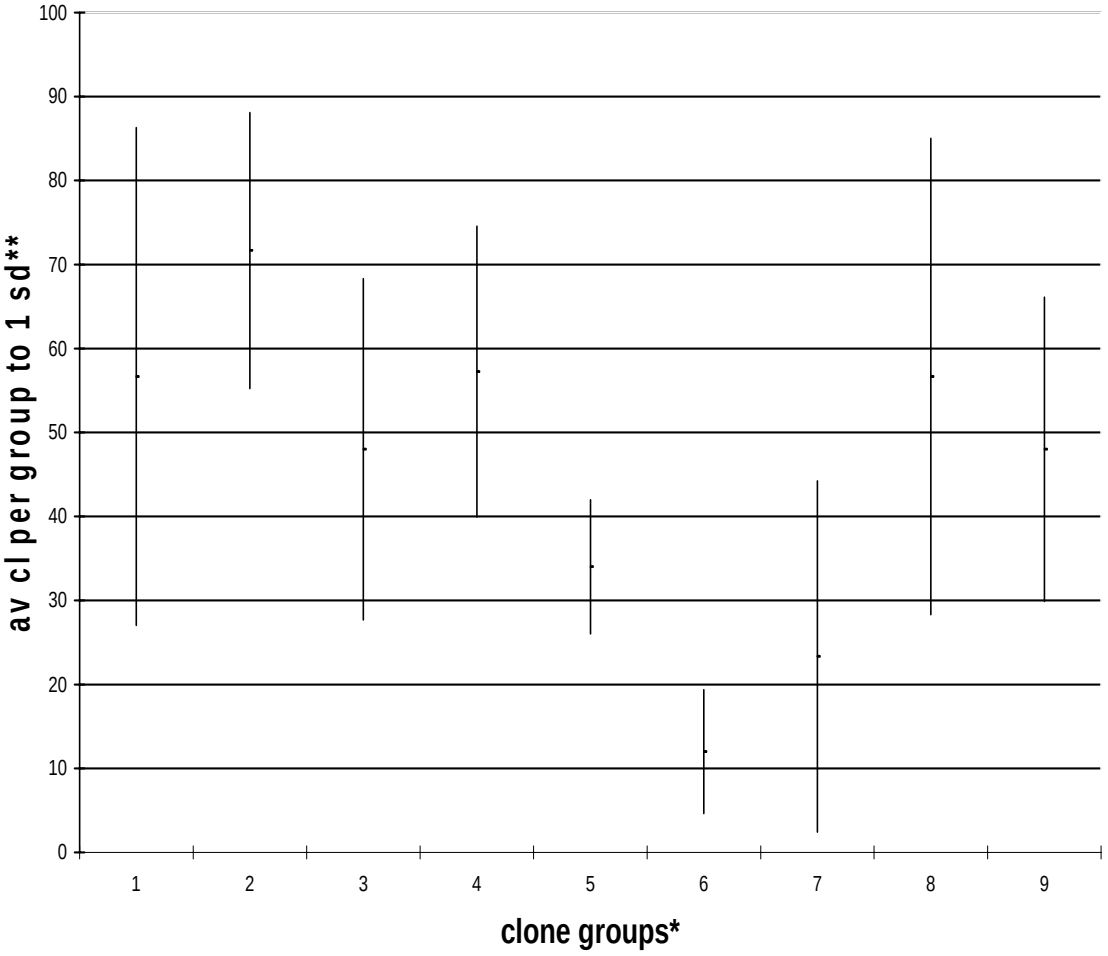

FIGURE 15 Comparison of Average Crown Losses of Clonal Groups. The clones were created from ramets from live oak trees at oak wilt disease sites. Each clone group contains at least three plants. The confidence interval is for one standard deviation. The individual crown loss percentages of the trees in the nine clone groups are as follows: group one (100, 70, 0); group two (95, 80, 40); group three (100, 90, 40, 5, 5); group four (99, 70, 40, 20); group five (50, 45, 40, 30, 5); group six (40, 10, 10, 0, 0); group seven (65, 5, 0); group eight (85, 85, 0); and group nine (95, 85, 30, 30, 0). Group number six was cloned from a post-epidemic tree that had 25 % crown loss. Group number five was cloned from a post-epidemic tree that had 30 % crown loss.

Comparison of latent period between inoculated live oak seedlings from pre- and post-epidemic parents: The mean latent period until symptom expression after inoculation for pre- and post-epidemic half-sib seedling groups (from the 1998 crop) were 30.8 and 28.6 days respectively. The post-epidemic population had a shorter average latent time using a t-test ($P = .058$). Comparisons of mean latent period for ramet producing seedlings versus non-ramet producers were significantly shorter for ramet producers in two separate trials of different populations of seedlings from the 1998 crop. In the greenhouse seedling population of 402 seedlings, the 295 ramet producing seedlings had a mean latent period of 28.9 days compared to 34.5 days for the 107 non-ramet producing seedlings. This difference was significant at $P = .0006$ using a t-test. In the growth chamber population of 162 inoculated seedlings, 65 ramet producing seedlings had a mean latent period of 20.7 days compared to 31.2 days for the 97 non-ramet producing seedlings. The difference was significant at $P = .0002$ using a t-test.

Comparison of ramet production between live oak groups: The percentage of seedlings producing ramets in the seedling groups from pre- and post-epidemic 1998 crop parents (from the Round Rock oak wilt research site) were compared and the results were: 96/138 (79.8 %) and 161/202 (69.6 %) respectively. Ramet production was significantly higher in the seedling population grown from acorns from the post-epidemic trees ($\chi^2 = .03$).

CHAPTER IV

POPULATION EXPERIMENTS

The experiments described in this chapter were designed to determine if allozyme markers would detect selection due to resistance in live oaks after exposure to oak wilt caused by *C. fagacearum*. Previous research that analyzed population genetics of live oaks in an oak wilt disease site found numerous differences between the genetic structure of the population of uninfected trees adjacent to an oak wilt center (pre-epidemic population) and the population of trees that survived the epidemic (post-epidemic population) (19, 72). They reported such post-epidemic population differences as: significantly different allele frequencies, lower gene diversity, the increase of multi-locus associations, and departure from Hardy-Weinberg equilibrium in some loci. Overall, they noted that there were "significant perturbations found for all four allozyme loci assayed" between the pre-epidemic and post-epidemic populations (19, 72). The genetic changes they found were interpreted as being the result of selection in the live oak population due to the plant pathogen interaction for resistant host genotypes.

The purpose of this experiment was to survey new oak wilt disease sites using population genetics analyses of allozyme markers to test for the same differences in population genetics dynamics that were previously reported McDonald et al. (72). If the same alleles increased or decreased in the post-epidemic population of this study as in prior research (72), then the previous hypothesis, that these alleles were associated with and possibly linked to resistance genes, would be supported.

The decision was made to conduct allozyme tests at two oak wilt disease sites to compare pre-epidemic to post-epidemic populations. This project was expanded to include a population genetics study using a greenhouse population of half-sib groups of live oak seedlings that were grown from acorns from the oak wilt site originally used in a previous study (72).

Materials and Methods

Locations of live oak leaf collections: Three separate oak wilt disease sites were tested in this part of the project (Austin, Izoro, and Round Rock). All sites consisted of two separate, adjacent populations: a post-epidemic population in which the disease had already killed and damaged live oak trees and a pre-epidemic area into which the disease had not yet spread. Trees in a transitional area between pre- and post-epidemic populations at least 100 feet from symptomatic trees were excluded. It was not possible to predict survivability of trees in the area.

Three types of comparison were done between the pre- and post-epidemic populations for the Austin and Izoro research sites. These comparisons were used to look for micro transitional evolutionary differences in allele frequencies and combinations. The first comparison "(Full Comparison") consisted of using leaf samples from all of the sample trees from each population. The second comparison ("Superior Comparison") used the same pre-epidemic trees as the full comparison, but used only post-epidemic trees that had no more than 30% crown loss. Therefore, the pre-epidemic populations for each comparison are identical, and will be simply referred to as such. The third comparison ("Clone Corrected Comparison") consisted only of the different multi-locus genotypes found within a subpopulation (pre- or post-epidemic). No genotype was used more than once, preventing the possibility of using clonal groups of trees in the population data.

The Austin and Izoro pre-epidemic populations were also compared to each other to determine if geographically distinct live oak populations in Texas show similar population dynamics, i.e. allele frequencies, gene diversity, and genotypic diversity.

The same population of live oak seedlings that were grown from acorns collected at Round Rock for inoculation screenings (Chapter III) was also evaluated for allozyme frequencies. In the seedling allozyme evaluations, the entire seedling population from the 1999 acorn crop (from the half-sib analysis in Chapter III) was designated as the pre-inoculation population. The fraction of this seedling population that was still alive one year after inoculation with *C. fagacearum*) was evaluated as the survivor population. The genetic characteristics of the pre-inoculation and survivor populations were compared. A

second seedling population comparison (superior comparison) was done between the pre-inoculation population and the superior survivors (no more than 25 % stem death). In a third comparison (ramet comparison), the sub-population of surviving seedlings that produced ramets was compared to the survivors that did not. In a fourth comparison, the allele frequencies were compared between seedlings with short latent periods (< 20 days) versus seedlings with long latent periods (> 20 days).

The Austin disease site is approximately 10 hectares in size and is located in the Balcones Canyon Lands Preserve which is located next to Highway 360 on the outskirts of Austin, in Travis County (Figure 2). Oak wilt was discovered at this site in the early 1980's. This site consisted of a post-epidemic area surrounded on 3 sides (approximately 270 degrees) by pre-epidemic trees.

The Izoro site is approximately 15 hectares in size and is located approximately 5 kilometers from the town of Izoro, in Lampasas County (Figure 1). Oak wilt was also discovered at this site in the early 1980's, and consisted of a post-epidemic area surrounded on 3 sides (approximately 270 degrees) by pre-epidemic trees.

Tree sampling: In the Austin and Izoro disease sites, trees were chosen from both pre- and post-epidemic areas for sampling leaf tissue for allozyme analysis. Because the trees were not uniformly distributed in either pre- or post-epidemic areas, the goal was to sample as evenly as possible. Live oaks can regenerate asexually by root sprouting, so a minimum of 20 feet was used as a standard between sampled trees to avoid nearby clones. The trees were labeled, their locations recorded, and the post-epidemic trees were evaluated for percentage of crown loss resulting from infection.

For the greenhouse population of seedlings the goal was to sample as many seedlings as possible to create a large population for allozyme analysis.

Allozyme procedure: Several leaves (10 to 20 per tree) where collected from live oaks, put in labeled zip lock bags, and immediately placed in a cooler. All leaves were transported to the lab, kept refrigerated at 4ºC and analyzed within three days of collection. The enzyme extraction procedure consisted of several steps. Prior to extraction, leaves were washed and blotted dry. Leaves were cut into small pieces, large veins were removed, and 300 mg samples were weighed. Each sample was then added to a mortar containing an equal volume of finely ground, abrasive sand and 1 gm of pvp-40.

Leaf samples were then ground inside an air flow hood in 1 ml of isozyme extraction buffer (Appendix A) while bathed in liquid nitrogen. Fluid from the samples was filtered through fine chiffon cloth placed over the slurry and used to saturate wicks made from Whatman filter paper. Wicks consisted of 2 layers of filter paper cut to 2 mm by 5 mm. The size of the wicks allowed for gels to be run with 20 samples and 2 control lanes. Prior to use in the gels, the wicks were placed on paper towels in an air flow hood to remove excess liquid and to allow mercaptoethanol in the extraction buffer to evaporate. Samples were prepared immediately prior to usage in gels, but extra wick samples were frozen for backup runs.

Electrophoresis gels were made from a mixture of 12 % v/v of potato starch (Sigma, St. Louis, MO) to water. Two different types of gels systems were used. A lithium borate gel system (Appendix B) was used to evaluate phosphoglucoisomerase (PGI) allozymes, and a histidine gel system (Appendix B) was used to evaluate phosphoglucomutase (PGM) and malate dehydrogenase (MDH) allozymes. This procedure required the mixture to be heated until viscosity was diminished (10 to 15 minutes) in an Erlenmeyer flask while shaking vigorously and then degassed under vacuum until large bubbles ceased to appear (30 to 60 seconds). The mixture was then poured into plastic gel molds for hardening and covered with plastic wrap. Gels required 6 hours to set at room temperature and could be kept for 2 days at room temperature until used for electrophoresis.

One hour prior to gel electrophoresis, the electrophoresis trays were placed into a refrigerator at 4º C to cool the electrode buffer (Appendix B). Fifteen minutes prior to running, the gels were also placed into the refrigerator for cooling. The cooling was necessary to prevent the gels and enzymes from being damaged by heat generated from high voltages used during electrophoresis. The cooled gels were prepared for sample insertion by cutting a vertical slit 4 cm from the anodal side of the gel. The end section of the gel was then pulled away, wicks were placed along the slit, and the gel was then rejoined. The gel was reinserted into the plastic mold with two soda straw spacers inserted at the anodal end to compress the gel to maintain electrical contact. After the gel was set onto the gel electrophoresis tray, electrical contact between the anodal and cathodal buffer tanks was created by cellulose towels that had one edge deep in a buffer

tank and the opposite edge laid over 1 cm of the gel surface on each side. A thin glass plate with an ice tray on top was placed over the gel for further cooling.

All gels were initially run for 15 minutes, stopped for wick removal, and electrophoresis was restarted. The lithium borate and the histidine gels were run using different voltages and times because of differences in allozyme migration speed and heating effects on the gels for the different buffer systems. The lithium borate gels were run for 2 hours total at 220 to 270 volts to give the desired amperage of 75 milliamps, and the histidine gels were run for a minimum of 8 hours at 280-320 volts to give the desired amperage of 50 milliamps.

Gels were stained immediately following electrophoresis. The gels were cut into slices 20 mm thick, placed into staining solutions (Appendices C and D), and put in the dark at room temperature. The gels were kept in the staining solutions until the alleles could be clearly differentiated. After staining, the gels were fixed in a 45% ethanol solution for 10 minutes. Finished gels were scored on a light box and photographed.

Gel evaluation: The gels were scored using the procedures of basic procedures set forth by Guttman and Weight (44). The models used for allozyme gel labeling are based on diploidy, knowing the quaternary structure of the enzymes, and previously documented numbers of loci (Figures E-1, E-2, E-3, and E-4). Live oaks are diploid and have 24 chromosomes, D'Emerico et al. (29). The trees at each research site were labeled, their allozyme genotypes were recorded and their allozyme phenotypes were recorded (Tables F-1, F-2, F-3, and F-4)

Statistical analyses: Allozymes were analyzed as alleles at individual loci. Therefore, the collection of all allozymes for each tree represents its genotype. Allozyme data from all of the trees for each site were entered into the software population genetics program "POPGENE-VERSION 1.31" that was developed and made available by Yeh and Boyle (125). POPGENE is a population genetics program that is adaptable to the use of both haploid and diploid data, and it can be used for co dominate markers such as allozymes. The POPGENE program can be used to estimate such individual population parameters as: allele frequencies*, genotype frequencies, genetic diversity (including expected genetic diversity) (82), Hardy-Weinberg equilibrium, effective allele number, observed and expected homozygosity and heterozygosity, and multilocus structure. POPGENE

can be used for multiple populations to compute Wright's F statistics (123), genetic distance and gene flow (N_m) (83). F statistics measure the loss of heterozygosity on three levels (46, 119, and 123). F_{IS} is a measure of the loss of heterozygosity in a population because of inbreeding; F_{ST} is a measure of the loss of heterozygosity in a population because of population subdivision, and F_{IT} is a measure of the total loss of heterozygosity because of the combination of inbreeding and subdivision. Gene flow (N_m) is a measure of the number of immigrants between two populations per generation and is calculated from the F_{ST} value. Genotype diversity was calculated using "Taylor's G" (106) using only trees with complete genotypes. Because two allozyme loci (PGM-1 and PGM-2) had skewed allele frequencies that resulted in small sample sizes for some alleles, additional tests of significance for some population genetics parameters were also tested with "exact tests" for significance. Exact tests for linkage disequilibrium and Hardy-Weinberg equilibrium were done using the population genetics software program PowerMarker, Version 3.25 (66, 118). Because of the skewed allele frequencies at some loci, direct count heterozygosities were used to measure genetic diversity. Exact tests for differences in the allele frequencies and for differences in direct count heterozygosity were carried out using SAS 9.1.3., 2006 (95) to run an exact test for independence. The direct count heterozygosity calculations use the actual number of heterozygotes and homozygotes in two populations and then compare them for significant independence. Use of direct count heterozygosities allow exact tests to be run, which are useful nonparametric estimators of significance between sample values when sample numbers are small or skewed. The exact test is from the works of Fisher (36, 37) and uses a hypergeometric distribution to determine probabilities. This is a discrete probability distribution used to determine the number of successes expected in a finite population when done without replacement. This is done by using permutations.

The probability of adjacent trees being clones was calculated by the methods of Parks and Werth (88). They denote the probability of two adjacent trees not being clones as P_{gen}, which is the probability that two trees with the same genotype will not be sampled consecutively. This calculation is based on the frequency of the alleles in each genotype and is the likelihood of picking the genotype in one trial. The P_{gen} concept can be expanded to groups of more than two adjacent trees, by squaring the P_{gen} value for three trees, or cubing it for four trees. This is based on multiplicative probability and is useful when the number of trees with the same genotype is small relative to the total population size. If P_{gen} is 0.01 the chance of two adjacent trees not being clones is 0.01. If a third tree with the same genotype is adjacent, then the chance of two of the three trees not being clones is $.01^2$ and is therefore .0001.

The crown loss averages were calculated for groups of trees (≥ 4) with the same genotype at the two research sites. Group crown loss averages were compared using a t-test.

In probability tests for overall frequency differences for each locus, chi-square and G^2 probabilities were analyzed by the POPGENE software program (125), and the Fisher's exact test probabilities were analyzed by the SAS 9.1.3, 2006 statistical program (95) using frequency contingency tables. In the tests for individual allele frequency differences (with all other alleles summed), the chi-squared and Fisher's exact test probabilities were generated using contingency tables in SAS 9.1.3., 2006.

Results

Allele frequencies of populations: The gels that were stained for PGI contained 2 different allozyme zones of activity (loci). The zone that migrated the furthest (PGI-1) was monomorphic and was not used for population genetics calculations. The second allozyme system (PGI-2) was polymorphic and was previously reported to have 6 alleles (C, D, E, F, G, and H) (19). All 6 PGI-2 alleles were found during this experiment. The PGI-2 enzyme shows expression of 3 gel bands when there is a heterozygote, which is consistent with enzymes that function as dimers (Figure E-1).

The gels that were stained for PGM consisted of 2 separate zones of activity (PGM-1 and PGM-2), which is consistent with previous research (19). The 2 loci were polymorphic and each had 3 alleles (A, B, C, and D, E, F.). The heterozygotes had 2 bands which indicated this enzyme functions as a monomer (Figures E-2, and E3).

The gels that were stained for MDH-3 had 3 zones of activity. The two furthest migrating zones (MDH-1 and MDH-2) were monomorphic and were not used in population calculations. The third zone of activity (MDH-3) was polymorphic and was previously reported to have 7 separate alleles (19). The MDH-3 heterozygotes had 5 bands, which is consistent with tetrameric enzymes (Figure E-4). Only 5 MDH-3 alleles were found during this study (C, D, E, F, and G). The MDH enzyme system was not used for the greenhouse seedling population during this project due to poor MDH-3 activity.

Izoro Research Site: Three population comparisons were done at the Izoro research site using allozymes: 1. the "Full Comparison" of pre-epidemic population trees with all post-epidemic trees, 2. the "Clone Corrected Comparison" of all pre-epidemic trees with all post-epidemic trees with unique genotypes, and 3. the "Superior Comparison" of all pre-epidemic population trees with a post-epidemic population consisting of trees with 30 % crown loss or less.

Of 8 possible alleles, 5 PGI-2 alleles (C, D, E, F, and G) were present at the Izoro site. The E allele was the most common allele with a minimum frequency of 0.50 in all populations (Tables G-1, G-2, and G-3). The C and G alleles were rare alleles (less than 0.05). The C allele was absent from the pre-epidemic population in all comparisons, but

present at low frequencies in the full, clone corrected, and superior post-epidemic populations. The F allele dropped in frequency in the post-epidemic populations of all comparisons. The common theme for all comparisons was a drop in the frequency of the F and G alleles in the post-epidemic populations**.** However, the PGI-2 allele frequency changes between the Izoro pre- and post-epidemic populations were not significant for any of the 3 comparisons (Tables G-4, G-5, and G-6).

The 3 previously documented alleles (A, B, and C) for the PGM-1 locus (19) were all found at the Izoro site. The B allele was the most common allele with a minimum frequency of 0.88 in all populations (Tables G-1, G-2, and G-3). The A and B allele frequencies remained essentially the same between the full pre- and post-epidemic populations. The B allele dropped slightly in frequency in the post-epidemic populations for the "Clone Corrected" and "Superior Comparisons". The A allele decreased in frequency in the "Clone Corrected" post-epidemic population from 0.07 to 0.05. The allele frequency changes between the pre- and post-epidemic populations for the PGM-1 locus were not consistent among the 3 comparisons except for the increase in the frequency of C. There were no significant differences in PGM-1 allele frequencies between pre- and post-epidemic Izoro populations (Tables G-4, G-5, and G-6).

The 3 previously documented alleles (D, E, and F) for the PGM-2 locus (19) were all found at the Izoro site. The E allele was most common with a minimum frequency of 0.87 in all populations of all comparisons. There was a common pattern in the post-epidemic populations of a decrease in the E and F alleles and an increase in the D allele. (Tables G-1, G-2, and G-3) The allele frequency differences between the pre- and post-epidemic populations were not significant for the any comparison (Tables G-4, G-5, and G-6).

Of the 7 alleles previously reported for the MDH-3 locus (19), 5 alleles were found at the Izoro site (C, D, E, F, and G) (Tables G-1, G-2, and G-3). The C allele was most common, with a minimum frequency of 0.64 in all populations. The D and E alleles were rare or missing in the populations. There were no consistent MDH-3 allele frequency differences between any of the comparisons between Izoro pre- and post-epidemic populations. There were no MDH-3 alleles significantly different between the pre- and post-epidemic populations (Tables G-4, G-5, and G-6).

Austin Research Site**:** PGI-2 locus comparisons: Five PGI-2 *alleles* were found at the Austin site (C, D, E, F, and H). The E allele was the most common allele in all populations with a minimum frequency of 0.50 (Tables H-1, H-2, and H-3). The C and H alleles were rare alleles in all populations. The constant theme for the Austin PGI-2 allele frequency changes between pre- and post-epidemic populations was an increase in the frequency of the E allele and a decrease in the frequencies of D and F in the post-epidemic population. In the "Full Population Comparison" there was a difference in the allele frequencies of the PGI-2 locus between the pre- and post-epidemic populations ($P = 0.07$ by chi-square test, $P = 0.04$ by G^2 test, and $P = 0.06$ by Fisher's exact test (Table 20). The significant difference was primarily due to the increase in the post-epidemic population of the frequency of the E allele with a decrease of the D and F allele frequencies. The PGI-2 E allele was at a significantly higher frequency in the full comparison post-epidemic population ($P = 0.034$ by chi-square test, and $P = 0.044$ by Fisher's exact test). In the clone corrected and superior comparisons, the overall allele frequency differences between the pre- and post-epidemic populations were not significant (Tables H-5, and H-6).

Three alleles (A, B, and C) were present for the PGM-1 locus at the Austin site. The B allele was most common with a minimum frequency of 0.95 in all populations (Tables H-1, H-2, and H-3) and was higher in frequency in the post-epidemic population of all comparisons. The C allele was present in all pre-epidemic populations, but was missing in all post-epidemic populations. The A allele stayed at the same frequency for the pre- and post-epidemic in the Full Populations Comparison", increased in the "Clone Corrected Comparison", and decreased in the "Superior Comparison". The constant theme at this locus was for a small increase in the B allele and a loss of the C allele in the post-epidemic populations. The pre- and post-epidemic allele frequencies for the PGM-1 locus were different in the "Full Comparison" ($P = 0.081$ by chi-square test, $P = 0.038$ by G^2 test, and $P = 0.058$ by Fisher's exact test) (Table H-4). In the "Full Comparison", the PGM-1 C allele was significantly higher in frequency in the pre-epidemic population ($P = 0.025$ by chi-square test and $P = 0.039$ by Fisher's exact test). There were no significant differences in allele frequencies between the pre- and post-epidemic

populations in the "Clone Corrected" and "Superior Comparisons" (Tables H-5, and H-6).

Three alleles (D, E, and F) were present for the PGM-2 locus at the Austin site. The E allele was most common with a minimum frequency of 0.82 in all populations (Tables H-1, H-2, and H-3). The E allele did increase in frequency in the post-epidemic population for the "Full Comparison", but remained the same in the "Collapsed" and "Superior" post-epidemic populations. The F allele was missing in all post-epidemic populations, but was present at low frequencies in the pre-epidemic populations. The constant theme for this locus was a loss of the C allele and an increase in the frequency of the D allele in the post-epidemic populations. The Austin PGM-2 allele frequencies were not significantly different between pre- and post-epidemic populations in any comparison (Tables H-4, H-5, and H-6).

Five MDH-3 alleles (C, D, E, F, and G) were present at the Austin site. The C allele was most common with a minimum frequency of 0.70 in all populations (Tables H-1, H-2, and H-3). The D and E alleles were rare alleles in all comparisons. The F allele had a frequency in the post-epidemic population that was 3 times the pre-epidemic frequency in each comparison. The constant theme at this locus was for a large increase in the frequency of the F allele in post-epidemic populations and a decreased frequency of the D and E alleles. There were significant overall difference in MDH-3 allele frequencies between the pre- and post-epidemic populations in the "Full Comparison" ($P = 0.042$ by chi-square, $P = 0.026$ by G^2 test, and $P = 0.034$ by Fisher's exact test (Table H-4). The MDH-3 F allele was more frequent in the post-epidemic population ($P = 0.053$ by chi-square test and $P = 0.059$ by Fisher's exact test). There were no significant differences in individual allele frequencies between the pre- and post-epidemic populations in the "Clone Corrected" and "Superior Comparisons (Tables H-5, and H-6).

Greenhouse half-sib seedling populations**:** PGI-2 locus comparisons: Six PGI-2 alleles were found in the greenhouse population (C, D, E, F, G, and H). The E allele was most common in all populations with a minimum frequency of 0.50 (Tables I-1, and I-2). The C, G, and H alleles were rare in all populations. In the "Full comparison" all alleles remained at almost the same frequencies in the post-inoculation population. The E allele decreased slightly in frequency from 0.67 to 0.50 in the "Superior" post-inoculation

population. The allele frequency comparisons for the PGI-2 locus were not significantly different between pre- and post-inoculation populations for either the "Full" and "Superior Comparisons" (Tables I-3 and I-4).

Three PGM-1 alleles were found at this site (A, B, and C). The B allele was most common with a minimum frequency of 0.93 in all populations (Tables I-1, and I-2**).** The A and C alleles were rare alleles. PGM-1 allele frequency changes between pre- and post-inoculation populations were small for all alleles. The PGM-1 A allele dropped from a frequency of 0.05 in the pre-inoculation population to 0.03 in the post-inoculation population in the "Full Comparison". In the "Superior Comparison", the A allele did not change in frequency between the pre- and post-inoculation populations (0.046 versus 0.044). No PGM-1 alleles had frequency differences between pre- and post-inoculation populations that were significant (Tables I-3, and I-4).

Three PGM-2 alleles (D, E, and F) were found at this site. The E allele was most common with a minimum frequency of 0.93 in all populations (Tables I-1, and I-2). In the "Full Comparison", the allele frequencies were almost identical between the pre- and post-inoculation populations. In the "Superior Comparison", the D allele increased in frequency from 0.04 in the pre-inoculation population to 0.07 in the post-inoculation population, and the E and F alleles decreased in frequency. There were no significant changes in PGM-2 allele frequencies between pre- and post-inoculation populations for either comparison (Tables I-3 and I-4).

Alleles compared for average crown loss: The average crown losses of post epidemic live oaks were compared for different alleles at each locus for the Austin, Izoro, and greenhouse populations.

Austin post-epidemic site: There were no significant differences in the average crown losses among the alleles from trees for any of the four loci (Table J-1). Trees with the MDH-3 G allele had the lowest average crown loss for any allele at any locus (28.2 %, n = 11), but this was not significantly different ($P = 0.2$, using a two-tailed t-test) than the MDH-3 C allele from trees with the highest average crown loss at that locus (41.1 %, n = 109).

Izoro post-epidemic site: The PGI-2 D allele had an average crown loss of 23.9 % (n = 22) that was significantly lower ($P = 0.04$) than the PGI-2 F allele, which had a

40.7 % crown loss (n = 51) (Table J-2). The PGI-2 E allele (39.1 % av. CL, n = 128) had an average crown loss which was different than the PGI-2 D allele (23.9 % av. CL, n = 22), with $P = 0.06$, (using a two-tailed t-test). No other Izoro alleles had significantly different average crown losses among alleles at the same locus for the full post-epidemic population. The Izoro post-epidemic population was split into 2 subpopulations (A and B) that were on opposite sides of a large pasture. There was a significant difference in the average crown loss between trees in the A and B post epidemic populations that carried the PGM-2 D allele. The A site trees that carried the PGM-2 D allele averaged 59.8 % crown loss (n = 5), compared to a 14.0 % average crown loss in the B site trees with the PGM-2 D allele, ($P = 0.03$, using a two-tailed t-test).

The average crown loss for trees with the PGM-2 E allele in the A site was not significantly lower (33.6 %, n = 81) than the average crown loss for trees with the same allele in the B site (42.0 %, n = 102), ($P = 0.1$, using a two-tailed t-test).

The average crown losses for Austin and Izoro post-epidemic trees were 41.9 % and 38.9 %, respectively, and not significantly different. The average crown losses between the Izoro post-epidemic A and B sites were 41.2 % and 35.9 % respectively, ($P = 0.42$) which was not a significant difference.

Greenhouse post-inoculation survivor population: There were significant differences detected between 2 sets of alleles at two of the three loci tested (Table J-3). At the PGI-2 locus, the average crown loss of the F allele (85.8 %, n = 116) was compared to the PGI-2 E allele (92.6 % CL, n = 429), and the difference was significant ($P = 0.02$, using a two tailed t-test). At the PGM-2 locus, the average crown loss of the E allele (90.3 %, n = 20) was compared to the PGM-2 F allele (98.2 % CL, n = 6), and the difference was significant ($P = 0.002$, using a two-tailed t-test). The small size of the F allele sample must be considered when accepting this small of a probability, but it can be inferred that the probability is $\leq .05$.

Genotype diversity and additional genotype characteristics: The calculations for multi-locus genotype diversity were made by using only trees for which complete genotypes with four loci were obtained. The genotype diversity for the live oak populations at the Austin and Izoro sites was very low. The genotype diversities of the Austin pre- and post-epidemic populations were G = 19.2 and G = 9 which represented

30% and 15% of maximum, respectively. The genotype diversity of the Izoro pre- and post-epidemic populations was G = 10.1 and G = 8.5 which represented 10% and 9% of maximum respectively. There was a substantial loss of genotype diversity at the Austin site in the post-epidemic population.

Austin site: The total sample population of live oak trees consisted of 122 trees that had 38 different genotypes (Tables F-1, and F2). Of the 38 genotypes, 15 were found in more than one tree (multiples) (Table K-1). Of the 14 multiples, three were found only in the pre-epidemic population and three were found only in the post-epidemic population. Seven of the 14 multiples occurred more frequently than expected from the population allele frequencies and assuming Hardy-Weinberg equilibrium. The most commonly occurring genotype (EEBBEECC) occurred 19 times, but was expected to occur 30.5 times based on individual allele frequencies. Eleven of the multi-locus genotypes had $P_{gens} \leq 0.05$. Six multi-locus groups with $P_{gens} \leq 0.05$ had at least two adjacent trees, and three groups had at least three trees in a cluster (Figure K-1). One Austin genotype (DEBBEECC) had a P_{gen} of 0.114, but contained two clusters of three adjacent trees. Based on the P_{gen} value and multiplicative probability, the probability of no clones in these groups of three was significant ($P \leq 0.05$). Four of the 15 groups of multiple genotypes had trees in clusters of three or more (Tables F-1, and F-2). In those clusters, they had significant probabilities ($P \leq 0.05$) of at least one clone in each cluster of three trees.

The average percentage crown losses were calculated and compared for those multi-locus genotype groups containing at least four trees in the Austin post-epidemic population (Table K-1). Five groups had at least four trees and their average percentages of crown loss ranged from 46.8 % to 23.9 %. The lowest average crown loss (for a genotype with 4 post-epidemic trees) was found in the EEBBEECC genotype (23.9 % CL, n = 9) and this was lower than the DEBBEECC genotype (40.9 % CL, n = 12), but not significant using a 2-tailed t-test for means ($P = 0.11$). The average crown loss for all Austin post-epidemic trees was 41.9 %. The EEBBEECC genotype did have a significantly lower average crown loss than all other Austin post-epidemic trees (49.8 %, n = 69) ($P = 0.012$ in a two-tailed t-test).

Izoro site: The total sample population consisted of 193 trees that had 50 different genotypes (Tables F-2, and F-3). Of the 50 unique genotypes, 16 were found in more than one tree (Table K-2). Of the 16 genotypes that occurred more than once, two groups were found only in the pre-epidemic population, and three groups occurred only in the post-epidemic population. Ten of the 16 multiple genotypes were found more often than expected based on population allele frequencies and assuming H-W equilibrium. The most commonly occurring genotype (EEBBEECC) occurred 53 times (expected 40.9 times). In a test of possible clonality, 12 of the 16 genotypes had P_{gen} values ≤ 0.05. Ten genotype groups with $P_{gens} \leq 0.05$ had at least two trees that were adjacent, and seven groups had at least three trees in an adjacent cluster (Tables F-3, and F-4) (Figure 16). The three most common groups had $P_{gens} > 0.05$ (not significant for two adjacent trees being clones), but they all contained at least one cluster of three adjacent trees. Fourteen of the 16 Izoro genotype groups had a significant probability ($P \leq 0.05$) of having at least one clone. The largest genotype groups contained several probable clones. The most common genotype (EEBBEECC) had a $P_{gen} = 0.212$ but had two large clusters of adjacent trees (eight trees and seven trees).

The average percentage crown losses were calculated and compared for the Izoro multi-locus genotype groups that had at least four trees in the post-epidemic population. Seven groups contained at least four trees, and their average percentage of crown loss ranged from 55.8 %, to 20.0 %. The lowest average crown loss, for a group with at least four post-epidemic site members, was found in the group with the DFBBEECC genotype (20.0 % CL, n = 4). This average crown loss was significantly less than the EFBBEECC genotype (55.8 % CL, n = 11) when compared with a 2-tailed t-test ($P = 0.028$). The average crown loss for Izoro post-epidemic trees was 38.7 %. The EEBBEECC genotype group at the Izoro site had a much higher average crown loss (41.3 %, n = 28) than it did at the Austin site (23.9 % CL, n = 9), but the difference was not significant ($P = 0.19$ using a two-tail t-test).

FIGURE 16 Aerial Photograph of Izoro Disease Center Clones. Clone groups are represented by different colors and size combinations. Group 1 is light green, Group 2 is small yellow, Group 3 is light blue, Group 4 is small orange, Group 5 is dark blue, Group 6 is large yellow, Group 7 is dark green, and Group 8 is large orange.

Hardy-Weinberg (H-W) equilibrium: Hardy-Weinberg equilibrium was calculated for both pre- and post-epidemic populations at the Austin, Izoro, and greenhouse populations. H-W equilibrium represents the allele frequencies that would be expected under the assumption of no selection, no migration (from populations with different allele frequencies), no drift, no significant mutations, and random mating.

Austin research site: There was a deviation from H-W equilibrium at the PGM-2 locus in the "Full Comparison", post-epidemic population ($P = 0.06$ by chi-square test and $P = 0.01$ by exact test) (Table L-1). This deviation from H-W expectations was caused by the appearance of two PGM-2 DD homozygotes in the post-epidemic population. Both trees with the PGM-2 DD homozygote genotype were in the same general area and both also had the PGI-2 EF genotype and they were probably clones. The MDH-3 genotype was incomplete for one of the trees, so it was not possible to use P_{gen} based on four loci to estimate the probability that these two trees were clones. The PGM-2 D allele was present at a frequency of only 0.09 in the "Full Comparison" post-epidemic population. In contrast, the PGM-2 locus was in H-W equilibrium in the Austin "Clone Corrected" post-epidemic population. There was one PGM-2 DD homozygote in the "Clone Corrected" population, but the D allele frequency (0.18) was higher than the "Full" population. All other loci at the Austin site were in H-W equilibrium.

Izoro research site: The PGM-1 locus deviated from H-W equilibrium in the pre-epidemic population for the full comparison ($P < 0.001$ by chi-square test and $P = 0.031$ by exact test) (Table L-1). This deviation was still significant in the pre-epidemic population of the "Clone Corrected" comparison according to chi-squared test ($P = 0.037$), but not according to exact test ($P = 0.183$). The deviation from H-W expectations at the PGM-1 locus was due to the presence of one AA homozygote for the low frequency A allele. This deviation from H-W equilibrium was still significant when the "Full" Austin pre- and post-epidemic populations were combined into one population ($P = 0.005$ by chi-square test). The PGM-1 A allele was present at a frequency of only 0.02 in the full post-epidemic population and the combined pre- and post-epidemic populations.

Greenhouse research site: There were no significant deviations from H-W equilibrium in any of the green house, seedling populations for the 3 alleles tested (PGI-2, PGM-1, and PGM-2).

Linkage-disequilibrium: Level of linkage-disequilibrium was calculated for the Austin, Izoro and greenhouse pre- and post-epidemic populations.

Austin site: There were no findings of linkage-disequilibrium between specific alleles or overall loci in the pre- and post-epidemic populations at the Austin site for any of the population comparisons ("Total", "Superior", and "Clone corrected") (Table M-1).

Izoro site: There was linkage disequilibrium detected in both the "Full" pre- and post-epidemic populations between individual alleles and between loci. In the Izoro, pre-epidemic population of the "Full Comparison" there were two cases of linkage disequilibrium. The PGM-1 A and MDH-3 F alleles were linked ($P = 0.032$ by chi-square and $p = 0.007$ by Burrows test (125). However, the PGM-1 and MDH-3 loci were not significantly linked by exact test ($P = 0.23$) (Table M-1) (65). PGM-1 A and MDH-3 F were both low frequency alleles. The genotypes containing both PGM-1 A and MDH-3 F were present in only two trees (p51 and p55), but PGM-1 A was homozygous in the genotype of one of the trees (EF<u>AA</u>EEC<u>F</u>). These two trees were located in close proximity. Three of the five pre-epidemic trees with the MDH-3 F allele (p51, p55, and p59) were located in the same area of the pre-epidemic population, and all of the pre-epidemic trees containing the PGM-1 A allele (p49, p51, and p55) were also located in this area (Figure 17)**.** The PGI-2 and MDH-3 loci were also in linkage disequilibrium in the pre-epidemic population of the "Full Comparison" ($P < 0.001$ by chi-square test and $P = 0.004$ by exact test) (66). No significant linkage disequilibrium was found after the pre-epidemic population was "Clone Corrected".

There were two significant cases of linkage disequilibrium detected in the "Full Comparison", post-epidemic Izoro comparison. In the first association, the PGI-2 C allele was linked to the PGM-1 A allele ($P = 0.015$ by chi-square test and $P = 0.005$ by Burrows test (125). These two loci were also in linkage disequilibrium by exact test ($P = 0.0002$) (Table M-1). Only three trees contained both the PGI-2 C and PGM-1 A alleles. Two of these trees had identical multi-locus genotypes (CEABEECC) and were adjacent.

The P_{gen} (probability of two trees being adjacent and not being clones) for the two adjacent trees (P51, and P55) was 0.001. Therefore, these two trees were identified as clones ($P = 0.999$). After one of the two "clones" was removed from the data set, the statistically significant linkage disequilibrium between PGI-2 C and PGM-1 A was lost.

In the second case of significant post-epidemic linkage disequilibrium, the PGI-2 D allele was linked with the MDH-3 F allele ($P = 0.005$ by chi-square test and $P = 0.016$ by Burrows test) (125). The PGI-2 and MDH-3 loci were also in significant disequilibrium by exact test ($P = 0.036$) (66). This association between PGI-2 D and MDH-3 F occurred in six post-epidemic trees. Three of the six trees (A43, A44, and A45) had the same multi-locus genotype (DEBBEECF) and occurred in a cluster (Figure 17). The P_{gen} for this genotype was 0.021, and the three adjacent trees were identified as clones. The data set was re-analyzed after two of the "clones" were removed and the association between PGI-2 D and MDH-3 F was still significant by chi-square test ($P = 0.01$), but not significant by Burrows test (125) ($P = 0.219$). The PGI-2 and MDH-3 loci were also in significant disequilibrium ($P = 0.024$ by exact test).

Greenhouse site: There were no findings of linkage disequilibrium in any of the greenhouse seedling pre- and post-inoculation populations for the three loci assayed (PGM-1, PGM-2, and PGI-2). The POPGENE Version 1.32 software was used for individual allele LD analysis in the linkage disequilibrium results section (analysis not shown here) (125).

FIGURE 17 Aerial Photograph of Izoro Disease Center Trees with the PGM-1 A and MDH-3 F Alleles. The three trees with the MDH-3 F allele are colored red. The two red trees that are located to the north also have PGM-1 A allele. The centrally located tree of the PGM-1 A group is homozygous for the allele.

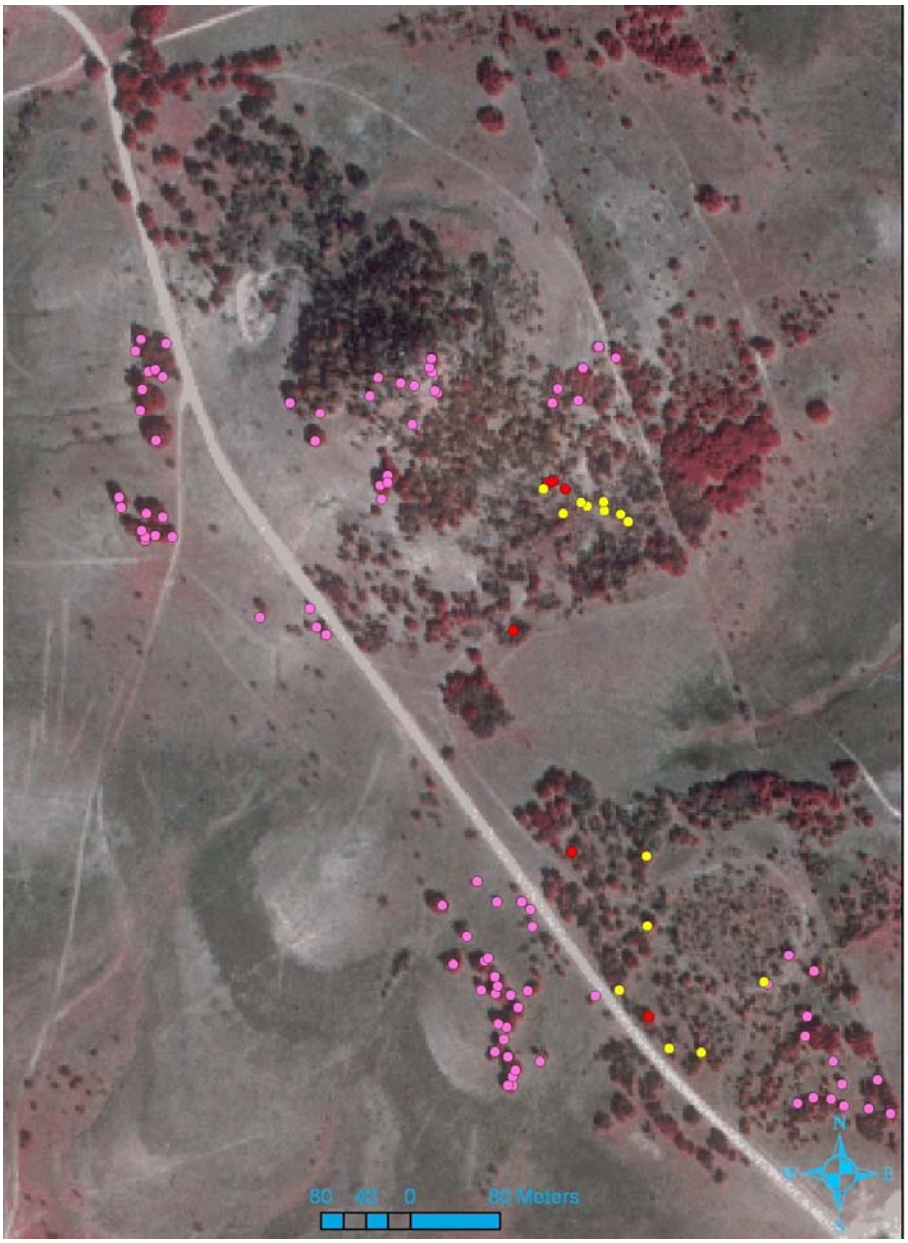

FIGURE 18 Aerial Photograph of Izoro Disease Center Trees with the PGI-2 D and MDH-3 F Alleles. Trees with the PGI-2 D allele are colored red. Trees with "only" the MDH-3 F allele are colored yellow. The three red trees at the top of the picture (appear as two dots) contain both PGI-2 D and MDH-3 F alleles and have an identical genotype. The pink dots are pre-epidemic trees.

F-Statistics: F-statistics were estimated for a three level sampling hierarchy using an approach by Weir (119). The F-statistics were calculated two types of pre- and post-epidemic comparison ("Full" and "Clone Corrected") at the Izoro, and Austin sites.

The F_{IS} values were calculated for the alleles of all loci for all subpopulations. If drift is the cause of population subdivision, then F_{IS} values should be the same at all alleles (123). The F_{IS} values varied between alleles in all Izoro and Austin subpopulation comparisons which suggested drift over generations was not a major factor in allele frequency differences between pre- and post-epidemic populations but does not rule out the possibility of allele frequency changes during severe bottlenecks.

The F_{ST} values were estimated for all loci for the "Full" population at each site (Tables N-1, and N-2). At the Austin and Izoro sites, the F_{ST} values were extremely low across all loci for all comparisons. For both the Austin and Izoro pre- and post-epidemic comparisons (for "Full" and "Clone Corrected" analyses), the individual loci F_{ST} values were all 0.0085 or less. The average F_{ST} of the Austin and the Izoro pre- to post-epidemic populations were calculated for both the "Full" and "Clone Corrected" comparisons. The small F_{ST} values indicate that there was little population subdivision due to genetic drift between the pre- and post-epidemic populations. The F_{ST} value was also calculated in a comparison of the "Full" pre-epidemic populations between Austin and Izoro (Table N-3) and was extremely low. This result indicates that the Austin and Izoro populations can be treated as one continuous population.

The average N_m values (estimates of migration) between the pre- and post-epidemic populations for both Austin and Izoro pre- and post-epidemic populations were high (Tables N-1 and N-2). These N_m values (Austin = 32, Izoro = 99) indicate that there was extremely high gene flow between the pre- and post-epidemic populations.

Genetic distance and similarity: At both the Austin and Izoro research sites the genetic distance was calculated between pre- and post-epidemic populations for all population comparisons ("Total", "Superior", and "Collapsed"). Genetic distance was tabulated using the method of Nei (82), and resulted in values D that were 0.006 or less for all comparisons. The value of D was also calculated between two greenhouse seedling subpopulations and was 0.003. This value of D was not significantly different than the value of D between the pre- and post-epidemic populations at Austin and Izoro.

Heterozygosity: Population gene diversity was estimated for all Austin, Izoro, and greenhouse populations of live oaks by measuring heterozygosity. At the Austin and Izoro sites, heterozygosity was compared between pre- and post-epidemic populations of all comparisons ("Full", "Superior" and "Clone Corrected"). Comparisons were made for mean heterozygosity across all loci and for individual loci. Two loci (PGM-1 and PGM-2) had small numbers of possible heterozygotes because of skewed allele frequencies (Table O-1). Therefore, comparisons for all loci were done using both Fisher's exact test and the chi-squared test based on direct count heterozygosity. Nei's unbiased heterozygosity (83) and direct count heterozygosity are compared (Table O-2).

Heterozygosity was low for the PGM-1 and PGM-2 loci in all populations because the major alleles at these loci (PGM-1 B and PGM-2 E) dominated allele frequencies. The observed PGM-1 and PGM-2 heterozygosities did not exceeded 0.3214 for any allele in any population. In contrast, the heterozygosities were high for the PGI-2 and MDH-3 alleles where several alleles had moderate frequencies. The lowest observed heterozygosity for any allele in any population for these two loci was 0.360. The major alleles for these two loci (PGI-2 C and MDH-3 C) made up less than 65 % of the loci allele frequencies in all populations. For the "Clone Corrected" populations, heterozygosities were higher for all loci. Clone correction resulted in a lowering of the frequencies of major alleles and therefore the elimination of more homozygous genotypes than heterozygous genotypes.

There were no significant differences of average heterozygosity between any pre- and post-epidemic populations at the Austin and Izoro sites (Table O-3). At the Austin site, the average heterozygosity was lower in the post-epidemic population for all comparisons. At the Izoro site, the average heterozygosity was virtually the same for the "Full" and "Clone Corrected Comparisons", but was higher in the "Superior" post-epidemic population.

For individual loci, there were no significant differences of observed heterozygosity in any pre- and post-epidemic comparisons at the two research sites when using Fisher's exact test. In the Austin full comparison, the PGM-1 locus had significantly higher heterozygosity in the pre-epidemic population (H = 0.0781) compared to the post-epidemic population (H = 0.0127) using the chi-square test (*P* =

0.05), but the heterozygosities were not significantly different by Fisher's exact test (P = 0.09). The Fisher's test is the appropriate test for comparing the PGM-1 and PGM-2 loci heterozygosities because of the skewed allele frequencies. The Austin PGM-1 locus also had less heterozygosity in the post-epidemic populations for the clone corrected and superior comparisons, but the differences were not significant at P = 0.05.

In the greenhouse population comparisons, there were no significant differences in the heterozygosities (individual loci or average loci) between pre- and post-inoculation populations at P = 0.05 (Table O-3). In the comparison between the pre-inoculation population and the superior survivors, the heterozygosity was higher for all individual loci in the survivor population. None of the heterozygosity differences between the pre-inoculation and survivors were significantly different.

There were no significant differences in heterozygosity found for any pre- versus post-epidemic population comparison done (Austin, Izoro, and greenhouse).

CHAPTER V
SUMMARY AND CONCLUSIONS

The main goals of the experiments described in the first chapter were: 1. to test previous findings that variable tolerance in live oak seedlings to *C. fagacearum* exists and is a result of heritable genetic control, and 2. to search for live oaks with superior genetics that can be used as sources of tolerance for breeding programs. We are not testing for resistance to oak wilt, because no live oaks are completely resistant to the fungus. With resistance, the fungus would not be able to live within a live oak and colonize parts of the tree. Tolerance, on the other hand, is the ability of the host to be infected with a pathogen and survive. Tolerance can be estimated quantitatively depending on how much of a diseased plant is injured or killed by the pathogen. In cases where a plant in nature does not show symptoms during the course of an epidemic infecting surrounding plants, then we call this an "escape." But we can not be sure if this plant was actually challenged with the pathogen in a natural setting. Three possibilities exist for such a plant in nature: it may have escaped inoculation of fungal spores, it may have been resistant to colonization by fungal spores after inoculation (due to resistance), or the plant could have tolerated the pathogen infection without discernable damage (due to complete tolerance). Resistance is immunity from infection by a pathogen and tolerance is the ability to survive a successful infection. In our inoculation experiments there were no escapes, because all seedlings that were inoculated with the fungus developed some level of crown loss.

We were able to confirm that significantly different levels of genetic tolerance to the oak wilt fungus exists among half-sib groups for two live oak crops when using average group survival as the indicator of tolerance. This finding was even more significant, because the two crops were subjected to very different environments before and after being inoculated. The 1998 crop of seedlings was grown in four liter pots. They had a branching, shrubby appearance with thick main stems. After inoculation in the spring of 1999, they were placed outdoors, probably resulting in the disease process going dormant in the summer heat. At the end of the summer they were given a second inoculation, after which disease symptoms returned and progressed to completion during

autumn. In contrast, the 1999 crop was grown in 0.7 liter pots and kept in the greenhouse through out the experiment. The 1999 seedlings did not branch but rather had one long main stem. Following inoculation once in the spring of 2000 the disease progressed maximally to completion through the summer. A limit of 25 % of crown loss was used as the estimator of tolerance, and significant differences were found in group tolerances for the 1999 crop, but not the 1998 crop. The use of crown death as an estimator was more difficult in the 1998 crop because of the shrubby appearance. Since significant differences among the half-sib seedling groups were obtained in both survival experiments, the group comparison data appears to be sufficient to demonstrate significant and heritable tolerance differences for the half-sib groups. We conclude that heritability exists for disease tolerance in live oak seedlings because we found significant differences among half-sib seedling groups for tolerance under invariant environmental conditions.

Although the significant differences in tolerance among half-sib groups supports the existence of genetic heritability for tolerance to *C. fagacearum* in live oaks, we were unable to quantitatively estimate heritability, as previously reported by Green and Appel (43) and Green (42). A quantitative estimation requires the use of ANOVA based on normal distributions. In the present project, the seedling crown death profiles had binomial distributions (either very little crown death or almost complete death). Therefore, non-parametric binomial tests were used to create confidence intervals for averages of successful trees in each group, so heritability could not be estimated quantitatively. The binomial distribution was unexpected because we modeled our seedling growth procedure after Green and Appel (43). We are aware of two differences between the two experiments. First, we used a razor blade to make the inoculation wound as opposed to their use of a syringe. This procedure certainly created a larger wound and possibly allowed a larger spore load into the seedlings. However, Fenn et al. (34) found that beyond a load of 10^4 *C. fagacearum* spores per seedling there was no change in the disease outcome for red oak seedlings. In both Green and Appel (43), and this project, seedlings were inoculated with at least 10^5 spores per seedling. The second difference is that we used a different strain of *C. fagacearum* in this project. It is possible

that we used a more virulent strain because we pre-tested five strains and chose the most virulent strain for this experiment.

We also compared the relative estimates of tolerance among half-sib seedling groups to the levels of crown loss of their post-epidemic parent trees. These comparisons were used to determine if the amount of damage suffered by live oaks is a good predictor of offspring performance. If so, then survival may be useful for identifying breeding stock. The 1998 live oak tree parents with less crown loss gave rise to seedlings with higher tolerance. However, only about 20 % of the variation in seedling performance could be accounted for by variation in prior parental performance (R^2). This indicates that parental performance has low predictably for their offspring performance as seedlings. This finding was not surprising because the 1998 half-sib seedling groups were not significantly different from one another in tolerance measured as crown loss, even when using only a 90 % confidence interval as the measure of significance. The 1998 seedling crop tolerance was again compared to parental tree crown loss using survival as the estimator for seedling tolerance, but there was no significant correlation between parental crown loss and seedling survival. The 1999 seedlings did not show a relationship between seedling tolerance and parental performance, even though several of the half-sib groups had significantly different tolerances from each other. However, when the tolerances of the 1999 half-sib seedling groups, estimated as percentage of the group that survived *C. fagacearum* were compared, increased seedling group tolerances correlated positively with parental tree performances. However, only 20 % of the seedling response variation could be attributed to the variation in parental tree crown loss. The wide variation about the least squares lines demonstrated that you could not significantly predict the average tolerance of individual half-sib groups based on previous crown loss percentage of the post-epidemic parent. Because all of the least squares lines had positive slopes in the comparison of higher average seedling group tolerances compared with higher parent tree performances, we concluded that there exists a positive relationship. Variables beyond our control in this experiment were unknown pollen sources for the seedlings and unknown environmental variation experienced by the parent trees during the course of the oak wilt epidemic. The inconsistent findings for the 1998 and 1999 seedling crops, when correlated against parental tree performance, demonstrates

that selection of live oak trees for a breeding program (based "only" on prior performance of the trees at oak wilt centers) would not be successful. We conclude that, based on our data, you may have a somewhat better chance of getting half-sib seedlings that survive more often if you take acorns from trees with less crown loss.

Previously, it has been hypothesized (72) that selection has occurred in post-epidemic live oak populations and should give rise to populations that are more resistant or tolerant to *C. fagacearum*. The same process has been hypothesized for post-epidemic populations in other plant species to other diseases such as anther smut disease in *Silene alba* by Alexander and Atonovics (4) and rust in *Linum marginale* by Burdon and Thompson (24). A recent lab inoculation experiment by Laine (62) found increased resistance within seedlings from areas of host populations "where encounters with the pathogen have been high". Laine suggests that this change was due to local selection. The hypothesis that oak wilt results in populations of live oaks that are more resistant to oak wilt (72) is disputed by results of the experiment in which we compared the average stem deaths of all 1998 seedlings from pre-epidemic parent acorns with all seedlings from the 1998 post-epidemic parent acorns. The seedlings that we grew from post-epidemic parents did not have a lower average crown loss than seedlings from pre-epidemic parents**.**

The results of: 1. the pre- versus post-epidemic seedling tolerance comparisons and 2. seedling versus parental tolerance comparisons, do not agree with the half-sib group data comparisons which suggest that a genetic basis of tolerance exists and is variable among half-sib seedling groups. One possible explanation of this disparity is that our model assumes that all surviving, post-epidemic trees have been challenged by *C. fagacearum*, and that the trees with the least crown loss have the highest tolerance to oak wilt. There is the possibility that several of the post-epidemic trees with very little (or no) crown damage are escapes that were never challenged by *C. fagacearum*. There is also the possibility that some of the variability in crown damage resulted from: 1. variation in inoculum load (34, 89, and 110), 2. length of time of inoculum challenge (89, 110), 3. favorability of the environment to fungal viability and growth at time of challenge (51, 89, and 110), 4. variation in the health of the trees prior to becoming infected, or 5. variation in the pre-existing microbial environment within the xylem of the

trees (23, 41, and 110) Furthermore, the disparity in the findings between the 1998 and 1999 half-sib tolerance comparisons strongly suggests that environment can effect major changes in the disease outcome in live oak seedlings (and probably in mature live oak trees). In the experiments where we compared half-sib groups from acorns from unknown pollen sources, we assumed that each acorn bearing tree was being pollinated from a mixture of sources that were no better or worse than pollinators of other acorn bearing trees. There were probably several pollen sources for each tree, but we can't discount the possibility that that some trees received more pollen from neighboring trees instead of an even mixture. It has been shown using numerous species of trees that most air dispersed pollen comes to rest in the general area of the disseminating tree in calm weather but still some pollen is dispersed over long distances, Colwell (26). Bacilieri (15) found high gene flow for two oak species but also detected population structure within oak stands. Douglas fir (*Pseudotsuga taxifolia* Mirb.) pollen was found at measurable levels out to 200 feet using radioactive pollen, but the highest concentrations were found within 20 feet (26). Oak pollination has previously been found to occur over substantial distances as determined by genetic markers to estimate high gene flow between different populations of oak species, including canyon live oak (*Quercus chrysolepsis* Liebm.) (31, 77).

In an attempt to better determine the genetic contribution to variation in the response of live oaks to infection with *C. fagacearum,* we created clones from ramets of post-epidemic trees with different levels of crown loss. This experiment provided several avenues to test the hypothesis that clones would respond to infection in a manner similar to the mature parent tree. This experiment also allowed us to compare the responses to *C. fagacearum* challenge of mature plants (clones) to the responses of juvenile plants (seedlings). Plant age has been shown to affect the response of elms when inoculated with *Ophiostoma novo-ulmi*, the Dutch elm disease pathogen (102). The hypothesis that live oak clones grown from ramets are composed of mature tissue was verified when several of the live oak clones flowered and made acorns during the first year in the greenhouse. None of the seedlings from acorns flowered.

In the comparison of tolerances among all clonal groups, crown loss data of the clonal live oaks to the half-sib seedlings indicates that juvenile and mature live oaks

differ in their responses to oak wilt. There has been tolerance and resistance reported in the red oak, *Q. rubra,* to *C. fagacearum* (90), but no tolerance or resistance has been reported in adult red oak trees. There has also been higher tolerance reported in sprouts of American elm to *Ceratocystis ulmi* than is seen in adult trees (52). It is possible that immature live oak seedlings are less tolerant than the clones because the cambium of mature plants has differences that better retard lateral movement of the fungus. Further support for this possibility is that longer latent periods have been documented in older red oak seedlings when inoculated by *C. fagacearum* (34). However, increased resistance in stump sprouts from dead elm trees to *C. ulmi* may be due to the affects from the prior disease (62). It is a possibility, although unlikely, that we may be seeing a post-disease immunity in the clones (52). The clonal groups did not show the extreme bimodal crown death response to *C. fagacearum* that was seen in the seedlings and only a small percentage of clonal plants from post-epidemic trees died. These more normal distributions allowed us to: 1. use average clonal group crown losses in comparisons of the tolerances among clonal groups, and 2. directly compare the crown losses of clones to performance of their parent trees. The differences in response to challenge by *C. fagacearum* between the clones, with semi-normal distributions of percentage crown loss, and the seedlings, with binomial distributions, called into question our research model of using one-year-old seedlings to search for genetically tolerant live oak trees for breeding stock.

In the comparison of tolerances, one clonal group was clearly superior with an average of only 12 % crown death for five clones. This group had an average crown loss at least one standard deviation lower than seven of eight other clonal groups (for groups with 3 plants or more). The two groups (n = 5) with the lowest average crown losses also had low variability in crown loss results. These two groups also had similar average crown deaths as their parents. Group number five with 35 % average crown loss is from a parent tree with 30 % crown loss and group number six with 12 % average crown death is from a tree with 25 % crown loss. The low variation in crown death profiles for these two groups is consistent with the hypothesis for a genetic component to tolerance to the oak wilt fungus in live oaks. But the large variation in the responses within some clonal groups suggests that either: 1. subtle environmental conditions may play a role in the

disease outcome, or 2. trees with less genetic tolerance vary more in phenotype. In a greenhouse environment, the only phenotypic variation within a clonal group should be due to genetic variation (14). The post-epidemic clones were all raised in the greenhouse in identically sized pots, but they were created at different times (as new ramets were found), and the clones varied somewhat in size and degree of branching. The only other possible environmental variables among the clones were microclimate soil and xylem ecosystems.

The comparison of the crown losses of the cloned live oak trees to that of their parent plants was not supportive for using degree of survival in post-epidemic live oaks to infer genetics for breeding. In two tests, we compared all clones' crown death percentages individually against their parent's prior crown loss percentages (n = 59) and we compared clonal group average crown deaths against their parent tree crown losses (n = 28). Neither comparison showed any relationship between clonal performance and parent tree performance. This suggests progeny testing should be done before entering a live oak into a breeding program based low levels of crown loss. The lack of any correlation between the disease tolerance of clonal progeny and the tolerance of their live oak parents suggests that environmental factors have effects on the disease outcome that are strong enough to partially mask genetically determined tolerance in mature trees. Because two clonal groups were superior to other groups, had low variation in crown loss, and had superior parents, we conclude that their post-epidemic parents may have survived and suffered less damage because of heritable traits.

The clones created from two TAMU campus live oaks that had never been subjected to oak wilt had only five percent survival after inoculation with *C. fagacearum*, but the clones from post-epidemic trees all survived. The poor performance of the clones from the two TAMU campus live oaks, when compared to the clones from the post-epidemic disease center is of interest, and may be due to several factors: 1. The campus clones are from a different species of live oak (*Q. virginiana Mill*) and the disease center clones are from *Q. fusiformis* or *Q. fusiformis* / *Q. virginiana* hybrids (86), 2. the two campus live oaks are not oak wilt survivors, and 3. campus clones were grown in smaller pots. Because the difference in performance is so striking, this serendipitous finding is intriguing. If the pot size is not the cause, then it is likely that the difference is either due

to the difference in species or to increased tolerance because the parent tree survived. It is doubtful that the pot size explains the difference totally because pot size did not result in a noticeable difference in the survivability of the two seedling crops. In the experiments of Green and Appel (43) seedlings groups were used from an area where *Q. virginiana* and *Q. fusiformis* live oaks hybridized regularly. They found these seedlings actually showed better tolerance than *Q. fusiformis* seedlings when challenged with *C. fagacearum*. Therefore, there is no evidence that the clones derived from campus live oaks did worse because they were *Q. virginiana*. We subjected several *Q. virginiana* campus live oaks to allozyme analyses and observed the same alleles as in the post-epidemic live oak trees. Also, the same major alleles predominated at all loci. These findings suggest they are closely related subspecies, and the difference in tolerances of their offspring is probably not due to taxonomic classification. An experiment should be undertaken to compare the clones of survivor trees with clones from trees never challenged with *C. fagacearum*.

From the totality of the clone and half-sib data, we conclude that a genetic basis of tolerance to *C. fagacearum* exists in some live oaks, but extensive progeny testing will be necessary to locate the best breeding stock for tolerance. We suggest that the comparison of clonal groups from different live oaks show promise to look for tolerance. Only a small proportion of post-epidemic trees produce viable ramets, only a few ramets are produced by most trees (usually less than five), and only a fraction of those ramets respond to present techniques to clone them. Therefore, it will take extensive testing to find trees that produce large numbers of ramets to produce sizeable clonal groups to be used in further tests. Because the clonal trees responded with little death and a seemingly normal range of symptoms (like mature live oak trees in disease centers), we suggest that the use of clones to look for resistant trees be continued using larger data sets than we were able to generate. Our observation that two campus live oaks were generating hundreds of ramets suggests that large clonal data sets can be created by canvassing a wide range of oak wilt centers. In addition, a method for using fully grown live oak clone groups is discussed in the allozyme experiment discussion.

Previous research found that the latent period after inoculation of live oak seedlings was significantly shorter for trees from an oak wilt epidemic site when

compared to trees not from the site, but the length of the latent period was not a good predictor for survival (42). Ramet production by live oak trees is a survival mechanism brought on by stress (personal communication, Dr. David Appel, Dept. Plant Pathology and Microbiology, TAMU, Sept. 20, 2006). It was hypothesized that a higher frequency of ramet production in inoculated seedlings would be associated with shorter latent times because faster symptom expression might trigger a higher level of stress and increase the likelihood of ramet production. There were significantly shorter latent times in two separate comparisons for ramet producing seedlings versus non-ramet producers to support the hypothesis. Working with red oaks and white oaks, Fenn et al. (34) reported that the highly tolerant white oaks have longer incubation periods than the highly susceptible red oaks. The knowledge of this association in live oaks may be helpful in understanding why some live oaks have increased tolerance to infection with the pathogen. For example, seedlings with shorter latent times may be producing tyloses more rapidly, or producing stress related hormones, or they may simply have xylem morphology less conducive to the spread of fungal spores. Assays could be used to search for differences in gene expression in those trees that produce more ramets under stress. It has been reported that fires result in large increases in ramet production in *Quercus spp*. (27) which supports the hypothesis that some ramet production can be a survival response to severe stress. If any such differences exist and can be found, they may have utility in creating more ramets for trees that we wish to clone because of desirable traits. The lack of prodigious ramet production by most diseased live oaks has limited the ability to create large clonal groups which can be used to better understand the roles that genetic and environmental factors play in oak wilt. The connection between early disease response and ramet production is of interest because ramet production is not just a reproductive mechanism, but is a survival mechanism as well. Numerous live oaks that suffer death of the bole still live on by generating ramets, some of which survive the disease.

Previous research found that different half-sib groups from live oak parents had significantly different averages in main stem height after one year of growth (43). The findings in this study support their finding at significant levels. This information suggests that breeding programs can successfully use live oak half-sib seedling progeny

to identify acorn sources for fast growing breeding stock. One of the goals of the Urban Tree Improvement Program in Texas was to produce trees with superior phenotypic traits including growth rate (42). Our findings, in support of the research of Green and Appel (43), verify the proposal to use half-sib progeny testing to identify breeding stock for superior growth rates.

In natural live oak centers, *C. fagacearum* is spread from tree to tree through the root systems. We wanted to be sure that our research model did not exclude the possibility that some difference between root systems due to genetics was a factor in differential survival among life oaks. From a test comparison of two groups of twenty seedlings from the same campus live oak tree, we concluded that the use of stem inoculations in the research model did not differ from results of root inoculated trees.

In the part of this research project described in Chapter II, we compared genetics of pre-epidemic live oak populations to post-epidemic populations. This comparison was made under the assumption that the genetic constitution of the pre-epidemic population represented the post-epidemic population before *C. fagacearum* spread through the site killing approximately 75% of the live oaks. Previous research compared the pre-epidemic population to: 1. the total post-epidemic sample and 2. the subset of the post-epidemic trees with less than 30 % crown loss (72). They found that the PGM-1 A allozyme allele was associated with increased tolerance in live oaks to *C. fagacearum* and that the PGI-2 C allozyme allele was associated with lower tolerance. If there are genes in a plant for tolerance or resistance to a pathogen, then it is reasonable to expect that there are markers linked to them (14). Under selection pressure, it is a valid hypothesis that allele frequencies will increase for any markers linked to alleles that increase fitness (46).

We found no evidence to support an association of the PGM-1 A allele or the PGI-2 C allele with tolerance to *C. fagacearum* in live oak (at the two natural disease centers or in the a large greenhouse population composed of half-sib seedling groups) from acorns from trees at the disease center previously used by Bellamy et al. (19). Because the PGI-2 C and PGM-1 A alleles were present in extremely low frequencies at the two natural sites, we cannot reject the previously reported findings (72) with these data. McDonald et al. (72) hypothesized that the nature of resistance in live oak was

most likely polygenic. It is possible that the alleles they found were not associated with enough additional resistance alleles to confer resistance at the other sites. With polygenically determined traits, there is usually a graded response in phenotype (human height being one example) (14, 46). In the greenhouse seedling population we did have a large enough number of seedlings containing the PGM-1 A and PGI-2 C alleles to run significant T-tests, and the two alleles demonstrated no significant association with seedling tolerance to *C. fagacearum*. As we previously demonstrated in Chapter I, the tolerance in greenhouse seedlings showed no significant association to tolerance of their parent trees, so the failure of the PGI-2 C and PGM-1 A alleles to have the same associations in the seedlings as previously reported (72) does not disprove their results obtained for mature trees.

We did find different allozyme alleles (and one allele combination) that were associated with tolerance at the Izoro and Austin disease centers. At the Izoro disease center, the PGM-2 D allele was at a significantly higher frequency in the superior survivor fraction of the post-epidemic population when compared to the pre-epidemic population. This allele was also at a higher frequency in the Austin post-epidemic population but the difference was not significant.

In the Izoro post-epidemic population, trees containing the PGM-2 D allele had less average crown loss than trees with the two other PGM-2 alleles (E and F).
At the Austin disease center, two different allozyme alleles were present at significantly different frequencies in the pre-epidemic and post-epidemic populations. The PGI-2 E allele was at a significantly higher pre- versus post-epidemic frequency, and the PGM-2 C allele was at a significantly higher pre-epidemic frequency.

In the greenhouse population of seedlings, there were no significant allele frequency differences between the pre- and post-inoculation populations, and no significant differences between the pre-inoculation and 'superior' post-epidemic seedlings.

In the comparisons of allele frequencies between pre- and post epidemic populations (and the pre-epidemic versus 'superior' post-epidemic populations) there was no significant continuity among different sites for the allele frequency differences between pre- and post-epidemic tree populations. If the allele frequency differences

between pre- and post-epidemic populations were a result of selection, then different alleles were being selected for at the two different sites. Furthermore, the allele frequency differences we detected between pre- and post-epidemic populations in this study were not found in the previous allozyme frequency research conducted by Bellamy (19) at the Round Rock site. This finding suggests that either different environmental factors at each site were affecting the fitness of different tolerance alleles or different genomic backgrounds at the different sites were complementary with different alleles that help confer tolerance.

There was one significant finding for the association of a two allele combination with lower average crown losses in trees at one of the two sites. Izoro post-epidemic trees that contained the PGI-2 DF allele combination had a significantly lower average crown loss (n = 7, 15.7 %) than all other Izoro post-epidemic trees (n = 94, 40.7 %), (P = 0.005). This significant result may have more to do with tolerance within four probable clones that were identified with the multilocus genotype DFBBEECC. Four trees with this genotype were located in a cluster and had an average crown loss of just 20 %. Because the $P_{gen} = 0.034$ for this genotype, we concluded the four trees were clones. While the PGI-2 DF genotype is common to these four clones and helped to identify them, the low levels of crown loss could be due to tolerance genes not linked to the PGI-2 locus. This could be the situation if the clones had high tolerance, but that tolerance was because of other alleles at loci unrelated to the PGI-2 locus. Or the lower average crown loss could be due to environmental factors at the common location of the four clonal trees. When three of the four putative clones with the DF combination were removed from the data set, the average crown loss for this allele combination was no longer significantly different than all trees at the site. This allele combination occurred three times in the Austin post-epidemic population and did not show lower average crown loss than expected (48.0 %). This suggests that even if the allele combination was instrumental in tolerance at the Izoro site, then it is not important in all environments. The evidence suggests that the group of four clones with the DF combination either represent one tolerant genotype unrelated to the PGI-2 locus or that local environment was a factor.

Although there were some significant differences in average crown losses among groups of trees with different alleles at Izoro, there were no significant differences among groups at Austin. If the Izoro trees are more tolerant because of tolerance genes linked to some allozyme alleles, then this association is not universal among all sites.

The finding that different allele frequency changes between pre- and post-epidemic sub-populations are seen at different disease sites does not rule out the possibility that we are seeing selection for tolerance genes linked to these markers. This finding indicates that if we are detecting the presence of tolerance genes for oak wilt, then their effectiveness, or perhaps their expression, are environmentally influenced, or influenced by genomic background (other genes in the genotype) (46).

One other possibility for differences in allele frequencies between the pre- and post-epidemic populations is drift. Genetic drift can lead to allele frequency changes between sub-populations and populations, but the small F_{ST} values we found in all population comparisons make this highly improbable over generations. Furthermore, if drift were causing the allele frequency changes we detected, then F statistics would be expected to be nearly equal for the different alleles at each locus (123). This was not found, so we ruled out drift over generations as a cause for the allele frequency differences. But, these post-epidemic populations were subjected to extreme bottlenecks by the oak wilt pathogen, and chance alteration of some allele frequencies not due to selection are probable due to short term drift.

However, when a plant species has a reproductive strategy that includes both sexual and clonal reproduction, then small patches of clones that have alleles that are rare in the larger population may cause significant changes in the frequencies for those alleles between sub-populations. Such differences in allele frequencies between sub-populations would be more probable for clonal plants that use a phalanx clonal strategy as opposed to a guerilla strategy. In a phalanx strategy the ramets are propagated near to the parent plant and in a guerilla strategy the ramets are propagated at extreme distances (36). In live oaks, the phalanx strategy is visually obvious in the Texas sites. Spatial patterns consistent with the phalanx strategy have been reported in other oak species such as *Quercus havardii* (71), and in both *Quercus macrocarpa* and *Quercus gambelli* (96).

We have observed that the allozyme alleles that appeared to be associated with tolerance by McDonald et al. (72), and alleles that appear to be associated with tolerance in this project do not show consistent associations to tolerance in different sites and have not been proven useful to base a breeding program. The PGI-2 DF allele combination that was associated with lower crown loss at the Izoro site may have been useful for identifying a tolerant clonal genotype, but the PGI-2 DF combination shows no significant evidence of being linked to genes for tolerance. Because most of the trees with this allele combination and low crown loss were adjacent, the local environment may be partially responsible for the uniform result.

It is well documented that environmental influences have profound effects of the cause of oak wilt, *C. fagacearum,* (7, 35, 51, and 110). Temperature, moisture, and competing saprophytes are the most commonly mentioned environmental variables that can affect the growth rate and survivability of the fungus (32, 41, and 64). Because the spread of the fungus through a stand of live oaks is randomly conducted through connected roots, there is the opportunity for variation in all of these environmental parameters among the infected trees. If a tree is infected at the beginning of weather favorable for fungal growth, the fungus can complete its damage to the tree within the season. But if this infection occurs just prior to the hot Texas summer, the fungus may be eradicated from most of the tree and go dormant for months before continuing its growth and plugging of the xylem (7). However, there are differences of opinion as to whether delays in the disease cycle due to temperature affect the final outcome for any individual tree (12, 110, and 111). Tainter and Ham (111) reported finding that *C. fagacearum* "loses virulence during the first growing season following inoculation" in red oaks. In Texas, all red oaks die in the first few weeks after showing symptoms of oak wilt unless infected just before the onset of hot weather, in which case they die soon after the weather is cool again (12).

There is also the unknown effect of variation in the amount of inoculum being introduced into a live oak's root system, the number of its roots being simultaneously infected, or the length of time during which inoculum is being supplied to the tree.

Another goal of this project was to use the two separate research sites at Izoro and Austin to search for common population genetic parameters, in order to predict what may

be expected in other Texas live oak populations. Because of the distance between these sites, the differences in topography, and the soil differences, we expected to see different population genetic parameters in the different sites.

Live oaks can reproduce clonally (78), but there has not been previous research that has tried to measure the strength of this component in live oak populations. The extent of clonality has been examined with allozyme analyses for other oak trees in Texas such as *Q. havardii, Q. margaretta, and Q. laevis*, (21, 71). Bellamy (19) did an analysis on a large population of live oaks in Texas, and found low levels of genotype diversity, but did not test for the presence of clones. In this project, we used recently published methods to determine the probabilities for adjacent trees being clones (88), to test for the presence of clones in our data set. This was made possible because of the potential for accurate mapping using GPS (Global Positioning System).

We found low levels of genotype diversity at the Austin and Izoro sites. We agree with Bellamy (19) in attributing some of the low genotypic diversity to skewed allele frequencies for two of the four polymorphic allozyme loci, but we also attribute part of the low levels of genotypic diversity to some clonal structure in both research sites. The genotype diversity in the Austin pre-epidemic site was double the diversity that we found in the Izoro pre-epidemic site. This was an important finding and contrary to the high genetic similarity measurement, that indicated the two pre-epidemic sites were so identical as to represent the same population. Both sites had about the same number of common genotypes, but the common multi-locus genotype groups at Izoro were more clearly clumped than at Austin**.** This clumping allowed us to identify more clonal mottes and larger clonal mottes at Izoro than at Austin using the P_{gen} calculation of Parks and Werth (88). A motte is a small, condensed stand of trees growing on a prairie. Furthermore, the most common genotype at Izoro made up almost 25 % of the total population of trees and occurred in several large clumps. This was the genotype that we expected to occur most commonly (based on allele frequencies) at both sites, but at Austin this genotype group did not occur more often than expected. The differences in genotype diversity and the larger presence of obvious clonal groups at Izoro suggest very different sites histories and dynamics between the Austin and Izoro sites. We found very high gene flow (N_m) between the sites, and this level of migration should prevent either

site from becoming different as long as some sexual reproduction is occurring. The genetic similarity was high because all major and medium frequency alleles for all loci were very close in frequency between the sites, even though they were approximately 70 miles distant**.** As sites age, drift should cause them to become monoclonal, if the forces of selection and migration are not significant. Because the two sites are so similar in allele frequencies (even having the same rare alleles) this suggests that gene flow has been high and differences in clonal frequency are probably not a result of a difference in the age of the sites. It is possible that the most common multi-locus genotype at both sites (EEBBEECC) has been selected for in the past at the Izoro site where it occurred at a much higher frequency than expected. It is unlikely that this genotype is being selected for because of an association with tolerance at Izoro, because it occurred less often than expected at the Austin site. Paradoxically, this genotype had an average crown loss that was significantly better than all other trees at Austin but at Izoro this genotype suffered more crown loss than the survivor average. We concluded that selection for resistance to *C. fagacearum* did not explain the clonality differences between the sites. In a comparison of the sites, the major identifiable environmental difference was topology. The Austin site sits down in a bowl, surrounded by hills on all sides, whereas the Izoro site is on a flat, open, plain with a small hill on one side. While this difference in topology does affect the air movement and therefore pollen flow, we found nothing to suggest an effect on clonal structure. It is our opinion that the extreme differences in clonality between Austin and Izoro must reflect very different population histories, but there is not enough information to determine the cause. Natural and man made disturbances, such as fires, can lead to accelerated rates of clonality in canyon live oak (77).

There were two significant violations of H-W equilibrium found in each of two sub-populations in this project. One significant violation was found for the PGI-2 locus in the Austin, "Full Comparison" post-epidemic population. This locus violated H-W because of the presence of two trees that were homozygous for the low frequency PGI-2 D allele. The unlikely presence of two trees homozygous for this allele could be caused by genetic drift, population subdivision, positive selection for additive tolerance linked to this allele, or to clonality. The two trees with this allele were adjacent and had identical

genotypes for the three loci that could be compared. One locus in one tree failed to give a readable gel result. The close location of the two trees strongly suggests that they are clones.

The second significant violation of H-W was found in the Izoro pre-epidemic, "Full Comparison" population at the PGM-1 locus. This locus violated H-W because of the presence of one tree that was homozygous for the rare PGM-1 A allele. Population subdivision within live oak populations seems unlikely because of the small F_{ST} values we found, but, in this case, all of the pre-epidemic trees with the PGM-1 A allele are located in close proximity to this tree. Although the population genetics data detected very little population subdivision, the best explanation for this homozygote is for a nearby tree heterozygous for the A allele pollinating another heterozygous tree. Given the clonal nature of live oaks, the clustering of trees with the PGM-1 A allele may have occurred in this area at one time.

Linkage disequilibrium can indicate the presence of two linked genes, or a selective benefit for the presence of two unlinked genes that together enhance fitness. We found no cases of two locus linkage disequilibrium in any of the populations at the Austin research site or in the greenhouse seedling populations.

There were three cases of Linkage disequilibrium found at the Izoro research site, one in the pre-epidemic population and two in the post-epidemic population. In the pre-epidemic population, the PGM-1 A with MDH-3 F association was significant by chi-square, $P = 0.03$. Both alleles are rare alleles and association only occurred in two trees, however one tree was homozygous for the PGM-1 A allele. The two trees were close together, all trees with the PGM-1 A allele were in this area, and three of the five trees also containing the MDH-3 F allele were near to these two trees. We concluded that this finding of linkage disequilibrium was probably due to local population structure because of increased likelihood of being pollinated by nearby trees. The two findings of linkage disequilibrium in the post-epidemic population were of greater interest, because they could signify a gene combination important in tolerance to oak wilt. The PGI-2 D with MDH-3 F association occurred in five trees, chi-square $P = 0.005$. When we compared this finding to the clonal analysis data, three of the trees with this allele association shared the same multi-locus genotype and were adjacent to one another. The probability

that two adjacent trees sharing this genotype would not be clones was $P_{gen} = 0.021$. In a cluster of three trees, they are all probable clones. We removed two of the three adjacent trees from the data and still had a significant linkage disequilibrium, chi-square, $P = 0.01$. We concluded that this allele represents a significant disequilibrium not due to clonality. Of interest is that the two of the three probable clones had crown losses of only 5 and 10 %. We believe that this allele combination may represent selection for tolerance at this site, but this two locus allele combination was not in linkage disequilibrium at the other site (Austin).

The post-epidemic PGI-2 C with PGM-1 A association (chi-square, $P = 0.015$) appeared significant only because two adjacent trees that were clones ($P_{gen} = 0.001$) had two rare alleles. This case of perceived linkage disequilibrium demonstrated how clonality in a population data set can lead to erroneous findings.

The average F_{IS} (inbreeding coefficient) values were negative for all populations, including the green house half-sib populations. This finding is consistent with species that are obligate out-crossing plants (46).

The F_{ST} values were extremely low between all pre- and post-epidemic populations, which indicated that each site was composed of one population with minimal subdivision. F_{ST} values were also calculated in a comparison of the Austin and Izoro pre-epidemic populations, and were not higher than pre- and post-epidemic comparisons. This finding indicates that even at a separation of approximately 70 miles, the two populations have not become different due to drift, or selection.

The values of N_m (migration, calculated from the F_{ST} values) were extremely large for the pre- and post-epidemic comparisons, and the Austin and Izoro comparisons. The discovery of the large N_m values between Austin and Izoro indicate that significant gene flow (via pollination) has taken place over long distances in south Texas. The allele frequencies of the two sites are remarkably similar. Both sites have the same major alleles for each locus; have the same medium frequency alleles, and the same rare alleles. It is possible that some alleles are under the same selection pressures, but the totality of similar allele frequencies indicates that high gene flow over long distances has been continuous. The same major alleles were also reported by Bellamy (19) for the PGM-1, PGM-2, and PGI-2 loci. (The MDH-3 locus data was analyzed differently than by

Bellamy in this project). The data from this experiment when combined with the Bellamy experiment indicates that, at least for three loci, we can predict that other south Texas live oak sites will have very similar allele frequency profiles. Although we are working from a small sample of polymorphic allozymes, it can be extrapolated that when new markers are found, they should be expected to have similar allele frequencies across south Texas, and that differences will suggest major selective events or recent severe bottlenecks. With such large values of N_m, it can be predicted that if this disease continues to exist across Texas several decades, then resistance genes that increase in frequency due to selection at disease sites will also be spread to increase the fitness of unchallenged sites. If, on the other hand, spread of this fungus is brought under control, then former disease sites with increased resistance will once again become more susceptible due to inward migration from sites with less resistance.

It was previously reported that a decline of heterozygosity in the post-epidemic area of an oak wilt center indicated that this disease had lowered the genetic diversity in the live oak tree population (72). We found no significant drops in heterozygosity in the post-epidemic populations at Austin and Izoro, and no significant loss of heterozygosity in the post-inoculation greenhouse seedling population. There was lower average heterozygosity in the Austin post-epidemic population, but the average heterozygosity was higher in the Izoro population of superior trees. We concluded that there is no evidence to support a significant loss of genetic diversity in large oak wilt sites resulting from the oak wilt epidemic. We must caution that we were looking at only four allozyme loci of which only two had significant heterozygosity. Because oak wilt kills almost 75 percent of the live oaks at most sites, the resulting bottleneck would be expected to result in lower genetic diversity at some loci.

The similarities in population genetics findings between the two distantly located sites, and the similar findings of Bellamy (19) in a third site have allowed us to make some predictions on the nature of natural live oak populations in south Texas. Because of the high levels of gene flow, most large sites should have the same major alleles at the four loci that we analyzed. Our data do differ from Bellamy (19) at the MDH-3 locus, but only in the names given to the alleles. At all sites we would expect the PGM-1 and PGM-2 loci to be monomorphic or nearly so, and the PGI-2 and MDH-3 loci to have

several alleles and high levels of heterozygosity. Until now, there was no consensus on the extent of the clonal structure in live oaks that had been tested genetically. We predict that all live oak sites will have low genotypic diversity because of many loci with low heterozygosity. Because the number of alleles tested will lead to greater findings of polymorphism, our estimates of genotypic diversity are under-estimates. Taylor's measure of genotypic diversity (106) is based on the most frequent genotypes, and these genotypes contained the most common alleles in our populations. If we had successfully tested more polymorphic loci then estimates of genotypic diversity may have dropped. We predict that some live oak sites will have large clonal mottes but different sites should be unique in regard to clonality based on our findings.

Our final conclusion is that there is no significant evidence that we have found any alleles to use as markers connected to major resistance alleles for the basis of a breeding program to date. This conclusion is based on not finding any alleles that had increased frequency in the post-epidemic populations for multiple disease centers or in our greenhouse survivor population. However, there is strong evidence for a genetic basis to tolerance to *C. fagacearum* based on the seedling data from this research and prior research.

It is important to ask: what is the genetic basis for tolerance in oaks that survive infection with the fungus? I believe that the ability of white oaks to limit lateral growth of the fungus and red oaks inability to limit lateral growth of the fungus is based both on xylem anatomy and a response elicited in the host. After inoculation entry into both white and red oaks the fungus has been shown to spread rapidly within vertical vessels. And the fungus can be recovered from both white and red oaks for weeks after inoculation. If there are genes for tolerance in live oak, and our seedling half-sib research indicates their presence, then it is my belief that they will have to do with the ability of the xylem to contain the fungus. There are several isolating mechanisms that are exhibited during infection of oaks that are tolerant to *C. fagacearum*. These factors include: the formation of tyloses and gums in xylem vessels (12, 107) the formation of a well defined zone of darkly stained parenchyma cells that surround colonized vessels (89, 108), and, in white oaks, the growth of an additional band of vessels adjacent to colonized vessels (61). The staining found in parenchyma cells has been shown to be the

result of oxidation of phenolic compounds and is believed to be a barrier to growth of the fungi into adjacent xylem vessels (18). Such compounds are associated with defense reactions against pathogens (18, 19). Pre-existing anatomical isolation factors found in oaks are tyloses, and they are more concentrated in the resistant white oaks than the susceptible red oaks (79, 120, and 121). All of these mechanisms, except the anomalous ring of xylem vessel formation, have been reported in both live oaks and red oaks. The difference between these red oaks and white oaks is that the tolerant white oaks have more preformed tyloses (120), have increased speed in formation of tyloses following infection (55), and have the well defined zone of darkly stained parenchyma cells (55). In the red oaks there is less parenchyma staining and no well defined zone (55). Gummosis is extensive in xylem vessels of all infected oaks (107), and no difference has been reported for tolerant species.

We reviewed research into the mechanisms believed to be of importance in tolerance and resistance to another fungal, vascular wilt disease, Dutch elm disease. Elm trees have many of the same reactions to infection with *Ophistoma ulmi* as oak trees have to *C. fagacearum*. Elm trees also react by forming tyloses, gum build-up, and dense staining of parenchyma cells that surround the xylem (17, 18). Gum build-up and differential tyloses production has been associated with localization of the infection in elm trees (17). Darker and better defined bands of stained parenchyma cells that are adjacent to colonized xylem vessels has been associated with increased resistance in elm trees (87). Smaller sized xylem vessels have also been correlated with higher resistance in elms (74, 101). It is hypothesized that large air pockets attributed to cavitation are responsible for stopping the function of large xylem vessels in elms, and therefore having smaller vessels confers tolerance (101). It has also been shown that elms can only be successfully inoculated with spores of *O. ulmi* during the brief period of time when spring wood vessels are being created which suggests smaller vessels may better isolate the fungus (74).

The common mechanism associated with higher resistance in elms and oaks to the two vascular wilts is the darker and more organized staining of adjacent parenchyma cells (87, 89). And this does seem important because tolerance in both live oaks and elms has been attributed to lateral confinement (55, 87, and 89). Lateral spread of the fungus is

through xylem pits into parenchyma cells layers and then through pits into adjacent vessels (55, 87, 89, and 94). The manufacture, release and oxidation of larger amounts of phenolic compounds in parenchyma cells should be under polygenic control (14) like other quantitative traits that separate the white and red oaks (120). The expression of a quantitative trait will normally approximate a bell curve. But, when considering resistance or tolerance, a binomial result in survival versus death of the host would not be unexpected. This is what we saw in seedling experiments. If the trait is expressed to a level to contain the pathogen, the host may not survive. If the expression of the trait does not reach that level the pathogen may kill the tree. The partial crown death of the live oak trees we reported in natural live oak stands may or may not correlate with the cumulative effects of a number of genes for partial tolerance because of the many environmental variables.

Recommendations for Future Research

We have discovered that there are differences in tolerance among half-sib seedling groups and among clonal groups of live oaks to challenge with *C. fagacearum.* We have found that apparent tolerance in post-epidemic survivor trees does not give good predictability in half-sib and clonal offspring with the methods we used. We also found that post-epidemic clones gave a more normal response curve for tolerance estimated by crown loss.

We suggest that future research should involve using both naturally occurring and artificially created live oak clones to look for heritable differences in tolerance. We have shown that there are numerous clonal groups of trees that can be determined with higher probability by using more genetic markers. Numerous isozymes have been used in other oak species with success. It is highly probable that with more refined techniques, more isozyme systems can be successfully used in live oaks to identify clones with higher probability. With identification of large clonal groups, numerous experiments can be done to alter the individual environments of individual trees to search for conditions that may retard fungal growth and limit time of viability within live oaks. There is a high consensus that this fungus is very susceptible to environmental differences.

We have found that it is possible to create clones from ramets with a fair degree of success. Because there are some live oaks that are prodigious ramet producers, it should be possible to find numerous post-epidemic trees that appear to have tolerance. The creation of large clonal groups from such trees should help identify genotypes that are highly tolerant for creation of clonal stock to use in breeding and as sources of clonal trees for high value locations. It appears that without creation of numerous markers, it is going to be difficult to identify enough minor genes conferring tolerance to realistically base a breeding program. It may be cheaper to identify genotypes that have high tolerance and to just clone them. If superior clonal groups can be found, it should be possible to compare the anatomical differences, if any, with groups that are highly susceptible.

We also conclude that our experimental model, of using live oak seedlings and clones with less than 2 years growth, may be inadequate to locate tolerance that is due to localization of a pathogen. Sinclair and Bremer (99) reached this same conclusion in using young root sprouts to look for tolerance in elms to Dutch elm disease.

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APPENDIX A

PROTEIN EXTRACTION BUFFERS

Byrne's Extraction Buffer (Bellamy, 1992)

100 ml Na phosphate buffer, pH 7.3
2.5 g PYP-40
0.25 ml Tween 80
1 ml mercaptoethanol

Na phosphate buffer, pH 7.3

Stock A: 27.8 g sodium phosphate monobasic /L distilled H_2O (0.2M)

Stock B: 28.4 g sodium phosphate dibasic/ L distilled H_2O (0.2M)

Mix 23 ml Stock A and 77 ml Stock B, then adjust pH to 7.3 with NaOH or HCL.

The extraction buffer is used within 1 day of preparation. The mixture is prepared by continuous stirring until the PVP-40 goes into solution. Mixing and storage are done inside a fume hood because of the toxicity of mercaptoethanol.

APPENDIX B

ELECTRODE AND GEL BUFFERS

Histidine Electrode Buffer pH 5.7 (Stuber et al., 1988)

0.065M L-histidine free base (10.09 g/L distilled H_2O

adjust pH to 5.7 with citric acid stock solution

(citric acid stock solution: 4 g citric acid/ 100 ml distilled H_2O)

Histidine Gel Buffer

1:6 dilution of electrode buffer in distilled H_2O

The Histidine Buffer System is use for the following isozymes: PGM, MDH

Lithium Borate Electrode Buffer pH 8.3 (Stuber et al., 1988)

0.19M boric acid (11.75 g/L distilled H_2O
adjust pH to 8.3 with lithium hydroxide stock solution

(lithium hydroxide stock solution: 10 g/100ml distilled H_2O)

Lithium Borate Gel Buffer

1:9 dilution of electrode buffer in Tris-citrate base stock solution
adjust pH to 8.3 with citric acid monohydrate stock solution

(Tris-citrate stock solution: 6.06 g Tris base/ L distilled H_2O)
(citric acid monohydrate stock solution: 1.47g/L distilled H_2O)

The Lithium Borate Buffer System is used for the following isozyme: PGI.

APPENDIX C

STAINING BUFFERS AND SOLUTIONS

Tris-HCl Buffers

.05M Tris-HCl Buffer: 12.1 g/ L distilled H_2O
pH adjusted to 8.0 with HCl for PGI stains

.1M Tris-HCL Buffer: 6.05 g/L distilled H_2O
pH adjusted to 8.5 with HCl for PGM stains
pH adjusted to 9.1 with HCl for MDH stains

Staining Solutions

The following 100 ml stock solutions are stored at room temperature:

$MgCl_2$: 50 mg/ml distilled H_2O
$CaCl_2$: 50 mg/ml distilled H_2O
1.0 Malic acid pH 7.0 (pH adjusted with HCl or NaOH)

The following solutions are stored in small amber dropper bottles at 4 degrees C and kept on ice during use.

NAD: 10 mg NAD/.7 ml distilled H_2O
MTT: 5 mg MTT/.7 ml distilled H_2O
PMS: 2mg PMS/.7 ml distilled H_2O

(One drop of water is approximately .7 ml).

APPENDIX D

STAINING RECIPES

MDH (malate dehydrogenase, E.C. 1.1.1.37), (Stuber et al., 1988) (Bellamy, 1992)
50 ml 0.1M Tris-HCl, pH 9.1
10 ml 2.0M malic acid pH 7.0
20 mg NAD
10 mg NBT
1.25 mg PMS
Mix stain ingredients and add to stain tray immediately. Add PMS last.
Pour the stain into a tray (that is used only for that stain) that contains the gel slice. Put the tray in a dark drawer at room temperature until desired allozymes become visible and readable. (1-2 hours) Remove staining solution from tray, rinse gel with distilled water, then put gel on a light box to interpret data and photograph the gel. Fix gel in 40% alcohol, wrap in saran wrap and refrigerate.

PGI (phosphoglucoseisomerase, E.C. 5.3.1.9), (Stuber et al., 1988) (Bellamy, 1992)
50 ml 0.05 M Tris-HCl, pH 8.0
50 mg D-fructose-6-phosphate
50 mg $MgCl_2$
5 mg MTT
5 mg NAD
1.5 mg PMS
10 units NAD dependent G6PD

Mix stain ingredients and add to tray immediately. Add PMS last.
Pour the stain into a tray (that is used only for that stain) and introduce gel. Put the tray into a dark drawer at room temperature until the allozyme bands become visible and readable (15 to 30 minutes). Remove staining solution, rinse with distilled water, then

put gel on a light box for interpretation and photograph. Fix gel in 40% alcohol solution, wrap in saran wrap, and refrigerate.

PGM (phosphoglucomutase, E.C. 2.7.5.1), (Stuber et al., 1988) (Bellamy, 1992)
50 ml 0.1 M Tris-HCl, pH 8.5
50 mg EDTA
300 mg a-D-glucose-1-phosphate
100 mg $MgCl_2$
1.5 mg MTT
10 mg NAD
1 mg PMS
37.5 units NAD dependent G6PD

Mix Tris-HCl and EDTA and put on high speed stir for 15 minutes before adding other ingredients. All other ingredients should be added immediately before use. Staining should be done in a dark drawer for one to two hours at room temperature. When bands are dark and clear, remove stain, rinse with distilled water, place gel on light box for evaluation and photograph. Fix gel in 40% alcohol solution, wrap in saran wrap, and refrigerate.

APPENDIX E

ALLOZYME GEL INTERPRETATION

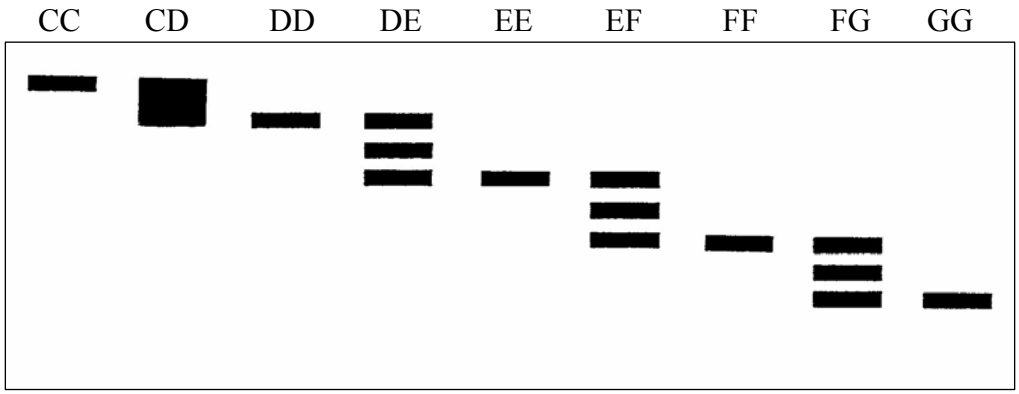

FIGURE E-1 PGI-2 Locus Allozyme Profile

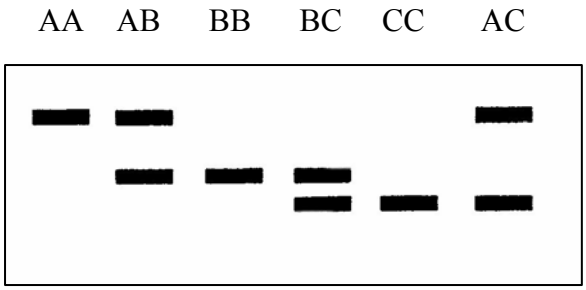

FIGURE E-2 PGM-1 Locus Allozyme Profile

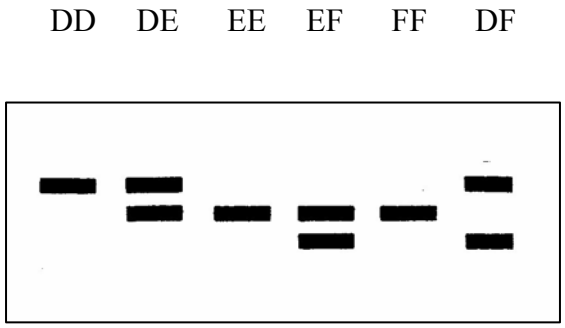

FIGURE E-3 PGM-2 Locus Allozyme Profile

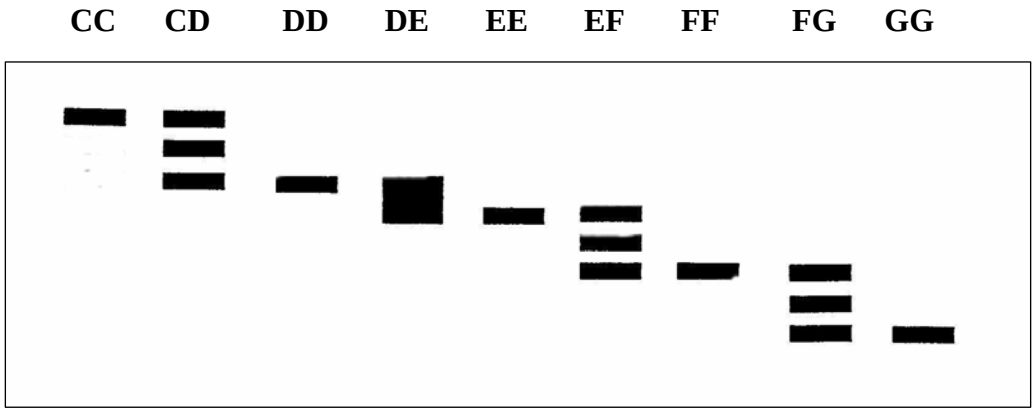

FIGURE E-4 MDH-3 Locus Allozyme Profile

APPENDIX F

OAK TREE ALLOZYMES, LOCATIONS, AND CROWN LOSS DATA

TABLE F-1 Austin Pre-epidemic Tree Data.

Tree	Allozyme* genotype 1 2 3 4	Genotype** group	GPS tree location longitude	latitude
P1	FF BB DE CC		-97.81857	30.26111
P2	--- BC EE CC		-97.81853	30.26127
P3	DE BB DE CC		-97.81844	30.26131
P4	EF BB EE CC	3	-97.81845	30.26158
P5	EE BB EE CC	5	-97.81839	30.26140
P6	EE BB EE CG	2	-97.81831	30.26152
P7	EE BB EE DG		-97.81844	30.26157
P8	EE BB EE CC	5	-97.81828	30.26166
P9	EE BC EE DG		-97.81805	30.26179
P10	DE BB EE CG	11	-97.81801	30.26197
P11	---- BC EE CG		-97.81781	30.26197
P12	DE BB EE CC	7	-97.81795	30.26200
P13	EE BB DE CG	10	-97.81788	30.26265
P14	EE BB EE CG	2	-97.81781	30.26267
P15	DE BB EE CC	7	-97.81807	30.26262
P16	---- BB EE CD		-97.81818	30.26271
P17	DE BB EE CF		-97.81828	30.26260
P18	DE BB EE CC	7	-97.81757	30.26268
P19	EF BD EC CG	6	-97.81750	30.26256
P20	DE BB EE CC	7	-97.81752	30.26251
P21	EE BB EE CC	5	-97.81757	30.26238
P22	DD BB EE CC	12	-97.81873	30.26064
P23	DD BB EE CC	12	-97.81888	30.26068
P24	DD BB EE CC	12	-97.81877	30.26053
P25	FF BB EE CC	14	-97.81897	30.26057
P26	EF BB EE CG	13	-97.81899	30.26055
P27	EE BB EE CC	5	-97.81934	30.26048
P28	EE BB EE CC	5	-97.81931	30.26048
P29	EE BB DE CG	10	-97.81957	30.26065
P30	EE BB EE CC	5	-97.81962	30.26073
P31	EF BB DE CD		-97.81946	30.26093
P32	EH BB EE CG		-97.81831	30.26164
P33	EF BB EE CC	3	-97.81921	30.26105
P34	EF BB EE CG	13	-97.81913	30.26100

TABLE F-1 (continued)

Tree	Allozyme* genotype 1 2 3 4	Genotype** group	GPS tree location longitude	latitude
P35	DE BB EE CC	7	-97.81922	30.26086
P36	DE BB DE CF		-97.81918	30.26097
P37	DE BB EE CG	11	-97.81897	30.26102
P38	DF BB EE CE		-97.81929	30.26129
P39	DE BB EE CC	7	-97.81927	30.26139
P40	DE BB EE CE		-97.81867	30.26148
P41	EE BB EE CG	2	-97.81864	30.26122
P42	DE BB EE CG	11	-97.81870	30.26120
P50	EE BB EE CC	5	-97.81680	30.26155
P51	EF BB DE CC		-97.81683	30.26167
P52	EF BB EE CG	13	-97.81686	30.26160
P53	EE BB EE CG	2	-97.81702	30.26152
P54	DE BB EE CC	7	-97.81724	30.26173
P55	EE BC EE CC		-97.81726	30.26181
P60	EH BB EE CC		-97.81511	30.26053
P61	FF BB EE CC	14	-97.81528	30.26054
P62	FF BB EE CC	14	-97.81512	30.26057
P63	EF BB EE CC	3	-97.81510	30.26054
P64	EE BB DF CC		-97.81517	30.26042
P65	EF BB EE CC	3	-97.81521	30.26026
P66	DF BB EF CC	15	-97.81516	30.26027
P67	EF BB EE CC	3	-97.81515	30.26017
P68	EE BB EE CC	5	-97.81518	30.26010
P69	DF BB EF CC	15	-97.81515	30.26010
P70	EE AB EE CC		-97.81511	30.26001
P71	EE BB EE CC	5	-97.81507	30.26006
P72	EE BB EE CC	5	-97.81496	30.25991
P73	DD BB EE CC	12	-97.81492	30.26011
P74	--- BB EE CC		-97.81487	30.26001
P75	DF BB EE CG		-97.81477	30.26005

* Allozyme genotype loci: 1 = PGI-2 locus, 2 = PGM-1 locus, 3 = PGM-2 locus, and 4 = MDH-3 locus.

** Trees with the same genotype group letter have identical genotypes at the Austin site. Trees without a genotype letter have a unique genotype or an incomplete genotype.

TABLE F-2 Austin Post-epidemic Tree Data.

Tree	Allozyme* genotype	Crown** loss	Genotype*** group	GPS tree location	
				longitude	latitude
	1 2 3 4				
q1-1	EE BB DE CC	99	1	-97.81927	30.26139
q1-2	EE BB EE CG	50	2	-97.81690	30.26027
q1-3	DF AB DE CC	85		-97.81694	30.26021
q1-10	EE BB DE CC	80	1	-97.81681	30.26040
q1-4a	EE BB DE CC	20	1	(20 meters n of q1-1)	
q1-11a	EF BB DD CF	50		-97.81668	30.26044
q1-11c	EF BB EE CC	99	3	(ne of q1-11b)	
q1-19a	EE BB EE CF	0	4	-97.81629	30.26025
q1-20a	EE BB EE CC	10	5	-97.81623	30.26039
q1-b	EF BB EE CC	5	3	(25 meters n of q1-10)	
q1-c	EF BB EE CC	15	3	(10 meters n of q1b)	
q2-1	EF BB DE CC	30	6	-97.81721	30.26005
q2-4	DE BB EE CC	85	7	-97.81695	30.26018
q2-5	EF BB EE CC	5	3	-97.81713	30.26021
q2-6 (tamu 1)	EF BB EE CC	75	3	-97.81720	30.26000
q2-7a	EE BB EE CF	99	4	-97.81685	30.26013
q2-9	DE BB EE CC	30	7	-97.81671	30.26002
q2-10	EF BB EE CC	10	3	-97.81667	30.25996
q2-11	EF BB DE CC	90	6	-97.81721	30.26082
q2-12	DE BB EE CG	0		-97.81689	30.25998
q2-13	CE BB EE CC	80	8	-97.81686	30.25990
q2-14	DE BB EE CC	40	7	(10 meters sw of q2-13)	
qs-1	DE BB EE CC	70	7	-97.81729	30.26111
qs-3	EF BB EE CC	10	3	-97.81721	30.26082
qs-4	EF BB DE CC	80	6	(near qs3)	
z-1	EE BB EE CG	90	2	-97.81671	30.26045
z-2	EE BB EE CG	5	2	(15 meters from z1)	
q3-3	DE BB EE CC	20	7	(near q3-2)	
q3-1a	DE BB EE CC	70	7	-97.81764	30.26048
q3-2	DE BB EE CC	85	7	-97.81733	30.26048
q3-3b	DE BB EE CC	0	7	(near q3-3)	
q3-4	DE BB EE CC	35	7	-97.81723	30.26068
q3-5	CE BB EE CC	10	8	-97.81712	30.26081
q3-6	DE BB EE CC	20	7	-97.81725	30.26060
q3-7	DE BB EE CC	20	7	-97.81725	30.26060
q3-8	DE BB EE CC	15	7	-97.81720	30.26069

TABLE F-2 (continued)

Tree	Allozyme* genotype 1 2 3 4	Crown** loss	Genotype*** group	GPS tree location longitude	latitude
q3-9	EF BB EE CG	0	9	-97.81725	30.26033
q3-11a	EF BB EE CF	50		-97.81660	30.25994
q3-12a	EE BB EE CD	99		-97.81713	30.26052
q3-10	EE BB EE CC	10	5	(near to q3-12a)	
q3-13	EE BB EE CF	90	4	-97.81684	30.26075
q3-14a	EE BB EE CC	20	5	-97.81682	30.26101
q3-15	EE BB EE CG	20	2	-97.81673	30.26097
q3-16	EE BB DE CG	20	10	-97.81673	30.26097
q3-17	EE BB EE CC	15	5	-97.81682	30.26091
q3-17a	DE BB EE CF	5		-97.81657	30.26092
q3-17b	FF BB EE CF	10		(10 meters from 17a)	
q3-18	EF BB EE CC	70	3	-97.81654	30.26082
q3-19	EF BB EE CC	50	3	(15 meters se from q3-18	
qa	EE BB EE CC	25	5	-97.81665	30.26119
qb	EE BB EE CC	25	5	-97.81656	30.26125
qd	EE BB EE CG	50	2	(20 meters w of qd)	
qe	EE BB DE CG	15	10	(20meters nw of qd)	
qf	EE BB EE CC	5	5	(30 meters w of qe)	
qc	EE BB EE CF	15	4	(near qd)	
q3-7a	EE BB EE CC	55	5	-97.81728	30.26076
q4-1	EE BB EE CC	50	5	-97.81769	30.26014
q4-2a	EE BB EE CF	30	4	(near q4-3)	
q4-5	EF BB EE CG	40	9	(near q4-9)	
q4-7	DF BB EE CF	10	11	-97.81785	30.26010
q4-12	DE BB DE CC	99		(near to q4-7)	
q4-9	DF BB EE CF	50	11	(near to q4-7)	
qs-2	EE BB -- --	?		(near to qs-1)	
q3-1	EE BB EE --	30		-97.81654	30.25943
q1-11	EE BB -- CC	25		(between q1-11 & q1-20a)	
q1-12	EE BB -- CC	99		(between q1-11 & q1-20a)	
q1-14	DD -- -- --	99		(between q1-11 & q1-20a)	
q1-18a	EF BB DD --	0		(between q1-11 & q1-20a)	
q1-ca	EE -- -- --	99		(between q1-11 & q1-20a)	
q3-1b	EE -- -- --	30		(near q3-1a)	
q4-2b	EE BB -- --	50		(near q4-2a)	
q4-3	DE BB -- --	99		(near q4-2a)	

TABLE F-2 (continued)

Tree	Allozyme* genotype	Crown** loss	Genotype*** group	GPS tree location	
				longitude	latitude
	1 2 3 4				
q4-2c	EE BB EE --	70		(near q4-2a)	
q4-6	DE BB -- --	20		(near q4-5)	
q4-11	-- BB EE CG	20		(near q4-10)	
tamu2	-- BB EE CC	5		(near tamu 1)	
q4-13	EE BB -- --	95		(near q4-7)	
q4-14	EE BB EE --	0		(area of q4-7)	
tamu4	DE -- -- --	?		(near tamu2)	
tamu-a	DE BB EE --	10		(20 meters w of q3-7)	

* Allozyme genotype loci: 1 = PGI-2 locus, 2 = PGM-1 locus, 3 = PGM-2 locus, and 4 = MDH-3 locus.

** Crown loss is the percentage of dead foliage.

*** Trees with the same genotype group letter have identical genotypes at the Austin site. Trees without a genotype letter have a unique genotype or an incomplete genotype.

TABLE F-3 Izoro pre-epidemic tree data.

Tree	Allozyme* genotype 1 2 3 4	Genotype** group	GPS tree location longitude	latitude
P1	EE BB EE CC	A	-98.10015	31.29029
P2	EE BB EE CC	A	-98.10059	31.28079
P3	EE BB EE CG	B	-98.10068	31.28976
P4	EE BB EE CC	A	-98.10094	31.28957
P5	EE BB EE CC	A	-98.10098	31.28957
P6	EE BB EE CC	A	-98.10094	31.28964
P7	EE BB EE CC	A	-98.10091	31.28969
P8	EE BB EE CC	A	-98.10098	31.28980
P9	EE BB EE CC	A	-98.10110	31.28984
P10	EE BB EE CC	A	-98.10102	31.28994
P11	EF BB EE CC	C	-98.10099	31.29004
P12	DE BB DE CC		-98.10107	31.29007
P13	EF BB EE CC	C	-98.10088	31.29020
P14	FF BB EE CC	K	-98.10095	31.29030
P15	EF BB EE CG	D	-98.10079	31.29033
P16	EF BB EE CF		-98.10071	31.29036
P17	EE BB EE CG	B	-98.10109	31.29031
P18	EE BB EE CG	B	-98.10107	31.29037
P19	EF BB EE GG		-98.10110	31.29045
P20	FF BB EE CG	E	-98.10123	31.29034
P21	EF BB EE CC	C	-98.10119	31.29058
P22	EF BB EE CC	C	-98.10116	31.29060
P23	EF BB EE CC	C	-98.10149	31.29055
P24	EF BB EE CG	D	-98.10136	31.29078
P25	EF BB EE CG	D	-98.10158	31.29103
P26	EF BB EE CG	D	-98.10159	31.29103
P27	FF BB EE CG	E	-98.10126	31.29122
P28	FF BB EE CG	E	-98.10107	31.29105
P29	EF BB EE CC	C	-98.29110	31.29110
P30	EF BB EE CC	C	-98.10084	31.29105
P31	FF BB EE CG	E	-98.10076	31.29099
P32	DF BB EF CG		-98.10074	31.29086
P33	EF BB DE CF		-98.10265	31.29326
P34	DE BB EE CG	F	-98.10275	31.29329
P35	DE BB EE CC	G	-98.10266	31.29323
P36	DE BB EE CG	F	-98.10281	31.29344

TABLE F-3 (continued)

Tree	Allozyme* genotype 1 2 3 4	Genotype** group	GPS tree location longitude	latitude
P37	FF BB EE CG	E	-98.10328	31.29337
P38	EE BB EE CC	A	-98.10410	31.29403
P39	EF BB EE CC	C	-98.10426	31.29404
P40	EE BB EE CC	A	-98.10436	31.29400
P41	EE BB EE CC	A	-98.10436	31.29403
P42	EE BB EE CC	A	-98.10439	31.29408
P43	EE BB EE CC	A	-98.10458	31.29427
P44	EE BB EE CC	A	-98.10434	31.29422
P45	EE BB EE CC	A	-98.10460	31.29435
P46	EE BB EE CC	A	-98.10419	31.29419
P47	EE BB EE CC	A	-98.10425	31.29481
P48	EE BB EE CG	D	-98.10439	31.29506
P49	EE AB EE CC		-98.10429	31.29504
P50	EF BB EE CC	C	-98.10437	31.29523
P51	FF AB EE CF		-98.10439	31.29531
P52	EE BB EE CC	A	-98.10431	31.29537
P53	EF BB EE CC	C	-98.10425	31.29539
P54	EE BB EE CC	A	-98.10418	31.29533
P55	EF AA EE CF		-98.10412	31.29539
P56	EE BB EE CG	B	-98.10414	31.29560
P57	EE BB DE CC	P	-98.10438	31.29563
P58	EE BB EE CG	B	-98.10443	31.29554
P59	EE BB EE CF		(between p58 & p60)	
P60	EE BB EE CG	B	-98.10183	31.29492
P61	DE BB DE CG		-98.10159	31.29518
P62	DE BB EE CE	H	-98.10164	31.29535
P63	DE BB EE CE	H	-98.10162	31.29520
P64	DE BB EE CE	H	-98.10167	31.29539
P65	--- --- BB EE		-98.10165	31.29546
P66	DE BB EE CC	G	-98.10181	31.29524
P67	EE BB EE CC	A	-98.10194	31.29526
P68	EE BB EE CG	B	-98.10215	31.29531
P69	EE BB EE CG	B	-98.10223	31.29516
P70	FG BB EE CC		-98.10270	31.29502
P71	DE BB EE CC	G	-98.10298	31.29511
P72	EE BB EE DG		-98.10275	31.29479
P73	EE BB EE CC	A	-98.10213	31.29432

TABLE F-3 (continued)

Tree	Allozyme* genotype 1 2 3 4	Genotype** group	GPS tree location longitude	 latitude
P74	DE BB EE CC	G	-98.10214	31.29443
P75	EE BB EF CG		-98.10207	31.29451
P76	EE BB EE CC	A	-98.10207	31.29445
P80	EE BB EE CG	B	-98.09826	31.28941
P81	EE BB EE CG	B	-98.09811	31.28945
P82	EE BB EE CG	B	-98.09794	31.28944
P83	EE BB EE CG	B	-98.09784	31.28956
P84	EG BB EE CC		-98.09782	31.28938
P85	EF BB EE FG		-98.09774	31.28921
P86	DF BB EE CC	I	-98.09759	31.28936
P87	EE BB EE CC	A	-98.09738	31.28932
P88	EE BB EE CG	B	-98.09750	31.28959
P89	EE BB EE CG	B	-98.09792	31.28974
P90	FF BB EE CG	E	-98.09818	31.28995
P91	DE BB EE CC	G	-98.09816	31.29011
P92	EE BB EE CC	A	-98.09809	31.29047
P93	EE BB DE CG	L	-98.09833	31.29060
P94	EE BB EE CC	A	-98.09853	31.29037
P95	DF BB EE CG	J	-98.10051	31.29509
P96	DF BB EE CG	J	-98.10046	31.29521
P97	DF BB EE CG	J	-98.10027	31.29511
P98	DF BB EE CC	I	-98.10022	31.29537
P99	DF BB EE CC	I	-98.10007	31.29554

* Allozyme genotype loci: 1 = PGI-2 locus, 2 = PGM-1 locus, 3 = PGM-2 locus, and 4 = MDH-3 locus.

** Trees with the same genotype group letter have identical genotypes at the Izoro site. Trees without a genotype letter have a unique genotype or an incomplete genotype.

TABLE F-4 Izoro post-epidemic tree data

Tree	Allozyme* genotype 1 2 3 4	Crown loss**	Genotype group***	GPS tree location longitude	latitude
A1	FF BB EE CC	90	K	-98.10126	31.29383
A2	EF BB EE CG	90	D	-98.10129	31.29346
A3	EF BB EE CG	5	D	-98.10118	31.29331
A4	EF BB DE CC	99		-98.10101	31.29312
A5	EE BB EE CC	99	A	-98.10081	31.29322
A6	DE BB DE CF	65		-98.10090	31.29325
A7	EE BB EE CC	50	A	-98.10096	31.29340
A8	EE BB EE CC	50	A	-98.10095	31.29346
A9	EE BB EE CC	50	A	-98.10100	31.29341
A10	EE BB EE CC	40	A	-98.10060	31.29342
A11	EE BB EE CC	60	A	-98.10059	31.29347
A12	EE BB EE CC	0	A	-98.10065	31.29362
A13	EE BB EE CG	0	B	-98.10044	31.29378
A14	EE BB EE CC	0	A	-98.10043	31.29380
A15	EE BB EE CC	30	A	-98.10030	31.29390
A16	EE BB EE CC	0	A	-98.10036	31.29393
A17	EF BB EE CC	5	C	-98.10034	31.29399
A18	EE BB EE ---	?		-98.10042	31.29387
A19	EE BB EE CC	0	A	-98.10048	31.29408
A20	EE BB EE CC	0	A	-98.10050	31.29405
A21	EE BB DE CG	25	L	-98.10042	31.29410
A24	EE BB EE CG	5	B	-98.10031	31.29419
A25	DF BB DE CC	30		-98.10042	31.29419
A26	EE BB EE CG	15	B	-98.10039	31.29427
A27	EE BB DE CG	?	L	-98.10038	31.29433
A29	DF BB EE CC	20	I	-98.10019	31.29425
A30	DF BB EE CC	20	I	-98.10003	31.29421
A31	DF BB EE CC	40	I	-98.10004	31.29428
A33	DE BB EE CC	5	G	-98.09988	31.29418
A34	DE BB EE CC	80	G	-98.09981	31.29412
A35	EE AB EE CG	10		-98.09974	31.29400
A36	CE AB EE CC	25	M	-98.09964	31.29418
A37	CE AB EE CC	80	M	-98.09961	31.29435
A38	EF BB EE CC	10	C	-98.10031	31.29438
A39	DF BB EE CC	0	I	-98.10025	31.29428
A41	EF BB EE CC	95	C	-98.10065	31.29432
A42	DF BB EE CG	0	J	-98.10060	31.29439

TABLE F-4 (continued)

Tree	Allozyme* genotype 1 2 3 4	Crown loss**	Genotype group***	longitude	latitude
A43	DE BB EE CF	5	N	-98.10056	31.29444
A44	DE BB EE CF	90	N	-98.10051	31.29445
A45	DE BB EE CF	10	N	-98.10040	31.29439
A51	EE BB EE CC	60	A	-98.10007	31.29354
A52	EE BC EE CC	15		-98.09946	31.29375
A53	EE BB EE CG	60	B	-98.09933	31.29370
A54	EE BB DE CG	30	L	-98.09952	31.29385
A55	EE BB DE CG	80	L	-98.09949	31.29395
B1	EF BB EE CC	99	C	-98.10056	31.29122
B2	EF BB EE CG	15	D	-98.10049	31.29118
B3	CD BB EE FG	15		-98.10036	31.29145
B4	EE BB EE CC	95	A	-98.10038	31.29134
B5	EE BB EE CG	90	B	-98.10030	31.29142
B6	EE BB EE CC	99	A	-98.10028	31.29140
B7	EE BB EE CG	99	B	-98.10023	31.29135
B8	EF BB EE CC	10	C	-98.29162	31.29162
B9	EE BB EE CG	80	B	-98.10012	31.29176
B10	EE BB EE CG	0	B	-98.10012	31.29168
B11	EF BB EE CC	50	C	-98.10033	31.29124
B12	EF BB EE CC	95	C	-98.10007	31.29116
B13	EF BB EE CC	95	C	-98.10004	31.29123
B14	EE BB EE CC	15	A	-98.10005	31.29098
B15	EE BB EE CC	0	A	-98.10012	31.29094
B16	EE BB EE CC	95	A	-98.09992	31.29086
B17	EE BB EE CC	95	A	-98.09990	31.29094
B18	EF BC EE CC	80		-98.09976	31.29082
B19	EF BB EE CC	80	C	-98.09966	31.29078
B20	DD BB EE CC	0		-98.09966	31.29085
B21	EE BB EE CC	30		-98.09950	31.29073
B22	EF BB EE DG	25		-98.09964	31.29059
B23	EF BB DE CG	30		-98.09975	31.29045
B24	EE BB EE CC	85	A	-98.09996	31.29051
B25	EE BB EF CC	95		-98.10002	31.29063
B26	EE BB EE CC	50	A	-98.10001	31.29049
B27	DE BB EE CG	5	F	-98.09995	31.29042
B28	DE BB EE CG	15	F	-98.09993	31.29033

TABLE F-4 (continued)

Tree	Allozyme* genotype 1 2 3 4	Crown loss**	Genotype group***	longitude	latitude
B29	DG BB EE CF	0		-98.09965	31.29012
B30	DE BB EE CC	10	G	-98.09946	31.28986
B31	EF BB EE CC	10	C	-98.09943	31.29105
B32	CF BB EE CC	70		-98.09952	31.29114
B33	FF BB EE CC	85	K	-98.09969	31.29116
B34	EE BB DE CC	20	P	-98.09976	31.29120
B35	FF BB DE CC	10		-98.09968	31.29128
B36	DE AB EE CC	40		-98.09966	31.29141
B37	EE BB EE CG	30	B	-98.09969	31.29138
B38	EF BB EE CC	65	C	-98.09947	31.29156
B39	EE BB EE CC	99	A	-98.09977	31.29102
B40	EE BB EE CD	30		-98.09990	31.29110
B42	EE BB EE CC	0	A	-98.09998	31.29112
B43	EE BB EE CG	10	B	-98.09927	31.29040
B44	EE BB EE CG	20	B	-98.09905	31.29033
B45	EE BB EE CC	20	A	-98.09890	31.29023
B50	EE BB EE CC	10	A	-98.09923	31.29025
B52	EE BB EE CC	35	A	-98.09898	31.29009
B53	EE BB EE CC	20	A	-98.09877	31.29009
B54	DF BB EE CG	0	J	-98.09916	31.28982
B55	EE BB EE CC	0	A	-98.09900	31.28999
B56	EF BC DE CC	0	O	-98.09859	31.29023
B57	DE BB EE CC	35	G	-98.09856	31.29039
B58	EF BC DE CC	10	O	-98.09840	31.29032
B59	EF BB EE CG	30	D	-98.09850	31.29010
B60	EF BC EE CG	25		-98.09865	31.28991

* Allozyme genotype loci: 1 = PGI-2 locus, 2 = PGM-1 locus, 3 = PGM-2 locus, and 4 = MDH-3 locus.

** Crown loss is the percentage of dead foliage.

*** Trees with the same genotype group letter have identical genotypes.

APPENDIX G

COMPARISON OF IZORO PRE- AND POST-EPIDEMIC ALLELE FRQUENCIES

TABLE G-1 Izoro "Full Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE N=191	POST N=202
PGI-2	C	0.0000	0.0196
	D	0.1053	0.1127
	E	0.6526	0.6863
	F	0.2316	0.1765
	G	0.0105	0.0049
PGM-1	A	0.0208	0.0196
	B	0.9635	0.9559
	C	0.0156	0.0245
PGM-2	D	0.0208	0.0539
	E	0.9635	0.9412
	F	0.0156	0.0049
MDH-3	C	0.7579	0.8081
	D	0.0105	0.0101
	E	0.0158	0.0000
	F	0.0368	0.0354
	G	0.1789	0.1465

TABLE G-2 Izoro "Clone Corrected Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE	POST
		N = 58	N = 60
PGI-2	C	0.0000	0.0500
	D	0.1552	0.1833
	E	0.5000	0.5167
	F	0.3103	0.2333
	G	0.0345	0.0167
PGM-1	A	0.0690	0.0500
	B	0.9138	0.8833
	C	0.0172	0.0667
PGM-2	D	0.0690	0.1167
	E	0.8966	0.8667
	F	0.0345	0.0167
MDH-3	C	0.6369	0.7167
	D	0.0345	0.0333
	E	0.0172	0.0000
	F	0.1207	0.0833
	G	0.1897	0.1667

TABLE G-3 Izoro "Superior Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE	POST
		N=191	N=112
PGI-2	C	0.0000	0.0179
	D	0.1053	0.1429
	E	0.6526	0.6818
	F	0.2316	0.1786
	G	0.0105	0.0089
PGM-1	A	0.0208	0.0179
	B	0.9635	0.9464
	C	0.0156	0.0357
PGM-2	D	0.0208	0.0714
	E	0.9635	0.9286
	F	0.0156	0.0000

TABLE G-3 (continued)

LOCUS	ALLELE	PRE N=191	POST N=112
MDH-3	C	0.7579	0.7589
	D	0.0105	0.0179
	E	0.0158	0.0000
	F	0.0368	0.0357
	G	0.1789	0.1875

TABLE G-4 Izoro "Full Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	5.822	0.213	7.369	0.118
PGM-1	3	2	0.400	0.819	0.405	0.817
PGM-2	3	2	4.037	0.133	4.210	0.121
MDH-3	5	4	4.080	0.396	5.233	0.264

TABLE G-5 Izoro "Clone Corrected Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	5.822	0.213	7.369	0.118
PGM-1	3	2	0.400	0.819	0.405	0.817
PGM-2	3	2	4.037	0.133	4.210	0.121
MDH-3	5	4	4.080	0.396	5.233	0.264

TABLE G-6 Izoro "Superior Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	4.067	0.397	5.233	0.264
PGM-1	3	2	1.910	0.384	2.037	0.361
PGM-2	3	2	1.118	0.572	1.134	0.567
MDH-3	5	4	1.800	0.773	2.185	0.702

APPENDIX H

COMPARISON OF AUSTIN PRE- AND POST-EPIDEMIC ALLELE FREQUENCIES

TABLE H-1 Austin "Full Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE	POST
		N= 126	N=150
PGI-2	C	0.0000	0.0123
	D	0.2083	0.1481
	E	0.5750	0.6975
	F	0.2000	0.1420
	G	0.0000	0.0000
	H	0.0167	0.0071
PGM-1	A	0.0078	0.0063
	B	0.9609	0.9937
	C	0.0312	0.0000
PGM-2	D	0.0703	0.0970
	E	0.9062	0.9028
	F	0.0234	0.0000
MDH-3	C	0.7891	0.8235
	D	0.0312	0.0074
	E	0.0156	0.0000
	F	0.0234	0.0809
	G	0.1406	0.0882

TABLE H-2 Austin "Clone Corrected Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE	POST
		N= 56	N=38
PGI-2	C	0.0000	0.0263
	D	0.1964	0.1579
	E	0.5000	0.5789
	F	0.2679	0.2368
	G	0.0000	0.0000
	H	0.0357	0.0000

TABLE H-2 (continued)

LOCUS	ALLELE	PRE N= 56	POST N=38
PGM-1	A	0.0179	0.0263
	B	0.9464	0.9737
	C	0.0357	0.0000
PGM-2	D	0.1429	0.1842
	E	0.8214	0.8158
	F	0.0357	0.0000
MDH-3	C	0.6964	0.7105
	D	0.0536	0.0263
	E	0.0357	0.0000
	F	0.0536	0.1579
	G	0.1607	0.1053

TABLE H-3 Austin "Superior Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE N=126	POST N=78
PGI-2	C	0.0000	0.0125
	D	0.2083	0.1375
	E	0.5750	0.7125
	F	0.2000	0.1375
	G	0.0000	0.0000
	H	0.0167	0.0000
PGM-1	A	0.0078	0.0000
	B	0.9609	1.0000
	C	0.0312	0.0000
PGM-2	D	0.0703	0.0769
	E	0.9062	0.9231
	F	0.0234	0.0000
MDH-3	C	0.7891	0.8194
	D	0.0312	0.0000
	E	0.0156	0.0000
	F	0.0234	0.0833
	G	0.1406	0.0972

TABLE H-4 Austin "Full Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	8.615	0.071	10.052	0.040
PGM-1	3	2	5.037	0.081	6.531	0.038
PGM-2	3	2	3.956	0.138	5.110	0.078
MDH-3	5	4	9.906	0.042	11.094	0.026

TABLE H-5 Austin "Clone Corrected Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	3.367	0.498	4.421	0.352
PGM-1	3	2	1.451	0.484	2.164	0.339
PGM-2	3	2	1.601	0.449	2.312	0.315
MDH-3	5	4	4.835	0.305	5.538	0.236

TABLE H-6 Austin "Superior Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	6.683	0.154	7.786	0.100
PGM-1	3	2	3.281	0.194	5.029	0.081
PGM-2	3	2	1.872	0.392	2.899	0.234
MDH-3	5	4	7.796	0.099	9.610	0.048

APPENDIX I

COMPARISON OF GREENHOUSE PRE- AND POST INOCULATION ALLELE FREQUENCIES

TABLE I-1 Greenhouse "Full Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE N=743	POST N=403
PGI-2	C	0.0125	0.0075
	D	0.0817	0.0825
	E	0.6731	0.6650
	F	0.2258	0.2375
	G	0.0055	0.0075
	H	0.0014	0.0000
PGM-1	A	0.0462	0.0297
	B	0.9367	0.9530
	C	0.0172	0.0173
PGM-2	D	0.0360	0.0322
	E	0.9560	0.9629
	F	0.0080	0.0050

TABLE I-2 Greenhouse "Superior Comparison" Allele Frequencies.

LOCUS	ALLELE	PRE N= 743	POST N=44
PGI-2	C	0.0125	0.0250
	D	0.0817	0.1250
	E	0.6731	0.5000
	F	0.2258	0.3250
	G	0.0055	0.0250
	H	0.0014	0.0000
PGM-1	A	0.0462	0.0435
	B	0.9367	0.9348
	C	0.0172	0.0217
PGM-2	D	0.0360	0.0652
	E	0.9560	0.9348
	F	0.0080	0.0000

TABLE I-3 Greenhouse "Full Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	2.894	0.576	3.278	0.512
PGM-1	3	2	0.730	0.694	0.769	0.681
PGM-2	3	2	1.630	0.443	1.777	0.411

TABLE I-4 Greenhouse "Superior Comparison" Allele Frequency Statistics.

LOCUS	ALLELES	D.F.	χ^2	P	G^2	P
PGI-2	5	4	6.882	0.230	5.858	0.320
PGM-1	3	2	0.059	0.971	0.056	0.972
PGM-2	3	2	1.370	0.504	1.550	0.461

APPENDIX J

CROWN LOSS AVERAGES FOR ALLELES FOR ALL DISEASE SITES

TABLE J-1 Crown loss averages for Austin Center Alleles.

LOCUS	ALLELE	N*	AV CL**	SD**
PGI-2				
	C	2	45.0	49.0
	D	23	46.3	36.6
	E	104	43.0	34.2
	F	22	38.4	
PGM-1				
	A	1	85.0	NA
	B	149	42.8	34.2
PGM-2				
	D	14	51.3	37.0
	E	120	36.5	32.0
MDH-3				
	C	109	41.4	33.2
	D	1	99.0	NA
	F	11	37.2	34.0
	G	11	28.2	27.2

* N = number of times this allele exists in the post-epidemic population. If the allele is expressed as a homozygote in a tree, then it is counted twice.

** AV CL = average crown loss for each allele.

*** Standard deviation about the mean.

TABLE J-2 Crown Loss Averages for Izoro Center Alleles.

LOCUS	ALLELE	N*	AV CL**	SD***
PGI-2				
	C	4	47.5	32.3
	D	22	23.9	27.1
	E	128	39.1	36.1
	F	51	40.7	34.3
PGM-1				
	A	4	38.8	30.1
	B	185	38.7	35.8
	C	5	26.0	31.5
PGM-2				
	D	10	36.9	33.1
	E	183	38.3	35.1
	F	1	95.0	NA
MDH-3				
	C	152	37.4	34.3
	D	2	27.5	3.5
	F	6	30.8	37.3
	G	27	30.0	31.1

* N = number of times this allele exists in the post-epidemic population. If the allele is expressed as a homozygote in a tree, then it is counted twice.
** AV CL = average crown loss for each allele.
*** Standard deviation about the mean.

TABLE J-3 Crown Loss Averages for Greenhouse Alleles.

LOCUS	ALLELE	N*	AV CL**	SD***
PGI-2				
	C	7	81.9	37.2
	D	50	87.5	26.2
	E	429	92.6	19.9
	F	116	85.8	29.3
	G	4	78.3	42.2
	H	2	99.5	NA
PGM-1				
	A	27	88.7	25.2
	B	616	90.8	23.3
	C	13	94.1	19.2
PGM-2				
	D	20	90.3	27.5
	E	601	90.3	23.7
	F	6	98.2	4.0

* N = number of times this allele exists in the post-epidemic population. If the allele is expressed as a homozygote in a tree, then it is counted twice.
** AV CL = average crown loss for each allele.
*** Standard deviation about the mean.

APPENDIX K

DESCRIPTION OF GENOTYPES OCCURRING MORE THAN ONCE

TABLE K-1 Austin Genotype Group Comparisons. The number of trees within each group (N) is shown for the total site and for the pre-and post-epidemic subdivisions. P_{gen} is the probability that two trees, with the same genotype, will be clones when they are found next to one another. When at least two genotype trees are found together, then 'Y' indicates a significant clone. When several genotype trees are found together, then a 'Y' indicates probable clonality. The average crown loss (Av. CL) is shown for the post-epidemic trees for each group.

Group	Allozyme Genotype	Total N	Pre N	Post N	Av. CL	Obs. N	Exp. N	P_{gen}.	Sig. clone	Prob. clones
1	EEBBDECC	3	0	3	66.7	3	5.8	0.040		
2	EEBBEECG	9	4	5	43.0	9	8.5	0.059		
3	EFBBEECC	14	5	9	37.6	14	15.8	0.110		Y
4	EEBBEECF	5	0	5	46.8	5	4.0	0.028		Y
5	EEBBEECC	19	10	9	24.0	19	30.5	0.212		Y
6	EFBBDECC	4	1	3	66.7	4	3.0	0.021		
7	DEBBEECC	19	7	12	40.0	19	16.4	0.114		Y
8	CEBBEECC	2	0	2	45.0	2	0.7	0.005		
10	EEBBDECG	4	2	2	12.5	4	0.1	0.001		
11	DEBBEECG	5	3	2	30.0	5	4.6	0.032	Y	Y
12	DDBBEECC	4	4	0	na	4	2.2	0.015	Y	Y
13	EFBBBECG	5	3	2	20.0	5	4.5	0.031		
14	FFBBEECC	3	3	0	na	3	2.0	0.014	Y	Y
15	DFBBEFCC	2	2	0	na	2	0.4	0.003	Y	Y

TABLE K-2 Izoro Genotype Group Comparisons. The number of trees within each group (N) is shown for the total site and for the pre-and post-epidemic subdivisions. P_{gen} is the probability that two trees, with the same genotype, will be clones when they are found next to one another. When at least two genotype trees are found together, then 'Y' indicates a significant clone. When several genotype trees are found together, then a 'Y' indicates probable clonality. The average crown loss (Av. CL) is shown for the post-epidemic trees for each group.

Group Letter	Allozyme Genotype	Total N	Pre N	Post N	Av. CL	Obs. N	Exp. N	P_{gen}.	Sig. clone	Prob. clones
A	EEBBEECC	53	26	27	40.9	53	40.9	0.212		Y
B	EEBBEECG	25	14	11	38.1	25	17.9	0.035	Y	Y
C	EFBBEECC	21	10	11	55.8	21	34.7	0.18	Y	Y
D	EFBBEECG	9	5	4	37.5	9	7.1	0.037	Y	Y
E	FFBBEECG	6	6	0	na	6	1.4	0.007	Y	Y
F	DEBBEECG	4	2	2	10.0	4	2.7	0.014	Y	Y
G	DEBBEECC	9	5	4	32.5	9	15.4	0.08	Y	Y
H	DEBBEECE	3	3	0	na	3	0.8	0.004	Y	Y
I	DFBBEECC	7	3	4	20.0	7	6.6	0.034	Y	Y
J	DFBBEECG	5	3	2	0.00	5	1.2	0.006	Y	Y
K	FFBBEECC	3	1	2	87.5	3	7.1	0.037		
L	EEBBDECG	5	1	4	45.0	5	0.4	0.002	Y	Y
M	CEABEECC	2	0	2	52.5	2	0.2	0.001	Y	Y
N	DEBBEECF	3	0	3	35.0	3	2.0	0.021	Y	Y
O	EFBCDECC	2	0	2	5.0	2	0.06	0.000	Y	Y
P	EEBBDECC	2	1	1	20.0	2	1.5	0.013		

APPENDIX L

HARDY-WEINBERG EQUILIBRIUM DATA

TABLE L-1 Hardy-Weinberg Equilibrium.

Austin full pre-epidemic population

Locus	x^2 Value	DF	x^2 Prob.	Fisher's exact Prob.
PGI-2	3.9442	6	0.684	0.565
PGM-1	0.1057	3	0.991	1.000
PGM-2	3.4999	3	0.321	0.240
MDH-3	6.6025	10	0.762	0.370

Austin full post-epidemic population

Locus	x^2 Value	DF	x^2 Prob.	Fisher's exact Prob.
PGI-2	2.2034	6	0.9001	0.947
PGM-1	0.0033	1	0.954	1.000
PGM-2	3.833	1	0.0503	0.010
MDH-3	2.9386	6	0.8165	0.716

Austin pre-epidemic population (clone corrected)

Locus	x^2 Value	DF	x^2 Prob.	Fisher's exact Prob.
PGI-2	2.8056	6	0.8328	0.880
PGM-1	0.0897	3	0.9930	1.000
PGM-2	2.673	3	0.4448	0.331
MDH-3	8.2165	10	0.6077	0.552

TABLE L-1 (continued)

Austin post-epidemic population (clone corrected)

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	1.5373	6	0.957	1.000
PGM-1	0.0139	1	0.9062	1.000
PGM-2	0.2941	1	0.5876	0.500
MDH-3	3.1536	6	0.7893	0.831

Austin superior survivors post-epidemic population

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	2.0898	6	0.91126	0.897
PGM-1	NA	NA	NA	NA
PGM-2	3.0093	1	0.0828	0.193
MDH-3	1.7478	3	0.6264	1.000

Izoro full pre-epidemic population

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	9.4734	6	0.1490	0.055
PGM-1	22.899	1	0.0000	0.031
PGM-2	0.1374	3	0.9870	1.000
MDH-3	10.960	10	0.3610	0.078

Izoro full post-epidemic population

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	13.278	10	0.209	0.118
PGM-1	0.2220	3	0.974	1.000
PGM-2	0.4832	3	0.923	1.000
MDH-3	4.6776	6	0.586	0.279

TABLE L-1 (continued)

Izoro pre-epidemic population (clone corrected)

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	1.9252	6	0.926	0.963
PGM-1	6.2486	3	0.100	0.183
PGM-2	0.2581	3	0.968	1.000
MDH-3	2.9970	6	0.981	0.950

Izoro post-epidemic population (clone corrected)

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	1.5373	6	0.597	1.000
PGM-1	0.0139	1	0.906	1.000
PGM-2	0.2941	1	0.588	0.500
MDH-3	3.1536	6	0.789	0.822

Izoro superior survivors post-epidemic population

Locus	x² Value	DF	x² Prob.	Fisher's exact Prob.
PGI-2	14.4430	10	0.1537	0.060
PGM-1	0.1794	3	0.9808	0.130
PGM-2	0.3314	1	0.5649	1.000
MDH-3	4.0689	6	0.6674	0.276

APPENDIX M

LINKAGE DISEQUILIBRIUM DATA

TABLE M-1 Linkage Disequilibrium

Austin full pre-epidemic population

Locus 1	Locus 2	DF	x^2 value	x^2 prob.	exact test P-value
PGI-2	PGM-1	6	1.9810	0.921	1.000
PGM-1	PGM-2	4	0.5383	0.970	1.000
PGM-2	MDH-3	8	8.0718	0.426	0.794
PGI-2	PGM-2	6	8.7003	0.191	0.454
PGM-1	MDH-3	8	4.9829	0.759	0.392
PGI-2	MDH-3	12	22.5048	0.033	0.107

Austin full post-epidemic population

Locus 1	Locus 2	DF	x^2 value	x^2 prob.	exact test P-value
PGI-2	PGM-1	3	2.4661	0.481	0.158
PGM-1	PGM-2	1	9.6856	0.002	0.089
PGM-2	MDH-3	3	1.1791	0.758	0.604
PGI-2	PGM-2	3	7.6914	0.052	0.998
PGM-1	MDH-3	3	0.2126	0.976	0.490
PGI-2	MDH-3	9	11.0493	0.272	0.191

TABLE M-1 (continued)

Izoro full pre-epidemic population

Locus 1	Locus 2	DF	x² value	x² prob.	exact test P-value
PGI-2	PGM-1	3	1.9680	0.579	0.414
PGM-1	PGM-2	2	0.1546	0.926	0.144
PGM-2	MDH-3	8	12.4562	0.132	0.175
PGI-2	PGM-2	6	4.1271	0.659	0.395
PGM-1	MDH-3	4	28.7162	0.000*	0.228
PGI-2	MDH-3	12	42.7389	0.000**	0.004

Izoro full post-epidemic population

Locus 1	Locus 2	DF	x² value	x² prob.	exact test P-value
PGI-2	PGM-1	8	58.6760	0.000***	0.000****
PGM-1	PGM-2	4	8.5683	0.073	0.455
PGM-2	MDH-3	6	4.1230	0.660	0.542
PGI-2	PGM-2	8	5.5245	0.700	0.782
PGM-1	MDH-3	6	1.0131	0.985	0.861
PGI-2	MDH-3	12	69.5809	0.000*****	0.036

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* x² prob. = .000001
** x² prob. = .000003
*** x² prob. = .00000000008
**** exact test P-value = .0002
***** x² prob. = .00000000004
This table shows data analyzed by the Power Marker, Version 3.25 software (66).
This table shows overall loci LD.

APPENDIX N

F-STATISTICS DATA

TABLE N-1 F-statistics for Austin.

Comparison between "Full" pre- and post-epidemic trees sampled

N	LOCUS	N_m*	F_{ST}	F_{IS}	F_{IT}
282	PGI-2	24	0.0105	-0.0010	0.0004
286	PGM-1	21	0.0115	-0.0300	0.0182
272	PGM-2	135	0.0018	0.1089	0.1106
264	MDH-3	41	0.0060	-0.1211	0.1143
276	MEAN	32	0.0077	-0.0263	0.0183

Comparison between the "Clone Corrected" pre- and post-epidemic populations

N	LOCUS	N_m*	F_{ST}	F_{IS}	F_{IT}
94	PGI-2	57	0.0043	-0.0141	0.0097
94	PGM-1	37	0.0067	-0.0380	-0.0310
94	PGM-2	100	0.0025	0.0323	0.0348
94	MDH-3	29	0.0085	-0.1851	-0.1750
94	MEAN	46	0.0054	-0.0607	-0.0550

* N_m = gene flow. Estimated from $N_m = 0.25\ (1\text{-}F_{ST})/F_{ST}$

TABLE N-2 F-statistics for Izoro.

Comparison between all pre- and post-epidemic trees sampled

N	LOCUS	Nm*	F_{ST}	F_{IS}	F_{IT}
394	PGI-2	107	0.0023	0.1008	0.1029
396	PGM-1	561	0.0004	0.1017	0.1021
396	PGM-2	53	0.0047	-0.0461	-0.0412
388	MDH-3	94	0.0027	-0.1250	-0.1222
394	MEAN	99	0.0025	0.0088	0.0113

Comparison between the clone-corrected pre- and post-epidemic populations

N	LOCUS	Nm*	F_{ST}	F_{IS}	F_{IT}
118	PGI-2	65	0.0038	0.0122	0.0160
118	PGM-1	50	0.0050	0.0964	0.1009
118	PGM-2	61	0.0041	-0.1136	-0.1091
118	MDH-3	59	0.0042	-0.0954	-0.0908
118	AVERAGE	60	0.0041	-0.0299	-0.0256

* N_m = gene flow estimated from Fst = 0.25(1-Fst)/Fst

TABLE N-3 F-statistics for Austin and Izoro Combined.

N	LOCUS	N_m*	F_{ST}	F_{IS}	F_{IT}
512	PGI-2	25	0.0099	0.0970	0.1059
522	PGM-1	277	0.0009	0.0331	0.0340
522	PGM-2	47	0.0053	-0.0110	-0.0056
518	MDH-3	295	0.0008	-0.0963	-0.0954
518	MEAN	43	0.0057	0.0161	0.0217

* N_m = gene flow estimated from $N_m = 0.25\ (1\text{-}F_{ST})/F_{ST}$

APPENDIX O

HETEROZYGOTE AND HOMOZYGOTE DATA

TABLE O-1 Numbers of Heterozygotes and Homozygotes for Austin and Izoro Loci.

POP.	STAT	PGI-2	PGM-1	PGM-2	MDH-3
AUST TL PRE	HOMO	27	59	53	62
	HETERO	33	5	11	10
AUST TL PST	HOMO	39	79	62	44
	HETERO	42	1	10	24
AUST CC PRE	HOMO	11	25	17	13
	HETERO	17	3	9	15
AUST CC PST	HOMO	7	18	14	8
	HETERO	12	1	5	11
AUST SP PST	HOMO	20	M	35	23
	HETERO	20	M	4	13
IZORO TL PRE	HOMO	52	91	89	53
	HETERO	43	5	7	42
IZORO TL PST	HOMO	57	93	90	63
	HETERO	45	9	12	36
IZORO CC PRE	HOMO	10	26	23	12
	HETERO	18	3	6	17
IZORO CC PST	HOMO	12	23	22	15
	HETERO	18	7	8	15
IZORO SP PST	HOMO	29	50	48	31
	HETERO	27	6	8	25

POP refers to population. "HOMO" and "HETERO" refer to the actual number of homozygotes and heterozygotes at each locus. TL refers to the population of all tree samples; CC refers to the population of tree samples after clone correction; and SP refers to post epidemic samples of superior trees (≤ 25% crown loss).

TABLE O-2 Heterozygosity Comparisons per Locus for Austin and Izoro.

POP.	N	PGI-2	PGM-1	PGM-2	MDH-3	MEAN
AUST TL PRE (H)	126	0.5857	0.0756	0.1732	0.3558	0.2976
AUST TL PRE (OBS)		0.5500	0.0781	0.1719	0.3906	0.2977
AUST TL PST (H)	150	0.4712	0.0126	0.1755	0.3074	0.2417
AUST TL PST (OBS)		0.5185	0.0127	0.1389	0.3529	0.2558
AUST CC PRE (H)	58	0.6384	0.1027	0.3036	0.4821	0.3817
AUST CC PRE (OBS)		0.6071	0.1071	0.3214	0.5357	0.3929
AUST CC PST (H)	60	0.5831	0.0512	0.3006	0.4584	0.3483
AUST CC PST (OBS)		0.6316	0.0526	0.2632	0.5789	0.3816
AUST SP PST (H)	78	0.4544	NULL	0.1420	0.3121	0.2271
AUST SP PST (OBS)		0.5000	NULL	0.1026	0.3611	0.2758
IZORO TL PRE (H)	191	0.5093	0.0709	0.0709	0.3919	0.2607
IZORO TL PRE (OBS)		0.4526	0.0521	0.0729	0.4421	0.2549
IZORO TL PST (H)	202	0.4848	0.0853	0.1113	0.3242	0.2514
IZORO TL PST (OBS)		0.4412	0.0882	0.1176	0.3636	0.2527
IZORO CC PRE (H)	56	0.6284	0.1599	0.1902	0.5410	0.3799
IZORO CC PRE (OBS)		0.6552	0.1034	0.2069	0.5862	0.3966
IZORO CC PST (H)	38	0.6422	0.2128	0.2350	0.4506	0.3851
IZORO CC PST (OBS)		0.6000	0.2333	0.2667	0.5000	0.4000
IZORO SP PST (H)	112	0.5225	0.1027	0.1327	0.3873	0.2863
IZORO SP PST (OBS)		0.4821	0.1071	0.1429	0.4464	0.2946
IZORO TL PST A (H)	94	0.5023	0.0822	0.1195	0.3136	0.2544
IZORO TL PST A (OBS)		0.4681	0.0851	0.1277	0.3696	0.2626
IZORO TL PST B (H)	110	0.4775	0.0874	0.1040	0.3280	0.2492
IZORO TL PST B (OBS)		0.4364	0.0909	0.1091	0.3519	0.2471

H = Nei's unbiased heterozygosity, OBS = direct count heterozygosity, and N = average number of alleles for all loci.

TABLE O-3 Statistical Comparisons of Heterozygosity between all Pre- and Post-Epidemic Sites. This table uses all disease site comparisons ("Full", "Clone Corrected", and "Superior" post-epidemic trees. This table also includes a comparison between the greenhouse pre- and post-inoculation populations.

"Full" Austin pre- and post-epidemic heterozygosity comparisons

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.711	0.736
PGM-1	0.052	0.090
PGM-2	0.595	0.640
MDH-3	0.654	0.720

Austin "Clone Corrected" pre- and post-epidemic heterozygosity comparisons

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.866	1.000
PGM-1	0.511	0.638
PGM-2	0.553	0.746
MDH-3	0.770	1.000

"Full" Austin pre-epidemic compared to Superior post-epidemic population

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.6236	0.685
PGM-1	mono*	mono
PGM-2	0.3334	0.400
MDH-3	0.8045	0.832

"Full" Izoro pre- and post-epidemic heterozygosity comparisons

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.872	0.887
PGM-1	0.321	0.410
PGM-2	0.286	0.339
MDH-3	0.265	0.306

TABLE O-3 (continued)

Izoro "Clone Corrected" pre- and post-epidemic heterozygosity comparisons

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.737	0.791
PGM-1	0.184	0.299
PGM-2	0.590	0.761
MDH-3	0.506	0.604

Izoro "Full" pre-epidemic compared to Superior post-epidemic population

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.725	0.739
PGM-1	0.206	0.215
PGM-2	0.163	0.127
MDH-3	0.959	1.000

"Full" Greenhouse pre- and post-inoculation heterozygosity comparisons

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.368	0.379
PGM-1	0.416	0.484
PGM-2	0.569	0.637

"Full" Greenhouse pre- and Superior post-inoculation comparisons

LOCUS	x^2 P-value	Fisher exact test P-value
PGI-2	0.240	0.259
PGM-1	0.835	0.742
PGM-2	0.835	0.742

* "mono" indicates a monomorphic locus.

VITA

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