

MANAGEMENT AND IMPACTS OF OAK WILT IN LAKE STATES FORESTS

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

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September, 2024

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Acknowledgements

In completing this dissertation, I learned a great deal about oaks and gratitude. First and foremost, I would like to thank my advisor, Dr. Rebecca Montgomery for her guidance and support throughout these past years. Rebecca, you not only showed me how to recognize strengths in others and in myself but also shaped my perspective as a scientist and woman, allowing me to embrace my multidimensional (n-dimensional, if you will) nature. Your mentorship has been invaluable. I am grateful to my graduate committee members: Dr. Jennifer Juzwik, Dr. Marcella Windmuller-Campione, and Dr. Jeannine Cavender-Bares. Your collective wisdom, thoughtful direction, constructive feedback, and words of encouragement have provided me a template for leadership that I hope to emulate in the future. I will forever be grateful for the joy and excitement you all conveyed when I shared the news of starting a family. Jenny, you went out on a limb to hire me when I was in my early twenties – and what a tremendous honor it has been to learn from you since then.

Thank you to the many generous sources of funding, including the Natural Resources Science and Management (NRSM) Program, Department of Forest Resources, Minnesota Invasive Terrestrial Plants and Pests Center (MITPPC), U.S. Forest Service State, Private, and Tribal Forestry, U.S. Forest Service Special Technology Development Program, U.S. Forest Service Pathways Program, and University of Minnesota Doctoral Dissertation Fellowship. I am especially grateful to the people who helped me survey vegetation (even when it was all poison ivy) and to Dave Anderson, who shares my curiosity for oak wilt and generously let me work on his property.

My gratitude also extends to my unofficial mentors and friends with the U.S. Forest Service, particularly Linda Haugen and Paul Castillo. Linda, thank you for letting me hang around like a pesky wood tick. You handed me so many incredible opportunities, including the chance to tell the oak wilt root rupture story. Paul, I have

learned so much from working alongside you – from measuring azimuths to working with landowners – your friendship means a great deal to me.

To my dear friends: Austin Yantes and Sam Reed. I truly do not know if I would have made it here without you. We navigated the winding road of graduate school together, through periods of motivation, doubt, inspiration, failure, and achievement. It is an honor to make it to the finish line with you both and I cannot wait to see where we go next.

Finally, thank you to my family. Mom, you have supported me from diapers to dissertation (and now back to more diapers) with grace and love. Llewyn and Robin, you were the best surprise I could have ever imagined. Everything that I learned while creating this dissertation pales to the magnitude of learning and growth I experienced with you in one short year. You are my greatest joy. To Paul – I could have written an entire chapter about how much I appreciate your unwavering support and the compromises you made for me to get here. You carry this family, and this achievement is as much yours as it is mine.

to the village

Dissertation Abstract

Oaks, one of the most ecologically important genera of trees in North America, are experiencing widespread declines. Oak wilt, caused by the fungus *Bretziella fagacearum*, is a major threat to oak ecosystems in the United States. My dissertation uses applied and theoretical approaches to understand how we can control oak wilt outbreaks while also examining how the disease and its management will shape the future of oak forests. In my first chapter, I describe the successful implementation of an oak wilt management technique (“root rupture” method) using data collected over ten years of operational disease control efforts in the Chequamegon-Nicolet National Forest and Menominee Forest in Wisconsin. Here, we found that the root rupture method achieved a high level of disease control and that the opportunity for successful control is highest when disease infection centers are small, providing forest managers with insights on how to prioritize disease management activities. The second chapter is an exploration of the long-term efficacy of oak wilt treatments and evaluates the longevity of *B. fagacearum* in root systems of diseased trees. Results from this study indicate that the pathogen and root systems may stay alive for longer periods of time (at least five years) than previously thought, putting remaining mature oaks and regenerating trees at risk for future infection. Finally, the third chapter explores how oak wilt alters forest resource availability, plant community composition, and plant diversity, with implications for long-term oak regeneration. Overall, we found that plant communities in oak wilt-affected sites shifted in composition and had higher diversity; yet, regenerating species in oak-wilt affected sites shifted away from dominance by oaks and towards shade tolerant tree species or nuisance species such as buckthorn. Taken together, the chapters of my dissertation illustrate that, while oak wilt can be successfully managed on the landscape, there may be substantial ecological implications of management that focuses on disease suppression without accounting for long-term regeneration.

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Introduction

Oaks (genus *Quercus*) are one of the most diverse groups of trees in temperate forests and are widely regarded as one of the most important clades of woody species in the northern hemisphere (Nixon 2006). They provide a staggering variety of ecosystem services such as sequestering carbon, absorbing air pollution, and regulating groundwater (Cavender-Bares 2016). These adaptable species have been intertwined with humankind for centuries: we explored the world in ships made of oak wood, we celebrate our happiest moments with wine aged in oak barrels, and we bury our loved ones in enduring oak caskets. It is within our nature, as both humans and scientists, to study oaks. Across regions of the eastern United States, oaks are failing to regenerate due to changes in historic fire stewardship that maintained them on the landscape, overabundant herbivores, and disease (Dey 2014, Nowacki and Abrams 2008). Oak wilt, caused by the fungus *Bretziella fagacearum* (Bretz) Z.W. deBeer, Marinc., T.A. Duong, & M.J. Wingf.), is the most important disease that contributes to this decline (Juzwik et al. 2011). It can infect all oak species regardless of age or size and cause tree death within one year for many species, particularly those of the red oak group (*Quercus* section *Lobatae*; Bretz 1955). Oak wilt has become increasingly destructive over the past several decades as it expands in geographic range (Jensen-Tracy et al. 2009; NAPPO 2023), creating potentially extensive consequences for biodiversity and ecosystem health.

The oak wilt fungus spreads from diseased to healthy trees by two methods: insects (predominantly sap feeding beetles of the *Nitidulidae* family) that fly from tree to tree carrying spores and belowground through the connected root systems between infected and healthy trees (Gibbs and French, 1980; Juzwik et al., 1985). Aboveground spread of *B. fagacearum* is needed to initiate new disease centers, but belowground spread is thought to be responsible for most new oak wilt infections in the Upper Midwest U.S. As such, oak wilt control programs use an integrated approach for

controlling both aboveground and belowground spread. Currently, belowground disease spread is controlled by creating a containment area where connected roots are separated by cutting roots or applying herbicides to kill root tissues (French and Steinstra 1978; Bruhn and Heyd 1992; Bronson et al. 2023). Managers in the Lake States have historically used vibratory cable plows to cut through connected root systems on sites with light textured soils; however, they are not appropriate in all circumstances. The blade on a vibratory cable plow is easily damaged, making their use inefficient in glacial till soils with buried rocks and boulders. Further, they can be challenging to use on steep slopes and are becoming increasingly difficult to source. Therefore, to prevent further expansion of oak wilt in the U.S., managers need disease control methods that disrupt root systems on landscapes with these limitations.

Despite decades of management, oak wilt continues to appear in stands where management previously occurred. While persistence of the disease on the landscape can occur from aboveground spread, belowground treatment failures are thought to be, in part, related our lack of knowledge regarding the presence and survival of *B. fagacearum* in root systems of asymptomatic, diseased, and dead trees. Limited evidence suggests that oaks trees can harbor the fungus in root systems for a year or more before the development of foliar symptoms (Skelly and Wood 1974) and that root systems continue to transmit the fungus through root grafts after tree death (Yount 1955; Rexrode 1978). Methods for establishing containment zones intrinsically account for some of the uncertainty created by asymptomatic trees (Menges and Loucks 1984; Bruhn et al. 1991); however, the lack of knowledge regarding *B. fagacearum* persistence after tree death remains a constraint to long-term management success. Characterization of *B. fagacearum* survival in root systems would improve containment efforts and increase the likelihood of stopping belowground spread in oak wilt-affected stands. Failure to contain oak wilt infection centers comes at great financial cost to land managers; moreover, it puts regenerating oak trees at risk for future infection and mortality.

Oak wilt creates openings in the overstory by killing mature oak trees and by triggering disease control efforts where land managers cut down infected oak trees. Such

openings, or canopy gaps, are disturbances that present an opportunity for ground-layer plants and young trees to thrive by freeing up resources such as light and nutrients (Canham et al. 1990; Denslow 1980; Sipe and Bazzaz 1995). Ecological theory predicts that large canopy gaps, such as those formed by tree death due to oak wilt or disease control efforts, promote plant diversity because they produce a mixture of high and low light conditions that suit a diverse array of plants (Denslow 1980). The pattern of plant growth that occurs after a canopy gap is formed will dictate which species grow to adult stature and the overall composition of the future forest. The dynamics of canopy gaps produced by tree diseases and disease management on forest plant communities are not well understood (Loo 2009). Many oak species in the eastern U.S. prefer medium-to-high light for regeneration and should therefore thrive in these canopy gaps. Disturbance characteristics (e.g., area affected, intensity, spatial pattern, frequency) can direct successional dynamics, but many other attributes of the forest (e.g., available seed, herbivore pressure, pre-disturbance vegetation) can override the effects of the disturbance itself (Brokaw and Busing 2000). Understanding how oak wilt-mediated canopy gaps change species diversity, tree succession, and ground-layer plant communities would allow us to respond effectively to the disease and sustain the health of our oak ecosystems.

The research presented in this dissertation is based in regions of Minnesota and Wisconsin that have been affected by oak wilt for over half a century. The history of oak wilt presence and management in these regions makes them uniquely suited to study long-term effects of the disease with observational studies. My dissertation addresses key knowledge gaps related to the short-term need for approaches to control the disease and long-term ecological implications of the disease and its management. In first chapter, I conduct a scientific evaluation of an oak wilt containment method (“root rupture” method) in regions of Wisconsin, USA that are characterized by glacial soils which preclude the use of vibratory plows. Chapter 2 then examines the longevity of *B. fagacearum* in roots of diseased trees over time to understand if pathogen persistence will affect management outcomes. Finally, Chapter 3 characterizes changes in the resource

dynamics, plant communities, and oak regeneration in oak wilt-affected gaps. Most forest ecosystems experience tree death from pathogens, but the long-term ecological impacts are often overlooked until after forest-wide change has already occurred. Using oak wilt as a case study, my research contributes to our broader theoretical understanding of how diseases, which are increasing in frequency and intensity due to climate change, drive ecological succession and shape forest ecosystems.

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Chapter 1 :
**Evaluation of the Root Rupture Method for Controlling Belowground Spread of the
Oak Wilt Pathogen (*Bretziella fagacearum*) in Northeast Wisconsin, USA**

Originally published as:

Yang, A., Haugen, L.M., Mausel, D.L., Lampereur, J., Stueck III, T., Montgomery, R.A. and Juzwik, J. 2024. Evaluation of the Root Rupture Method for Controlling Belowground Spread of the Oak Wilt Pathogen (*Bretziella fagacearum*) in Northeast Wisconsin, USA. *Journal of Forestry*, fvad055. <https://doi.org/10.1093/jofore/fvad055>

Abstract

Oak wilt, caused by the pathogen *Bretziella fagacearum*, is a destructive disease on oak (*Quercus*) species in the United States. Established methods to control the belowground spread of oak wilt are not appropriate in all circumstances; thus, forest health managers have a strong need for alternative methods for disease control. We evaluated the effectiveness of operational stump excavation treatments (i.e., “root rupture” method) applied between 2004 and 2014 to control belowground spread of oak wilt on two forests in northeast Wisconsin, USA. The root rupture method achieved a high level of disease control ($\geq 85\%$ of evaluated infection centers) for 5 years by harvesting red oak trees within infection centers and uprooting the stumps and associated root mass. The likelihood of disease control success depended on the initial size of the infection center that was treated, with larger infection centers being less likely to be contained with a single treatment than small infection centers. This study highlights the importance of early detection and prompt management in oak wilt suppression programs.

Forest management agencies and organizations in the North Central United States continue to face oak wilt epidemics. No single tool or treatment method ensures complete containment of oak wilt; thus, most managers carefully consider all the tools and techniques available to them and determine what best suits their budget and land use priorities. Stump excavation (i.e., “root rupture” method) is a cost-effective treatment for halting belowground spread of the oak wilt fungus, *Bretziella fagacearum*, that can achieve a high level of disease control. In northern red oak (*Quercus rubra*) stands with high economic value, this treatment can be applied as a group selection, with the value of the timber potentially offsetting the cost of treatment. The opportunity for complete disease control decreases as oak wilt infection centers increase in size; thus, monitoring and early control actions are critical.

Introduction

Oak wilt is among the greatest threats to oak (*Quercus*) species in North America, particularly the North Central states and Texas (Juzwik et al. 2011). The causal pathogen, *Bretziella fagacearum* (Bretz) Z.W. deBeer, Marinc., T.A. Duong, and M.J. Wingf. (syn. *Ceratocystis fagacearum* (Bretz) Hunt), can infect all oak species; however, members of the red oak group (*Quercus* section *Lobatae*) experience the highest annual rates of infection and mortality, often dying within weeks to months after the appearance of initial symptoms. Disease infection centers are established when contaminated beetles of the *Nitidulae* family carry spores from diseased trees to fresh wounds on healthy trees (Juzwik, French, and Jeresek 1985; Ambourn, Juzwik, and Moon 2005; Jagemann et al. 2018). Once introduced to a healthy tree, the fungus may spread through root grafts between neighboring oaks, resulting in the outward expansion of areas of wilting trees (Gibbs and French 1980). Transmission through connected root systems is thought to be responsible for most new infections in the Lake States and can result in rapid disease spread, thus a large focus of oak wilt control programs is to prevent belowground spread of *B. fagacearum* among neighboring trees (Juzwik 2009). However, established methods to prevent belowground spread are not suitable for all disease suppression circumstances or all affected landscapes. As the range of oak wilt continues to slowly expand, there is a need to build upon existing methods of controlling belowground spread to suit a wide variety of landscapes (New York Department of Environmental Conservation Bureau of Invasive Species and Ecosystem Health 2019; Haugen et al. 2022).

Belowground spread of *B. fagacearum* can be prevented by establishing a containment zone around diseased trees, either by severing or killing root systems between healthy and diseased oaks. One of the main challenges to preventing belowground disease spread is that the pathogen can colonize in the roots of a healthy-appearing tree prior to the onset of symptom development. Early research on the movement of *B. fagacearum* in roots documented a lag between root inoculations and foliar symptom development that ranged from 45 to 547 days (Yount 1958). Similarly, a

study in West Virginia found that only 50% of oak trees grafted to diseased trees developed symptoms within one year, while the remaining trees developed symptoms up to three years later (Rexrode 1978). To account for the potential delay in symptom development, the outermost boundary of the treated area is established beyond the location of the diseased tree(s) and encompasses healthy oak trees that may be infected but are not yet displaying symptoms. Before forest managers implement disease treatments, they first determine 1) how far the boundary of the treatment area should be placed from the diseased tree(s) and 2) what the best available options are for disrupting root systems. Because belowground spread contributes to most new infections, understanding where and how to place treatment areas is critical to effectively controlling the disease.

The placement of treatment boundaries is based on the likelihood that root grafts will occur in the stand and the level of risk for future oak wilt that forest managers are willing to accept. While root grafts can form between different oak species (e.g., between northern red oak and bur oak), they are most common between individuals of the same species (Parmeter, Kuntz, and Riker 1956; Boyce 1957; Gibbs and French 1980). The likelihood of root graft formation is also affected by related factors such as local tree diversity, inter-tree distance, basal area of oaks, soil depth, and soil type (Bruhn, Pickens, and Stanfield 1991). As a result, stands with a high density of red oak species tend to have a high frequency of grafted root systems and an elevated risk for rapid oak wilt transmission (Parmeter, Kuntz, and Riker 1956; Gibbs and French 1980; Menges and Loucks 1984). Various models for treatment boundary placement, based on either experience or mathematical theory, are available for forest managers to use. The “rule of thumb” model developed by French and Stienstra (1978) involves placing the boundary beyond two “rings”, or layers of visually healthy oak trees. Mathematical models, based on statistical estimations of how far the pathogen moves within one year, calculate treatment boundaries based on tree diameter, inter-tree distance, soil type, and desired confidence in disease control (Bruhn and Heyd 1992). The likelihood of disease control is further improved when root disruption is coupled with the removal of red oak species

within the treatment area, as they have the potential to produce the fungal spore mats that drive overland disease transmission (Wilson 2005; Juzwik et al. 2011). After a containment area is established, managers can choose to either remove all red oak species within the enclosed area (“cut to the line” option) or remove diseased trees on an annual basis (“monitor and remove” option) (Juzwik et al. 2010).

Selecting an appropriate method to sever root systems and establish the disease treatment area depends on human land use, soil type, equipment availability, cost, and availability of experienced operators. A successful treatment stops the movement of *B. fagacearum* by disrupting the continuous xylem between neighboring trees that have grafted root systems. Approaches to disrupting connected root systems include cutting roots with a long blade, digging a trench, or applying herbicides to kill the root tissue (Jones 1971; French and Stienstra 1978; Bruhn, Wetteroff, and Haugen 2002; Wilson and Lester 2002; Bronson et al. 2023). Managers in the Lake States have historically used vibratory cable plows (with a 60-inch blade) to cut through connected root systems (French and Stienstra 1978; Juzwik et al. 2010). The blade on a vibratory plow is inserted into the soil and pulled along the outermost boundary of the treatment area, functionally separating the root systems within the treatment area from the surrounding forest; however, this method is most effective when used on soils with sandy-loam or loamy-sand textures. Oak wilt infections located on glacial till soils with buried rocks and boulders present a challenge to the use of vibratory plows because of the damage that can occur to the blade from hitting boulders and interruptions in the line from “skipping” over impassible boulders. Sites with rolling topography or steep slopes also limit the safe use of traditional vibratory plows, which can require specialized tracked plows. Beyond physical site limitations, vibratory plows and operators who are skilled in oak wilt management are not readily available in all regions where the disease is present, creating a need for a simple, alternative method to contain belowground spread of *B. fagacearum*.

Stump excavation (i.e., “root rupture” treatment) was proposed in the early 2000’s as an alternative treatment for preventing the spread of oak wilt in settings that cannot be treated with a traditional vibratory plow. The practice of uprooting stumps following

harvest has been successfully used for managing root rot diseases such as *Armillaria* root disease in the Pacific Northwest U.S., with the added benefit that it requires neither specialized equipment nor deep experiential knowledge of the disease to implement (Roth, Rolph, and Cooley 1980). The treatment strategy involves harvesting all infected oaks and healthy oaks (> 4 or 5-inches diameter at breast height; dbh) in the treatment area around the infection center using a mathematical model for treatment boundary placement (Bruhn and Heyd 1992). The stumps are then pulled completely out of the ground with an excavator, severing any root grafts within the treatment area.

This case study aims to document the efficacy of the root rupture method for controlling oak wilt in sites where traditional vibratory plowing is not feasible. Root rupture treatments were implemented in the mid- to late-2000's as an operational treatment on oak wilt infection centers in Wisconsin on the Chequamegon – Nicolet National Forest (CNNF) and Menominee Forest (MF), where uneven topography and buried boulders restrict the use of vibratory plows. The CNNF, acquired and established by the USDA Forest Service in 1933, has many northern red oak stands that originated in the early 1920's following widespread exploitative harvest of red and white pine (*Pinus resinosa* and *P. strobus*, respectively) and repeated fires (Elliot 1977; Stearns 1997; Dunham, Moffat, and Howell 2021). As a result, many of the region's hardwoods remained and stands are now dominated by large diameter northern red oak. Oak wilt was identified in 1997 at a campground in the CNNF and the disease was treated with a vibratory plow; however, many infection centers were subsequently found scattered in active timber sale units where the topography and soil conditions precluded the use of vibratory plows.

Neighboring the CNNF is the Menominee Forest (MF), which is managed by Menominee Tribal Enterprises (MTE) and located on the 235,560-acre Menominee Indian Reservation. This piece of the ancestral lands of the Menominee Nation was protected from outside timber liquidation that occurred during the 19th century; the present-day MF remains host to diverse forest types that offer a rare glimpse of the landscape that once covered this region of Wisconsin (Pecore 1992). Tribal members

have managed timber resources using a sustained-yield concept since the late 1880's, which emphasizes maintaining diverse species and habitats to provide simultaneous economic, cultural, and ecological benefits (Pecore 1992; Mausel, Waupochick, and Pecore 2017). Sections of the MF where red oak species are dominant or mixed with other hardwoods were designated for oak wilt treatments beginning in 2009.

Despite several years of disease treatment and collection of monitoring data on the CNNF and MF, a formal evaluation of the root rupture method was never completed. Although the root rupture treatments were implemented as operational treatments rather than as part of a scientific study, the data present an opportunity for retrospective evaluation. The objectives of this study were to utilize the monitoring data to 1) evaluate the success rate of the root rupture method in preventing oak wilt spread beyond established infection centers five years after operational treatment and 2) determine whether infection center size influences treatment outcomes.

Methods

Study Areas

The operational treatments were applied in northeast Wisconsin on approximately 10,000 acres of the Menominee Forest and the 344,000-acre Lakewood-Laona Ranger District of the Chequamegon-Nicolet National Forest in Menominee and Oconto counties (Figure 1-1). These forests are situated on the eastern region of the Forest Transition Ecological Landscape, which spans approximately 200 miles east to west across Wisconsin (Wisconsin Department of Natural Resources 2015). The two forests are directly adjacent to each other, with the treated regions of the forests being approximately 27 miles apart from center to center in a straight line. Forests in this part of the ecological landscape are dry-mesic to mesic with varied soils that are generally characterized as sandy loam to loam with buried and surface boulders. These forests commonly feature a mix of pine species, red maple, oak, and aspen. Red oak stands on the CNNF that received root rupture treatments were primarily on the AVb (*Acer-Viburnum*) habitat type

with relatively few hardwood associates (Kotar, Kovach, and Burger 2002). Oak wilt pockets on the MF were in stands of red oak and other mixed hardwood species. Common habitat types featuring red oak on the MF include PArVAa – Vb (*Pinus-Acer-Viburnum*), AVb, AFVb (*Acer-Fagus-Viburnum*), ATM (*Acer-Tsuga-Mianthemum*), and AFAd (*Acer-Fagus-Adiantum*) (Kotar, Kovach, and Burger 2002; Menominee Tribal Enterprises 2012).

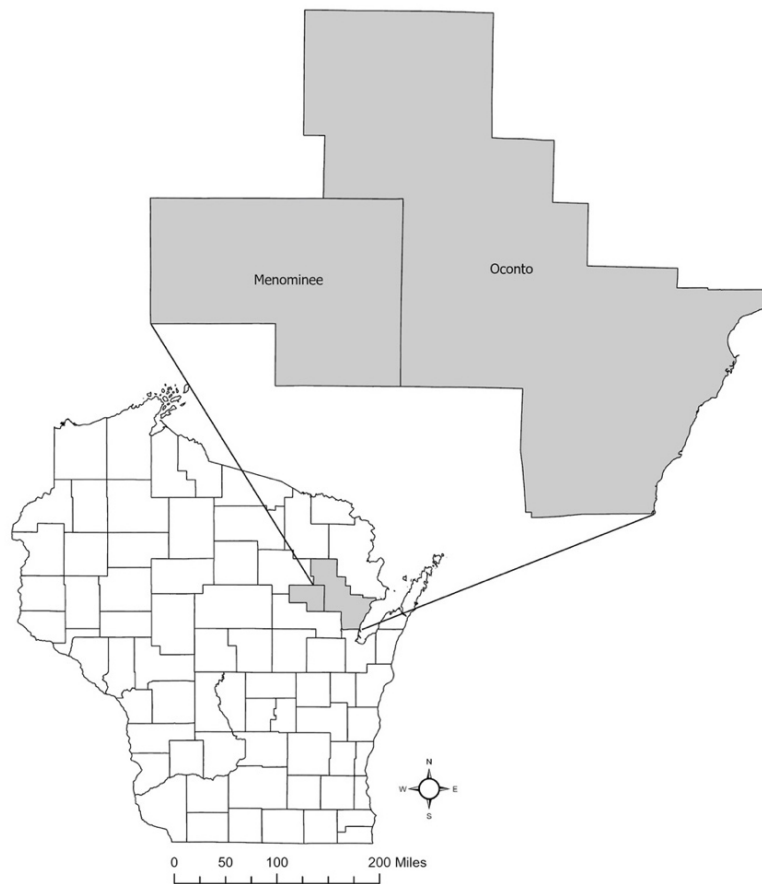


Figure 1-1. The Lakewood – Laona Ranger District of the Chequamegon – Nicolet National Forest (CENN) and the Menominee Forest (MF) are in northeast Wisconsin in Oconto and Menominee Counties, respectively.

Root Rupture Treatments

We examined treatments that were applied between 2004 through 2012 on the CNNF and between 2009 through 2014 on the MF. For both forests, field crews surveyed for diseased trees annually from August through September. They identified wilting trees by the appearance of leaf bronzing from the margin to the midrib, downward progression of symptoms in the crown, and premature leaf abscission. In the same year, they marked and flagged boundaries for the treatment areas based on the 95% probability Grayling soil equation from the statistical model developed by Bruhn et al. (1991). Within the treatment area, they marked (banded with paint and butt marked) all diseased oaks and healthy red oaks larger than four (CNNF) or five (MF) inches dbh for future removal (Carlson, Martin, and Scanlon 2010). Any remaining small diameter trees were left standing as they were presumed to be seed-origin trees (not sprout-based or coppice regeneration) that were not grafted to the larger trees. During winter months (generally between October and December), but prior to March 15 of the following year, logging contractors harvested red oak trees (including oak wilt-killed trees) within the marked boundary. Following tree harvest, they extracted stumps and root masses out of the ground using a mid-sized excavator equipped with a bucket designed for digging through frost or compacted soil (frost bucket) and thumbs for grabbing the stumps out of the ground (Figure 1-2). The stumps were overturned and pushed into the hole from which they were removed. During treatment operations, staff inspected the sites to ensure all stumps and root masses were fully excavated, flipped upside down, and severed from the remaining underground roots. Loggers removed all merchantable wood from the site and the wood was processed prior to April 1 to prevent the formation of fungal spore mats. Non-merchantable wood (e.g., large branches) was cut into 4 ft. lengths to expedite drying time, which also prevents the formation of spore mats.



Figure 1-2. Root rupture treatments were implemented on the Chequamegon – Nicolet National Forest (CNNF) and Menominee Forest (MF) during winter months before March 15. *Q. rubra* trees (> 4-in dbh for CNNF; > 5-in dbh for MF) were harvested and the stumps and root masses were dug out with an excavator. The excavator was equipped with a narrow bucket (frost bucket) that is used for digging into frost and/or compacted soil and thumbs for grabbing stumps. Top) Photo from the MF of stump and root mass being pulled from the ground and flipped upside down. Bottom) Photo from the CNNF of oak wilt infection center after completed root rupture treatment.

Monitoring and Data Collection

Field crews monitored the treated sites annually between July and late August on the CNNF and between August and September on the MF for the occurrence of newly symptomatic trees near the treated areas. New oak wilt infections were identified by the same suite of symptoms described previously. When newly infected trees were identified near previously treated sites (within approx. 66 ft from the treatment boundary), the crew assessed the tree(s) for evidence of potential overland transmission (e.g., recent wounding or broken limbs). Knowledge of separate, active oak wilt infections present in the vicinity was also used to judge whether the newly infected tree(s) likely resulted from insect spread rather than belowground through connected roots. If field crews suspected the new infection was the result of insect spread, the tree(s) were considered a new infection and the site was marked for treatment. If evidence suggested belowground spread was more likely, the crew marked the site for re-treatment using the same method and statistical model previously described. In general, forest managers monitored the sites for a minimum of five years. Current management guidelines recommend a five-to-six-year monitoring period which reflects the duration of documented vibratory plow treatment effectiveness, time in which root grafts are thought to reform, and the estimated belowground survival of *B. fagacearum* in roots (Yount 1955, Wilson and Lester 2002, Juzwik et al. 2010, Wisconsin Department of Natural Resources 2023). If disease infection centers were re-treated, a new five-year monitoring period was initiated. Field crews and managers collected annual data on the status (healthy or diseased) of treated sites and the number of trees removed during treatments. In addition to ground surveys of previously treated sites, MTE foresters conducted part of the annual oak wilt survey with the assistance of unmanned aerial vehicles (UAVs).

Study Design

A total of 202 oak wilt infection centers on the CNNF and 293 centers on the MF were treated with the root rupture method between 2004 and 2011 and between 2009 and

2014, respectively (Table 1-1). For our evaluation, we selected for analysis 186 treated sites from the CNNF and 292 treated sites from the MF. Although forest managers generally monitored treated sites for five years, interruptions in monitoring occasionally occurred. Sites selected for this analysis had at least five continuous years of annual disease monitoring data after the most recent root rupture treatment. For our evaluation, we considered infection centers that had coalesced into a single infection center as an individual site. We also considered several large infection centers (≥ 300 oak trees removed) on the CNNF as a stand replacing harvest and excluded them from the study. These stands did not contain any red oak species directly adjacent to treated areas that could develop future disease via root graft spread. Because data for the number and diameters of wilting trees were not available, we used the number of trees removed as a proxy and refer to this an infection center size. As previously stated, boundary of the treatment area was delineated using a mathematical model for the 95% Grayling soil equation for which the tree diameters, inter-tree distances, soil type, and confidence level were variables (Bruhn, Pickens, and Stanfield 1991).

Table 1-1. Number of sites with oak wilt centers on the Chequamegon – Nicolet National Forest (CNNF) and Menominee Forest (MF) treated using the root rupture method. Sites were selected for study if they were monitored for at least five years after the first treatment or subsequent re-treatment and if they contained fewer than 300 trees removed.

Location	Year of First Treatment	No. of Sites Treated	No. of Sites Selected for Analysis
CNNF	2004	88	84
	2005	23	23
	2006	16	14
	2007	10	10
	2008	7	7
	2009	19	17
	2010	9	5
	2011	17	14
	2012	13	12
MF	2009	9	9
	2010	37	36
	2011	94	94
	2012	98	98
	2014	55	55

Efficacy of Root Rupture Treatments

We calculated the overall success of the root rupture treatment as the proportion of infection centers at each forest where treatments prevented disease spread within five years. To understand the likelihood of disease control after root rupture treatments, we analyzed treatment outcomes as a function of the number of trees removed and treatment year using a generalized linear mixed effects model. We ran the model using the lme4 package in R with the number of trees removed during the first treatment as a continuous fixed effect and treatment year as a categorical fixed effect (R Core Team 2021; Bates et al. 2015). Due to the operational nature of the oak wilt treatments, we believed that disease identification and treatment precision improved annually as forest health staff gained experience. Our analysis was designed to examine differences in treatment

outcome over time, therefore we treated year as a fixed effect rather than a random effect that would control for disease spread due to interannual variation in weather. Infection centers differed in size between the two forests, resulting in a high correlation between the forest location (CNNF and MF) and number of trees removed in the initial infection centers ($X_1^2 = 287.84, p = 1.971 \times 10^{-15}$); thus, the forest location was treated as a random effect. The model was checked for zero-inflation and residuals were checked for assumptions of normality (Hartig 2022). Post-hoc pairwise comparisons were calculated using the emmeans package and a Sidak p-value adjustment was used to control for multiple comparisons between treatment years (Lenth et al. 2022).

Results

Overall, the infection centers treated at the CNNF were larger than those at the MF. The average number of trees removed for treatment was 74.41 ($SE = 4.49$) and 18.53 ($SE = 0.91$) for the CNNF and MF, respectively (Figure 1-3). Following the initial root rupture treatments, 63% of the infection centers on the CNNF and 93% of the infection centers on the MF did not develop further disease symptoms within five years of the treatment (Table 1-2). The infection centers that did not develop further disease symptoms after a single root rupture treatment were, on average, smaller in size than those that developed new disease symptoms. The mean number of trees removed on infection centers where a single treatment was successful was 32.17 ($SE = 1.84$), while the average for infection centers that were not contained was 72.78 ($SE = 7.43$). The likelihood of successful disease control after one root rupture treatment decreased as the size of the infection center increased ($p < 0.05$; Table 1-3; Figure 1-4). The odds of treatment success decreased by 0.81% for each additional tree that needed to be removed within the calculated treatment boundary. The likelihood of disease control also varied by year, with the highest estimated treatment success in 2010, 2011, 2012, and 2014 (Table S1; Figure 1-5).

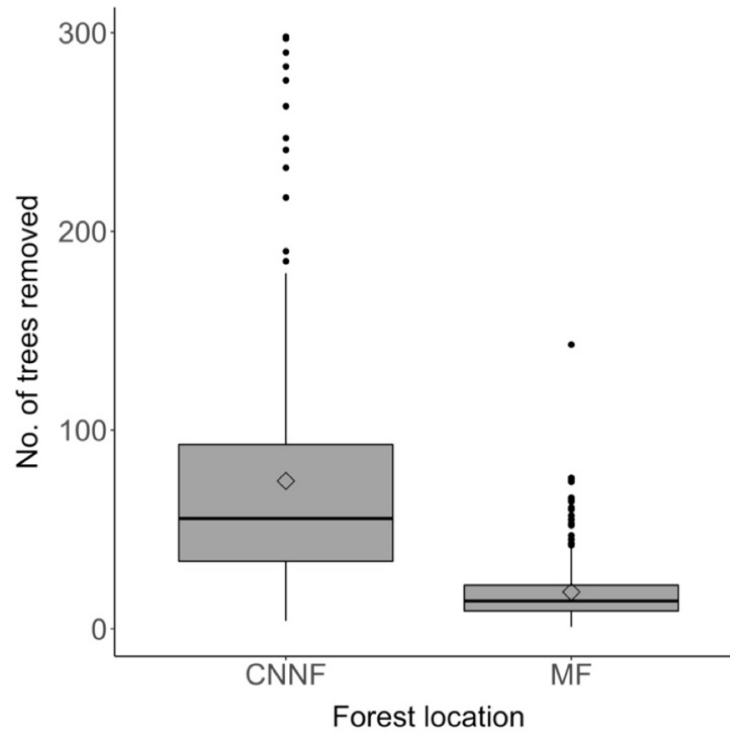


Figure 1-3. Boxplot of the number of trees removed during root rupture treatments on the Chequamegon-Nicolet National Forest (CNNF) and Menominee Forest (MF) for all years of the observational study. The average number of trees removed (open diamonds) for oak wilt treatment was higher in the CNNF than MF. Median number of trees removed is represented by solid horizontal lines and whiskers depict the interquartile range.

Table 1-2. Number and proportion of total infection centers where belowground spread of oak wilt was controlled following one or more root rupture treatments at the Menominee Forest (MF) and Chequamegon – Nicolet National Forest (CNNF) in Wisconsin.

No. of treatments applied	CNNF		MF	
	No. of sites	Proportion of sites	No. of sites	Proportion of sites
One	117	0.629	272	0.928
Two	32	0.172	9	0.031
Three	7	0.038	0	0.000
Four	2	0.011	1	0.003
Cumulative success	-	0.850	-	0.962
Not re-treated ^a	28	0.151	11	0.038

^a Symptomatic trees were identified after the initial treatment, but the infection center was not re-treated during the timeframe of this analysis. Data were not included in the cumulative success calculation.

Table 1-3. Results of generalized linear mixed effects model testing the effect of the number of trees removed and year on the success of the initial root rupture treatment. Forest location (Chequamegon – Nicolet National Forest or Menominee Forest) was treated as a random effect. The reference level for comparison is 2004.

Variable	Estimate	Std. Error	z-value	p-value
Intercept	0.773	0.271	2.846	< 0.001
No. of trees removed	-0.008	0.003	- 3.092	0.002
2005	-0.561	0.499	-1.123	0.261
2006	0.298	0.615	0.485	0.628
2007	0.222	0.697	0.318	0.750
2008	0.661	0.886	0.745	0.456
2009	0.192	0.468	0.410	0.682
2010	1.174	0.479	2.451	0.014
2011	1.996	0.437	4.571	< 0.001
2012	2.621	0.513	5.108	< 0.001
2014	1.967	0.650	3.028	< 0.001

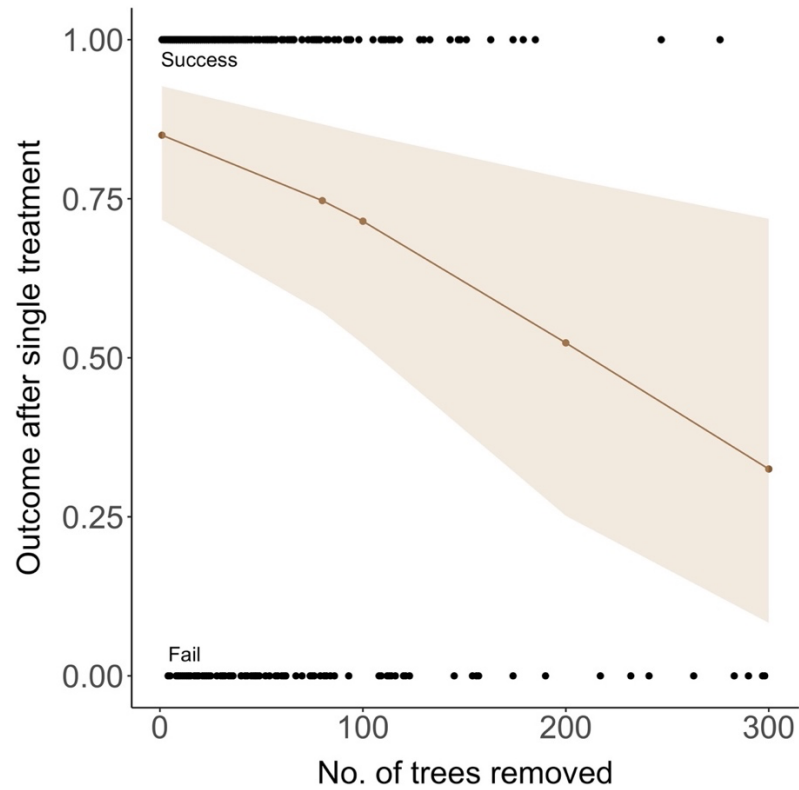


Figure 1-4. Predicted outcome (brown line) of disease treatments following a single root rupture treatment based on infection center size and 95% confidence interval (brown envelope). The predicted outcome is based on generalized liner mixed effects model estimates and are plotted against binary experimental data (black circles; treatment success or failure). Treatment “success” denotes that a new belowground infection did not occur adjacent to the treated area within five years of initial treatment. The number of trees removed was indirectly determined based on perimeter treatment line placed using the Bruhn 95% confidence Grayling soil model (Bruhn, Pickens, and Stanfield 1991; Carlson, Martin, and Scanlon 2010).

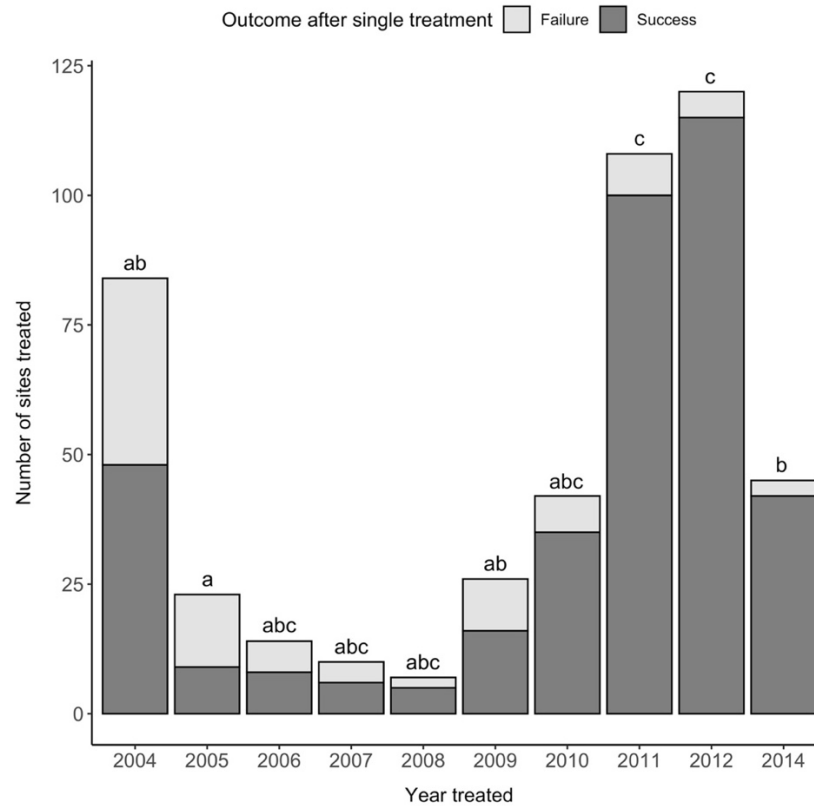


Figure 1-5. Outcomes (success or failure) after a single root rupture treatment on oak wilt infection centers in the Chequamegon – Nicolet National Forest (CNNF) and Menominee Forest (MF) for each year of this observational study. Treatment “success” indicates that a new infection did not occur (or was not detected) adjacent (within 66 ft.) to the treatment area within five years of initial treatment. Letters represent differences ($\alpha = 0.05$) based on the Sidak p -value adjustment to control for multiple comparisons.

The majority of the infection centers in which treatments were not successful developed new symptoms within two years after the initial treatment ($n = 44$ of 77 treatment failures on the CNNF; $n = 12$ of 22 treatment failures on the MF). Of the 77 infection centers on the CNNF for which the root rupture treatment failed, 71 were re-treated and were, on average, similar in size to the originally treated sites ($F_{1,69} = 0.905$, $p = 0.302$). Due to the low number of re-treated infection centers on the MF, we could not compare the size of re-treated infection centers to the size of the original sites. After the application of additional root rupture treatments, the cumulative success of disease control was 85 and 96% for the CNNF and MF, respectively.

Discussion

Strategies to control forest diseases, such as oak wilt, require a tailored approach that carefully balances landscape constraints, land manager needs, and economic costs. In this study, we found that stump extraction (i.e., “root rupture” treatment) resulted in a high level of oak wilt control in infection centers across the Chequamegon-Nicolet National Forest and Menominee Forest, particularly if repeated treatments were applied. In addition to demonstrating success of the root rupture treatment, this study revealed the complex considerations managers face when approaching disease treatment and the implications of historic stand management on present-day disease incidence and disease management outcomes.

Differences in Treatment Success Between Forests

Across the CNNF and MF, belowground spread of oak wilt was contained in the majority of infection centers (>62%) with one treatment, yet treatment outcomes were different between the two forests. In general, infection centers on the CNNF were larger than those on the MF, contributing to a lower likelihood of treatment success based on our model estimates. Despite the proximity and large red oak component of the two forests, management over the last circa 180 years varies substantially between the two forests, which now differ in terms of species and age class diversity. The land on which the CNNF is located was subjected to exploitative, large-scale lumbering in the mid-1800’s after the Menominee Tribe were forced to cede much of present-day central Wisconsin (Elliot 1977; Stearns 1997). After the U.S. Forest Service established the CNNF in 1933, management shifted to focus on high-quality northern red oak, thus many present-day stands are dominated by large, even-aged red oak. In contrast, stands on the MF were, and continue to be, managed by the Menominee Tribe and Menominee Tribal Enterprises with sustained-yield silvicultural prescriptions that simultaneously maximize diversity (e.g., species composition, age class distribution, structural diversity) and

cultural benefits (Pecore 1992; Menominee Tribal Enterprises 2012). The belowground spread of *B. fagacearum* is, in part, related to the density and size of red oak species on a stand (Menges and Loucks 1984). Thus, the lack of species diversity and uniformly large size-class of red oak on the CNNF increases the likelihood of root grafting and the rate of disease spread.

Differences in oak wilt treatment outcomes between the CNNF and MF may also be attributed to differences in disease detection methods between the two land managers. In general, annual oak wilt monitoring had two components: ground surveys of areas adjacent to previously treated sites and a broad survey for new infections that resulted from aboveground insect spread. New infections from aboveground spread are especially challenging to detect as they can occur up to 0.6 miles, or occasionally further, from an infected tree (Menges and Loucks 1984, Juzwik et al. 1985, Shelstad et al. 1991). As a result, new infection centers can remain unnoticed for several years until additional trees become infected and die. The assistance of UAVs in annual surveys likely allowed MTE foresters to identify and treat small infection centers, thus increasing the opportunity of successful treatment.

Understanding Why Treatments Fail

There are several reasons why treatments may fail (or appear to fail) that arise from 1) difficulty recognizing diseased trees and 2) wrongly identifying the source of new infections. Theoretically, treatment area boundaries calculated using the statistical model should include all grafted trees with less than 95% probability of survival for at least one year (Bruhn, Pickens, and Stanfield 1991). However, only 63% and 93% of treated infection centers (CNNF and MF, respectively) showed no evidence of further belowground spread after one treatment. One of the largest challenges in accurately identifying oak wilt is that *B. fagacearum* can infect root systems for up to three years before the foliar symptoms are evident (Rexrode 1978). Consequently, trees infected with the oak wilt fungus that are not yet displaying symptoms may not be accounted for when

calculating where to place the treatment boundary. Similarly, the survival of *B. fagacearum* in root systems after tree death is not well understood, thus older oak wilt-killed trees may be overlooked when placing treatment boundaries (Yount 1955; Skelly and Wood 1974). Other biotic and abiotic stressors (e.g., two-lined chestnut borer damage, drought) are known to cause symptoms that appear similar to that of oak wilt and can occur simultaneously with the disease (O'Brien et al. 2011). If symptoms on diseased trees are not visible or are mistaken for other disorders, then the resulting treatment boundary will not account for the true extent of the infection center. In this way, such treatment “failures” result from a failure to accurately diagnose oak wilt rather than a failure of the root rupture treatment itself.

Despite best efforts of forest health professionals to differentiate between new overland introduction by Nitidulid beetles and root graft spread, field technicians may have trouble determining whether a new symptomatic tree is truly the result of a treatment failure. Incorrectly attributing a new wilted tree outside of the treatment boundary to root graft spread would inflate the proportion of treatment failures, particularly in years with damaging spring storms that lead to spikes in the frequency of overland infection (Juzwik and Schmidt 2000; Ostile 2020). Success of oak wilt containment efforts on the CNNF and MF lands increased over time for the evaluated infection centers, which likely reflects an improvement in oak wilt identification by managers and field crews.

Our study revealed that the disease was more likely to be controlled in infection centers treated during the later years (2010 through 2014) of the study compared to early years (2005 through 2009). Oak wilt was identified on the CNNF nearly ten years before the conception of the root rupture control program, thus forest health managers faced many large disease infection centers that had remained unmanaged for several years. Though difficult to quantify, it is reasonable to suspect that large infection centers also presented greater opportunity for human error when marking treatment boundaries compared to small infection centers. Minor errors in delineating treatment boundaries may have failed to encompass the full extent of the belowground infection. It is likely

that the accuracy of oak wilt identification and root rupture treatments improved as control programs matured, resulting in smaller infection centers and fewer treatment failures in later years.

Practicality and Cost Considerations for Forest Managers

Uprooting stumps for disease control creates a high visual impact to the landscape and substantial soil disturbance. Typical root rupture treatments resemble a large canopy gap or a small group selection with root- and soil-exhumed mounds. Characteristics of these treatments resemble small-scale wind events and may be aligned with natural disturbance-based management, creating an unexpected silver lining of oak wilt management in this region (Seymour, White, and deMaynadier 2002; Kern et al. 2019). Anecdotally, managers on the CNNF and the MF have observed diverse advanced and seed-origin regeneration including red oak, paper birch, and white pine in root rupture-treated sites (Figure 1-6). MTE foresters have also used the treated infection centers as an opportunity to plant climate-adapted tree species to diversify the forest and reduce the risk imposed by future climate conditions (Janowiak et al. 2014).



Figure 1-6. Seed-origin and advanced regeneration in a treated infection center on the Menominee Forest (MF) and Chequamegon-Nicolet National Forest (CNNF). Top) Regeneration two years after root rupture treatment on MF. Bottom) Regeneration 14 years after root rupture treatment on the CNNF.

Treatment cost is an important factor to consider when deciding how to best manage diseases on the landscape. Estimating the cost of root rupture treatments is complex for several reasons. First, land managers may either choose to contract with a logging company to harvest the trees and pull the stumps or the procedure can be done in-house, incurring little cost beyond the hourly rate to rent an excavator. Second, in regions with high quality red oak, the cost of implementing treatments can be greatly offset by profits made from wood utilization. In the case of the CNNF, trees removed were packaged into Stewardship Contracts, a USDA Forest Service legislative authority that enables timber managers to bundle timber sale contracts within service contract work. In this arrangement, the CNNF offers high value timber with a requirement that the contractor uproot the stumps after harvest, thus infection centers on the CNNF were usually treated with an overall financial net gain. MTE also has a unique advantage in that they can harvest trees and implement the treatment with both their own loggers and equipment. Further, the harvested wood can be processed internally at a mill owned by the Menominee Indian Tribe of Wisconsin, where the lumber can either be used or sold.

Comparison to Established Oak Wilt Treatments

There are limited reports on the efficacy of belowground oak wilt containment efforts on comparable terrain. In this study, individual root rupture treatments resulted in a lower rate of disease control than published rates of the “monitor and remove” strategy (84% of evaluated disease centers) on similar soil types and slopes in Minnesota (Juzwik et al. 2010). However, the infection centers in the vibratory plow evaluation were, on average, smaller (based on the theoretical number of trees removed) than the infection centers in this study. Reported success rates of other oak wilt control methods are varied. For example, evaluations from the 1970’s in Pennsylvania and West Virginia describe >84% containment over four years after felling oaks and treating the stumps with herbicides, while a recent trial testing the use of double frill girdle and herbicide applications found that the disease was only contained 55% of treated infection centers

(Jones 1971; Bronson et al. 2023). To our knowledge, this is the first oak wilt control evaluation to relate the size of infection centers to treatment outcomes. This observational study provides valuable insight on the relationship between infection center size and treatment outcome; however, our analysis would be strengthened by including basal area or density of infected and healthy red oak trees removed rather than using number of trees removed as a proxy for infection center size. While such data were not available for this study, future evaluations of belowground disease control should consider reporting basal area of diseased oaks and of oaks removed to allow for more direct comparisons between methods.

As the range of oak wilt continues to slowly expand, there is a pressing need to expand upon existing disease control strategies by developing creative, effective alternative management tools. The root rupture method achieved a high degree of disease control with the potential of encouraging diverse regeneration that is less susceptible to widespread disease outbreak. Our evaluation of operational treatments indicates that the likelihood of treatment success decreases as infection centers increase in size. Thus, the best approach to managing oak wilt may be a proactive management program aimed at early disease detection followed by prompt treatment.

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Supplementary Materials

Supplemental Table 1-1. Estimated marginal means of a single root rupture treatment each year based on a generalized linear mixed effects model. Forest location (Chequamegon – Nicolet National Forest or Menominee Forest) was treated as a random effect. Lower and upper confidence limits (LCL and UCL) represent the lower and upper boundaries of a 95% confidence interval. Means are given on the logit scale without back-transformation.

Year	Estimated Marginal Mean	Std. Error	LCL	UCL
2004	0.446	0.230	-0.004	0.897
2005	-0.114	0.452	-1.001	0.772
2006	0.745	0.582	-0.396	1.886
2007	0.668	0.664	-0.633	1.970
2008	1.107	0.860	-0.578	2.792
2009	0.639	0.414	-0.172	1.449
2010	1.621	0.422	0.794	2.447
2011	2.443	0.369	1.719	3.167
2012	3.068	0.458	2.170	3.966
2014	2.413	0.602	1.233	3.593

Chapter 2 :

**Survival of *Bretziella fagacearum* over time in roots of diseased *Quercus ellipsoidalis*
trees in Minnesota, USA**

Abstract

Oaks (genus *Quercus*), considered some of the most culturally, ecologically, and economically valued trees in North America, are experiencing widespread declines across their range due to many factors including disease. Oak wilt, caused by *Bretziella fagacearum*, is a destructive disease of oaks that contributes to their decline in the United States. Despite decades of implementing methods to control the belowground spread of the disease, it sometimes re-emerges in sites that were previously treated. One underlying reason for failed oak wilt treatments arises from an incomplete understanding of *B. fagacearum* survival over time in root systems of oak wilt-killed trees. To better understand this phenomenon, we characterized *B. fagacearum* presence and viability, as well as root viability in oak trees that were: 1) killed by oak wilt between 2018 and 2021 (n = 11 trees), 2) displaying active oak wilt crown symptoms (n = 8 trees), or 3) herbicide-treated stumps of healthy red oaks within grafting distance of diseased oaks (n = 7 trees). We found that *B. fagacearum* viability diminished quickly in the years after complete crown wilt, but that some viable pathogen remained (2 out of 44 roots assayed) five years after crown wilt. We also found that *B. fagacearum* viability was positively correlated with root viability, with roots remaining viable for at least five years after tree wilt. From herbicide-treated stumps, 38.8% of root sections remained viable four years after treatment. We conclude that roots of diseased oaks may harbor viable *B. fagacearum* for longer periods of time than previously reported, which may put stands at continued risk for belowground disease spread.

Introduction

Oaks (genus *Quercus*) are one of the most abundant and diverse group of trees in North American forests (Cavender-Bares, 2019). Oak forest types constitute over 50% of forest land in the U.S, yet they are declining in eastern U.S. forests due to changes in historic disturbance regimes that maintain oaks, pests, and pathogens (Dey 2014). Oak wilt is widely considered the most important disease of oaks in the eastern U. S. and Texas (Appel 1995; Juzwik et al. 2011). The causal fungus, *Bretziella fagacearum*, induces a systemic vascular wilt in most, if not all, oak species and is fatal to species of the red oak group (*Quercus* section *Lobatae*; Bretz 1955). Red oak species often die within weeks to months after the first appearance visual of symptoms, while species within the white oak lineage (*Quercus* section *Quercus*) exhibit varying degrees of disease resistance (O'Brien et al. 2011). The pathogen can spread from diseased to healthy trees aboveground, carried by insect vectors (predominantly sap feeding beetles of the *Nitidulidae* family), and belowground through root grafts (Gibbs and French 1980; Juzwik et al. 1985). While aboveground spread of *B. fagacearum* is critical to initiation of new disease centers, belowground spread is thought to be responsible for most new oak wilt infections in the Upper Midwest U.S. (Juzwik et al. 2011). Altered disturbance regimes in this region, stemming from exploitative timber harvesting and European settlement, gave rise to dense stands of trees within the red oak group with wide networks of interconnected root systems that allow for rapid disease spread (Bruhn et al. 1991; Menges and Loucks 1984). As a result, many federal, state, and private oak wilt control efforts focus on preventing belowground spread of *B. fagacearum*.

Control of belowground spread of *B. fagacearum* involves severing or killing grafted root systems between healthy and infected oaks, thereby establishing a containment zone around disease centers. Containment zone boundaries are positioned such that they surround healthy-appearing trees that may be infected but are not yet displaying symptoms (Bruhn et al. 1991; French and Steinstra 1978). There are various models for determining the placement of containment zone boundaries; the most common approaches are based on statistical predictions of how far *B. fagacearum* moves in one year and on practitioner experience (Bruhn et al. 1991; French and Steinstra 1978). After determining where to place containment zone boundaries, managers select the most appropriate option for disrupting root systems within the marked line. Methods for disrupting root systems include cutting roots with a vibratory cable plow, uprooting stumps, digging a trench, or applying herbicides to stems or stumps to kill root tissues (Bronson et al. 2023; Bruhn et al. 2002; French and Steinstra 1978; Jones 1971; Wilson and Lester 2002; Yang et al. 2024). The likelihood of disease containment is improved when spore-producing red oak trees are removed within the containment zone area, thereby reducing the potential for aboveground spread of *B. fagacearum* (Juzwik et al. 2011; Wilson 2005). Depending on land manager goals and resources, managers either remove all red oak species in the containment zone (“cut to the line”) or annually remove oak wilt-killed trees (“monitor and remove”) (Juzwik et al. 2010).

Herbicide treatments are a cost-effective method of controlling oak wilt infection centers; however, results from evaluations of their use in killing oak roots and preventing *B. fagacearum* transmission are mixed (Bronson et al. 2023; Bruhn et al. 2002;

Jones 1971). The application of herbicides to kill root tissues in infection centers is done in at least two ways: 1) felling all red oak species within the containment zone and immediately applying herbicide to the cut stump surfaces (Bruhn and Heyd 1992; Jones 1971), or 2) creating stem-encircling cuts (frill-girdle) to standing red oaks and applying herbicides to the cuts (Bronson et al. 2023). Whether applied to stump surface or to cuts into the trunk, herbicides are generally presumed to be translocated through the symplast (via transpiration) and apoplast (via phloem source-sink dynamics) with the intended goal to kill the root collar tissue and distal roots beyond the collar (Stein 1998). Assessments of pathogen survival in herbicide-treated oak roots and stumps is limited to trials in Pennsylvania in the 1950's, where the authors isolated viable *B. fagacearum* up to three years after herbicide (ammate) treatment of cut stumps (Yount 1955). A recent evaluation of frill-girdle herbicide (trichlopyr and trichlopyr mixed with imazapyr) treatments demonstrated that herbicides can be used to limit belowground spread of the pathogen, but significant questions remain regarding longevity of the pathogen in herbicide-treated trees and roots, the distribution of herbicide in the treated root systems, and the amount of time needed to achieve complete root mortality (Bronson et al. 2023). Studies in Texas and West Virginia evaluated pathogen survival in aboveground portions of girdled trees without herbicide application, but data were based on the incidence of spore mat formation and did not extend to belowground tissues (Gillespie et al. 1957; Greene et al. 2008). Altogether, evidence suggests that herbicides have the potential to be an important tool for oak wilt management, but gaps in research currently limit their practicality in operational settings.

Despite decades of management, oak wilt continues to re-appear in stands where management previously occurred. While persistence of the disease on the landscape can occur from aboveground spread, belowground treatment failures are commonly attributed to 1) inaccurately establishing containment zone boundaries around infection centers, and 2) disease re-emergence due to pathogen survival in infection centers (Bronson et al. 2023; Juzwik et al. 2010; Yang et al. 2024). Inaccurate placement of the outer boundary of the containment area occurs when managers fail to identify the full extent of the disease in a stand. This type of error can result from overlooking stumps of older, oak-wilt killed trees when determining where to place the boundaries of containment zones or from shortcomings of the line placement models themselves. Models for barrier line placement are derived from observations of aboveground symptom development in infection centers (Bruhn et al. 1991; French and Steinstra 1978; Juzwik et al. 2010). Such models have been used for decades with a high level of success (> 80% disease containment for four to six years) in containing belowground spread of oak wilt (Bruhn and Heyd 1992; Juzwik et al. 2010), yet they do not predict the location of *B. fagacearum* in roots nor do they account for the potential for the pathogen to be present in root systems of trees with no foliar symptoms. Rather, the statistical model is based on predictions of which trees are likely to die within one year (Bruhn et al. 1991). Re-emergence of the disease outside of containment zone boundaries several years (> 4-5 years) after treatment is particularly perplexing and may be attributed to several factors such as incomplete separation of root grafts (either mechanical separation or functional

separation of the xylem from root tissue death), survival of residual root systems within containment zones, and formation of new root grafts across containment zone boundaries.

The lack of tylose development in red oak roots may explain, in part, why halting root graft transmission remains a challenge for forest health managers. Extensive tylose formation, or balloon-like defense structures that obstruct the xylem, in secondary xylem vessels of stems and branches of infected trees is induced in response to *B. fagacearum* infection as a means to restrict pathogen movement through the xylem (Sachs et al. 1970; Struckmeyer et al. 1954, 1958). Very few studies have characterized tylose formation in roots of hardwood species and observations of *B. fagacearum* in root systems of red oak species suggest that tylose formation is absent or uncommon in roots during wilt and after tree death (Struckmeyer et al. 1954; Yelenosky and Fergus 1959). The spatial pattern of tylose production in stems is related to pathogen infection and presence, resulting in clustered regions of the xylem that have restricted xylem function and regions that could continue to allow movement of fungal spores (Sapes et al. 2024). Without a host response that limits pathogen movement in roots of wilting trees, the pathogen may potentially persist in roots and even move through the xylem of grafted roots after tree death. Beyond that, there is speculation that roots from diseased trees may survive for several years after tree death via an influx of water and nutrients from root grafts with healthy trees (Yount 1955) or from stumps with sprouts. When exploring the occurrence of root grafts in West Virginia, Rexrode (1978) injected dye mixed with *B. fagacearum* spores into healthy trees to identify nearby trees with connected root systems and monitor disease development. Some grafted trees did not develop symptoms until three years after

death of the initial tree, suggesting that pathogen movement through grafted roots may continue for several years after tree death and/or that the pathogen can survive in root systems of asymptomatic trees for several years before development of aboveground symptoms. Root inoculations of *B. fagacearum* have also resulted in delayed symptom development compared to stem inoculations, with some trees exhibiting delays in symptom development for a year or more after artificial inoculation (Skelly and Wood 1974; A. Yang personal observation). Models for placement of root graft barrier lines intrinsically account for some of the uncertainty created by asymptomatic trees; however, the lack of knowledge on how long roots remain a suitable substrate for *B. fagacearum* after a tree dies or is cut down is a constraint to accurately establishing containment zone boundaries around disease centers. Failure to contain oak wilt infection centers comes at great financial cost to land managers; moreover, it puts regenerating oak trees at risk for future infection and mortality.

Characterization of *B. fagacearum* survival in root systems would likely improve barrier line placement and increase the likelihood of success in stopping belowground spread in oak wilt-affected stands. Failures to prevent disease re-emergence outside of containment areas puts remaining healthy oak trees and saplings at risk and comes at great financial cost to managers. Therefore, we need to understand how long *B. fagacearum* persists in root systems following tree death and the extent to which herbicide treatments impact root and pathogen survival. The objectives of this study were to 1) characterize the duration of root tissue survival in oak-wilt killed trees and in roots of herbicide treated stumps of felled, apparently healthy trees within root-grafting

distance of an infected tree, and 2) assess the presence and viability of *B. fagacearum* in the roots of oak-wilt killed trees compared to those of actively wilting trees and herbicide-treated stumps. Results from this study may lead to refinement of current oak wilt management recommendations by improving placement of root graft barrier lines and implementation of disease treatments.

Methods

Site Characteristics and Sample Collection

Our study site was located within a 163-ha conservation easement in Orrock, Minnesota, USA with ongoing prairie restoration and oak wilt management. The site is situated within the Anoka Sand Plain region in Central Minnesota, which was once characterized by large stretches of prairie and oak savanna that were maintained by large ungulate grazing, Indigenous fire management, and drought-prone soils (Dey et al. 2017). European colonization forced the removal of Indigenous peoples, transforming much of the land to agricultural fields. In the absence of fire stewardship, oak savannas shifted into dense woodlands dominated by *Q. ellipsoidalis* and *Q. rubra*, which are both in the red oak group. The rate of oak wilt spread is, in part, related to the density of susceptible species in a stand (Menges and Loucks, 1984), thus many oak woodlands on the Anoka Sand Plain currently have extensive pockets of disease mortality.

We collected root samples in early September 2022 and early October 2023 to characterize root and pathogen survival in oak root systems. Roots were collected from

trees that were 1) killed by oak wilt between 2018 and 2021, 2) displayed active oak wilt crown symptoms in July 2022 and 2023, or 3) herbicide-treated (25% triclopyr [Garlon 4A] and 75% basal oil) surface of a red oak stump whose crown appeared healthy at time of felling in 2019 (Table 2-1). In 2022, we excavated root systems using an air-vacuum excavation truck (Vacmasters System 1000, Arvada, CO, USA), which combines high pressure air with a vacuum to remove the loose soil. Commonly used to carefully expose underground utilities, this specialized equipment loosens soil with a high level of precision while a vacuum lifts the loosened soil from the excavated area into a holding tank. This method allowed us to select and slowly excavate individual roots radiating from the root collar. In 2023, classic excavation equipment was used rather than an air-vacuum excavator. Specifically, root systems were cut approximately two meters from the root collar and the root ball mass extracted from the soil. Several (4 to 13) roots (52.4 to 72.5 cm in length) were cut from the exposed root system, placed in plastic bags, and stored in a walk-in cold room (3.3 °C) until further processing occurred.

Table 2-1. Size and characteristics of *Quercus ellipsoidalis* trees and roots sampled in Orrock, MN, USA. We identified diseased *Q. ellipsoidalis* trees based on appearance of leaf bronzing from the margin to midrib, downward progression of symptoms in the crown, and premature leaf abscission. Roots were harvested from trees and cut into root sections (20 to 30-cm long). Root sections were assayed for viable *Bretziella fagacearum*, presence of *B. fagacearum* DNA, and root vitality.

Source	Tree Condition	Years Since Wilt or Felling	No. Sampled ^b	Mean Basal Dia. (cm) ^c	No. of Roots Harvested	Root Sections	
						No. Assayed	Mean Dia. (cm)
Diseased	Active Wilt	0	8	42.5	11	109	4.4
Oak	Dead	2	3	46.9	13	73	4.8
	Dead	3	3	38.7	5	16	10.5
	Dead	4	1	39.4	11	16	4.5
	Dead	5	4	51.1	9	44	7.0
	Dead	5	4	51.1	9	44	7.0
Healthy +	Felled	3	2	40.2	4	15	9.2
Herbicide	Felled	4	5	28.9	10	67	5.3

^a The cut surface of healthy-appearing *Q. ellipsoidalis* trees within root grafting distance of disease trees was treated with herbicide (triclopyr) shortly after tree felling.

^b Number of trees or stumps sampled.

^c Basal diameter was measured on each tree or stump 20 to 30 cm above the soil line.

Vitality Staining

From each of the roots, we cut 20-cm sections to assess viability of root tissue and test for presence and viability of *B. fagacearum*. We assessed vascular cambium vitality of each root section using the vital stain 2, 3, 5-triphenyltetrazolium chloride (TTC), a common redox indicator that differentiates between metabolically active and inactive tissues (Bruhn et al. 2002; Joslin and Henderson 1984; Ruf and Brunner 2003). We removed a small piece of vascular cambium (1.5 long x 1.5 wide x 0.5 cm deep) from each root section using a sterile chisel and covered it in a 1% aqueous solution of TTC (1% TTC dissolved in 0.1M potassium phosphate buffer [pH 7.0] and 0.05% Tween 20) in a glass screw-cap vial. The submerged wood pieces were incubated in the dark for up

to four days at ambient room temperature (20 to 22 °C), but samples were generally read within 24 hours. The cambium of living samples turned fuchsia, while the cambium of dead root samples remained unchanged in color. Roots were assigned a categorical vitality status (0, no stain; 1, faint stain; 2, bright stain; Fig. 2-1) by the same individual to maintain consistency among the samples. Roots sampled in 2022 were not included in the vitality assay.

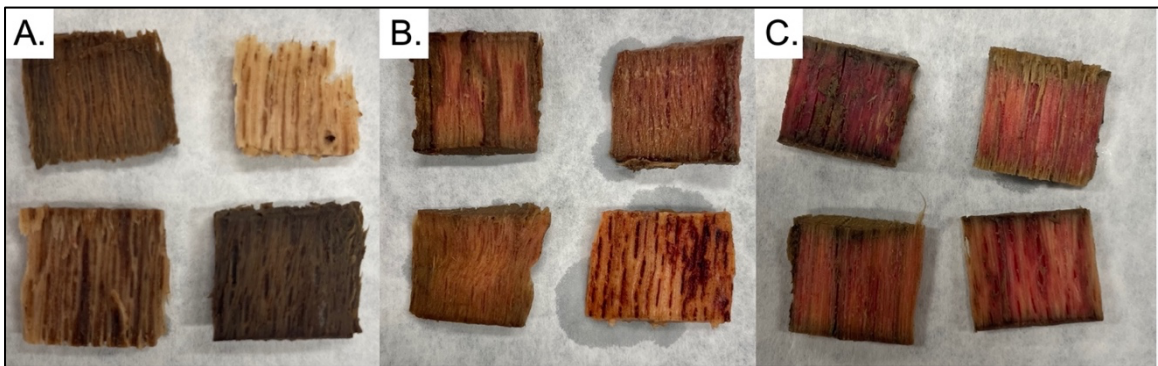


Figure 2-1. Vitality status of root sections sampled from *Quercus ellipsoidalis* trees was based on the development and visual intensity of the fuchsia stain that developed after submerging small pieces of root sections in vital stain 2,3,5-triphenyltetrazolium chloride (TTC). Vitality categories were as follows: A) 0, no stain, B) 1, faint stain, and C) 2, bright stain.

Pathogen Assays

To assay the root sections for living *B. fagacearum*, we carefully removed the outermost layer of the epidermis and cortex with a sterile draw knife and inspected the vascular cambium for blue-grey discoloration that is typical of *B. fagacearum* colonization. Using a sterile wood gouge, we flame-sterilized the root surface, excised small wood chips from four locations on each root section, and placed them onto petri dishes containing a semi-selective oak wilt growth medium (Barnett 1953). We

monitored the plates for *B. fagacearum* growth for up to 21 days and identified *B. fagacearum* based on colony color, microscopic features including the presence of endoconidia and characteristic wavy hyphal growth, and characteristic fruity odor. We considered a root section to be positive for *B. fagacearum* if at least one of the four wood chips yielded the fungus. To assay for pathogen DNA, we collected drill shavings from undisturbed locations on the same root sections following the protocol of Yang and Juzwik (2017). Briefly, we used a drill bit (0.32 cm diameter) to drill a shallow hole (approx. 0.5 cm deep) into the root and collected the wood shavings that were produced by the drill bit. The wood shavings were funneled into a 2.0 mL microcentrifuge tube, covered with cetyltrimethylammonium bromide (CTAB) lysis buffer, and stored at -20 °C. We extracted DNA from the wood shavings, amplified the DNA with nested polymerase chain reaction (PCR), and sequenced the PCR product following the methods described in Yang and Juzwik (2017).

Statistical Analysis

Individual root sections were the experimental unit for all analyses. We collapsed the stain categories into two groups for analysis, with 0 representing root samples that did not yield any color (dead roots) and 1 representing root samples that yielded a faint or bright stain (living roots). We used a Chi-square test for independence to test for relationships between tree type and vitality stain outcome, with the null hypothesis that tree type was independent of vitality stain outcome. We calculated post-hoc pairwise tests

for significance with a Bonferroni adjustment using the *chisq.theo.multcomp* function in the RVAideMemoire package in R (Herve 2023).

To understand root survival over time, we analyzed root vitality in root sections sampled from oak wilt-killed trees using generalized linear mixed effects models with a binomial distribution. Fixed effects included years since initial wilt, position relative to root collar, root section diameter, and tree basal diameter (20 to 30 cm above soil level). Random effects were included for individual trees where multiple roots were sampled. We determined the most important variables for predicting root survival through a backwards elimination process. First, we constructed a full model with all possible fixed effects and then created sequentially smaller models by removing variables with the highest *p*-values until all variables in the model had a *p*-value of < 0.10. Each of the models retained the same random effect structure. We then compared models using Akaike's Information Criterion (AIC). We selected the best model based on the lowest AIC and with the fewest fixed effects. We ran the models using the lme4 package in R with a binomial distribution and a Type II analysis of variance (Bates et al. 2015; R Core Team 2022). We checked assumptions of normality and homoscedasticity using the *simulateResiduals* function in the DHARMA package (Hartig 2022). We also checked the models for zero inflation using the *testZeroInflation* function in the same package.

To evaluate pathogen viability and presence, we calculated the proportion of root sections assayed where at least one of the four sapwood chips was positive for *B. fagacearum* and the proportion of root sections that yielded pathogen DNA. To understand the effect of time on the likelihood of pathogen persistence in the oak wilt-

killed trees, we analyzed pathogen viability (i.e., culture result) as a function of time since initial wilt using a generalized linear mixed effects model with the following form:

$$\text{Logit } P(Y_{ijk}) = \mu + T_i + \alpha_j + \beta_{k(j)}$$

where Y_{ijk} is the assay result (positive or negative; 0 or 1) of a root sample follows a Bernoulli distribution with P_{ijk} . In the model, μ is the overall mean, T is the years since wilt, α is the error associated with tree, and β is the error associated with root. We initially tested the same model with an added fixed effect for stain category (0, 1, or 2) to explore the relationship between root survival and pathogen viability. However, years since wilt and stain category were highly correlated ($\chi^2 = 54.1$, $df = 6$, $p < 0.001$), therefore we adjusted the model so that stain category was the only fixed effect and added a random effect for years since wilt. We examined model residuals for normality, homoscedacity, and zero inflation in the same manner described above.

Results

Root Survival in Diseased Trees and Herbicide-Treated Stumps

The source of root sections assayed (active wilt, dead, felled + herbicide) was significantly associated with vitality stain outcome ($\chi^2 = 35.5$, $df = 2$, $p < 0.001$), with roots from actively wilting trees yielding the highest frequency of live root sections from each tree (Fig. 2-2). In healthy herbicide-treated stumps, 38.8% of the root sections were living; however, only 6% (four samples) yielded a bright fuchsia stain (Fig. 2-3). Based on analyses of root vitality data for diseased trees only, the best fit model included years

since initial wilt and position of assayed root section from root collar, which were both significant ($p > 0.05$; Table 2-2). Root vitality was found to decrease over time for these trees and was highest in root sections that were closest to the root collar (Fig. 2-4).

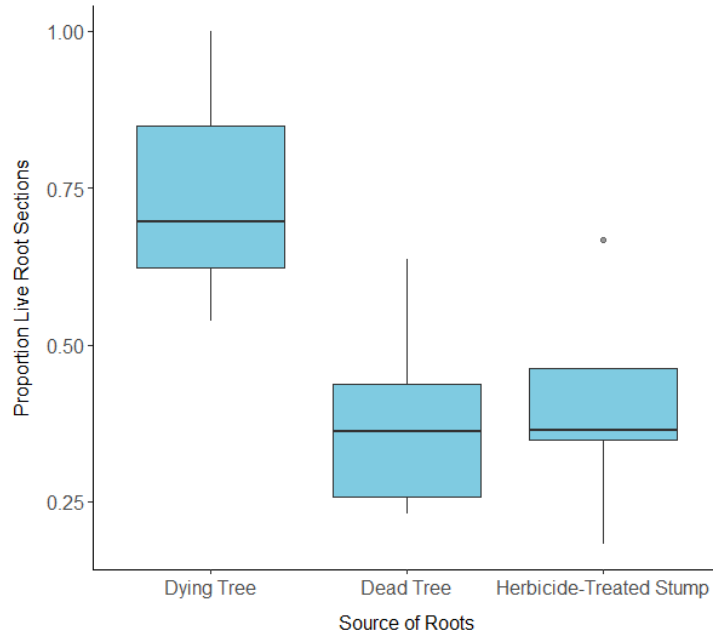


Figure 2-2. Proportion of live root sections sampled from *Quercus ellipsoidalis* with active crown wilt (dying tree) or killed by oak wilt (dead tree) and from herbicide treated stumps of felled, healthy appearing oaks. Proportion living is based on binary 2,3,5-triphenyltetrazolium chloride (TTC) vitality stain outcome.

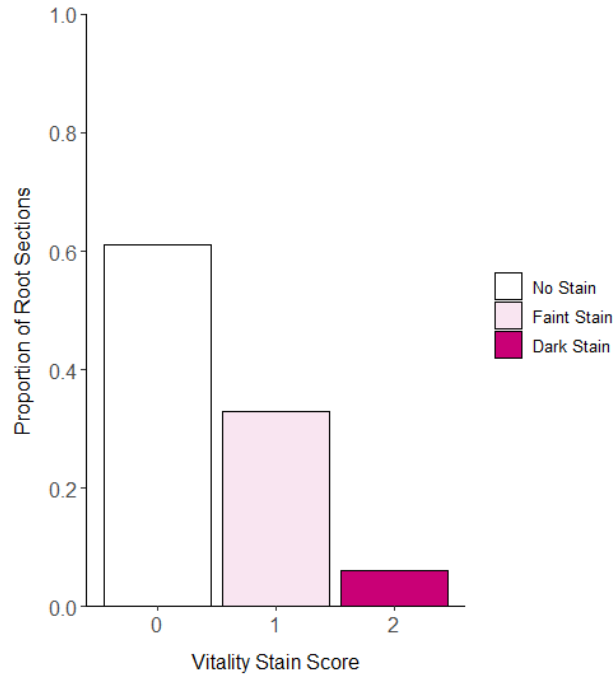


Figure 2-3. Proportion of live root sections obtained from herbicide-treated stumps of *Quercus ellipsoidalis* trees four years after application. Results based 2,3,5-triphenyltetrazolium chloride (TTC) vitality stain assay of 67 root sections sampled from ten roots.

Table 2-2. Comparison of Akaike Information Criteria (AIC) of generalized linear mixed effects fit to root vitality data for root sections sampled from actively wilting and dead *Quercus ellipsoidalis* trees and stumps. We constructed the models using backwards model selection method and used AICc to correct for a small sample size.

Model	Fixed Effects				Degrees of Freedom	AICc	Log Likelihood	$\Delta AICc$
	Years Since Wilt	Position from Root Collar ^a	Root Section Diameter	Tree Basal Diameter ^c				
1	x	x	x	x	7	293.6	-139.5	0
2	x	x	x		6	291.7	-139.7	1.9
3	x	x			5	291.2	-140.5	2.4

^a Proximity of root section relative to the root collar.

^b Main stem diameter 20 – 30 cm above the root collar.

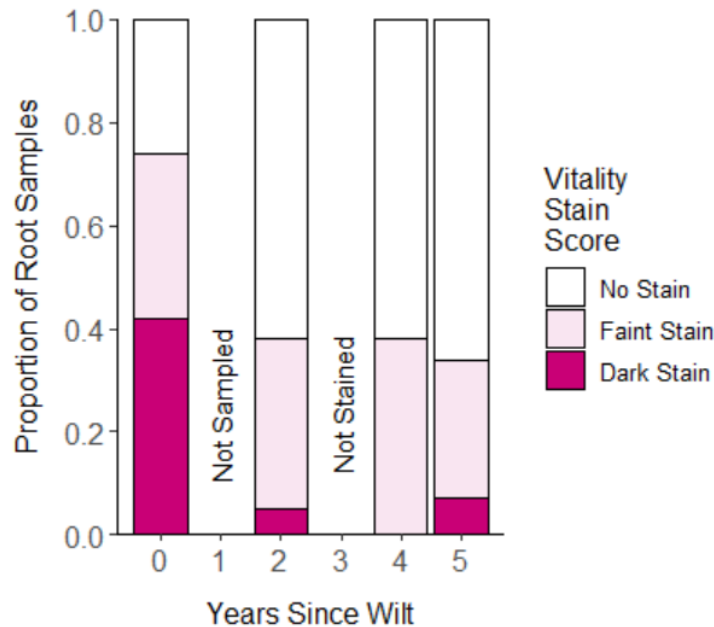


Figure 2-4. Proportion of live root sections ($n = 230$) based on 2,3,5-triphenyltetrazolium chloride (TTC) vitality stain assay where fuchsia stain indicates living root tissue. Roots were obtained from *Quercus ellipsoidalis* trees that either had active crown wilt symptoms or were killed by oak wilt. Root sections that were collected in 2022 were not assayed.

*Persistence of *B. fagacearum* in Roots*

The likelihood of isolating *B. fagacearum* was highest in roots of actively wilting trees (68% of root sections; $n = 109$) and decreased as time from wilt (up to five years) progressed ($p < 0.001$; Fig. 2-5). Based on model results, the odds of isolating *B. fagacearum* decreased by 41% each year after tree wilt (Table 2-3). Root vitality (i.e., stain category) was a marginally significant ($p = 0.06$) predictor of pathogen viability. The pathogen was not isolated from any of the roots sampled from healthy trees treated with herbicide. We detected the pathogen DNA in at least 35% of root sections from all trees with active wilt symptoms. For root sections sampled from dead trees, we detected pathogen DNA in 13% of root sections sampled from roots of 9 of the 11 trees.

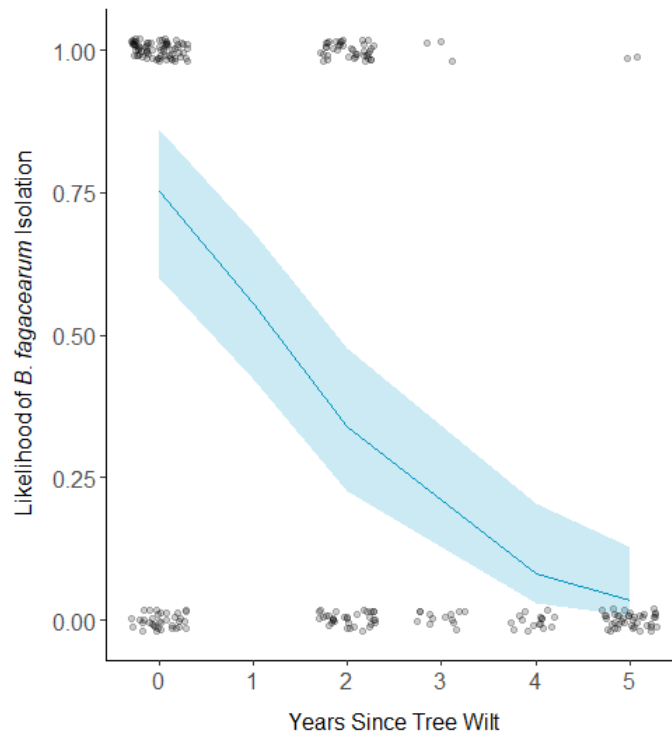


Figure 2-5. Predicted likelihood of *Quercus ellipsoidalis* root sections yielding *B. fagacearum* based on fungal isolation assay (blue line) and 95% confidence interval (blue envelope) based on generalized linear mixed effects model estimates. Predicted likelihood is based on generalized linear mixed effects model estimates and are plotted against experimental data (grey circles; jittered for clarity).

Table 2-3. Coefficients of the generalized linear mixed effects model fitted to the *Bretziella fagacearum* isolation data for sections sampled from roots of actively wilting and dead *Quercus ellipsoidalis* trees and stumps.

Variable	Coefficient Estimate	Std. Error	z-value	P-value
Intercept	1.11	0.36	3.09	0.002
Years Since Wilt	-0.89	0.18	-4.90	<0.001

Discussion

Oak wilt contributes to substantial oak mortality in the Upper Midwest and management costs have been predicted to exceed \$18 million over the course of a decade (Haight et al. 2011). Belowground spread, which is considered responsible for most new infections each year, is a major focus of many oak wilt management programs. In this study, we sought to obtain information that may improve existing methods of controlling belowground spread of the pathogen. Results of this study provide documented persistence of *B. fagacearum* in red oak roots over time, longevity of live root tissue after tree wilt, and the effect of herbicide stump treatment following tree felling on root tissue survival over time in an east central Minnesota landscape. We found that *B. fagacearum* viability quickly diminished in the years after complete crown wilt, but that some pathogen remained viable in root systems for several subsequent years. For the maximum time period evaluated (5 years), 2 out of 44 roots yielded the fungus in standard isolation assays but DNA was not detected using nested PCR methods. The very low levels of pathogen detection five years after tree wilt may help to explain the occasional re-emergence of the disease outside of treated areas after the five-to-six-year monitoring period.

Persistence of Pathogen in Roots

Our observations of pathogen survival in roots for longer durations than previously reported may have implications for aspects of current management, particularly regarding the placement of and durability of treatments within the root graft barrier line (or boundary of containment zone). For decades, managers in the Lake States

have managed the belowground spread of *B. fagacearum* by severing connections of grafted root systems with the vertical blade of a vibratory cable plow at the marked boundaries of containment zones encompassing infection centers (Bruhn and Heyd 1992; French and Steinstra 1978; Juzwik et al. 2010). To achieve effective management, forest health managers identify oak trees with active oak wilt symptoms and trees that are suspected to have recently died from the disease. Occasionally, trees that appear to have died several years previous, especially when they are not the nearest neighbor of trees displaying wilt symptoms, are excluded from the containment zone. In these cases, the trees are considered low risk because managers assume the cause of death was not oak wilt (e.g., density-related mortality, drought, or two-lined chestnut borer mortality) or that the tree was killed by oak wilt, but that the roots are unlikely to still harbor viable *B. fagacearum*. The justification for the latter is generally attributed to the limited saprophytic capabilities of *B. fagacearum*, although saprophytic survival of the fungus has only been characterized on aboveground portions of diseased trees (Bretz and Morrison 1953). However, our research reveals that both the roots and pathogen remain viable for at least five years following tree death, suggesting that excluding older dead trees from containment zones may put a stand at continued risk for belowground pathogen spread.

Following treatment, managers generally revisit infection centers annually for five-to-six years to monitor for newly wilted trees occurring outside the treated zone boundary. This generally accepted monitoring period is based, in part, on assumptions regarding the time in which root grafts are believed to re-form and the number of years

after treatment when the boundary appears to fail to contain the disease (Juzwik et al. 2010). Research on trench inserts as a means to stop root graft spread in Texas reported that roots of live oak (*Quercus fusiformis* and *Q. virginiana*) began to regenerate three years after being severed by a mechanical trencher that penetrated 1.5m into the ground (Wilson and Lester 2002). Neither the likelihood nor the timing of re-grafting across the narrow zone of severed roots created by vibratory plow are known, making the durability of vibratory plow lines somewhat of a mystery. While our results suggest that belowground survival of the pathogen may exceed the generally accepted monitoring window, the likelihood of new root grafts across root graft barrier lines likely depends on the method used to create the boundary of the containment zone. Pathogen survival appears to be related to root survival ($p = 0.06$). It follows that methods that result in faster root deterioration would decrease the duration of pathogen survival and the likelihood of re-grafting across containment zone boundaries. For example, it is reasonable to suspect that roots may be more likely to re-graft across a root graft barrier line that was established by a vibratory plow blade than one established by excavating and overturning root masses (Yang et al. 2024).

Effects of Herbicides on Root Mortality

Managers across the Midwest and Northeast U.S. operationally use herbicides, applied either to a frill girdle of a standing oak or the surface of a recently cut stump, with the goal of accelerating root tissue death and containing belowground spread of *B. fagacearum*. This may or may not be coupled with use of root severing by a vibratory plow, a trenching machine, or a mini excavator along a pre-determined boundary line.

Although a variety of herbicides have been tested, triclopyr ester (e.g., Garlon 4; Element 4), is currently the most commonly used. In this study, we found that nearly 40% of root sections remained viable four years after treatment to a cut stump. This was somewhat surprising, as Bruhn et al. (2002) reported full root mortality 30.5 months after applying herbicide to a frill girdle. Reasons for the differences in root mortality between this study and previous reports are unclear, but may be related to differences in herbicide uptake based on application method (i.e., application to cut stump versus frill girdle). Various herbicides have been tested and used as a tool to suppress oak stump sprouts during pre-commercial thinning (Campbell 1985; Groninger et al. 1998; Stein 1998); however detailed characterizations of herbicide translocation in plants are currently limited to herbaceous species. Therefore, the spatial and temporal dynamics of herbicides in trees stems and roots remains largely unknown (Gorrell et al. 1988; Humburg et al. 1989). Further exploration into the persistence and translocation of herbicides in oak stems and roots with various application methods is warranted to justify their practicality in containing belowground spread of *B. fagacearum*.

Herbicide treatments would be a valuable addition to the toolkit of validated oak wilt treatments, particularly in situations where traditional methods of separating root systems (e.g., vibratory plow, chain trencher) are not available or not feasible due to landscape constraints (Bronson et al., 2023). Our observations were limited to red oak trees on the Anoka Sand Plain region in Minnesota, which has light textured soil that likely presents a “worst case scenario” in terms of root graft frequency. Nevertheless, inference from our results may be useful to managers across the Upper Midwest and

Northeast U.S. who are weighing management options. Further work will aid in our understanding of pathogen survival in herbicide-treated root systems and the extent to which herbicide treatments can contain belowground disease spread.

Oak wilt is slowly expanding its geographic range (Jensen-Tracy et al. 2009; NAPPO 2023) at a time when many oak species are failing to regenerate across North America (Dey 2014). Re-appearance of oak wilt in stands that previously received disease management is of concern to land managers who seek to control oak wilt infections and sustain oaks on the landscape. Our study reveals that disease re-emergence may be related to the belowground survival of *B. fagacearum* in root systems. Further work is needed to better understand the effects of pathogen persistence in disease centers on oak regeneration.

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Chapter 3 :

Impacts of oak wilt and disease management on successional dynamics in northern forests

Abstract

Oak forests provide a suite of ecosystem services, including important habitat for wildlife species, understory plant diversity, carbon sequestration, aesthetic value, and forest products. Oak wilt, a disease caused by a long-established fungal pathogen, *Bretziella fagacearum*, threatens oak forests across North Central U.S.A. Decades of experience and research offers natural resource managers with information and tools to prevent and manage oak wilt spread. Still, the impacts of oak wilt on forest dynamics remains largely unknown. As the geographic range of oak wilt continues to slowly expand, we must understand how the disease, and disease management, are changing forest diversity and impacting regeneration of oak species. To examine the early impacts of oak wilt and various management strategies used to combat oak wilt on plant communities, we surveyed three types of oak wilt infection centers: 1) unmanaged disease, 2) removal of diseased trees, 3) stem-girdle treatment of diseased trees, as well as 4) healthy stands with an intact oak overstory. Compared to healthy stands, infection centers with unmanaged disease or where diseased trees were girdled had higher overall plant diversity. However, the same infection centers also had the highest relative abundance of common buckthorn. In general, the composition of seedlings and saplings in all types of infection centers appeared to shift away from abundant red oak species (*Quercus* section *Lobatae*) towards more shade tolerant species. These results suggest that red oak species may fail to reach pre-disease levels without further intervention.

Introduction

Oaks are widely recognized as some of the most diverse and ecologically important woody species in North American forests (Smith et al. 2009; Dey 2014; Cavender-Bares 2016). Oaks are keystone species that provide essential ecosystem services such as creating habitat and food for thousands of wildlife species and pollinators, sequestering carbon, and absorbing pollution (Cavender-Bares 2016). Climate projections for North America predict favorable conditions for many oak species,

yet oaks are declining and failing to regenerate across many North American forests (Juzwik and Schmidt 2000; Dey 2014; Peters et al. 2020). Oak regeneration failure is a complex issue with several interrelated causes that largely stem from altered disturbance regimes that promote dominance of other woody species (Abrams 1992; Nowacki and Abrams 2008). Oak regeneration is further complicated by threats from herbivores (e.g., insects, mammals) and diseases.

Oak wilt, caused by the purported introduced fungus *Bretziella fagacearum* (Bretz) Z.W. deBeer, Marinc., T.A. Duong, & M.J. Wingf.), is among the most important diseases of oaks in the United States (Juzwik et al. 2011). All oak species are believed to be susceptible to the disease (Bretz 1955), but species in the red oak group (*Quercus* section *Lobatae*) succumb to the disease most rapidly and often die within a single growing season because they fail to contain the disease once infected. Oak wilt infection centers are formed when spore-laden sap beetles (*Coleoptera: Nitidulidae*) fly from diseased trees to feed on fresh wounds on healthy trees, passively depositing spores onto the exposed xylem in the process (Juzwik et al. 1985). Once a tree is infected, the fungus may spread through grafted root systems to neighboring oaks, thereby creating an expanding pocket of disease mortality (Gibbs and French 1980). Efforts to control oak wilt involve belowground and aboveground management actions (French and Steinstra 1978; Bruhn and Heyd 1992; Juzwik et al. 2010). Belowground spread is prevented by cutting root connections in a buffered area around diseased trees because *B. fagacearum* can infect root systems prior to the appearance of foliar symptoms (French and Steinstra 1978; Rexrode 1978; Bruhn et al. 1991). After belowground containment is established, there are various options for limiting spore mat formation and reducing aboveground spread. These options vary in the magnitude of overstory removal from treatment of symptomatic trees with a stem-girdling cut that leaves standing dead trees to complete removal of all trees in the red oak group within the containment area (Greene et al. 2008; Bronson et al. 2023).

By killing or removing overstory trees, both oak wilt and efforts to contain it lead to canopy gaps that vary in size and structure depending on the extent of the infection

center and the management approach. In temperate forests, gap formation influences the composition and diversity of ground layer communities and regenerating tree species by altering the availability and distribution of understory resources (Denslow 1980; Runkle 1982; Canham et al. 1990; Gray and Spies 1996; Coates and Burton 1997). Solar radiation, one of several key understory resources, increases as the extent of overstory removal increases (Poulson and Platt 1989; Canham et al. 1990; Gray and Spies 1996), therefore establishment and growth of understory is greatly influenced by gap size and species adaptation to light availability. This general framework forms the basis for gap-based management systems that aim to regulate tree regeneration and forest diversity by manipulating harvest gap size (Gray and Spies 1996; Coates and Burton 1997; Webster and Lorimer 2005; Kern et al. 2017). For example, small gaps may favor shade-tolerant and mid-tolerant species through their ability to establish in a low light environment or through the release of advanced regeneration (Runkle 1982; Dahir and Lorimer 1996; Lhotka 2013). Conversely, large gaps with high light environments tend to favor early successional, shade intolerant species (Leak 2003; Lhotka 2013). While gap size is a major factor in determining species composition, other characteristics of the gap-forming disturbance and preexisting forest stand also affect plant community response.

Several studies have explored the extent to which ecological theory explains the composition of plant communities in temperate forests following gap-forming disturbances, although results have been varied (Sipe and Bazzaz 1995; Kern et al. 2013; VanderMolen et al. 2021). Numerous factors such as pre-gap vegetation, herbivore pressure, fluctuations in seed dispersal, and disturbance characteristics can exert powerful influence over species performance and coexistence within gaps (Brokaw and Busing 2000). Many oak wilt infection centers are similar in area to a mid-sized canopy gap (100 to 1000 m²; Muscolo et al. 2014), but the magnitude of overstory removal varies based on management approach, potentially creating within gap differences in light distribution that vary from harvest or windthrow canopy gaps. Theory from the intermediate disturbance hypothesis (IDH), suggests that the scale of a disturbance (e.g., intensity, frequency) plays an important role in community composition and that the highest levels

of diversity are maintained at intermediate scales (Connell 1978). In this way, variations in the light environment within oak wilt-mediated gaps may have important implications for plant diversity in successional communities. However, successional dynamics in gap-based management systems commonly defy predictions derived from theory, suggesting that succession in disease-mediated gaps could deviate substantially from theoretical predictions (Madsen and Hahn 2006; Bolton and D'Amato 2011; Kern et al. 2017).

To date, there have been only marginal efforts to evaluate the ways in which disease-mediated canopy gaps influence resources, species composition, or successional pathways (Loo 2009; Wardle and Peltzer 2017). Dynamics in canopy gaps formed by diseases likely differ from those in gaps formed by harvest or other abiotic disturbances in several ways. First, in the absence of management, disease infection centers are characterized by trees in various stages of mortality. In this way, gaps formed by pathogens are dynamic in space and time, with spatial heterogeneity that changes as the disease spreads (Menges and Loucks 1984). Second, compared to disturbances such as wind, many nonnative pathogens that cause widespread mortality in North America kill trees based on species rather than age or stature, thereby applying a unique filter to overstory and understory species pools. Studies relating disease to canopy gaps are generally limited to either the etiology of gap formation or role of native root diseases in late successional forests (Worrall et al. 2005; van der Kamp 1991; Holah 1997). The scarcity of research on dynamics within canopy gaps formed by nonnative pathogens (e.g., chestnut blight, Dutch elm disease) is arguably related to the reality that we can often only evaluate the impacts of disease after landscape-scale alterations have already occurred (Huenneke 1983). Oak wilt provides a unique opportunity to examine the dynamics of disease-mediated gaps because, although it causes widespread mortality, it has not yet expanded its range into all oak-growing regions of North America.

The objective of this study was to understand the impacts of oak wilt and how oak wilt-mediated canopy gaps influence plant community composition and diversity in central Minnesota, USA. To evaluate vegetation dynamics across a range of disease management approaches, we selected treatments that were representative of common oak

wilt control strategies: 1) girdle of infected trees, 2) annual removal of infected trees, and 3) no control. Using light as a proxy for altered resource environments we expected that plant community diversity and composition would vary based on changes in the availability of understory resources. Specifically, we expected that 1) infection centers with moderate light would support the highest diversity of plant species and that 2) community composition within infection centers would vary based on shade tolerance, where infection centers with moderate light would support species with a mix of light tolerance preferences (Fig 1-1). Inference from this study will be relevant to managers who seek to control oak wilt while simultaneously sustaining oaks on the landscape. Moreover, our results will contribute to the theoretical understanding of how pathogens shape structure and composition of temperate forests.

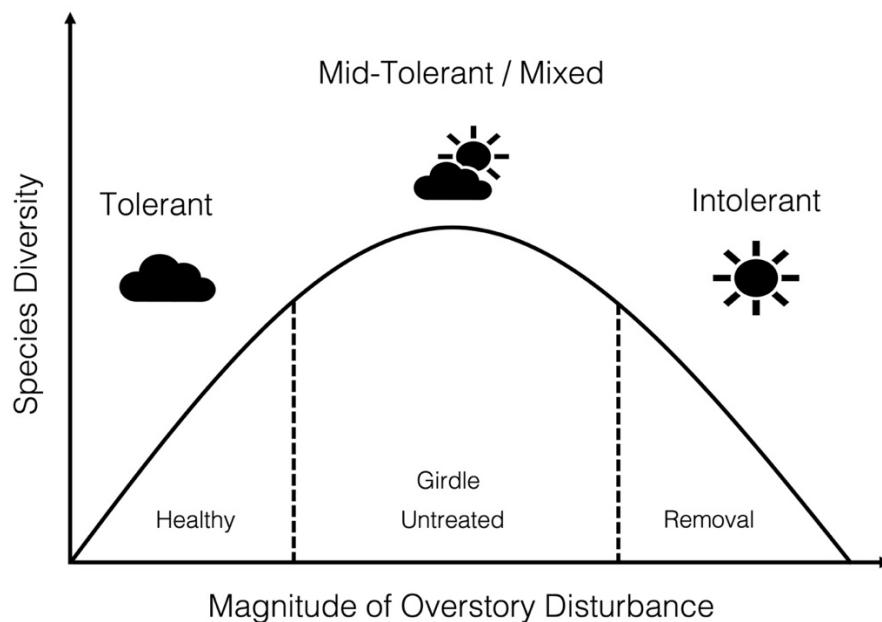


Figure 3-1. Conceptual diagram illustrating predicted effects of canopy disturbance on plant community diversity and composition in oak wilt-mediated gaps based on shade tolerance and the intermediate disturbance hypothesis.

Methods

Study Area and Site Selection

Our study sites were located within the Anoka Sand Plain region in central Minnesota, USA. Topography in this region is flat to gently rolling, with deep and excessively drained outwash soils that remained following the last glacial period (USDA NRCS 2019). Historically, this region was characterized by large stretches of oak savanna that were maintained by indigenous fire stewardship and large ungulate grazing (Guyette et al. 2003). Forced displacement of Indigenous peoples, European settlement, and urban expansion caused much of the remaining oak savanna to transform into closed-canopy oak woodlands (Nowacki and Abrams 2008). Forested areas within this region are currently dominated by *Quercus ellipsoidalis* (northern pin oak) and *Q. rubra* (northern red oak) mixed with *Q. macrocarpa* (bur oak) and *Acer rubrum* (red maple). The frequency of root grafts between adjacent trees in the red oak group varies among regions and on local scales and is, in part, related to soil type and tree density (Gibbs and French 1980; Menges and Loucks 1984). It is generally assumed that the sandy soils and high density of species in the red oak group in this region leads to high root graft frequency among these trees and rapid rates of disease spread.

Table 3-1. Site size and treatment year for the eleven oak wilt-affected gaps (infection centers) and four sites with a healthy oak overstory as a reference. All sites were located on coarse textured sandy soils in central Minnesota, USA. Vegetation assessments were conducted in June through August 2021 and 2022.

Treatment ^a	No. of Sites	Years Treated	Mean Gap Area (ha) ^b
Healthy	4	-	0.13
Girdle	4	2017, 2018	0.14
Untreated	4	-	0.10
Removal	3	2017, 2018	0.08

^a Healthy sites had intact, visually healthy oak overstory that did not receive any disease treatment. Girdle sites were monitored annually during the summer and diseased trees were treated with a stem-encircling cut (girdle) at the time of monitoring to prevent the formation of spore mats. Sites with untreated infections displayed symptoms of oak wilt beginning in 2018. Removal sites were monitored annually, and diseased trees were harvested in the winter following initial symptom appearance.

^b Gap area for healthy sites represents the area within a hypothetical treatment boundary estimated using the “rule of thumb” method (French and Steinstra 1978).

In 2021 and 2022, we selected eleven sites that were representative of common oak wilt control strategies: 1) girdle of infected trees (n = 4 sites), 2) annual removal of infected trees (n = 3 sites), and 3) no control (untreated; n = 4 sites; Table 3-1). Where overstory manipulation occurred, the perimeter of each site was treated with a vibratory plow with a 152-cm long blade (2.54 cm wide) to mechanically sever grafted root systems. Sites where symptomatic trees were girdled or removed were treated in 2017 or 2018. We used land manager records and local knowledge to identify and select sites with untreated oak wilt infection centers that began displaying crown wilt symptoms in 2018. We also selected four sites with an oak-dominated overstory that did not show signs of previous or current oak wilt infections to serve as undisturbed references. To mimic the size of treated sites, we marked hypothetical containment perimeters around untreated and healthy sites based on the “rule of thumb” method for containment boundary placement to delineate boundaries for plot placement. In short, the “rule of thumb” method places the containment boundary two rings (or tiers) of healthy trees away from the symptomatic trees (Juzwik and French 1999). For healthy sites, we

selected four trees to serve as hypothetical infected trees to determine plow line placement. We estimated the area in each site by calculating the area of an ellipse (Runkle 1992).

Community Composition

Within each site, we laid transects in the four cardinal directions radiating from the center and extending 5 m beyond the treated area (usually 5 m beyond the vibratory plow line). We placed a 1/1000-ha fixed radius plot every five meters along each transect to measure sapling and shrub (<12.54 cm diameter and ≥ 50 cm in height) communities. For shrubs and saplings, we recorded species, the number of living stems, and number of dead stems. We also recorded diameters at 1.3 m height (DBH) for saplings that were >1.3 m in height. *Corylus americana* and *Zanthoxylum americanum* were recorded as shrubs regardless of height. We placed 1-m² plots in the center of the 1/1000-ha plots to measure woody seedling and herbaceous communities. For each meter square census, we counted woody seedlings (<50 cm in height) by species and assigned herbaceous species to one of six cover classes (1 = 1%, 2 = 2–5%, 3 = 6–25%, 4 = 26–50%, 5 = 51–75%, and 6 = 76–100% estimated area covered). Additionally, we recorded the diameter, species, and condition for trees (>12.54 cm DBH; alive, declining/wilting, and dead) for the entire site within the vibratory plow line. Juvenile *Quercus rubra* and *Q. ellipsoidalis* are difficult to distinguish from one another and commonly hybridize in this region, therefore we combined them into a single species. All surveys were conducted in late-June through August of each survey year.

Light Estimation

We measured light availability for each plot using hemispherical canopy images captured on a DSLR camera (Sony Alpha-6000, Tokyo, Japan). The camera was fitted with a 180-degree fisheye lens and fixed on a self-leveling tripod mount. We used the

default aperture width and shutter speed for all photos. Images (4,000 x 6,000 pixels) were taken from a height of 1 m in the center of each 1-m² plot. Canopy photos were analyzed using WinSCANOPY (Regent Instruments, Quebec, Canada) to obtain estimates of the average total (direct and diffuse) daily understory photosynthetically active photon flux density (PPFD). For images taken with a uniformly overcast sky, pixel classification was based on gray scale using an automatic threshold. For images with solar flares or a high degree of solar reflection, pixel classification was based on a color library constructed from representative images from each site.

Data Analysis

We conducted all analyses on light, plant species diversity, vegetation structure, and plant community composition in R (R Core Team 2022). We used linear mixed effects models to identify differences among treatments in photosynthetically active photon flux density, shrub and sapling stem density, sapling basal area, and herbaceous cover (fixed effects) using the lme4 package (Bates et al. 2015). Each model had a random effect for site and was tested for significance with a Type II Wald Chi-Square test with $\alpha = 0.05$. We checked all model residuals for assumptions of normality and homoscedacity using the DHARMA package (Hartig 2022). When vegetation metrics did not meet assumptions of normality, we used either a square root transformation or arcsine square root transformation. When models indicated that there were significant differences among treatments, we conducted post-hoc pairwise means comparisons with a Tukey adjustment using the emmeans package (Lenth 2024).

To understand how taxonomic diversity differed among treatments, we calculated the effective number of herbaceous and woody species (Hill diversity) for each treatment using sample size and coverage-based rarefaction/extrapolation techniques in the iNEXT package (Chao et al. 2014; Hsieh et al. 2016). We calculated Hill diversity with $q = 1$, which accounts for the number of species observed in each treatment and their relative abundance. This estimation of Hill diversity is commonly referred to as Hill-Shannon

diversity or the exponential of Shannon entropy. Because this method combines all plots within treatments, we accounted for differences in the number of plots surveyed among treatments by comparing asymptotic estimates of Hill-Shannon diversity. We considered plant species diversity to be significantly different if 95% confidence intervals between treatments did not overlap. We also calculated species diversity for individual plots. Individual plots were too small to estimate asymptotic diversity, therefore we calculated diversity (Shannon entropy index) for each plot using the *vegan* package. We used a generalized mixed effects model to determine if average plot-level diversity of woody and herbaceous species and understory light could be predicted by a quadratic (unimodal) curve. We fit the models with a quadratic term for understory PPFD and random effect for treatment.

We visualized herbaceous and woody species composition of each treatment using nonmetric multidimensional scaling (NMDS) with Bray-Curtis distances based on the abundance of each species with the *metaMDS* function in the *vegan* package (Oksanen et al. 2022). For herbaceous and woody species matrices, rows consisted of individual plots and columns consisted of individual species. The matrix for herbaceous species was based on calculated midpoints of the recorded cover class, while the matrix for woody species was based on seedling, sapling, and shrub stem counts. To reduce variability in Bray-Curtis dissimilarity imposed by rare species, we eliminated herbaceous and woody species that occurred in fewer than three plots ($\leq 1.15\%$ of all plots). For both communities, we chose a three-dimensional solution to reduce stress and rotated the axes to maximally differentiate treatment groups in two dimensions. We tested for differences in community composition among treatment centroids using permutational multivariate analysis of variance (PERMANOVA) with Bray-Curtis distances with the *adonis* function in the *vegan* package. If PERMANOVA indicated differences in communities ($\alpha = 0.05$), we conducted pairwise comparisons using the *pairwise.adonis* function in the *pairwiseAdonis* package (Martinez 2017). We also tested for homogeneity of group dispersion (variance) using the *betadisper* function in the *vegan* package. To understand how communities clustered along light gradients, we fit

PPFD measurements with the ordination axes using the *envfit* function in the *vegan* package. We identified herbaceous and woody species associated with each treatment group using the *indicspecies* package and considered species to be significant based on a threshold of $\alpha = 0.05$ (De Cáceres 2009).

Results

Overall, understory light (PPFD) increased as the magnitude of overstory removal increased (Fig. 3-2; $\chi^2 = 24.9$, $df = 3$, $p > 0.001$). Sites where oak wilt was unmanaged or where symptomatic trees were removed had 54% and 1216% higher average understory light compared to healthy sites, respectively. The maximum average daily PPFD was higher in girdle sites than in healthy sites, though the difference was not statistically significant.

Altogether, we found only minor differences in vegetation (woody and herbaceous) structure among treatments (Table 3-2). Seedling density was at least 55% lower in oak wilt-affected sites compared to healthy sites, but the difference was not significant ($p = 0.14$). Shrub stem density was highest in healthy and removal sites, but again we found no significant differences among treatments ($p = 0.20$). Compared to healthy sites, oak wilt-affected sites had a higher overall density of sapling stems (<12.54 cm diameter and > 50 cm in height), but lower basal area of saplings that were >1.3 m height ($p = 0.002$). Removal sites had 28% less sapling basal area compared to healthy sites, followed by untreated sites (75% less), and girdled sites (83% less). Overall, herbaceous cover was highest in removal sites (110% increase from healthy sites) and increased with magnitude of overstory disturbance, but there were no statistical differences among treatments ($p = 0.21$).

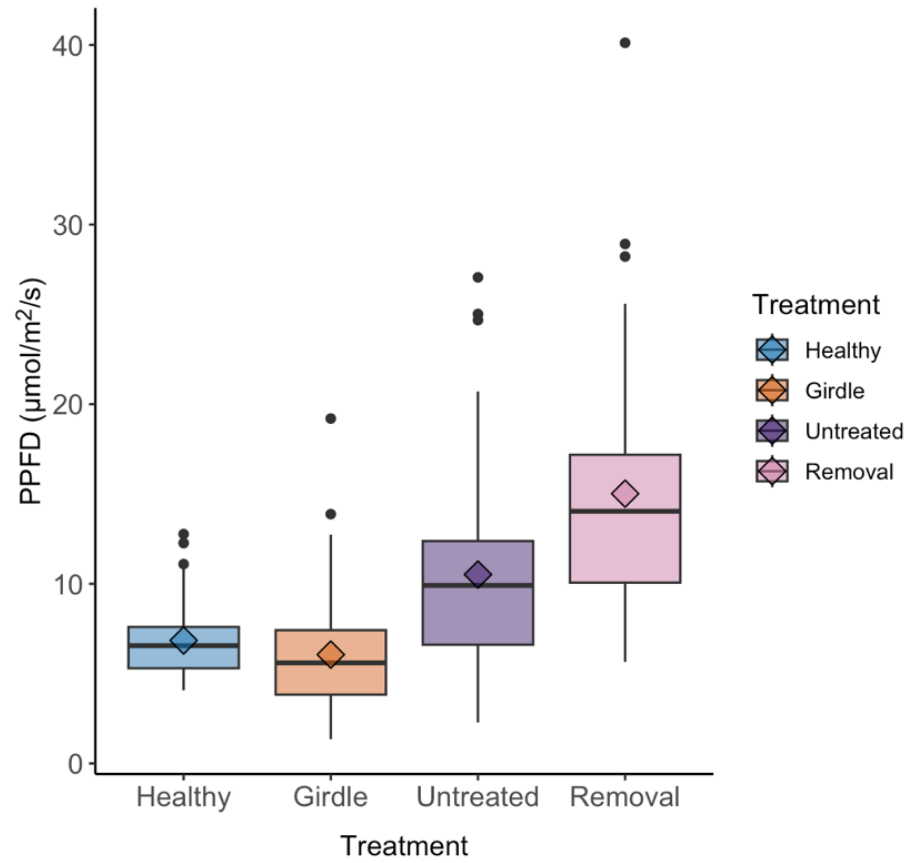


Figure 3-2. Average daily understory photosynthetic photon flux density (PPFD) increased with overstory removal in oak wilt-affected sites compared to sites with a healthy overstory. Solid horizontal lines represent medians and diamonds represent means.

Table 3-2. Metrics for woody and herbaceous species among oak wilt and healthy reference sites. Data represents raw means and standard error, with superscript letters indicating significant differences in mean sapling basal area ($\alpha = 0.05$) among treatments based on post-hoc means comparison of linear mixed effects models. Seedlings included all stems of woody species < 50 cm in height. Sapling density represents all tree saplings (<12.54 cm diameter and > 50 cm in height); sapling basal area represents the tree saplings that were > 1.3 m in height.

Site Type	Seedling Density x 10000 (stems ha ⁻¹)	Sapling Density x 1000 (stems ha ⁻¹)	Sapling Basal Area (m ² ha ⁻¹)	Shrub Density x 1000 (stems ha ⁻¹)	Herbaceous Cover (%)
Healthy	6.6 ± 0.8	1.3 ± 0.2	11.6 ± 2.1 ^a	8.2 ± 0.8	21.4 ± 2.7
Girdle	3.0 ± 0.3	3.0 ± 0.6	1.9 ± 0.3 ^b	5.3 ± 0.5	30.1 ± 3.3
Untreated	3.3 ± 0.2	3.5 ± 0.5	2.9 ± 0.7 ^{ab}	6.1 ± 0.5	32.6 ± 3.8
Removal	2.8 ± 0.2	3.2 ± 0.5	8.3 ± 2.5 ^{ab}	8.1 ± 0.8	44.9 ± 5.0
Model Output	$\chi^2 = 5.5$, df = 3, $p = 0.14$	$\chi^2 = 7.1$, df = 3, $p = 0.07$	$\chi^2 = 14.8$, df = 3, $p = 0.002$	$\chi^2 = 4.7$, df = 3, $p = 0.20$	$\chi^2 = 4.5$, df = 3, $p = 0.21$

In general, the mean relative abundance of red oak group species was higher in diseased sites than in sites with a healthy oak overstory (Fig. 3-3). The average density of seedlings in the red oak group (*Q. rubra* or *Q. ellipsoidalis*) did not vary significantly among the treatments ($\chi^2 = 2.3$, df = 3, $p = 0.51$). Seedlings in the red oak group were found in 9.6% of plots (n = 62) in healthy sites in 12% of plots (n = 200) in diseased sites (n = 200). Saplings in the red oak group were present in 6.4% of plots (n = 62) in healthy sites and 4.8% of plots (n = 200) across oak wilt-affected sites. Healthy sites had the highest mean basal area of red oak saplings (7.9 m² ha⁻¹), while plots in girdled sites had no red oak group saplings (Fig. 3-4). Healthy sites had the highest density of *Quercus macrocarpa* seedlings and saplings. Across all treatments, *Q. macrocarpa* was present in 11.1% of sapling plots.

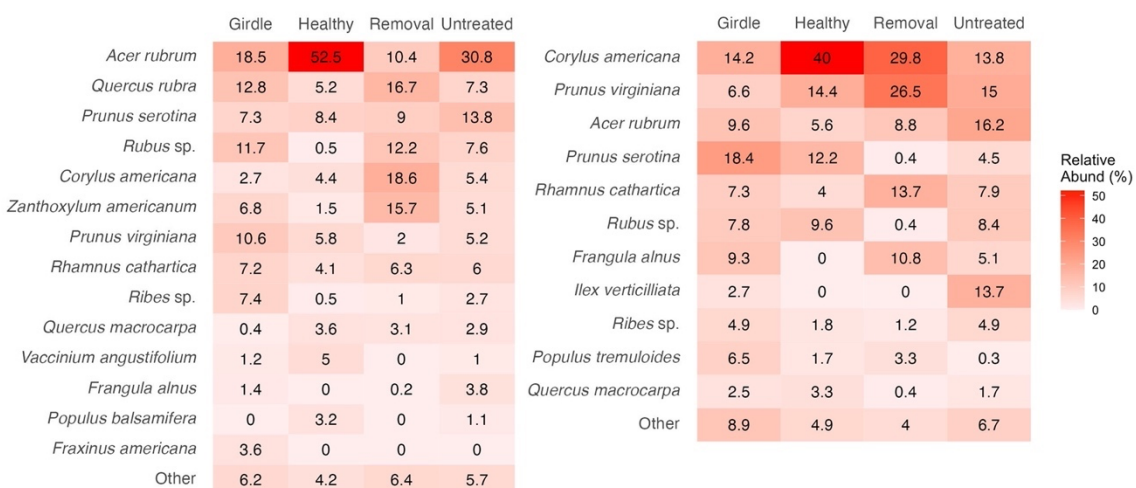


Figure 3-3. Heatmap showing the mean relative abundance of woody species regenerating in oak wilt-affected and healthy sites. Seedlings (left) includes stems of all woody species < 50 cm in height. Saplings (right) represents all tree and shrub stems <12.54 cm diameter and > 50 cm in height. Species grouped into the “Other” category had a maximum mean relative abundance of $\leq 3\%$.

Overall, our woody communities consisted of 36 genera and 51 species with an estimated sample coverage of >99% for all treatments. Herbaceous communities consisted of 71 genera and 79 species with an estimated sample coverage of at least 95% for all treatments. Diversity of herbaceous and woody communities varied among treatments, with asymptotic estimates of Hill-Shannon diversity (effective number of species based on Shannon entropy index; $q = 1$) following the same relative order for both assemblages (Fig. 3-5). Sites with the highest and lowest magnitude of overstory removal (i.e., removal and healthy, respectively) corresponded with the lowest estimated diversity, while sites with intermediate overstory removal (girdle and untreated) corresponded with the highest diversity. We found a quadratic correlation between mean plot-level diversity (Shannon entropy; non-asymptotic estimate) of woody species and average daily understory PPFD ($p = 0.004$ and 0.03 ; Supplemental Table 3-1) but did not find the same relationship for herbaceous species diversity ($p = 0.28$ and 0.23 ; Fig. 3-6).

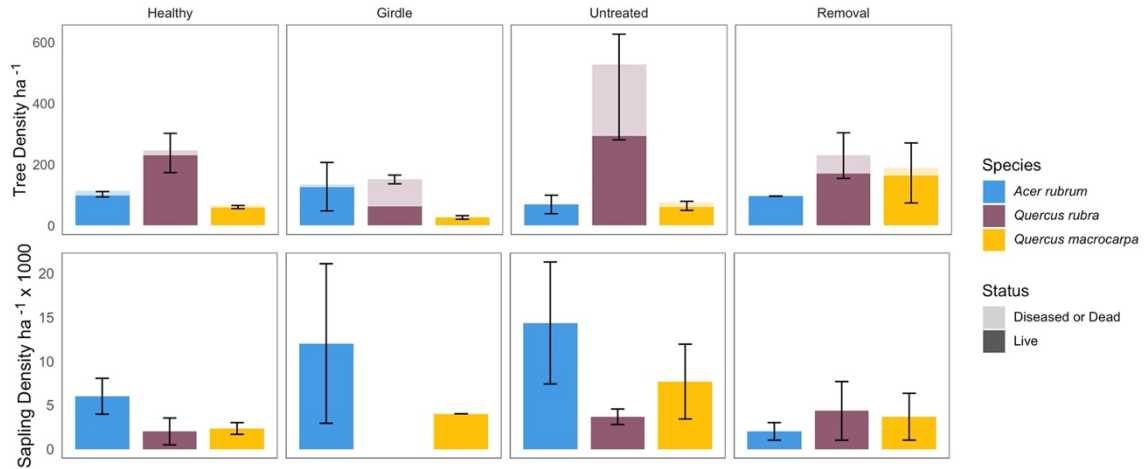


Figure 3-4. Mean density of *Acer rubrum*, *Quercus rubra*, and *Quercus macrocarpa* saplings (left) and canopy trees (right) among treatments. Sapling density represents all tree saplings <12.54 cm diameter and > 50 cm in height and tree density represents all trees > 12.54 cm diameter. Saplings were censused in 1/1000 ha fixed radius plots and trees were censused across the entire site (extending 5m beyond the boundary of the vibratory plow line or hypothetical line boundary). Species within the red oak group (*Quercus* section *Lobatae*) were combined to a single species (*Q. rubra*) because they are difficult to distinguish in young trees and can hybridize in this region. Diseased and dead oaks encompass all causes of mortality, but infection from *B. fagacearum* was generally assumed to be the cause of death for trees in the red oak group. Error bars represent standard error of the mean for stem density regardless of disease status.

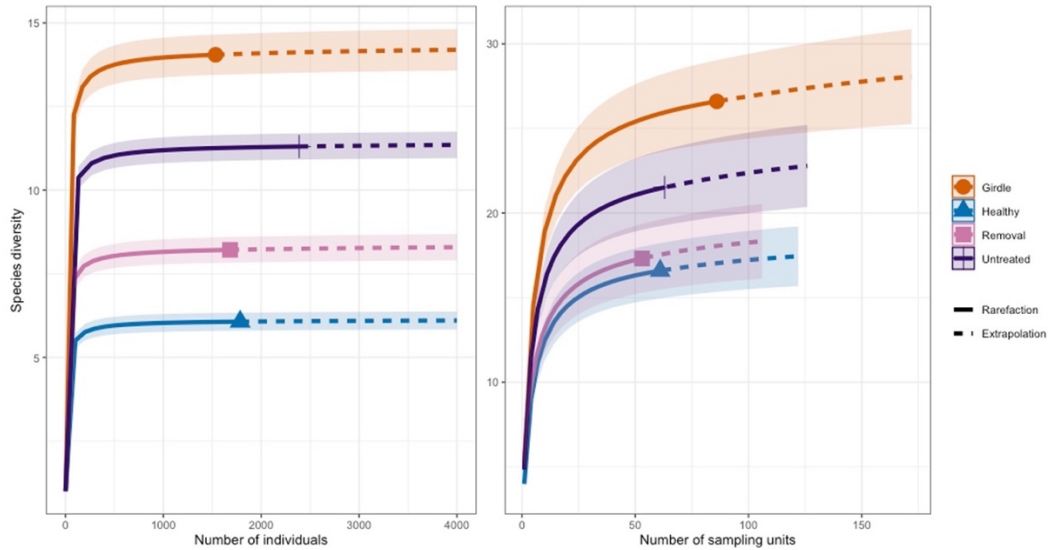


Figure 3-5. Sample-sized based rarefaction/extrapolation curves ($q = 1$) of woody (left) and herbaceous (right) species. Curves for woody species developed based on abundance; curves for herbaceous species were constructed based on presence/absence of species in plots. Solid circles represent observed Hill numbers (effective number of species) for each reference sample and shaded envelopes represent 95% confidence intervals.

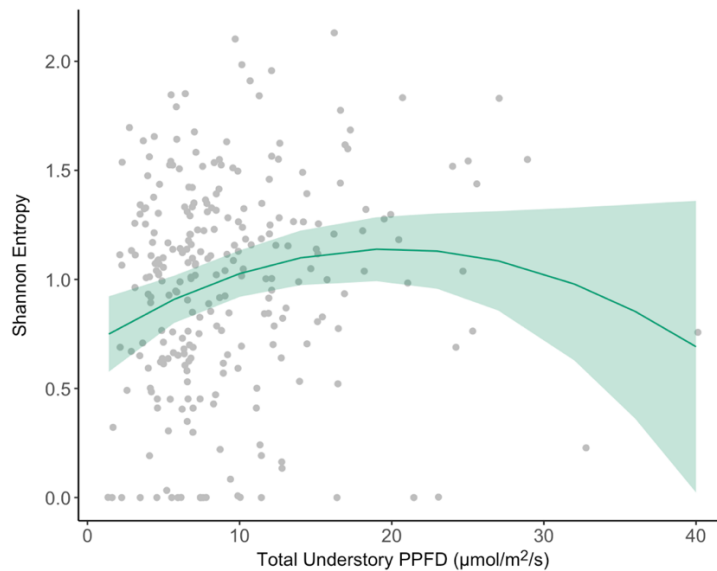


Figure 3-6. The relationship between average daily understory light (PPFD $\mu\text{mol}/\text{m}^2/\text{s}$) and taxonomic diversity of woody species. Plot-level diversity (Shannon entropy index; gray circles) was generally higher in plots with moderate understory light compared to plots with the lowest and highest light. Predicted mean diversity (green line) across the light gradient and 95% confidence interval (green envelope) were calculated based on mixed effects model results.

Community composition of woody and herbaceous species varied among treatments ($p < 0.001$ for woody and herbaceous species; Fig. 3-7). All pairwise comparisons of treatment centroids were statistically significant for woody (adj. $p = 0.006$) and herbaceous communities (adj. $p = 0.006$). We also found significant dispersion of points relative to treatment centroids ($p < 0.001$) for both communities, meaning that differences in treatment centroids may be due to difference in variance of the data among treatments. In both ordinations, PPFD corresponded to changes in treatment communities ($p < 0.001$). Indicator species analysis identified 15 woody and 18 herbaceous species that were associated with individual or multiple treatments (Supplemental Table 3-2). For woody species, *Acer rubrum* and *Pinus strobus* were indicators for healthy sites. There were various shared indicator species among oak wilt-affected sites including *Cornus racemosa*, *Prunus virginiana*, *Rubus* spp., *Quercus macrocapa*, and *Vitis riparia*. Girdled sites were characterized by *Fraxinus nigra*, while untreated sites were characterized by *Ulmus americana* and *Frangula alnus*. For the herbaceous species, healthy sites were characterized by *Anemone quinquefolia* and *Maianthemum canadense*. Shared indicators among various oak wilt treatments included *Phryma leptostachya*, *Galium triflorum*, and *Aralia nudicaulis*. Species such as *Arisaema triphyllum*, *Osmunda claytoniana*, and *Polygonatum biflorum* were common in sites with girdled trees. Indicators for the untreated sites included species such as *Osmorhiza claytonii*, *Circaea alpina*, and *Solidago canadensis*. Sites where diseased trees were removed had indicator species such as *Toxicodendron rydbergii*, *Carex pennsylvania*, and *Hackelia virginiana*.

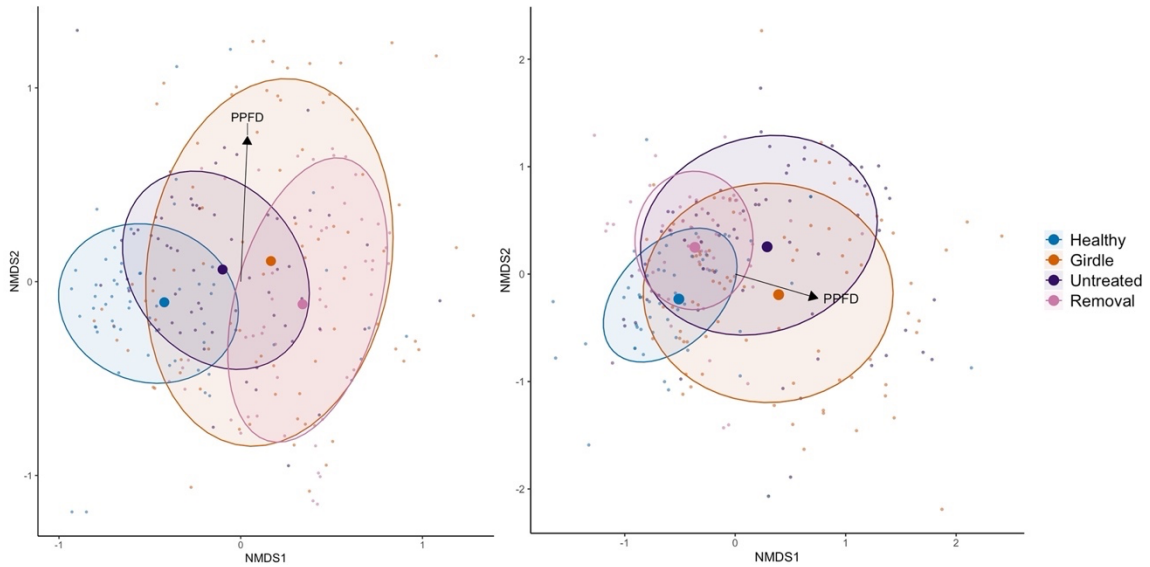


Figure 3-7. Non-metric multidimensional scaling (NMDS) ordination visualizing dissimilarity among plots (colored points) from woody (left; stress = 0.174) and herbaceous (right; stress = 0.158) communities. Ordination for woody species is based on abundance and herbaceous species based on the midpoint of cover classes. Colored ellipses encompass plots that are within one standard deviation of the treatment centroid. Black arrows indicate the direction of the most rapid change in average daily understory light (PPFD). Axes of three-dimensional ordinations were rotated to visually maximize differentiation between treatment groups in two dimensions.

Discussion

Efforts to manage oak wilt stem from the desire to maintain oak-dominated forest communities, yet very few studies have evaluated whether oak wilt management helps to achieve the long-term goal of oak regeneration. By examining canopy gaps formed by oak wilt and its management, we sought more generally to expand our understanding of how diseases shape forest ecosystems and more specifically to understand how disease impacts oak regeneration. Oak wilt and its management were correlated with increases in woody and herbaceous plant diversity; however, woody regeneration tended to shift away from species in the red oak group and towards shrubs and other shade tolerant and mid-tolerant tree species. Extensive mortality of the oak overstory makes the immediate impact of oak wilt striking; however, our results suggest that the disease may also trigger changes in plant dynamics that will have negative implications for long-term oak regeneration.

Shifts in Community Composition

In this study, we found that plant communities shifted with changes in available light among treatments and generally aligned with predictions from gap-based theory. Treatments with the lowest and highest light tended to support shade-tolerant and intolerant plant communities, respectively, while treatments with intermediate light supported species with a variety of light preferences. Herbaceous communities shifted in response to variations in understory light and the greatest differences in community composition was between healthy and oak wilt-affected sites. Healthy sites were characterized by species adapted to shade, while oak wilt-affected sites were characterized by a range of shade intolerant to tolerant species. For woody communities, healthy sites with closed canopies were characterized by seedlings and saplings of the shade tolerant *Acer rubrum*, while shade intolerant *Corylus americana* was an indicator for sites where diseased trees were removed. Oak wilt-affected sites (unmanaged, girdled, and removal) were associated with a variety of seedlings and saplings of mid-tolerant tree species including *Cornus racemosa*, *Fraxinus pennsylvanica*, and *Ulmus americana*. Interestingly, *Q. macrocarpa* was an indicator for both untreated and removal treatments. Although this species is not in the red oak group, it is a key component in temperate oak savannas and its regeneration in oak wilt pockets is promising for managers seeking to restore degraded savanna remnants (Yantes et al. 2023). Overall, saplings of species in the red oak group were not well-represented in successional communities and were not indicators for any of the treatments.

Canopy gap size can be an important determinant in recruitment of oak species (Lhotka 2013). To date, only a handful of studies have explored oak regeneration and community dynamics in disease gaps (Tryon et al. 1983; Collada 1998; Pellegrini et al. 2021), with even fewer studies integrating the added influence of disease containment efforts (Meunier et al. 2019; Abella et al. 2021). Here, we observed similar trends in oak regeneration to those documented in small gaps (removal of one to three trees) in Ohio,

where Abella et al. (2021) found abundant of black oak (*Quercus velutina*) seedlings but no saplings. On the other end of the spectrum, infection centers treated with larger clearcut harvests in Wisconsin exhibited regeneration of *Q. ellipsoidalis* from stump sprouts and acorns ten years after treatment (Meunier et al. 2019). The infection centers in our study represent an intermediate size between previous studies and a similar, albeit somewhat earlier, time since treatment. Predicting the long-term potential for oak recruitment is challenging, but gaps in our study were similar in area to harvest openings in Central Hardwood Forests that were large enough (0.16 ha) to encourage recruitment of a range of shade intolerant and tolerant species, including several mid-tolerant oak species 48 years after treatment (Lhotka 2013). Woody communities in oak wilt-affected sites in our study partially match regeneration patterns in the harvest openings assessed by Lhotka (2013). Although our oak wilt-affected sites contained very little regeneration of oaks in the sapling size class, they did contain several small-diameter, mid-tolerant tree species. However, we found that shade tolerant tree and large shrub species such as *Acer rubrum*, *Prunus serotina*, *Rhamnus cathartica*, and *Frangula alnus*, tended to have the highest basal area regardless of overstory treatment and understory light, indicating that light availability resulting from gap size is only one of several predictors of species performance.

Several studies have pointed to other agents and mechanisms that might outweigh the effects of light availability on plant survival and dynamics (Royo and Carson 2006; Montgomery et al. 2010; Kern et al. 2012). We found a relatively high abundance of ground layer species that can behave as a filter for tree seedlings and persist for decades after a disturbance, thereby influencing the growth and survival of tree species and altering successional trajectories (Royo and Carson 2006; Powers and Nagel 2009; Reed et al. 2022). In particular, *Carex pennsylvanica*, *Osmunda claytoniana*, and *Rubus* spp. were indicators for sites with unmanaged disease and with removal treatments and have been implicated in tree regeneration issues (George and Bazzaz 1999; Donoso and Nyland 2006; Powers and Nagel 2009). In addition to ground layer species that influence tree seedling survival, we found midstory species that may affect both seedling and

young sapling survival of oaks and other mid-tolerant tree species. Specifically, buckthorn species (*Rhamnus cathartica* and *Frangula alnus*) were present in many of the infection centers, which create dense, shaded thickets that impede the growth of young red oaks and other tree species (Hamelin et al. 2016). The relative abundance of *Rhamnus cathartica* seedlings and saplings in healthy stands was similar to oak-wilt affected stands, but it remains unclear whether buckthorn establishment occurred prior to disease establishment, or if buckthorn establishment was facilitated by the establishment and spread of oak wilt (Simberloff and von Holle 1999). The presence of these influential species in the ground and mid-story layers may be important determinants of future tree species composition, particularly for shade intolerant species or mid-tolerant oaks.

Variations in Light and Diversity Among Treatments

This study begins to characterize the subtle, indirect effects of oak wilt disease on plant communities that may stem from the patchy nature of the overstory in disease infection centers (Menges and Loucks 1984). Transmission of *B. fagacearum* through grafted roots can be sporadic and not all neighboring red oaks on a stand are grafted to one another (Blaedow and Juzwik 2010). These characteristics contribute to an irregular pattern of disease transmission that results in patchy overstory mortality rather than a uniform canopy opening. In this way, the overstory in our infection centers may be more akin to shelterwood systems than a uniform harvest opening. Irregular shelterwood systems in balsam fir (*Abies balsamea*) – yellow birch (*Betula alleghaniensis*) stands were shown to diversify light distribution and intensity, seedbeds, and regenerating species compared to clearcut harvests (Raymond and Bédard 2017), but similar characterizations of light and microsite variation for comparable treatments in oak-dominated stands are scarce (Patterson et al. 2022). In our study, oak wilt-affected sites that were girdled or untreated were characterized by standing dead oak trees, coarse woody debris from dead trees that fell over, or trees that were in various stages of crown wilt. In this way, the heterogeneous overstory may have contributed to a diverse mosaic

of light environments and microsites within the infection centers, allowing for coexistence of more species than untreated or removal sites with less heterogeneity.

Across our treatments, we found that relationship between plant diversity and understory light followed a unimodal pattern for woody communities, but not for herbaceous communities, partially aligning with predictions derived from the intermediate disturbance hypothesis (IDH). The widescale applicability of the IDH is controversial due to imprecise definitions within its theoretical framework and mixed empirical evidence (Bongers et al. 2009; Fox 2013; Sheil and Burslem 2013). Yet, the general framework from the IDH remains a tool that is used to examine diversity-disturbance relationships in many ecosystems (Sheil and Burslem 2013). Studies have proposed that the lack of unimodality in diversity-disturbance relationships is related to difficulties in sampling across the full disturbance gradient (Huston 2014). By restricting the spatial (medium-sized gaps) and temporal (time since disease establishment) scale at which we measured diversity, we were able to sample across a broad gradient of disturbance intensity, thus allowing us to capture a wide snapshot of the disturbance-diversity relationship. Our findings underscore the notion that, while that the IDH is a helpful tool to frame disturbance research, it is difficult to test in practice.

Species diversity metrics are often used to guide and evaluate forest restoration decisions and conservation efforts. However, our study reaffirms the importance of considering plant community composition and time since disturbance, in addition to diversity, when evaluating management outcomes. We surveyed oak wilt sites during an early window (three to four years) after overstory removal when the understory undergoes a pulse of growth and plant diversity increases (Donoso and Nyland 2006). Reliance on diversity metrics alone may fail to account for the presence of individual species that are considered a hindrance to conservation or restoration efforts. For example, our early snapshot of diversity does not offer insight regarding long-term buckthorn impacts on plant diversity in the years to come. In order to fully characterize the influence of oak wilt and management efforts on plant diversity, it will be important

to re-evaluate plant communities after longer periods of time to observe which, if any, tree species recruit to larger size classes.

Implications for Oak Regeneration

Oak wilt interferes with essentially all pathways for red oak regeneration: death of mature oak trees reduces the availability of acorns for seed-origin regeneration, death of saplings reduces opportunity for advance regeneration, and root system infection eliminates stump sprouts as a viable method to regenerate oaks. Further, little is known regarding the persistence and spread of *B. fagacearum* in grafted root systems after tree death, potentially putting regenerating oaks at continued risk of infection. Most evidence points to *B. fagacearum* being an introduced pathogen, therefore the disease presents a challenge to which oak species are not necessarily adapted and trees die regardless of age, stature, or other traits (Juzwik et al. 2008). In contrast, many oak species exhibit fire-adapted traits such as thick bark and resprouting ability that allows them to exhibit variable mortality based on the intensity and frequency of fires and persist on the landscape (Abrams 1992).

Present-day oak woodlands located within former oak savannas arguably hold the greatest potential for oak regeneration in the Upper Midwest because they tend to have sandy, nutrient-limited soils that preclude establishment of less tolerant tree species (Schwartz and Demchik 2012; Dey 2014). Paradoxically, these sites also exhibit the highest frequency of root grafts among red oaks, making them very susceptible to widespread oak wilt epidemics (Menges and Loucks 1984). Evidence from our study adds to an extensive body of literature that points towards oak replacement by shade-tolerant species across many oak-growing regions of North America (Nowacki et al. 1990; Lorimer et al. 1994; Taylor and Lorimer 2003; Richards and Hart 2011). Despite the uptick in understory light in oak wilt infection centers, the shade tolerant species may be more likely to eventually reach dominant canopy positions and limit the future development oak species (Taylor and Lorimer 2003). As shade-tolerant plants begin to

dominate landscapes where oaks were once maintained by fire, they modify the environment (cooler, damper, shadier conditions) in a way that favors mesophytic species and suppresses the establishment of oaks (Nowacki and Abrams 2008). Although the rate of mesophication depends on site conditions, it can be accelerated in landscapes like those in our study that house mesophytic species in the understory (Abrams and Nowacki 1992). In this manner, oak wilt creates yet another avenue to initiate the process of mesophication, potentially overriding predictions of favorable future conditions for oak regeneration in this region.

Conclusions

Efforts to manage oak wilt are undertaken with the goal of preserving oaks on the landscape. Our study finds that there may be substantial ecological implications of management that focuses solely on disease suppression without accounting for long-term regeneration goals. Advance regeneration, arguably the single most important component to successful oak regeneration (Johnson et al. 2019), was present in seedling size classes, but limited or absent in the sapling size class. Disease treatments may serve as an important window of opportunity for intervention, as plant communities are highly susceptible to management actions in the brief period of time following a disturbance (Seidl and Turner 2022). Explorations of additional management actions such as removing shade tolerant species or planting advanced oak regeneration may improve disease mitigation strategies and the likelihood of maintaining oak-dominated ecosystems (Dey 2014; Graboski et al. 2020). By continuing to characterize the unique ways that oak wilt affects oak regeneration and stand dynamics, we will gain a deeper understanding of how to sustain oaks across the Upper Midwest.

Supplementary Materials

Supplemental Table 3-1. Parameters for quadratic mixed effects models of plot-level diversity (Shannon entropy index) as a function of understory light (photosynthetic photon flux density; PPFD). The quadratic models were used to test for a unimodal relationship between disturbance intensity (PPFD) and diversity.

Community	Coefficient Estimate	Chi-Square	Degrees of Freedom	p - value
Woody	PPFD	8.254	1	0.004
	PPFD ²	4.968	1	0.026
Herbaceous	PPFD	1.166	1	0.280
	PPFD ²	1.462	1	0.227

Supplemental Table 3- 2. Indicator species identified for oak wilt-affected sites and healthy reference sites using indicator species analysis. Analysis was conducted using the *indicspecies* package in R (De Cáceres and Legendre 2009).

Woody Species				
Treatment	Species	Common Name	Stat	p - value
Healthy	<i>Acer rubrum</i>	Red maple	0.433	0.001
	<i>Pinus strobus</i>	White pine	0.182	0.023
	<i>Vaccinium angustifolium</i>	Lowbush blueberry	0.163	0.044
Girdle	<i>Fraxinus pennsylvanica</i>	Green ash	0.296	0.001
	<i>Sambucus racemosa</i>	Red elderberry	0.197	0.006
	<i>Ribes sp.</i>	Gooseberry/currant	0.194	0.013
	<i>Cornus racemosa</i>	Gray dogwood	0.172	0.031
Untreated	<i>Frangula alnus</i>	Glossy buckthorn	0.354	0.001

	<i>Ulmus americana</i>	American elm	0.211	0.005
	<i>Prunus virginiana</i>	Chokecherry	0.256	0.001
	<i>Rubus sp.</i>	Brambles	0.228	0.001
	<i>Quercus macrocarpa</i>	Bur oak	0.230	0.002
	<i>Vitis riparia</i>	Riverbank grape	0.170	0.037
Removal	<i>Corylus americana</i>	American hazelnut	0.351	0.001
	<i>Lonicera morrowii</i>	Morrow's honeysuckle	0.169	0.039
	<i>Cornus racemosa</i>	Gray dogwood	0.172	0.031
	<i>Prunus virginiana</i>	Chokecherry	0.256	0.001
	<i>Rubus sp.</i>	Brambles	0.228	0.001
	<i>Quercus macrocarpa</i>	Bur oak	0.230	0.002
	<i>Vitis riparia</i>	Riverbank grape	0.170	0.037

Herbaceous Species

Healthy	<i>Anemone quinquefolia</i>	Wood anemone	0.316	0.001
	<i>Maianthemum canadense</i>	Canada mayflower	0.171	0.028
	<i>Phryma leptostachya</i>	American lopseed	0.178	0.029
Girdle	<i>Arisaema triphyllum</i>	Jack in the pulpit	0.284	0.001
	<i>Laportea canadensis</i>	Wood nettle	0.207	0.007
	<i>Osmunda claytoniana</i>	Interrupted fern	0.206	0.008
	<i>Eurybia macrophylla</i>	Large leaf aster	0.189	0.015
	<i>Polygonatum biflorum</i>	Smooth Solomon's seal	0.174	0.015
	<i>Phryma leptostachya</i>	American lopseed	0.178	0.029
	<i>Galium triflorum</i>	Fragrant bedstraw	0.170	0.028
Untreated	<i>Circaea alpina</i>	Alpine enchanter's nightshade	0.308	0.001
	<i>Osmorhiza claytonii</i>	Hairy sweet Cicely	0.234	0.002
	<i>Parthenocissus quinquefolia</i>	Virginia creeper	0.208	0.006
	<i>Solidago canadensis</i>	Canada goldenrod	0.165	0.036

	<i>Aralia nudicaulis</i>	Wild sarsaparilla	0.179	0.020
Removal	<i>Toxicodendron rydbergii</i>	Poison ivy	0.377	0.001
	<i>Carex pennsylvania</i>	Pennsylvania sedge	0.239	0.005
	<i>Hackelia virginiana</i>	Virginia stickseed	0.157	0.006
	<i>Galium asprellum</i>	Rough bedstraw	0.142	0.021
	<i>Galium triflorum</i>	Fragrant bedstraw	0.170	0.028
	<i>Aralia nudicaulis</i>	Wild sarsaparilla	0.179	0.020

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Conclusions

This dissertation explores considerations for oak wilt management and offers insights about the long-term impacts of the disease and management on northern oak forests. Chapter 1 provided an evaluation of an oak wilt “root rupture” treatment on landscapes that have historically posed a challenge for managers in Wisconsin. In this chapter, I discuss the difficulty in containing belowground spread in large disease infection centers and the “silver-lining” of root rupture treatments regarding forest regeneration. Chapter 2 builds on questions from Chapter 1. Namely, I examine the survival of *B. fagacearum* in root systems of diseased oaks to understand how the longevity of pathogen survival impacts belowground containment efforts. We found that *B. fagacearum* was capable of surviving in root systems of dead trees for at least five years, which helps to explain, in part, why managers continue to see re-emergence of the disease in treated stands. Survival of *B. fagacearum* and re-emergence of oak wilt in treated stands puts regenerating oaks at continued risk of infection and holds the potential to alter forest dynamics. Chapter 3 illustrates how the unique patterns of overstory mortality from oak wilt and disease management can shape plant communities. We found that plant community diversity and composition varied based on the presence of the disease and disease treatments, and that the disease may be leading to dominance of non-oak species in Minnesota forests.

Oak wilt presents an interesting, long-term challenge for forest management in the Upper Midwest U.S. The introduction of invasive pests and pathogens can have rapid, devastating consequences to forest ecosystems. For example, the establishment and spread of diseases such as chestnut blight or Dutch elm disease (caused *Cryphonectria parasitica* and *Ophiostoma novo-ulmi*, respectively) functionally eliminated species from the overstory across millions of urban and forested hectares in North America. Unlike other introduced pests and pathogens, rapid, widespread decimation of oak species after the introduction of oak wilt never occurred. Rather, the disease has persisted on the landscape for nearly a century by intensifying in regions where it already occurs and slowly expanding its geographic range. As a result of the longstanding presence of oak

wilt on the landscape, managing agencies have developed a myriad of effective strategies to control the disease based on manager resources, landscape constraints, and land use priorities. Until recently, little attention has been paid to whether oak wilt management helps to achieve the long-term goal of oak regeneration. My dissertation builds on more than fifty years of oak wilt research and continues the process of integrating forest pathology with the broader forest ecosystem.