Environment and Natural Resources Trust Fund

Research Addendum for Peer Review

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Project Title: **Finding Disease Resistant Elm Trees in Minnesota**

Project number: **019-B**

1. Abstract

We propose to find elms [American (*Ulmus americana*), red (*Ulmus rubra*) and rock (*Ulmus thomasii*) elms)] from across Minnesota with resistance to Dutch elm disease, test them rigorously and have genetically diverse trees with resistance available so that this magnificent shade tree and important forest species will be brought back to its previous grandeur.

2. Background

Dutch elm disease (DED) is a vascular wilt caused by two closely related fungi, *Ophiostoma ulmi* and *Ophiostoma novo-ulmi,* and is vectored by several elm bark beetles. This devastating wilt disease has killed millions of elms in Minnesota over the past five decades. So many trees have died people have just about given up hope for this tree. Losses from Dutch elm disease have been devastating, and have literally caused an ecological disaster. There is no tree more beautiful or well suited for urban conditions than the American elm. They tolerate salt, pollution and other stresses better than any other tree currently planted in urban environments. As Dutch elm disease has attacked our urban elms, removing and disposing of these diseased trees and conducting other control programs have become very expensive. Municipal budget constraints over recent years have meant that fewer funds are available for Dutch elm disease control, leading to more diseased elms remaining for bark beetles to breed in and to attack healthy elm tree. We now find other introduced pests, such as the Emerald Ash Borer, threatening Minnesota ash trees and so the need for new trees to be used for replanting in urban areas is greater than ever. Replanting with a diverse population of tree species including resistant elms is essential and elms that are hardy in Minnesota with high levels of resistance are needed. Elms also play a vital part in the ecology of Minnesota's forests and they are an important tree throughout Minnesota. Their seed provides important food for wildlife, they are excellent trees for wetlands and add to the biodiversity of a healthy forest (for example, 213 species of moths and butterflies are supported by elms) (Tallamy 2007). Elms can also be used for making furniture, boxes, crates and other wood products.

There is new hope for the elm. Previous work has established that resistance to Dutch elm disease occurs in nature in a small number of American elms (Smalley and Guries, 1993; Townsend 2000; Townsend et al. 2005). Some of the most notable that have been identified are "Valley Forge" and "Princeton", which we have repeatedly tested and found them to have a high tolerance to the disease. Some hybrid crosses of Asian, European and American elms have also proven to be highly resistant, mainly due to the resistance found in Asian varieties. In an effort to combat Dutch elm disease and keep American elms in our landscapes, these resistant selections are being used with increasing frequency in urban areas. This has been a positive trend toward the reestablishment of the American elm. However, there are a few pitfalls to planting only a few varieties of resistant elms. The vulnerability lies in the pathogens ability to mutate or hybridize as it has in the past and become more virulent (Brasier and Buck 2002 ; Brasier and Kirk 2010). The resistance of particular elms to Dutch elm disease will last only as long as the virulence of the pathogen remains the same or lower. For this reason, it is important to have a variety of genotypes of resistant elms to protect against losing large populations of trees with similar genetic background. Furthermore, it is exceedingly important that newly-identified trees with putatively-resistance have good growth characteristics and be hardy under Minnesota conditions. Many of the resistant elms currently available are not hardy in Minnesota and have poor growth form.

We have observed that some elms have survived the disease in Minnesota and these survivors appear to have special characteristics making them resistant to Dutch elm disease. In our preliminary work, seedlings were grown from a few of these trees and were injected with the fungus. Results show that some trees survive and even after repeated inoculations remain free of the disease. Although we have a few trees that appear resistant, having a large genetic stock of hardy and resistant native Minnesota elms is vital to fight this deadly fungus. The objective of our research is to identify and test putative resistance in selected elms from throughout the state of Minnesota in an effort to bring this majestic tree back to our landscape.

3. Hypothesis

Some elm trees that have survived decades of heavy pressure from Dutch elm disease in Minnesota may have a high level of Dutch elm disease resistance. Screening clones of these trees in the greenhouse and in field trials by inoculating with the fungus that causes Dutch elm disease will determine if these trees have resistance to the disease.

4. Methodology

Survey and identify resistant elm trees. From preliminary research supported by the Minnesota Turf and Grounds Foundation, we identified and tested several elms and found that there appears to be different levels of resistance in the remaining elm population. In addition to the elms already identified but not tested, we have information from arborists and city foresters about many candidate elms from throughout Minnesota that appear to be resistant. These trees survive where all other elms have died. Screening (injecting with the fungus that causes Dutch elm disease in greenhouse and field trials) will identify which of these trees are truly resistant. Once trees are selected they need to be propagated so a sufficient number of saplings are available for our testing. Investigations into resistance are supported by our ability to vegetatively reproduce these elms. Through our past funding from the Minnesota Turf and Grounds Foundation and other sources we have developed methods for propagating many elms through stem cuttings. We have discovered many differences between elm species and varieties and so we continue to fine-tune these methods for each individual elm that we reproduce. Our goal is to propagate cuttings from each elm that we select with over 80% success. The ability to clone each elm of interest with a high rate of success is a crucial component to its commercial viability and ultimate utility of these trees to the green industry. We will our work with urban foresters, arborists, and private owners from throughout the state of Minnesota to identify elms that appear to be resistant (selecting trees that have survived in areas where most if not all the elms have been killed).

Screen selected trees. To determine if trees are resistant, rigorous testing is needed. This is done by injecting replicated blocks of trees with the fungus in greenhouse studies followed by additional inoculation studies in the field on older trees. The preliminary data shows that elms resistant to Dutch elm disease exist in Minnesota. Our testing will determine which trees are resistant and with state wide screening we expect to find a large number of them. Several different strains of the fungus that causes Dutch elm disease, *Ophiostoma novo-ulmi,* have been obtained from diseased trees in Minnesota. These isolates have been shown to be very pathogenic to susceptible American elms in previous experiments. Three strains will be used in preparing the inoculum for these experiments. The strains have been collected from different trees dead/dying from Dutch elm disease in the metro area and are representative strains that ensure aggressive disease inoculum. Inoculum will be prepared by growing the fungus in a minimal liquid media and shaking for several days to induce the yeast-like stage growth form. Following the shaking period, 100 µl of the spore suspension, having a spore concentration of approximately 1.0×10^5 per mL, is pipetted into a small (2.4 mm dia.) hole drilled into the stem of the seedlings and wrapped with parafilm. Holes are placed in the main stem of seedlings 10 cm up from the soil. Controls will be inoculated with sterile water. We anticipate having nine experimental units of each genotype divided into three blocks with three replicates for each block. After inoculation, seedlings are evaluated every 4 weeks using a 0 - 4 rating scale based on the percentage of the crown that showed wilt symptoms (0 = no symptoms to 4 = complete wilt). Tree height and stem diameter are also recorded at this time and will used for final statistical analysis.

Field testing of elms. Our goal is to obtain a diverse selection of elms from Minnesota that are genetically different but all have resistance. Field testing for resistance in elms is essential and will be done on trees from our preliminary research as well as new selections made from this project. Field testing will also be used to identify trees with the best growth and hardiness characteristics.

Clones of elms that survive the greenhouse inoculation trials will be planted in the tree nursery located on the St Paul Campus of the University of Minnesota in nine experimental units of each genotype divided into three blocks with three replicates for each block. Seedlings will be allowed to become established and will be inoculated with the Dutch elm disease pathogen, *Ophiostoma novo-ulmi*. The Inoculum will be prepared in the same way that it was for greenhouse treatments by growing the fungus in a minimal liquid media and shaking for several days to induce the yeast-like stage growth form. Following the shaking period, 100 µl of the spore suspension, having a spore concentration of approximately 1.0×10^5, will be pipetted into a small (2.4mm dia.) hole drilled into the main stems of the trees and wrapped with parafilm. Holes are to be placed approximately 30 cm below the bottom of the crown (the first major branches with leaves). Controls will also be inoculated with just sterile water. Susceptible elms will also be inoculated for comparison. Seedlings will be evaluated at 4 and 8 weeks post inoculation and after 12 to 15 months. Trees will be scored on a percentage scale according to overall crown wilt. Wilt scores are based on a 0 to 4 point scale: 0 = no visible wilt, 1 = 1 to 25%, 2 = 26 to 50%, 3 = 51 to 75%, and 4 = 76 to 100%. Trees that were fully wilted (100% wilt) received a wilt score of 4 and were also noted as “fully wilted” in a separate binary score (0 or 1). Additionally, trees will be assessed for mortality and recovery 12 to 15 months after inoculation.

Study defense mechanisms in resistant trees. What makes an elm resistant to disease? This complicated process is not fully understood, however we do know that chemical and physical barriers are produced by trees to stop invading pathogens (Rioux and Ouellette 1991; Martín et al. 2005a; Martín et al. 2005b; Martín et al. 2007). Although the funds allocated from the LCCMR were less than requested and not sufficient to carry out this basic research, we hope to use other funds from the University of Minnesota to provide a fellowship for a graduate student to take part in this work. This research will identify the various mechanisms in operation by selected resistant elms. Once this is known, these characteristics can be looked for in new selections resulting in a more rapid method of screening trees for resistance. The mechanisms of resistance in American elms to DED are not well understood and investigations are needed. It is clear that resistance is being observed in certain trees and information from prior research suggests a number of factors play a role in resistance. However, a rapid genetic screening method of determining disease-resistance would be very valuable to future work. One method that has shown promise is using acid fuchsin to stain functional xylem tissue. With this method, sections of stem from inoculated seedlings are placed in a 1% stain solution. As the solution is drawn up through the sample, xylem tissue that is still functional (able to conduct water) will stain red. This was used with several samples and showed distinct differences between susceptible and non-susceptible elm selections tested. We hope to use this method to quantify differences between field inoculated selections and relate those findings to resistance. Also, this will aid in identifying functional and non-functional tissue for histological examination. Once these areas are identified comparisons can be made between susceptible and resistant selections to better understand and document morphological and chemical mechanisms that promote resistance. Staining, resulting from a natural host defense mechanism, will also be assessed using water conductivity to determine if any correlation can be made to disease severity.

Recent research has described elms in the United States as a polyploidy complex that ranges from diploid to tetraploid (Whittemore and Olsen 2011). It is not clear how ploidy contributes to resistance, although one resistant clone, "Jefferson" is triploid. The ploidy range of elms in Minnesota is unknown. By determining the ploidy of the trees that are currently being tested and have shown resistance to DED, correlations can be made as to the significance of ploidy as it relates to DED resistance. In addition to our own elm selections currently being tested, other previously released elms like "Valley Forge" have unknown ploidy. This information would be useful for comparison. If a positive correlation is made between ploidy and resistance, this could be a rapid screening test for newly identified putatively resistant trees. The ploidy of plants can be determined by cytological methods, where chromosomes are stained, visualized and counted using microscopy. There is also evidence that ploidy can be inferred by the size of pollen. Initial results show that elm chromosomes are small, but are distinguishable. We would continue this work to determine the ploidy using cytological methods. In addition, pollen size will also be determined and tested for ploidy determination.

References:

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5. **Results and Deliverables**

Survey and identify resistant elm trees. Trees from different regions of Minnesota that have possible resistance to Dutch elm disease will be located and cuttings made. These cuttings will be propagated at the University of Minnesota and clones grown for disease screening experiments. We are expecting that up to 15 trees will be selected the first year and 10 trees selected in the second year.

Screen selected trees. Selected trees will be inoculated in the greenhouse and screened for resistance to Dutch elm disease. Clones of these resistant trees will be propagated, grown in the greenhouse and prepared for planting in replicated field trials.

Field testing of elms. Trees that have been found to resist the inoculations in the greenhouse will be further tested in the field. After becoming established, trees will be inoculated under field conditions. Trees found resistant in these field trials will be available for out planting and propagation by the nursery industry in Minnesota.

Study defense mechanisms in resistant trees. The studies undertaken on trees selected with resistance will provide new information on the mechanisms of tree defenses that elms use to resist infection. This basic information should prove valuable information to develop more rapid screening procedures for finding additional trees with resistance in Minnesota and other regions of the United States. This work depends on obtaining additional funding to that allocated by the LCCMR.

6. Timetable

Year 1 and 2- Survey and obtain resistant elms and propagate selected elms for resistance testing. The first set of trees will be ready for testing by September 2014. Additional trees will be added to the program as they are ready.

Years 2 and 3- Screen selected elms for resistance in greenhouse trials. The first screenings will be done by March 2015.

Years 2 and 3- Increase clones of elms surviving greenhouse tests and out plant. The first trees will be ready for out planting by June 2015.

Year 3- Begin disease testing in the field and monitoring growth characteristics of resistant trees.

7. Budget

Budget Category	$ Amount	Explanation
Personnel: Scientist - pathology Scientist – horticulture Undergraduate students (3)	 $ 92,000 $ 48,000 $ 40,000	 50% time 71% salary, 29% fringe, for 3 years) 25% time (71% salary, 29% fringe, for 3 years) 100% salary, $5000/year per student. Three students will be involved with the project during years 1 and 2 and two students involved in year 3.
Professional/Technical/Service Contracts:	$0	
Equipment/Tools/Supplies: Greenhouse and field supplies Laboratory Supplies	 $6,500 $6,500	 pots, stakes, pruning supplies, fertilizers, container substrates. Supplies are for 3 years. microbiology and inoculation materials, general laboratory materials, fungal genotyping, growth hormone for cuttings, propagation supplies. Supplies are for 3 years.
Capital Expenditures over $3,500:	$0	
Fee Title Acquisition:	$0	
Easement Acquisition:	$0	
Easement – Long-term Monitoring, Management, and Enforcement	$0	
Professional Services for Acquisition:	$0	
Printing:	$0	
Travel Expenses in MN:	$4,500	Survey and collection of Resistant Elms
Other: Greenhouse maintenance costs	 $2500	 Maintenance fees $1000 per year for 2 years
TOTAL ENRTF BUDGET:	**$200,000**	

Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
Minnesota Turf and Grounds Foundation	$15,000	$	These funds will be applied for in 2013 and are expected but we have

			not been granted these funds yet.
State			
University of Minnesota University of Minnesota	$41900 $36900	$	Blanchette (co-PI) salary and fringe for one month per year for 3 years Gillman (co-PI) salary and fringe for one month per year for 3 years. These funds will be allocated for this project.
TOTAL OTHER FUNDS:	**$93,800**	**$**	

8. Credentials

Dr. Robert Blanchette (Co-PI) is a professor in the Department of Plant Pathology. He has been involved with research and teaching of forest and landscape trees at the University for 30 years. His research accomplishments include 2 books, over 200 publications, 14 US Patents and numerous foreign patents. He has received several honors for research accomplishments including Fellow of the American Association for the Advancement of Science, Fellow of the American Phytopathological Society, Fellow of the International Academy of Wood Science, Hans Merensky Fellow for Wood Science and Distinguished Service Award from the American Society of Microbiology. He teaches undergraduate and graduate classes at the University of Minnesota on forest and shade tree diseases. Research interests are in the area of forest pathology and wood microbiology with research in tree defense mechanisms, deterioration processes of wood and biotechnological uses of forest fungi. Projects involve novel, interdisciplinary approaches to solving tree disease problems and understanding the biology and ecology of forest microbes. In 2011, a selection of white pine that had been identified by his research group with disease resistance to white pine blister rust was released by the University of Minnesota.

Dr. Jeff Gillman (Co-PI) is an Associate Professor in the Department of Horticultural Science where he researches tree growth and transplanting. He holds a Ph.D. in horticulture and a master's degree in entomology. He is the author of five books and numerous scientific papers on a variety of tree and shrub related topics. He is also co-author of the book Pruning Young Elms by Chad Giblin. He teaches courses on plant propagation and plant production. He directs the University research nursery (TRE nursery) located on the St. Paul campus where research focuses on improved nursery practices for maximizing growth and improving long term tree health in the urban environment takes place. He is also a member of the University of Minnesota's Urban Forestry & Horticulture Research Institute.

The Department of Plant Pathology and the Department Horticultural Science are in the College of Food, Agricultural and Natural Resource Sciences at the University of Minnesota St. Paul Campus. Modern research laboratories are available for this work and a 10 acre research field site on Campus can be used for the field trials proposed for this project. Professor Blanchette and Gillman will take an active part in this research and their salaries will be paid by the University of Minnesota. All equipment needed for this work is available in the PI's laboratories.

9. Dissemination and Use

Results will be published in general articles for the public as well as scientific journal articles. Presentations will be given at field days, workshops and other gatherings of the horticultural and arboricultural communities.