PCR vs. Isolation and the War Against Oak Wilt

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ABSTRACT

Bretziella fagacearum is a devastating disease. In no place in the United States are the losses of oaks in both quantity and diversity worse than in Central Texas. Current management strategies employed in Texas have not proven extremely effective. There is a desperate need to advance every tool possible that can assist those of us who dedicate our lives to the care of oaks. Recently, DNA analysis using polymerase chain reaction (PCR) was proven to be the best standard laboratory protocol in oak-wilt diagnostics (Yang and Juzwik 2017). Specifically, two PCR methods of testing for oak wilt have proven more consistent in finding the oak-wilt pathogen in various species within subgenus *Quercus* section *Quercus* as well as in section *Lobatae* (though in this section the results are equivocal). By extension, a reasonable expectation is that this method could more effectively diagnose oak wilt accurately from samples throughout the family Fagaceae. One of the unique and most useful aspects of this method is its ability to detect the fungus in wood samples that are as much as one year old. The fact is this fungus doesn't stand a chance under the scrutiny of PCR.

Keywords: *Bretziella fagacearum*, *Ceratocystis fagacearum*, polymerase chain reaction

Introduction

Photo 1/ *Quercus fusiformis* trees dying from oak wilt.

In the heart of Texas it is not hard to become a quercophile whether you live here or are just passing through. The Hill Country region, in the southeast part of the Edwards Plateau of Central Texas, is my home as well as the home of many *Quercus* species – *Q. fusiformis*, *Q. buckleyi*, *Q. laceyi*, and *Q. sinuata* to name but a few. As an ISA Board Certified Master Arborist (TX-3347B), it is my distinct privilege to allocate much of my time to the care of this beloved genus that plays such a vital role to the people, the animals, and the Edwards Plateau ecosystem in general.

There is a daunting foe laying waste to our beautiful oaks by the millions – and so I find myself in the middle of a losing battle with this "killer of beech", *Bretziella fagacearum* (the new name for *Ceratocystis fagacearum*). We are not only losing this battle miserably with our most prevalent oak species – *Q. fusiformis* and *Q. virginiana* (section *Virentes*) and *Q. buckleyi* (section *Lobatae*), but also with our White Oak species (section *Quercus*) (Denk et al., 2017). For numerous reasons, the indigenous White Oak species (e.g., *Q. laceyi*, *Q. sinuata*, *Q. muehlenbergii*, *Q. polymorpha*, *Q. macrocarpa*) were thought to have a high tolerance and therefore that we wouldn't have to defend them aggressively from this fungus. This assumption has been proven wrong. In the decades-long absence of inoculation research trials, we have had to resort to field observation and lab testing to help ascertain what the actual tolerance level of these White Oaks is. It is this lab testing that I look further into here.

The primary public agencies in Texas leading the fight against this disease are the Texas Forest Service and the Texas AgriLife Extension Service who recommend using the latter's Texas Plant Disease Diagnostic Laboratory for oak-wilt diagnosis. This lab uses the traditional, standard isolation and culture method to diagnose the disease.[1] For a commercial, consulting arborist the need to send comparatively sizeable wood samples far away in a cooler with ice and then wait anywhere from 3-5 weeks for the results is not an ideal proposition economically, nor does it ensure expedient care of trees in dire need of immediate therapeutic treatment and likely implementation of a complex oak-wilt infection-center management plan.

On the recommendation of an exceptional peer, Jerry Pulley,[2] I researched the PCR

1. This method involves isolating the pathogen from infected tree tissue to obtain pure cultures.
2. American Society of Consulting Arborists, Registered Consulting Arborist #329.

methods (both nested and real-time[3]) of diagnostics vs. culture isolation and specifically the PCR real-time quantitative method used by Research Associates Laboratory (Yang and Juzwik 2017; Luley and Lytle 2018). Both of these papers clearly demonstrate the benefits of the latter; it is therefore surprising to me that it is not recommended by public agencies.

What benefits do the PCR approaches offer?[4]

1. Superior results for all species in finding oak wilt even where only small levels of the pathogen are present. Real-time PCR, also known as quantitative PCR (qPCR), can even specify the amount of fungus present in the sample by determining the cycle threshold value (Ct value)[5] even in compromised samples from which little DNA can be extracted, thus safeguarding against false positives.

2. PCR is considerably more effective diagnosing the disease in White Oak species. In my professional assessment, the limitations of the traditional isolation method have likely played a considerable part in the unfortunate historical position of the Texas Forest Service, to wit, that White Oak species are all more resistant or less succeptible than, say, section *Virentes* oaks.

Photo 2/ Oak-wilt resistant *Quercus polymorpha*.

3. It is effective on dried-out samples as much as one year old.

4. Secondary microorganisms don't compromise the ability to detect the pathogen DNA.

5. From personal experience working with Dr. Chad Lytle, Director of Research Associates Laboratory (RAL) in Allen, Texas, results are communicated in most instances the day the samples are received by the lab or, at most, within 3 days for nested PCR analysis results versus 3-5 weeks with the traditional method.

6. Samples can be reused for further testing at a later time

7. Small samples (e.g., wood-shavings rather than huge wood chunks of wood) are all that is needed.

8. No blue ice or dry ice, or indeed any kind of ice or cooler, is needed. Only a mailing

3. Nested PCR is a technique that reduces nonspecific amplification of the DNA template. Real-time PCR is a method used for the identification of specific, amplified DNA fragments by analyzing their melting temperature.

4. For more details, see the author's website: https://arborcareandconsulting.com/oak-wilt/oak-wilt-diagnostics

5. The cycle threshold (Ct) value of a reaction is defined as the cycle number at which the fluorescenece of a PCR product can be detected above the background signal.

Photo 3/ *Quercus laceyi* "on fire" with oak wilt.

envelope is needed.

9. Real-time PCR techniques offer more plant health test options than the traditional methods.[6] This has huge implications for all green industry diagnostic needs well beyond oak wilt in that any disease type, whatever the origin or pathogen, can be diagnosed.

10. PCR analysis is evolving and improving all the time while the traditional method is "stuck" in its ways without any prospect of improving speed of analysis or reducing the labor required.

11. Cost for DNA testing is much lower: $20 vs. $35 (respectively, RAL and Agrilife published charges, August 2019). Though cost is of course an important consideration, due to what's at stake, I put this benefit last because the information is so critical and costs of waiting potentially so very high.

Discussion

The fact that the traditional culture isolation method is incapable of consistent, accurate diagnostics of oak wilt in White Oak species is quite troubling. It is not a far stretch of the imagination to presume that this deficiency has a significant correlation with the often-expressed idea that White Oaks rarely die from oak wilt. With recourse to the real-time PCR method, I am now capable of determining that infection centers of both pure stands of White Oaks and mixed stands exhibit high mortality rates due to this disease.

Though it is both true and unfortunate that this method of diagnostics has revealed that many more oaks have succumbed or are succumbing to infection (and likely mortality) it is imperative that all Texans know this.[7] Adjustments in the Texas Forest Service position on the tolerance of Texas White Oaks, more indicative of the reality of the situation, were made in mid-November 2019.[8] Following a formal meeting I had with the directors of the Texas Oak Wilt Suppression Project,[9] I believe that efforts in this direction will continue and that an adequate communication strategy will be developed.

Consistent, repetitive symptomology of oak wilt in White Oak species in both mixed and pure stands would seem to indicate that disease transmission by intersectional root grafting is more than a reasonable conclusion. The absence of more research on various aspects of grafting is quite unfortunate. In much of Central Texas the shallow soil depth,

6. Personal experience working with Dr. Chad Lytle, Director of Research Associates Laboratory, Allen, Texas.
7. For more details, see https://arborcareandconsulting.com/oak-wilt/oak-wilt-resistant
8. For more details see www.texasoakwilt.org
9. With funding from the US Forest Service, Texas A&M Forest Service initiated a Cooperative Oak Wil Suppression Project in Central Texas in 1988.

Photo 4/ *Quercus muehlenbergii* leaves with oak wilt symptoms.

with horizons just above the bedrock, support the hypothesis that the roots grow in extremely close proximity, with trees growing in clumps.[10] It is my hope that the use of PCR testing will shed enough light upon this particular issue to spur research projects regarding root grafting of oaks in Central Texas. The dual-infection experiences of Guy Sternberg at the Starhill Forest Arboretum may very well call into question much of the presumed infrequency of grafting between oak species in general (Sternberg 2009, 2019).

My results of PCR testing for oak wilt in *Q. sinuata*, *Q. laceyi*, *Q. stellata*, *Q. muehlenbergii*, *Q. polymorpha*, and *Q. macrocarpa* have, I believe, contributed significantly to the Texas Forest Service's change in position on White Oak species' tolerance to oak wilt. PCR testing has enabled me to hone my understanding of the symptomology of this disease in section *Quercus* oaks – whether they have been treated or not.[11] For clients who wish to save an infected tree with an effective long-term therapeutic management plan that involves fungicide treatment, PCR testing provides prompt and reliable results for the rapid elaboration of such a plan. As regards containment options (i.e., trenching with herbicide or herbicide alone), from the above discussion it would seem clear that any such decision must take into account the below-ground vectors (root grafting), considering all oak species as possible suspects in the transmittal of the disease. Failure to do so would be a very costly oversight: in my experience, re-grafting of roots is a given and, indeed, a subject worthy of an article on its own. PCR testing will help to tighten up both of these containment approaches in

10. For more details see https://arborcareandconsulting.com/oak-wilt/how-does-oak-wilt-spread
11. Fungicide treatment saves the tree from death, but does not prevent infection. Symptoms of treated and untreated trees are different.

order to avoid the dreaded “breakout” events or, worse, the unchecked progression of this all-consuming fire.

Finally, I utilize the real-time PCR method frequently to determine the cause of death of a tree. The ability to analyze samples of dead wood is one of the astounding benefits of this technique.

Conclusion

Real-time PCR used for plant pathology is only in its infancy. It is possible that the entire green industry will soon use this method regularly throughout the spectrum of integrated pest management (IPM) services. Likely, the primers will be improved, the number of service providers will increase, and prices may possibly decrease. All of this will enhance the abilities of those committed to serving as “tree herders”[12] as well as of those fulfilling the vocation of plant caretakers.

Photographer. Title page: Kevin Belter (*Quercus muehelenbergii* with symptoms of oak wilt). Photos 1-4: Kevin Belter.

Works cited

Denk, T., G.W. Grimm, P.S. Manos, M. Deng, and A.L. Hipp. 2017. An updated infrageneric classification of the oaks: review of previous taxonomic schemes and synthesis of evolutionary patterns. In *Oaks Physiological Ecology. Exploring the Functional Diversity of Genus Quercus L.* Cham, Switzerland : Springer International Publishing AG.

Luley, C.J., and C. Lytle. 2018. Landscape Diagnostics : DNA and RNA Testing for Landscape Pests. *The Arboriculture Consultant* 51(3) : 3-6.

Sternberg, G. 2009. Oak Wilt Rears Its Ugly Head. *Oak News & Notes* 13(2).

Sternberg, G. 2019. Managing Oak Wilt. *International Oaks* 30: 209-216.

Yang, A., and J. Juzwik. 2017. Use of Nested and Real-Time PCR for the Detection of *Ceratocystis fagacearum* in the Sapwood of Diseased Oak Species in Minnesota. *Plant Disease* 101(3): 480-486.

12. Reference to characters (Ents) in J.R.R. Tolkien’s *Lord of the Rings* trilogy as well as a common theme in his other writings and undeniable personal commitments.