PUTTING A NEW SPIN ON AN OLD DISEASE: USING MOLECULAR TOOLS TO IDENTIFY BEETLES CARRYING SPORES FROM THE OAK WILT FUNGUS IN GILLESPIE COUNTY, TEXAS, USA

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PURPOSE AND SCOPE

Oak wilt fungus, *Bretziella fagacearum* (Bf), affects oak populations in the Midwestern and Northeastern United States. Red oaks (*Quercus* subgenus *Erythrobalanus*) are most impacted by oak wilt, with mature trees declining and dying within one season. Bf also occurs in central Texas, infesting and killing live oaks (*Q. virginiana*), spreading through root grafts. Red oak species, such as Spanish oak (*Q. texana)* and blackjack oak (*Q. marilandica*), may form mycelial mats that attract nitidulid beetles (Coleoptera: Nitidulidae) that serve as vectors to aboveground spread across longer distances. Previous studies have identified nitidulid beetles associated with oak wilt infested areas (Appel et al. 1986), including *Cryptarcha concinna* and *Colopterus maculatus*. In prior work, beetles were assayed for Bf spores by crushing and growing fungi on culture (Appel et al. 1990). This method required time and space to grow identifiable cultures. Recent advances in pathogen testing by using molecular detection and improved protocols for quantitative polymerase chain reaction (qPCR) have decreased time requirements and increased diagnostic sensitivity. Our lab has developed Bf-specific simple sequence repeat (SSR) markers, which, when combined with a TaqMan probe, can be used with qPCR to screen beetles quickly for presence of fungal DNA.

METHODS AND APPROACH

Wind-orienting fermentation and lure-baited traps were deployed in an oak wilt-infested, wooded landscape in Gillespie County, TX from March 2022 to April 2024. Beetles were removed at 2-week intervals from traps, then frozen and shipped to The University of Tennessee, Knoxville. Thawed specimens were visually identified to species based on morphology. By date and trap, species

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were refrozen in vials until DNA was extracted. Individual beetles were placed in lysis buffer and sonicated to dislodge fungal structures from the beetle body surface. Extracted DNA was assayed using novel Bf-specific SSR markers with a TaqMan probe developed in our lab using qPCR. Because of small amounts of recovered pathogen DNA and high fungal species specificity enabled by the SSR marker, beetles were considered positive for the fungus if at least one of three replications amplified.

FINDINGS AND IMPLICATIONS

Screening beetles in advance of finding mycelial mats in a forest or urban landscape can give managers and integrated pest management scouts advance warning that Bf inoculum is present. Our study site had been heavily impacted by oak wilt, so we expected to find evidence of fungal spores associated with bodies of sap beetle species. Eleven different sap beetle species and one bark beetle were captured in baited wind traps. Across seasons, the most abundant beetles were *Carpophilus mutilatus* followed by *Colopterus maculatus, Cryptarcha concinna*, and *Carpophilus sayi* and a few *Colopterus truncatus*. Beetles were captured from March through May, and then from October through November. Similarly, Appel et al. (1986) found peak flight times of sap beetles in Texas occurring from February through June with little or no beetles captured from November through January.

From the more than 300 body wash samples from sap beetles that we have assayed over 2 years, six species tested positive for Bf DNA presence (table 1). Most beetles were captured from March through May 2022, which corresponds with expected timing of mycelial mat formation in Texas (Appel et al. 1987). So far, no beetle species have tested positive in 2023 or 2024, even during expected peak mat formation. These negative results could indicate that new mats did not form on oaks in the area during the past 2 years. Consequently, in years when peak flight times of sap beetle species correspond with mycelial mat formation, sap beetles are most likely to associate with Bf inoculum that may be transmitted to new oak trees.

Table 1—Sap beetle species (Colopterus: Nitidulidae) that were tested for *Bretziella fagacearum* DNA

Sap beetle species	Positive qPCR results			Total beetles	Collection dates from positive results
	3 of 3	2 of 3	1 of 3		
Carpophilus corticinus	0	1	0	7	Apr. 5, 2022
Carpophilus mutilatus	2	6	16	207	Apr. 5, 2022; Apr. 21, 2022; May 5, 2022; July 27, 2022; Nov. 2, 2022
Carpophilus sayi	2	2	2	22	Apr. 5, 2022; Apr. 21, 2022
Colopterus maculatus	0	1	0	36	Apr. 5, 2022
Colopterus truncatus	3	1	1	17	Apr. 5, 2022
Cryptarcha concinna	2	0	1	26	Apr. 5, 2022
Other species[1]	0	0	0	11	
Total	9	11	20	326	

Samples were tested in triplicate and considered positive if the base threshold was reached.

[1] includes specimens of *Carpophilus hemipterus*, *Colopterus unicolor, Cryptarcha strigulata*, *Lobiopa undulata*, and *Phenolia grossa*, as well as a *Xylobiops basalaris* bark beetle (Coleoptera: Bostrichidae).

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