An enhanced strategy for molecular detection of thousand cankers disease

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An enhanced strategy for molecular detection of thousand cankers disease
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Juglans nigra (black walnut) is native throughout the eastern U.S. and used in the western U.S. as an ornamental plant and rootstock. The current estimated value of standing Juglans nigra timber exceeds $539 billion dollars (USD) across its native range. All members of the Juglans genus, as well as some Pterocarya spp., are threatened by Thousand Cankers Disease (TCD). This disease complex includes the fungal pathogen, Geosmithia morbida, the primary insect vector, Pityophthorus juglandis, and susceptible host trees. We have used microsatellite loci in a rapid molecular detection protocol that confirms presence of either pathogen or vector DNA in suspect samples. However, the existing protocol requires expensive specialized equipment and technically trained personnel. Our goal was to optimize and simplify the existing molecular detection protocol to enable faster adoption by end-users. The modifications will allow easier visualization of positive samples by utilizing a conventional gel protocol, thus reducing the equipment cost by about $36,000. Results presented here demonstrate successful amplification of G. morbida and P. juglandis DNA from drilled bark shaving samples and bark sample archetypes. Assays were also performed to determine the sensitivity of the new conventional gel protocol, yielding detectability for positive identification with very low amounts of target DNA; the equivalent of about 6 conidia or hyphal cells. State and federal regulators and disease diagnosticians will be able to confirm disease presence in suspect samples in less than 12 hours greatly enhancing the ability for preventing further spread of TCD.