# Identification of invasive shot hole borers by targeting fungal DNA in field samples

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## Background:

Invasive ambrosia beetles were discovered in Los Angeles County[1] (2012) and San Diego County[2] (2014) causing damage a wide host range of California wildland trees, as well as urban landscape trees and to an agriculturally important commodity (Avocado). The two distinct ambrosia beetles named Polyphagous shot hole borer[1] (PSHB) and Kuroshio shot hole borer[3] (KSHB) from Southeast Asia vector unique symbiotic fungi that are plant pathogens on multiple hosts and cause Fusarium Dieback (FD). The fungi are cultivated by the beetles within the host and block nutrient transport systems while supporting beetle development[1]. Invaded areas include Los Angeles, Orange, Riverside, San Bernardino, Santa Barbara, San Diego, San Luis Obispo, and Ventura counties. The spread of their pathogenic fungi ultimately results in dieback, and tree mortality in severe cases. They can attack and cause severe damage on at least 64 trees species where the beetles can reproduce and spread to other areas[1].

## Novel Detection Method:

The invasive beetles are difficult to distinguish by morphology and the fungi they vector are similarly indistinguishable in culture. However, real time polymerase chain reaction (qPCR) technology allows for distinguishing the fungi the beetles vector by targeting unique fungal DNA signatures within wood, fungal, and beetle samples. These samples are not reliant on the beetle being present, which allows for identification of the invasive pest is present in a suspect area.

## Survey in Orange, Riverside, and San Diego Counties:

Results from our field studies from attacked sycamore, cottonwood, and willow across Orange, Riverside, and San Diego Counties found PSHB and/or KSHB in 17 out of 26 city parks in surveyed areas. Fungi from both invasive beetles were found to be present within a 4 square mile area within three parks surveyed in Orange County and San Diego County (Figure 1).

## Outlook:

The novel method is currently being utilized for early detection of the pest when suspicious attacks resembling invasive ambrosia beetles are observed and sampled. In addition, the movement of the two invasive beetles and their pathogenic fungi through southern California can be monitored using this targeted detection method. Samples of trees that appear to be infested should be reported to county Agriculture Departments and CDFA Plant Pest Diagnostic Center and UCCE Farm Advisors office for confirmation of the invasive pests through detection of their fungi.


Unknown sample

DNA extraction and qPCR

BIORAD® CFX96

Analyze and identify fungi


PSHB

or

KSHB

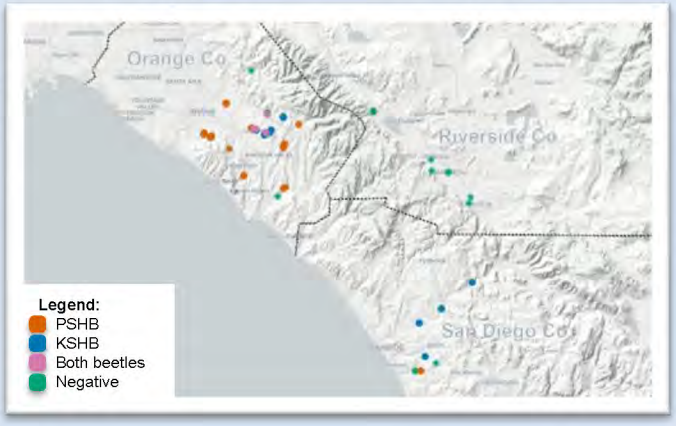


**Figure 1.** Results from surveys in Orange, Riverside and San Diego Counties using qPCR technology.

**References:**
[1]Eskalen, A., Stouthamer, R., Lynch, S.C., Rugman-Jones, P.F., Twizeyimana, M., Gonzalez, A., Thibault, T., 2013. Host Range of Fusarium Dieback and Its Ambrosia Beetle (Coleoptera: Scolytinae) Vector in Southern California. Plant. Dis. 97, 938–951.
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[3]Stouthamer, R., Rugman-Jones, P., Thu, P.Q., Eskalen, A., Thibault, T., Hulcr, J., Wang, L.J., Jordal, B.H., Chen, C.Y., Cooperband, M., Lin, C.S., 2017. Tracing the origin of a cryptic invader: phylogeography of the E uwallacea fornicatus (Coleoptera: Curculionidae: Scolytinae) species complex. Agri. Forest. Entomol. 19, 366-375.