Topics in Subtropics Newsletter
University of California Cooperative Extension
Fresno, Kern, Madera, Riverside, San Bernardino, San Diego, San Luis Obispo, Santa Barbara, Tulare, & Ventura Counties
News from the Subtropical Tree Crop Farm Advisors in California

Volume 14 No. 4 June 2016

Water-Based Latex Paint as a Means to Track Ambrosia Beetle Activity on Infested Trees

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Ambrosia beetles are known to cultivate their symbiotic fungi as a nutrient source inside the various woody hosts they bore into and colonize. In their natural tropical environment, the beetles serve an ecological purpose by accelerating degradation of decaying wood. However, when introduced to a non-native environment, the beetles can cause significant damage in living hosts (Hulcr and Dunn, 2011). Since 2012, in southern California, a disease called Fusarium Dieback has been causing significant damage to landscape trees, native tree species, and agriculturally important crops, such as avocado (Eskalen et al., 2013). This disease is the result of activity from two closely related invasive ambrosia beetles and their associated mutualistic fungi. These two Shot-Hole Borers (SHB) are morphologically indistinguishable, but have been found to be genetically distinct along with the fungal symbionts they cultivate. Currently there are limited options to treat affected trees which too often result in complete removal of infested trees.

Figure 2: Shows a female shot-hole borer blocking the entry hole with her abdomen (A) and in cross section (B).

To establish inside a host, a single female beetle bores through the bark to initiate gallery construction in the wood. Soon after the initial attack, the female beetle inoculates the wall of the galleries with fungal spores of their associated symbiotic fungi (*Fusarium* spp., *Graphium* spp., *Paracremonium pembeum*) which they use as a food source (Lynch et al., 2016, Freeman et al., 2015). After the female has completed constructing and inoculating the gallery, and laying eggs, the female beetles were observed blocking the entry point of the gallery with their abdomen. If the entry hole is obstructed by a foreign object, then the beetles will clear the entrance of the obstruction and continue to block the entry point of the gallery with their abdomen which indicates that the beetle is alive and active. However, upon close inspection of individual entry holes, it is apparent that beetles are not present or active in *all* entry holes inspected. In this study we took advantage of the blocking behavior of the beetle to determine if galleries are active in order to determine the efficacy of pesticides applied to affected trees in an attempt to manage this pest.

Figure 3: A-D shows the process of counting the initial area for beetle attacks (A), applying water-based latex paint with brush (B), All the holes covered by the paint

We are currently testing various pesticides along with evaluation methods to accurately assess the efficacy of applied treatments, with the ultimate goal of managing these pests and their symbiotic fungi. When conducting field trials to investigate pesticide efficacy on reducing SHB activity, it can be difficult to determine which galleries are active or inactive. Close inspection can help determine if a given entry hole is active, but in heavily infested trees there can be over 100 entry holes to inspect in a square foot, which can be time consuming and increase the possibility of miscounting. To easily distinguish between whether the beetle is alive or dead in the gallery, we have developed a method using water-based latex paint to cover a designated area on an infested tree and compare activity between areas within the same tree and also between different trees. This method is particularly useful to compare pesticide treatments from one treated tree to another in order to quantify the efficacy of experimental treatments on SHB activity.

Figure 4: Progression of beetle reemergence after paint application. After paint application has begun to dry (A), the female beetle will begin to re-establish the entry point with her abdomen (B), break through the paint obstruction (C), and continue to maintain the gallery (D).

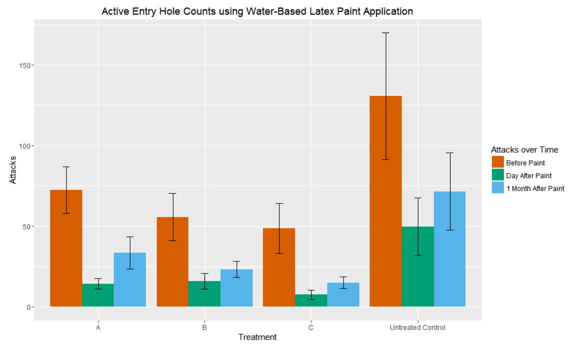

Figure 1: Counts of SHB attacks over a one-month period treated with various applied chemicals after application of water-based latex paint.

The method was as follows: An 8" x 11.5" (size of an A4 paper sheet) area was outlined in vertical orientation with a paint marker at each cardinal direction, on the trunk, at a consistent height, for a total of four replications on each tree per treatment. Before painting the marked area, entry holes were counted. After initial counting, the off-white water based latex paint was applied with a paint brush. The painted areas were revisited and counted again the next day to look for visible entry holes; the areas that have holes visible after the paint application has dried are indicative of active entry holes (Figure 2D). These painted areas were revisited the following month to track the activity. If new entry holes were visible on the painted area, this indicates an active beetle gallery. In our trial, we compared three chemical treatments to an untreated control to observe effects on beetle activity. In Figure 1, there is a significant decrease in SHB attacks from "Before Paint" to "Day After Paint" since all holes counted initially are not truly active. Treatments B and C were shown to have a statistically significant lower increase in active entry holes within a month's time when compared to treatment A and the untreated control. This method is a useful evaluation method to monitor beetle activity as a response over time to applied treatments in field trials.

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