BOXWOOD (*Buxus sempervirens 'common'*)

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Quantification of flutriafol in boxwood leaves after drench application, 2024.

In previous experiments that tested the efficacy of multiple triazole fungicides (flutriafol, tebuconazole, and prothioconozole) to manage boxwood blight, flutriafol was found to be systemic and the most efficacious (Sacher PhD thesis, 2023). Therefore, we continued our study of this active ingredient, designing a trial to test how long flutriafol remains detectable and/or quantifiable within leaf tissue following a single drench application of TopGuard. In this report, we describe the results from a 36-week long study that provides meaningful insight into the longevity of this fungicide within treated boxwood plants, providing further implications for managing boxwood blight.

Methods:

TopGuard (flutriafol, FRAC 3) fungicide and a no-fungicide water control were applied to small *Buxus sempervirens* 'common' plants grown in 1-gallon pots on 20 Nov 2023. Each fungicide treatment was applied directly to the media (Sungro Metro-Mix 840) as a soil drench on 3 replicate plants per leaf collection time point. Drench treatments consisted of 100 µl of formulated fungicide diluted in 40 ml of tap water (equivalent to 32 fl oz fungicide/100 gal water), or 40 ml of water for the no-fungicide control. Pot media volume averaged 170 in^3. Treatments were arranged in a randomized complete block design with pots spaced 4 inches apart. Plants were placed on saucers to catch solution run-through and watering was done by hand to minimize splash between neighboring plants.

The first leaf collection time point was 48 hours post fungicide application (PFA). At 48 hr, three leaves were collected from each of three replicate plants in the fungicide treatment and from each of three replicate plants in the no-fungicide control. One leaf was collected from the lower third, one from the middle third, and the final leaf from the upper third of each plant. The three leaves were bulked per each replicate plant and homogenized as one sample during later steps in the extraction process. Leaves were frozen immediately in a -65 °C freezer for later analysis. Leaf collection was repeated at an additional 18 time points PFA: 1 wk, 2 wk, 3 wk, 4 wk, 6 wk, 8 wk, 10 wk, 12 wk, 14 wk, 16 wk, 20 wk, 22 wk, 26 wk, 28 wk, 30 wk, 32 wk, 34 wk, and 36 wk. Plants were 4 x 3 inches at the start of the experiment and 7.5 x 5 inches at the end with a flush of growth in early spring from 16 to 18 weeks PFA.

Leaves were homogenized via bead beating using methanol as the suspension medium. Leaf weight was quantified such that later fungicide quantification could be normalized by the amount of leaf tissue analyzed. Homogenized samples were then analyzed for flutriafol content via mass spectrometry/liquid chromatography (LC/MS) at the Oregon State University Mass Spectrometry Center. Methods were developed based on reagent grade standards of the fungicide molecule. The limit of detection was 0.1 ng/ml, while the limit of quantitation was 0.5 ng/ml flutriafol. Limits were calculated for each collection adjusting for the weight of leaf material analyzed, with an average limit of detection calculated as 0.6 pg/mg and an average limit of quantification calculated as 2.9 pg/mg.

Results & Discussion:

Flutriafol was detected at all time points except in non-treated plants at 3 and 4 weeks PFA (Figure 1). Flutriafol was quantifiable at all time points except in non-treated plants at 1 to 4 and 10 weeks PFA. For the collections in which flutriafol was quantifiable, flutriafol (pg/mg) was significantly greater in leaves of boxwoods drenched with TopGuard for all collection points except the initial 48 hr PFA collection. Quantities of flutriafol (pg/mg) within leaves of boxwoods drenched with TopGuard showed a steady increase over time with a peak detection at 18 wks PFA, then a slight decrease at 26 wks PFA with quantities remaining relatively constant until the last collection at 36 wks PFA. Our study provides insight into the capacity of flutriafol to remain elevated in the foliage of potted boxwoods for at least nine months PFA. We suspect that non-treated plants picked up flutriafol from vapor action of the fungicide. A second trial of the experiment is in process at the time of this writing. Evidence for the longevity of this chemical provides meaningful input to be considered in regards to the management of boxwood blight, while

also suggesting the need for additional experimentation to be conducted in order to better understand the efficacy over longer time scales and with varying application rates.

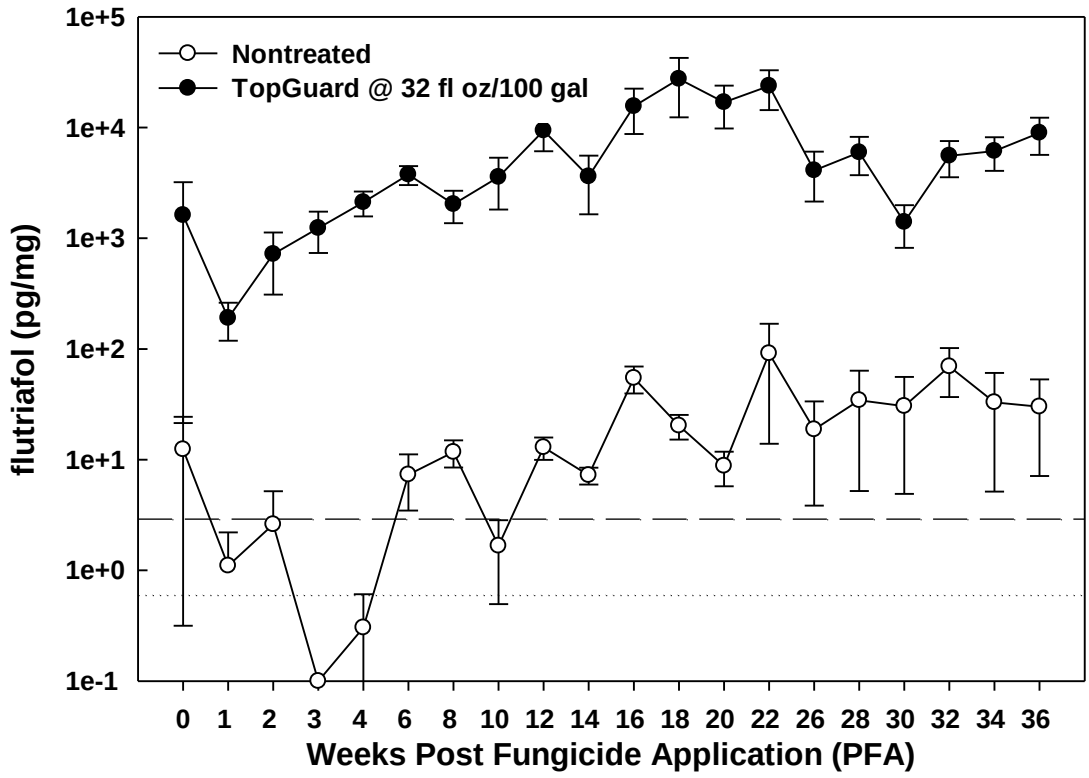

Figure 1. Quantities of flutriafol (pg/mg) present in leaves of Boxwood collected at multiple time points following a single drench application of TopGuard fungicide. The limit of detection (0.6 pg/mg) is indicated with a dotted line, while the limit of quantification (2.9 pg/mg) is indicated with a dashed line. Analysis of variance is based on log (x+1) transformation with significance based on Fisher's protected LSD (*P=0.05*). For the collections in which flutriafol was quantifiable, flutriafol was significantly greater in leaves of boxwoods for all collection points except the initial 48 hr PFA collection.