BOXWOOD (*Buxus sempervirens 'common'*)

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Quantification of flutriafol and tebuconazole in boxwood leaves after drench application, 2023.

Systemic triazole fungicides have been shown to effectively manage boxwood blight. Among these fungicides, flutriafol is considered the most systemic and was also the most efficacious when drench applied in our previous experiments. There are many questions about how these fungicides translocate throughout boxwood plants. In this report, we describe preliminary data collected using analytical chemistry to quantify the translocation of flutriafol (Topguard), tebuconazole (Torque), and prothioconazole (Proline), following drench applications in boxwood liners.

Methods:

Each of 3 triazole fungicides and a no-fungicide water control were applied to liners of *Buxus sempervirens* 'common'. Each treatment was applied directly to the media as a soil drench on 6 replicate plants. 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. Plants were then separated into two groups, with 3 replicate plants per treatment per group. Plants were placed on saucers to catch run-through and contain the fungicide. Plants were watered by hand by filling the saucers with water and allowing the media to take up water by capillary action.

After 48 hr, 3 leaves were collected from each plant in the first group. One leaf was collected from the lower third, one from the middle third, and the final leaf from the upper third of each plant. Leaves were bulked by treatment and collection location. This collection was conducted again 1 week post fungicide application (PFA) using plants from the second group, and the leaves were bulked separately from the first collection. Leaves were frozen immediately in a -80 °C freezer for later analysis.

Leaves were homogenized via bead beating using methanol as the suspension medium. Leaf weight and methanol volume were quantified such that later fungicide quantification can be normalized by the amount of leaf tissue analyzed. Homogenized samples were then analyzed simultaneously for flutriafol, tebuconazole, and prothioconazole 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 each fungicide molecule. The limits of detection were: prothioconazole 1 ng/ml, tebuconazole 0.1 ng/ml, and flutriafol 0.1 ng/ml. The limits of quantitation were: prothioconazole 5 ng/ml, tebuconazole 0.5 ng/ml, and flutriafol 0.5 ng/ml. Data analysis was conducted using Fisher's exact test to determine if fungicides were detected more frequently in plants treated with the same fungicide as compared to plants treated in any other treatment group. This analysis uses only the data from 1 week PFA. Other analyses were not conducted due to low detection and quantification frequency.

Results:

In leaves collected 48 h PFA, flutriafol was not detected in any leaves (Fig. 1). In contrast, tebuconazole was detected in 22% (2 of 9) of leaves from tebuconazole treated plants, and 7% (2 of 27) of leaves from all other treatments (Fig. 1). No fungicide detections were made above the level of quantification (data not shown).

In leaves collected 1 week PFA, flutriafol was detected in almost every leaf. Flutriafol was detected in 89% (8 of 9) of leaves collected from plants drenched with flutriafol and in 100% of leaves from plants in any other treatment (Fig. 1). Flutriafol was not detected at a significantly different frequency between leaves from plants drenched with flutriafol and leaves from any other treatment ($p = 0.25$). Tebuconazole was detected in 89% (8 of 9) leaves from tebuconazole-drenched plants, significantly more frequently than in all other treatments where it was detected in 41% (11 of 27) of leaves ($p = 0.02$; Fig. 1). Flutriafol was detected at a quantifiable level in leaves from flutriafol drenched plants (8 leaves, mean 280 pg/mg) and in a single leaf from another treatment (4.32 pg/mg; Fig. 2). Tebuconazole was only quantifiable in leaves from the tebuconazole drenched plants (5 leaves, mean 12.1 pg/mg; Fig. 2).

Prothioconazole was never detected in any leaves from any treatment from either time period.

Discussion:

While only preliminary, our data has revealed several important aspects of triazole fungicide systematics in boxwood plants. First, consistent with previous experiments that revealed no disease control 48 h PFA, few positive detections were made during the same timepoint in this experiment, and none above the level of quantification. In addition, previous experiments showed variable disease control 1 week PFA, which could be explained by our high levels of detection but low and variable quantification values (Sacher 2023). While triazole fungicides are predicted to have low volatility, some studies have suggested that vapor translocation is an important source of systemicity in this fungicide class. While our experiment was not specifically designed to look for vapor translocation, the detection of flutriafol in nearly 100% of leaves 1 week PFA, despite our efforts to minimize splash dispersal, indicates that vapor translocation might be a likely mechanism for the fungicide to disperse between plants. Finally, prothioconazole was never detected in any sample. A metabolic product of prothioconazole breakdown is known to be the active compound against plants, and it is possible that our samples only contained this altered form, which was not quantified.

Our preliminary data has expanded our knowledge of the dynamics of triazole fungicides in boxwood plants. Given the large differences between our 48 h and 1 week PFA samples, further work should expand on our data by extending the timeframe of the experiment. It is hoped that by quantifying the movement of triazole fungicides in woody plants, more precise application schedules can be determined.

Literature cited: Sacher, G. 2023. Diseases of Rhododendron and Boxwood: Survey and Virulence of Phytophthora Root Rot and Management of Boxwood Blight with Systemic Fungicides. Ph.D. Thesis, Oregon State University.

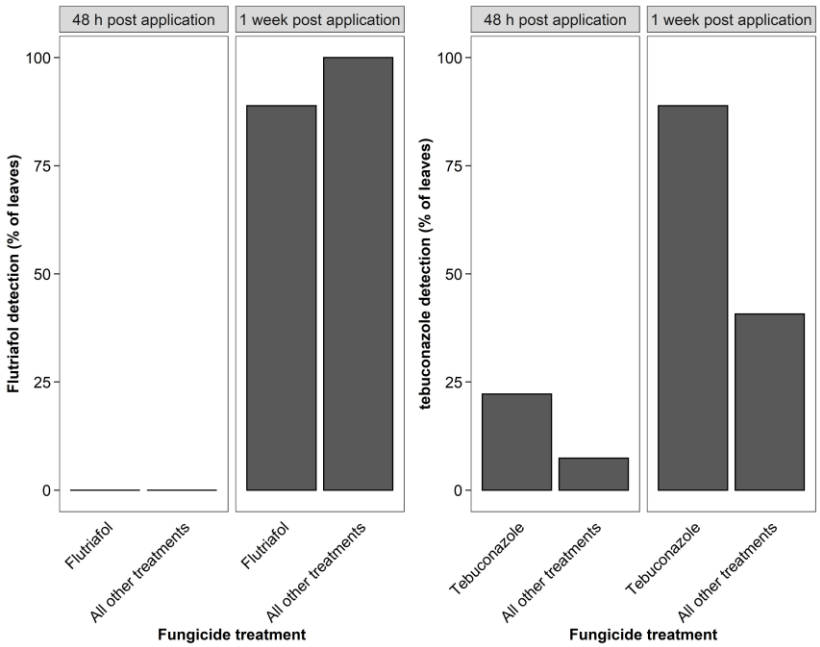

Figure 1: The frequency (percent of leaves) of detection of flutriafol (left two panels) or tebuconazole (right two panels) using LC/MS at two timepoints following fungicide drench applications. Four treatments were applied in this experiment: flutriafol, tebuconazole, prothioconazole, and no-fungicide control. For each time period, 3 replicate plants were treated, and 3 leaves were collected from each plant. For each fungicide, the detection frequency was compared between leaves from plants treated with the same fungicide (n=9), vs. leaves from plants treated with any other treatment (n=27). Prothioconazole was never detected, and its data is not shown.

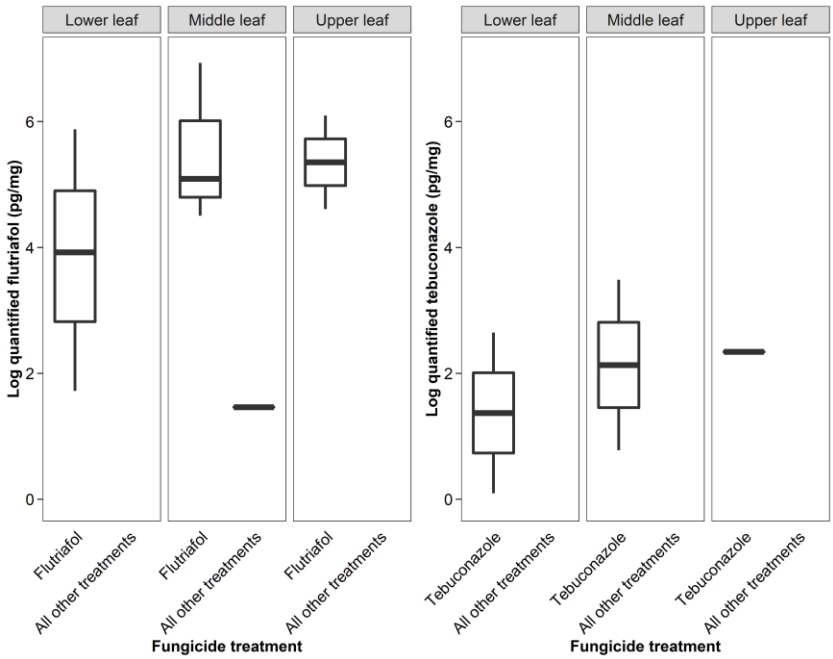

Figure 2: The log quantity (pg fungicide/mg leave tissue) of flutriafol (left two panels) or tebuconazole (right two panels) using LC/MS 1 week post fungicide application. Four treatments were applied in this experiment: flutriafol, tebuconazole, prothioconazole, and no-fungicide control. For each treatment, 3 replicate plants were used, and 3 leaves were collected from each plant (one each from the lower, middle, and upper third of each plant). For each fungicide and leaf location, the quantification was compared between leaves from plants treated with the same fungicide (n=3) vs. leaves from plants treated with any other treatment (n=9). Prothioconazole was never detected, and its data is not shown.

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