BOXWOOD (*Buxus microphylla cv. 'Winter Gem'*)
Boxwood Blight; *Calonectria pseudonaviculata*

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Using a detached leaf assay to determine efficacy of flutriafol against boxwood blight, 2021.

In order to test the efficacy of the FRAC group 3 fungicide "flutriafol", fungicide treatments were applied in a complete randomized block design to field grown *Buxus microphylla* cv. 'Winter Gem' at the BPP Field Laboratory in Corvallis, Oregon. Plants were heavily irrigated with approximately 11 cm of water on 3 Jun 2021 and 9 Jun 2021 to rewet dry soil. Irrigation then continued weekly throughout the experiment with 2.5 to 5 cm of water. Fungicide applications were made on 10 Jun 2021 (trial 1) or 21 July 2021 (trial 2) 24h after an irrigation event. Four fungicide treatments were investigated: a flutriafol drench, a flutriafol sprench, a propiconazole sprench as a positive control, and a no fungicide water sprench as a negative control. Flutriafol treatments were applied at a rate of 4 ml TopGuard (11.6%) per gallon while propiconazole was applied at a rate of 7 ml BannerMaxx II (15.6%) per gallon. One gallon of the appropriate treatment was applied directly to individual boxwood plants as either a drench, where the treatment was applied directly to the ground surrounding each plant, or a sprench, where the treatment was applied foliarly at a high rate such that most of the liquid flowed off the plant and onto the ground. Soil was mounded up around each treated plant to catch excess solution/water and minimize application runoff. Nontreated spacer plants separated each treated plant. Each treatment was replicated 6 times. The experiment was conducted twice.
Leaves were collected on 6 dates post fungicide application (PFA): 48 h PFA and each week for 5 weeks PFA. Each leaf collection consisted of collecting 2 leaves from the interior and exterior sections of the upper and lower canopy on each of the 6 plants and bulking the 12 leaves by canopy location and fungicide treatment. Leaves were immediately brought back to the lab at Research Way for inoculation. Leaves were surface sanitized with bleach (0.525% NaClO), separated by treatment and canopy location and allowed to dry. A stock spore suspension was made from sporulating *Calonectria pseudonaviculata* grown on potato dextrose agar plates. This stock spore solution was quantified by counting in a hemocytometer 6 times and averaging the results. A 2ml dilute spore suspension was made at a concentration of 80,000 spores/ml water (trial 1) or 40,000 spores/ml water (trial 2). The collected leaves were inoculated with 10 ul of either the dilute spore suspension or distilled water. Inoculated leaves were then organized in a complete randomized block design into 12-well low-evaporation microwell plates which were placed in low-evaporation vegetable crisper boxes with damp paper towels lining the bottom. Leaves were incubated for 2 weeks and visually rated 3 times per week for presence or absence of symptoms and percent diseased area.

Prior to analysis an area under disease progress curve (AUDPC) for each leaf was calculated by using the area under disease progress stairs method and the percent diseased area data. This method calculates disease severity by weighting the observed percent diseased area at each observation date by the number of days between the previous and following observation date then adding together each of these weighted measurements (Simko & Piepho, 2012). Each trial was analyzed separately due to large differences between trials trial 1 and 2. Incidence was evaluated using a logistic regression and AUDPC was evaluated using a general linear model. Model selection for each response variable in each trial was performed in a stepwise manner starting from the null model and again from the full model. The null model included only the fungicide treatment as an explanatory term while the full model included fungicide treatment × canopy height × canopy depth × week PFA + assay block. Estimated effect sizes were calculated using least-squared means. Significant differences were determined with an alpha level of 0.05 using Tukey's HSD test.

Results:

In trial 1 the leaves collected from propiconazole treated plants had the lowest incidence of infection and AUDPC among all treatments and were significantly different overall from the water sprench treatment (Table 1A and 1C). However, while the propiconazole treatment had significantly lower AUDPC than the water sprench for all leaf collection dates the incidence was only significantly lower 3 weeks PFA and earlier. Overall, the flutriafol sprench treatment resulted in significantly lower AUDPC, but not incidence, than the water sprench treatment. When analyzing individual leaf collection dates, significant differences in AUDPC were observed between the flutriafol sprench and water sprench treatments 3 to 5 weeks PFA but were never observed for incidence. Throughout trial 1

the flutriafol drench treatment was never significantly different from the water sprench treatment in incidence or AUDPC.

Overall, in trial 2 all three fungicide treatments had significantly less incidence and AUDPC than the water sprench treatment (Table 1B and 1D). However, only the propiconazole sprench was significantly different for all leaf collection dates. The flutriafol drench treatment had significantly less incidence than the water sprench treatment at 48h, 1 week, and 5 weeks PFA and significantly less AUDPC at every leaf collection date except 4 weeks PFA. The flutriafol sprench treatment only resulted in significantly less incidence and AUDPC than the water sprench treatment 1 week PFA.

All treatments including the water sprench resulted in less disease in trial 2 compared to trial 1. In trial 2 all fungicide treatments developed less disease as a percentage of the water sprench treatment than in trial 1 and significant differences between the water sprench and fungicide treatments were more common in trial 2. This difference in disease pressure may be related to the lower spore concentration used in trial 2. Propiconazole resulted in the lowest levels of boxwood blight among the tested fungicides at all leaf collection dates. Depending on the leaf collection date and the trial both flutriafol treatments were of similar efficacy, both resulted in less overall disease than the water sprench control but more disease than the propiconazole sprench treatment.

In both trials the location in the canopy of leaf collection and time PFA had similar effects on disease development. Across all fungicide treatments inoculated leaves from the lower and interior canopy developed lower incidence and AUDPC than leaves collected from the upper and exterior canopy (data not shown). Increasing the time PFA that leaves were collected resulted in a significant increase in incidence (trial 2) or AUDPC (trial 1 and 2; analysis not shown). The greatest difference in incidence PFA between fungicide treatments and the water control treatment is in the first week or less (table 1A and 1B). There are interactions between fungicide treatment and canopy location as well as fungicide treatment and time PFA. These interaction effects vary in significance week by week and may be related to the difference in efficacy of the fungicide treatments. A larger trial with more replicates will be needed to investigate these interactions further.

No boxwood blight was ever found on water-inoculated leaves, though occasionally Volutella blight was found in both inoculated and non-inoculated detached leaves. No disease of note was found on treated plants in the field. Phytotoxicity and/or plant growth regulation was never observed on treated plants.

Literature cited:

Simko, I., and Piepho, H.-P. 2012. The Area Under the Disease Progress Stairs: Calculation, Advantage, and Application. *Phytopathology*, *102*(4), 381–389. https://doi.org/10.1094/PHYTO-07-11-0216

Table 1

A

Trial [1]	Fungicide and application method [2]	Mean Incidence (%) [5] 48 h [3]		Week 1		Week 2		Week 3		Week 4		Week 5		Overall [4]	
1	Flutriafol drench	83.33	B	100.00	B	95.83	AB	91.67	AB	96.00	A	100.00	A	95.10	B
1	Flutriafol sprench	75.00	B	87.50	B	100.00	B	87.50	AB	87.50	A	83.33	A	87.50	B
1	Propiconazole sprench	29.17	A	54.17	A	83.33	A	79.17	A	83.33	A	83.33	A	70.80	A
1	Water sprench	83.33	B	95.83	B	100.00	B	100.00	B	100.00	A	91.67	A	95.80	B

B

Trial [1]	Fungicide and application method [2]	48 h [3]		Week 1		Week 2		Week 3		Week 4		Week 5		Overall [4]	
2	Flutriafol drench	4.17	AB	33.33	B	70.83	B	45.83	AB	83.33	B	25.00	AB	43.80	B
2	Flutriafol sprench	29.17	BC	45.83	B	79.17	B	75.00	B	79.17	B	45.83	BC	59.00	C
2	Propiconazole sprench	0.00	A	0.00	A	8.33	A	16.67	A	12.50	A	12.50	A	8.33	A
2	Water sprench	50.00	C	83.33	C	83.33	B	70.83	B	87.50	B	58.33	C	72.20	D

C

Trial [1]	Fungicide and application method [2]	Mean AUDPC [5] 48 h [3]		Week 1		Week 2		Week 3		Week 4		Week 5		Overall [4]	
1	Flutriafol drench	41.99	B	62.32	B	58.73	B	55.67	BC	51.85	BC	48.92	AB	53.20	C
1	Flutriafol sprench	29.53	B	52.64	B	56.10	B	49.64	B	43.17	AB	41.67	A	45.50	B
1	Propiconazole sprench	9.01	A	18.37	A	33.51	A	31.85	A	37.01	A	37.58	A	27.90	A
1	Water sprench	42.06	B	59.80	B	66.50	B	65.26	C	59.77	C	55.95	B	58.20	C

D

Trial [1]	Fungicide and application method [2]	48 h [3]		Week 1		Week 2		Week 3		Week 4		Week 5		Overall [4]	
2	Flutriafol drench	0.98	A	17.23	B	31.39	B	22.75	B	45.33	B	9.31	AB	21.20	B
2	Flutriafol sprench	12.84	B	27.67	B	46.38	C	36.70	BC	42.56	B	20.22	BC	31.10	C
2	Propiconazole sprench	0.07	A	0.18	A	0.69	A	4.32	A	2.86	A	2.06	A	1.70	A
2	Water sprench	19.22	B	51.59	C	47.65	C	40.54	C	50.16	B	24.34	C	38.90	D

[1] Treatments were applied on 10 Jun 2021 (trial 1) and 21 July 2021 (trial 2)

[2] Fungicide was applied as 4ml TopGuard (flutriafol) or 7ml BannerMaxx II (propiconazole) in 3.76 liters of water

[3] Leaves were collected 48 hours, 1, 2, 3, 4, and 5 weeks post fungicide application.

[4] Overall disease is calculated using data from all leaf collection dates within a trial.

[5] Means of each fungicide treatment for each leaf collection date (or overall trial) after 2 weeks of incubation after inoculation. Treatments in the same trial and column with the same letter are not significantly different based on linear models and a post hoc Tukey's HSD test at $P = 0.05$.