Leaf Discoloration in *Rhododendron* Species Exposed to *Phytophthora Cinnamomi* Corresponds with Future Mortality[1]

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Abstract

Phytophthora cinnamomi, which causes the disease root rot, is an oomycete pathogen that is damaging to woody plants, including many horticulturally important groups, such as Rhododendron. Infecting the root of plants, *Phytophthora cinnamomi* inhibits water uptake, leading to root damage, wilting, and increased rates of plant mortality. Some observations suggest that *P. cinnamomi* infection corresponds to changes in leaf coloration, though whether this indicates a plant stress response or plant damage is generally unknown. We used leaf color analysis to test for differences in leaf discoloration between plants inoculated with the pathogen and control plants. We demonstrate a significant link between leaf discoloration in *Rhododendron* species and *Phytophthora cinnamomi* inoculation. This method was most useful when mortality was not exceptionally high, and analyzers must consider mortality as well as leaf damage in quantifying effects of the pathogen. Plants with leaf discoloration were 3.3 times more likely to die 2 weeks from our leaf census than plants with no leaf discoloration ($P = 0.005$). This method is particularly inexpensive to implement, making it a valuable alternative to multi-spectral or hyperspectral imaging, especially in contexts such as horticulture and citizen science, where the high speed and low-cost nature of this technique might prove valuable.

Species used in this study: root rot disease pathogen (*Phytophthora cinnamomi* Rands); *Rhododendron atlanticum* (Ashe) Rehder; *Rhododendron brachycarpum* D.Don ex G.Don; *Rhododendron kiusianum* Makino; *Rhododendron maximum* L.; *Rhododendron minus* Michx.; *Rhododendron calendulaceum* (Michx.) Torr.; *Rhododendron kaempferi* Planch.; *Rhododendron keiskei* Miq.

Chemicals used in this study: Fosal Select Aliette/aluminum phosphite.

Index words: image analysis, ImageJ software, leaf discoloration, Phytophthora root rot.

Significance to the Horticulture Industry

Plant pathogens present unique challenges within the horticulture industry, as they cost billions of dollars in plant mortality annually. The ease with which pathogens of the genus *Phytophthora* are spread within nursery environments severely compromises the health and production of *Rhododendron* and many other woody plants. Thus, the ability to quickly identify diseased plants within large populations of *Rhododendrons* is crucial to nurseries. Our results indicate that rapid photo assays using a cell phone camera and free ImageJ software correlates with experimental pathogen inoculation. Furthermore, our work confirms the use of phosphite treatment as a strategy to reduce leaf discoloration when *Rhododendrons* are exposed to *Phytophthora cinnamomi*. While identification of root rot and other pathogens in *Rhododendrons* is already possible, our work provides a tool for rapid leaf discoloration quantification that is repeatable, quantitative, and inexpensive. The high degree of accessibility of these tools, including cell phone cameras and free software, suggest that this tool might be useful in applications such as citizen science projects, industry, and horticulture.

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Introduction

Phytophthora cinnamomi, which causes the disease root rot, is an oomycete plant pathogen, infecting roughly 5,000 different types of woody plants (Hardham and Blackman 2018). *Phytophthora cinnamomi* infects the roots of plants, killing the inner bark and leading to browning of the sapwood (Perry 2006). This causes inhibition of water uptake, wilting, and sometimes death. Plants with root rot disease often show changes in leaf coloration, including dull green, yellow, red, or purple coloration (Perry 2006).

Leaf coloration is a complex trait that can vary with plant light exposure, water availability, disease status, herbivory risk, and many other factors (e.g. Lev-Yadun 2006, Barbedo 2016). For example, plants can reduce the production of chlorophyll in the presence of stressors to optimize the photosynthetic apparatus, leading to leaf discoloration (Adams et al. 2004). Leaf color in the visible spectrum has often been used as a non-invasive measure of plant health, though with caveats about lighting, camera exposure, and other covariates (Jhuria et al. 2013, Barbedo 2016). Because experiments in the greenhouse control for many covariates of leaf coloration, such as light and water availability, we propose that leaf color analysis might provide a simple, quantitative measure of leaf discoloration (Pride et al. 2020) that may correspond with plant health in the presence of the pathogen, *Phytophthora cinnamomi*. Here, we test the predictions that leaf discoloration will correlate with experimental pathogen inoculation and future mortality in a greenhouse experiment.

Our experiment included pathogen inoculation, phosphite, and live soil biota treatments (see also Liu et al. 2023). Phosphite has commonly been used to treat

Phytophthora root rot in different species of plants, particularly in citrus seedlings (Orbović et al. 2008). The use of phosphite as a treatment for root rot works by attacking the oomycete itself and boosting the defense system of the host plant (Smillie 1989). Live soil biota are also thought to improve plant health, especially in the presence of harmful pathogens (Alexander 1998). Soil biota is highly biodiverse and contains many different types of fungi, bacteria, and other microorganisms, some of which may contribute to nutrient availability and plant health (Yang et al. 2018). The increase in nutrients facilitated by soil biota may allow plants to become more resilient to stress (Stachowicz 2001). Soil biota can also inhibit the spread of invasive pathogens, such as harmful fungi, promoting plant health (Liao et al. 2015). Components of live soil biota can also function as competition for harmful pathogens, such as *P. cinnamomi*, decreasing the harm that can be done by these pathogens (Lekberg et al. 2018). Live soil biota may also contain mycorrhizal fungi, which have symbiotic relationships with the plant, leading to more positive outcomes for plant survival (Smith and Read 2017).

In order to determine how plant leaf discoloration might respond to the pathogen *Phytophthora cinnamomi*, we analyzed the relationship between experimental pathogen inoculation and leaf discoloration across eight species of Rhododendron. With this experiment, we addressed the following two questions. First, we ask (1) *Does the addition of Phytophthora cinnamomi lead to increases in leaf discoloration and is discoloration reduced by phosphite or live soil biota?* Previous literature notes that *Phytophthora cinnamomi* causes significant root damage that inhibits water uptake (Hardham and Blackman 2018). Because plants with water relations problems often have discolored leaves (Perry 2006), *P. cinnamomi* inoculation might lead to observable leaf discoloration. Live soil biota have been shown to reduce plant mortality in the presence of pathogens, suggesting that it might reduce leaf discoloration in *Rhododendron* species that have been infected with *Phytophthora cinnamomi* (Alexander 1998, Liu et al. 2021). Phosphite has previously been used as a responsive treatment against root rot in several other species (Orbović et al. 2008). Since different *Rhododendron* species have differing susceptibility to *Phytophthora cinnamomi* (Liu et al. 2021), we anticipate that the effect of phosphite on *Rhododendrons* infected with *Phytophthora cinnamomi*, and thus on measures of leaf discoloration, will be species-specific. Second, we ask *(2) Does leaf discoloration correlate with plant survival?* Leaf discoloration could reflect damage to the leaves caused by disease or could reflect plant stress responses (e.g. Adams et al. 2004). We therefore tested whether leaf discoloration predicts future plant survival in these experimental plants, as a test of the underlying assumption that leaf discoloration reflects leaf damage.

Materials and Methods

Experimental design. The greenhouse experiment was conducted using seeds and soil from 8 *Rhododendron* species across four sub-genera, collected from the Holden Arboretum (see Figures). Three soil samples were taken for each species of *Rhododendron*. We also used two different types of soil treatment (live and sterile), two *Phytophthora* treatments (*Phytophthora*-present and *Phytophthora*-absent), as well as three phosphite treatments (no phosphite-added, before pathogen, and after pathogen phosphite addition). The experimental design was 8 *Rhododendron* species × 2 soil treatments × 3 soil collections × 2 *Phytophthora* treatments × 3 phosphite treatments × 3 replicate pots per treatment for a total of 576 pots. Each pot contained one plant to avoid overcrowding. We randomly assigned each pot a block, nine of which contained *Phytophthora*-present treatment and nine with *Phytophthora*-absent treatment, to prevent *Phytophthora* from infecting the uninfected pots. Pots were randomly assigned blocks to prevent differences in treatments based on positioning in the greenhouse. For more details about the experimental design, please see Liu et al. (2023).

Due to the ease with which *Rhododendron* hybridizes, controlled pollination was ensured through the process of bagging *Rhododendron* buds, to guarantee species-true seed production. We hand-pollinated the *Rhododendron* for our experiment at Holden Arboretum in Kirtland, Ohio, using conspecific pollen. Seedlings were cultivated in the University Farm greenhouse complex in Chagrin Falls, Ohio. All flats were filled with a 50% peat moss and 50% horticultural perlite mixture. Seedlings were then transferred into 6.4 centimeter by 25.4 cm (2.5 × 10 in) pots for experimentation in June 2021.

Soil treatment. Once the seedlings were transplanted into the pots, they were allowed to grow for two weeks to avoid conflating effects of transplant mortality and disease treatment. Each species of *Rhododendron* was treated with species-specific soil, and half of the plants received sterilized soil. It has been suggested that live soil biota can result in the addition of nutrients, which could increase plant survival in the context of disease (Nijjer et al. 2008). In order to separate soil biota from nutrient availability, a small (5 mm layer) of live soil was used to add soil microbial communities without significantly altering the amount of nutrients available (Brinkman et al. 2010). In June 2021, we collected soil samples from three different locations beneath each *Rhododendron* species, with each sample being taken from the root zone at Holden Arboretum. Field samples were taken at least 1 m apart and from independent plants within a species, whenever possible (see Liu et al. 2023 for details). Sterilized tools were used for soil collection.

Each soil type was kept separate from the others throughout the duration of the experiment to prevent cross-contamination. Preventing cross-contamination of soil samples allowed for a more accurate assessment of the effect of each species' soil biota, since mixing soil samples, even from the same plant species, leads to unpredictable changes in the makeup of the soil biota (Rinella and Reinhart 2018). Each soil sample was dried in the University Farm greenhouse for five days and was then sieved to ensure consistent soil structure. We then placed half of the soil in an autoclave for two hours at 121 (250 F) for sterilization. The other half of the soil was untreated so that we could assess the influence of soil biota on plant growth. The soil was then inoculated into each pot by forming a

Table 1. Binomial analysis of whether or not leaf discoloration was present across the experiment with eight Rhododendron species.

	Df	Deviance Residual	Df	Residual Deviance	P-value
Species	7	92.391	459	490.91	< 0.0001
Soil (live vs. sterilized)	1	0.221	458	490.69	0.64
Phytophthora presence	1	38.437	457	452.26	< 0.0001
Phosphite presence	2	22.133	455	430.12	< 0.0001
Species × soil	7	4.232	448	425.89	0.752711
Species × phytophthora presence	7	17.906	441	407.98	0.012
Soil × phytophthora presence	1	0.599	440	407.39	0.44
Species × phosphite presence	14	21.346	426	386.04	0.093
Soil × phosphite presence	2	0.979	424	385.06	0.61
Phytophthora presence × phosphite presence	2	0.462	422	384.6	0.79
Species × soil × phytophthora presence	7	6.803	415	377.8	0.45
Species × soil × phosphite presence	14	30.25	401	347.55	0.007
Species × phytophthora presence × soil	14	21.529	387	326.02	0.089
Soil × phytophthora presence × phosphite presence	2	0.567	385	325.45	0.75
Species × soil × phytophthora presence × phosphite presence	14	2.427	371	323.02	0.999

Null DF 466, Residual DF 583.3.

five-millimeter layer on top of the previous soil mixture, using 5 mL (one teaspoon) of the soil sample.

Pathogen inoculation. We performed our *Phytophthora* inoculations using the same methods as Krebs and Wilson (2002). The *Phytophthora cinnamomi* used in this experiment were strains BDW, LA-7, and MAD-C, and they were cultivated on Lima Bean agar plates (Schmitthenner and Bhat 1994). Pieces of agar that had grown mycelium were then removed from the plates and mixed with sterile long-grain rice, which we mixed periodically to ensure an even spread of mycelium. The mycelium had fully spread and covered the rice after two weeks. The experimental plants were then inoculated with the *Phytophthora cinnamomi* rice, with six grains of rice per pot. The *Phytophthora*-absent plants were then treated with sterile rice to ensure they received similar treatment to the *Phytophthora*-present plants.

Phosphite application. We applied Fosal Select Aliette/Aluminum Phosphite to the plants at two different periods: either simultaneously with disease inoculation or directly after plants started to show disease symptoms. Each plant that received phosphite treatment only had phosphite

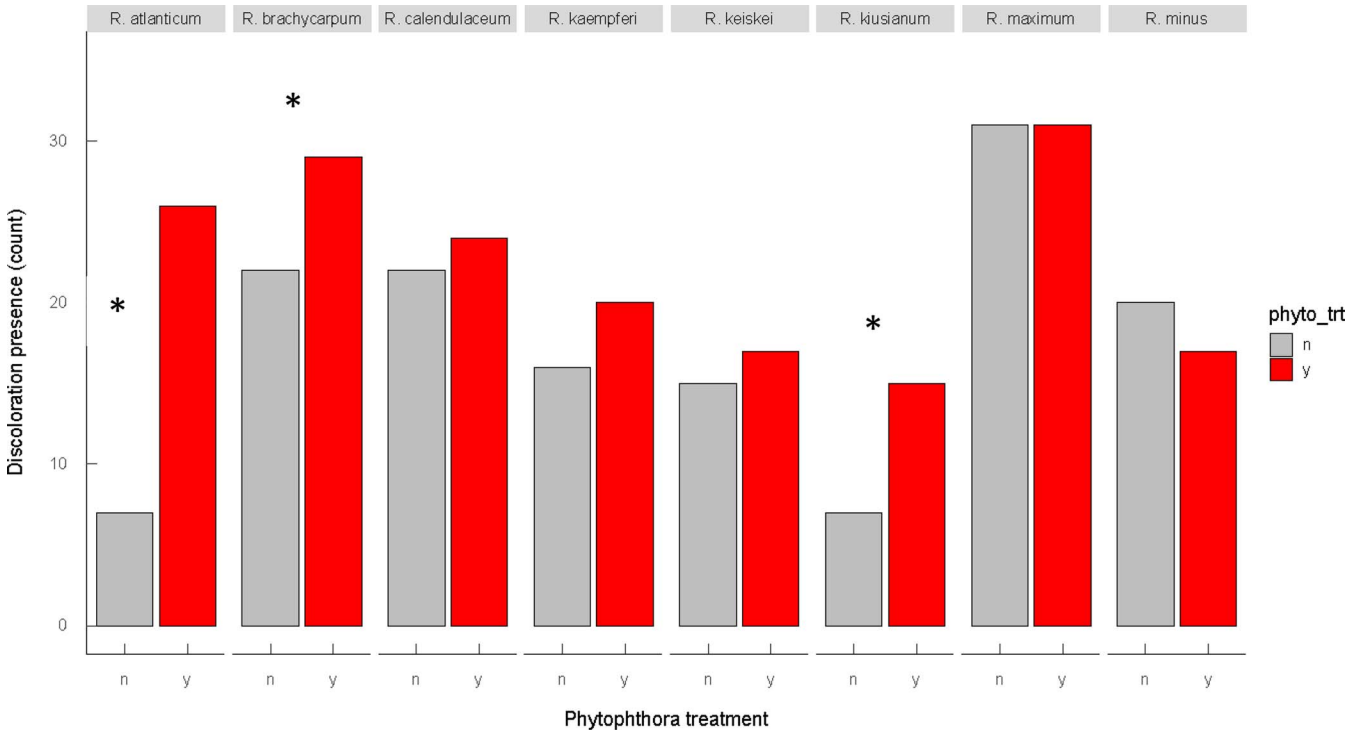

Fig. 1. Number of leaves per species in which discoloration was present. Only one leaf per plant was analyzed. 72 total leaves were analyzed per species, 36 per *Phytophthora* treatment group. Pathogen inoculation is indicated in red, no inoculation in gray. Species include *R. atlanticum, R. brachycarpum, R. calendulaceum, R. kampferi, R. keiskei, R. kiusianum, R. maximum,* and *R. minus*, respectively. phyto_trt n refers to no Phytophthora added (gray bar), phyto_trt y refers to Phytophthora added (red bar). Asterisks indicate statistically significant ($P < 0.05$) intraspecific contrasts with Tukey adjustment for multiple comparisons.

Table 2. Generalized linear model statistical analysis for the proportion of leaf discoloration (when present) for different experimental treatments Null: 318, 2210.01.

	Df	Deviance Residual	Df	Residual Deviance	F	Pr(>F)
Species	7	531.47	311	1678.53	17.9482	< 0.0001
Soil	1	5.26	310	1673.27	1.2436	0.27
Phytophthora presence	1	5.89	309	1667.38	1.393	0.24
Phosphite presence	2	141.13	307	1526.25	16.6811	< 0.0001
Species × soil	7	79.98	300	1446.27	2.7009	0.01
Species × phytophthora presence	7	53.55	293	1392.72	1.8085	0.087
Soil × phytophthora presence	1	20.42	292	1372.3	4.8277	0.029
Species × phosphite presence	14	93.46	278	1278.84	1.578	0.086
Soil × phosphite presence	2	0.47	276	1278.37	0.0551	0.95
Phytophthora presence × phosphite presence	2	40.91	274	1237.46	4.8358	0.0088
Species × soil × phytophthora presence	7	15.6	267	1221.86	0.5267	0.81
Species × soil × phosphite presence	14	118.77	253	1103.1	2.0054	0.018
Species × phytophthora presence × phosphite	12	99.77	241	1003.33	1.9654	0.028
Soil × phytophthora presence × phosphite presence	2	4.15	239	999.18	0.4904	0.61
Species × soil × phytophthora presence × phosphite presence	10	30.46	229	968.72	0.72	0.71

applied once. Phosphite was applied using a soil drench method, with 1.14 grams of phosphite per 3.8 L (1 gal) of drench.

Data collection and ImageJ analysis. Photographs of the *Rhododendron* leaves were taken after disease inoculation and treatment application, once plants began showing symptoms of disease such as wilting, in August of 2021. Photographs were taken using a 12 MP Apple iPhone 8 inside the greenhouse using a white sheet of paper as a high-contrast background and a ruler for scale (Pride et al. 2020). We photographed a single leaf per plant. To control for potential confounding factors, such as developmental stage and light availability during development, we selected the topmost fully expanded leaf.

After images were collected, they were exported to ImageJ for analysis. The ImageJ application allowed for a quantitative measure of leaf discoloration, which provides more objective and accurate information on overall plant health compared to visual assessment (Laflamme et al. 2016). First, the program was calibrated for image size using a ruler included in every photo. We then converted the images to black and white to remove the background of the photo, leaving the outline of the leaf (Pride et al. 2020), and measured the overall area of the leaf. The color was then returned to the original state so that the

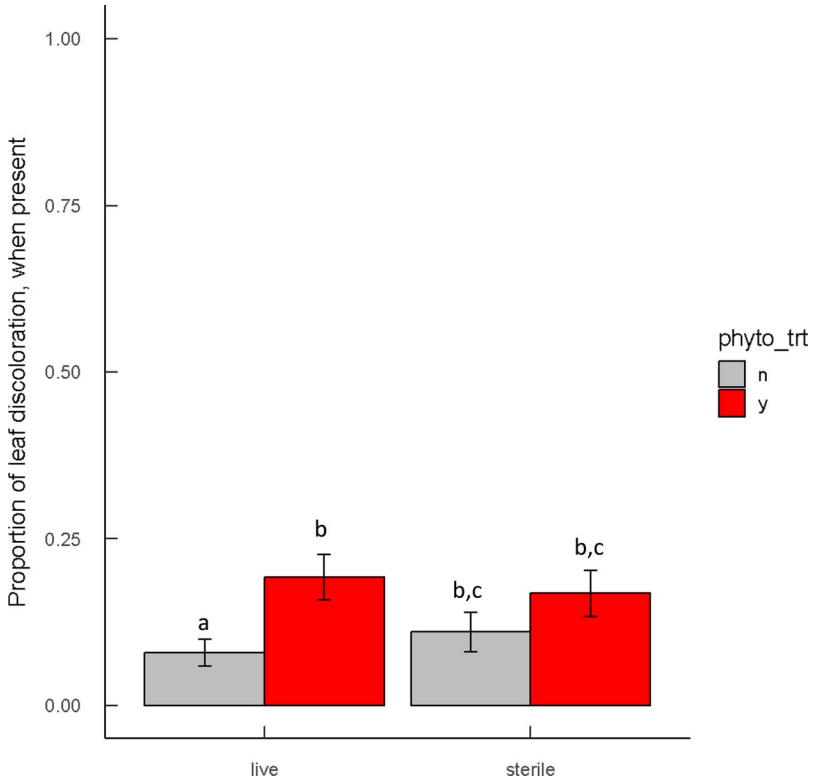

Fig. 2. Soil and *Phytophthora* interaction in context of proportion of leaf discoloration when leaf discoloration is present. Pathogen inoculation is indicated in red, no inoculation in gray. Soil treatment is broken down into live and sterile categories. phyto_trt n refers to no *Phytophthora* added (gray bar), phyto_trt y refers *to Phytophthora* added (red bar). Bars that share a letter are not statistically different in post-hoc comparisons. Means are averaged across eight *Rhododendron* species ± 1 SE.

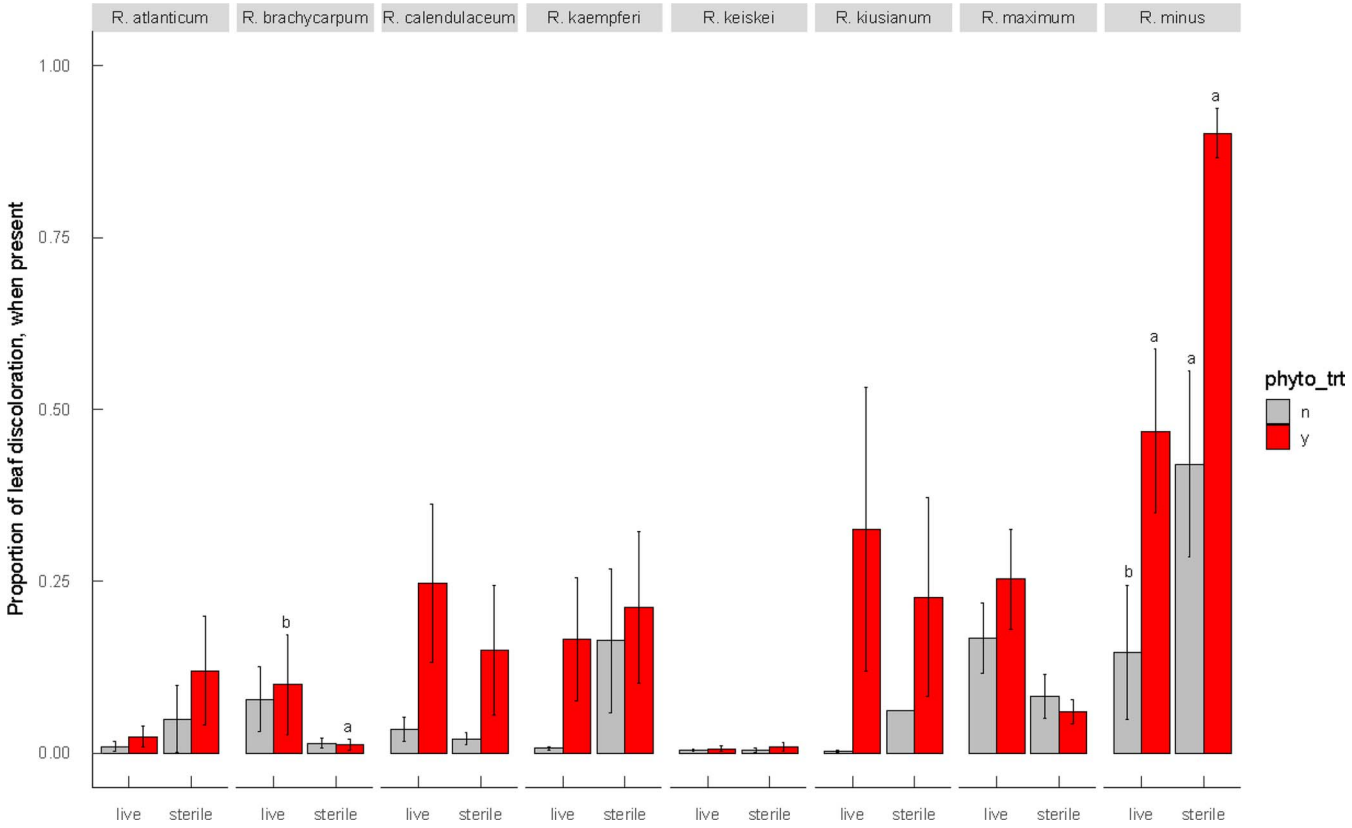

Fig. 3. Species by soil by *Phytophthora* interaction for the proportion of leaf discoloration, when present. Soil treatment is broken down into live and sterile conditions. phyto_trt n refers to no *Phytophthora* added (gray bar), phyto_trt y refers to *Phytophthora* added (red bar). Different letters within a species indicate means that are significantly different ($P < 0.05$) in a contrast analysis with Tukey correction of multiple comparisons. Bars without letters are not significantly different within a species. Means ± 1 SE.

discolored sections of the leaf could be identified. Hue values of less than 40 were identified as discolored, following Pride et al. (2020). This was then confirmed through a visual analysis of the hue histogram provided by ImageJ that determined green hues of the leaves occurred above the cutoff point of 40, as shown by a minimal presence of hues between approximately 40 and 50, giving a clear distinction between discolored and green leaf tissue. The hues that do not match that of the green leaf, with a cutoff value of 40 (following Pride et al. 2020), were then selected, and the photo was again transformed to black and white, and the excess noise (small specks that are not a part of the leaf) was removed using the "Process > Noise > Remove Outliers" filter in ImageJ. We used the Measure tool to measure the discolored area of the leaf, which we then compared to the total leaf area to calculate the percentage of discolored leaf.

Data analysis. The distribution of percentage of leaf discoloration was zero-inflated, containing more zeros than expected, and could not be transformed to normality. Therefore, we broke the data up into two variables for analysis: (1) a binomial variable, discoloration present, where values of 0 indicated no discoloration and values of 1 indicated the presence of some leaf discoloration and (2) the percentage of leaf discoloration, when discoloration was present, which was NA for leaves with no discoloration and continuous for leaves with any discoloration present.

We then addressed question one: *Does the addition of Phytophthora cinnamomi lead to increases in leaf discoloration and is discoloration reduced by phosphite or live soil biota?* We used the natural log transformation on the percentage of leaf discoloration, when present, and the data was normally distributed after transformation. The binomial data was analyzed using a generalized linear model with a binomial error structure and a chi-square test for significance. The continuous data was analyzed using a linear model with gaussian error structure and an F-test for significance.

For both response variables, the predictor variables were Rhododendron species, *Phytophthora cinnamomi* treatment, live or sterile soil treatment, phosphite treatments, and the interactions among these treatments. When statistical interactions were significant, we followed these interactions with posthoc tests for significance using the *glht* function with Tukey's method in the multcomp package (Hothorn et al. 2008).

We next addressed question two: *Does leaf discoloration correlate with plant survival?* We conducted two analyses with plant survival as the response variable and either the presence of discoloration or the amount of discoloration when present as predictor variables. We used generalized linear models with a binomial error distribution and a chi-square test for significance.

All analyses were done in the R Program (Version 4.0, R Core Team 2022). Data is archived at the Open Science Framework.

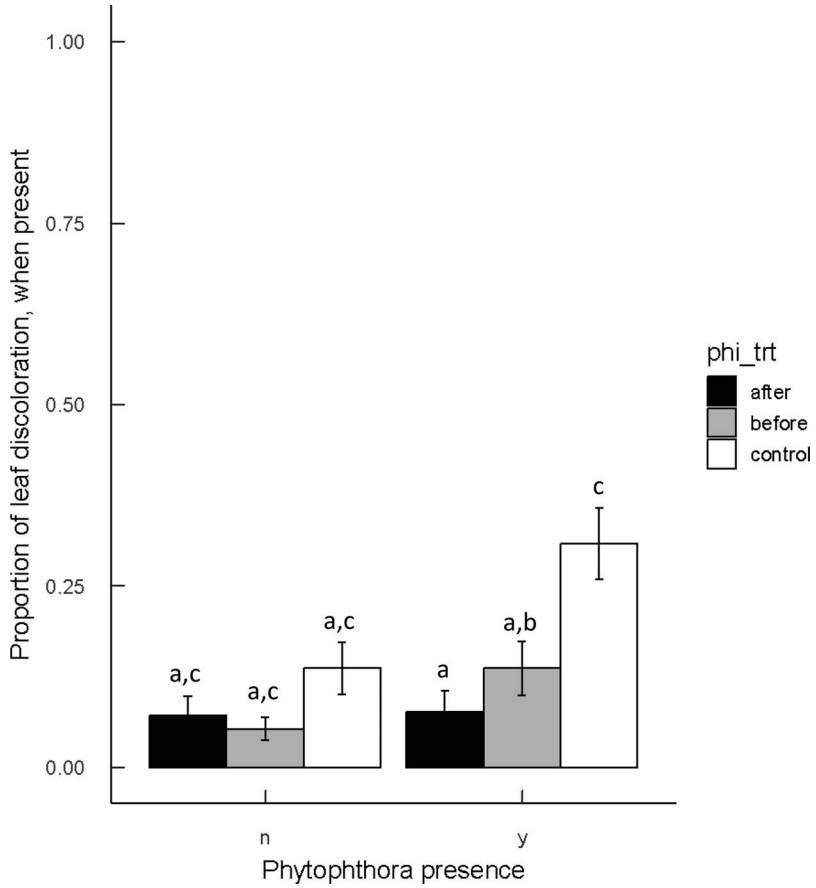

Fig. 4. Phosphite and *Phytophthora* interaction in proportion of leaf discoloration when leaf discoloration is present. Phosphite treatment was applied either before or after symptoms developed, or not at all, which is indicated by black, gray, and white, respectively. n indicates that *Phytophthora* is not present, while y indicates that it is. Means that share a letter are not statistically different in post hoc tests. Data is averaged across eight *Rhododendron* species. Means ± 1 SE.

Results and Discussion

Here we tested the prediction that (1) experimental inoculation with *Phytophthora cinnamomi* will lead to more leaf discoloration in Rhododendron. We also asked whether leaf discoloration was influenced by phosphite or live soil biota, known modifiers of disease response in Rhododendron. Further, we test the underlying assumption that (2) leaf discoloration correlates with plant health, tested via survival.

(1) *Does the addition of Phytophthora cinnamomi lead to increases in leaf discoloration and is discoloration reduced by phosphite or live soil biota?*

As predicted, leaf discoloration was more likely to be present when plants were inoculated with the pathogen, *Phytophthora cinnamomi*. The binomial analysis in this experiment analyzed the number of leaves that were found to contain any amount of discoloration. There were 72 leaves per species analyzed, 36 of which were inoculated with *Phytophthora cinnamomi*. These results were species-specific, in that presence of discoloration was increased in the *Phytophthora* present groups in species *R. atlanticum*, *R. brachycarpum*, and *R. kiusianum* (Table 1, Fig. 1). *Rhododendron maximum*, *R. minus*, *R. calendulaceum*, *R. kaempferi*, and *R. keiskei* showed no significant difference in the number of plants with discolored leaves between *Phytophthora* present and absent groups. The addition of *Phytophthora cinnamomi* was shown to increase the number of leaves discolored in all but two of the *Rhododendron* species; *R. maximum* and *R. minus*. However, the sample sizes for both of these species when inoculated with *Phytophthora cinnamomi* were greatly reduced due to plant death ($n_{max} = 25$, $n_{min} = 17$). We believe that plant mortality explains why *R. maximum* and *R. minus* did not follow the trend of *Phytophthora cinnamomi* increasing the number of leaves discolored. Thus, overall, the presence of discoloration was increased with pathogen inoculation, but only significantly for some species.

Live soil biota did not have a significant effect on whether or not leaves were discolored in the presence of *Phytophthora cinnamomi* (Table 1). There was also no significant species by soil treatment by *Phytophthora* treatment interaction. Soil biota effects were generally species-specific and did not lead to consistently higher or lower proportions of leaf discoloration. The interaction between soil treatment and pathogen presence was statistically significant for amount of discoloration (Table 2). However, leaf discoloration was only significantly greater with pathogen addition in live, not sterile soils, likely as a result of high variability in leaf discoloration in sterile soils (Fig. 2). When broken down by species, some species had significant

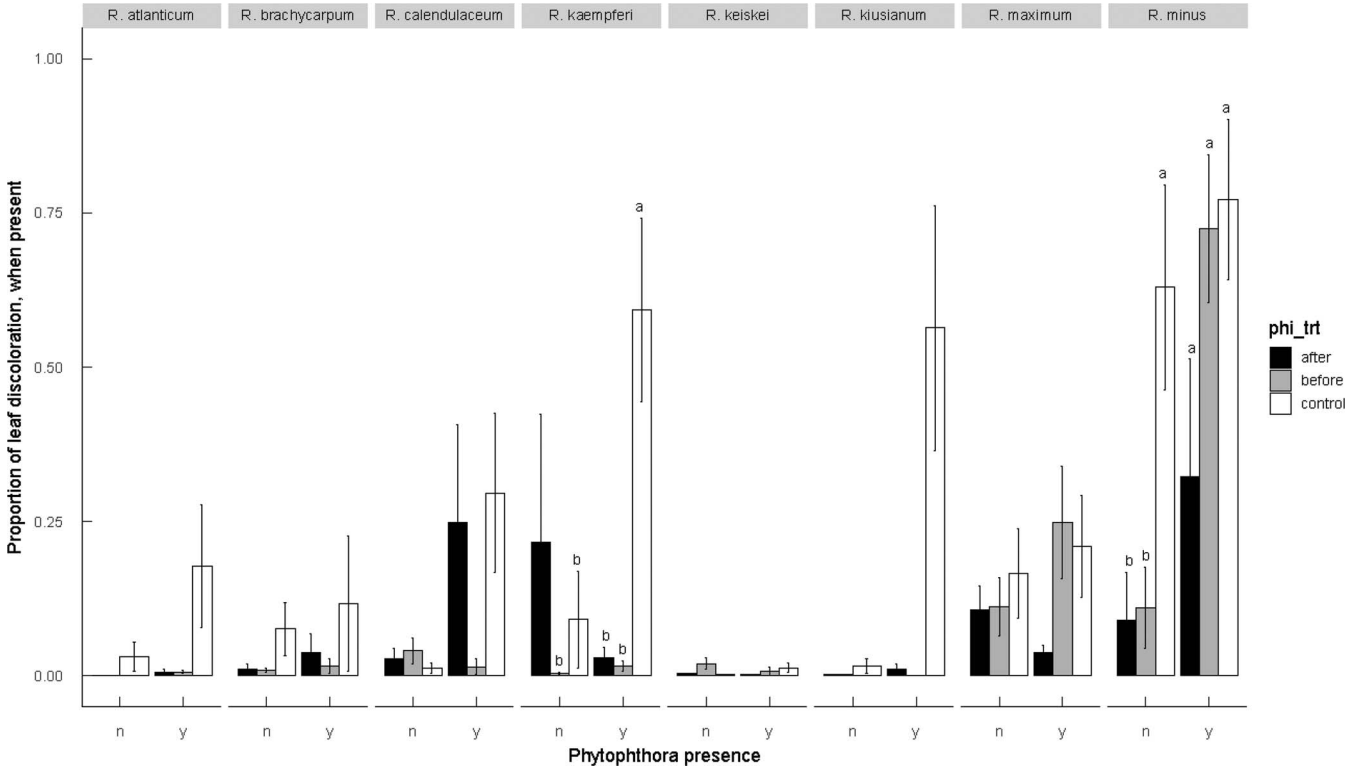

Fig. 5. Species by phosphite by *Phytophthora* interaction in terms of proportion of leaf discoloration when present. Different letters within a species indicate means that are significantly different ($P < 0.05$) in a contrast analysis with Tukey correction of multiple comparisons. Bars without a letter are not statistically different within a species. Means ± 1 SE.

soil by pathogen treatment effects (Fig. 3). *Rhododendron brachycarpum* showed an increase in proportion leaf discoloration when given live soil biota compared with sterile soil when the pathogen was inoculated (Fig. 3). *Rhododendron minus*, conversely, showed decreased proportion leaf discoloration in live soil than in sterile soil with no pathogen, again likely because high morality in *R. minus* likely limited statistical power (Fig. 3). Overall, the presence of live soil biota from conspecifics had minimal effects on leaf discoloration.

As predicted because phosphite is a known treatment for root rot disease (e.g. Havlin and Schlegel 2021), phosphite generally lead to lower levels of leaf discoloration across treatments, though this effect was also dependent on Rhododendron species and soil treatment. Phosphite and *Phytophthora* had a significant interaction on the proportion of leaf discoloration (Table 2), as predicted. Phosphite treatment applied both before and after root rot symptoms occurred decreased the amount of leaf discoloration in plants inoculated with *Phytophthora* (Fig. 4). Phosphite also had a species-specific effect with *Phytophthora*. In *R. kaempferi*, phosphite applied both before and after root rot symptoms greatly decreased the proportion of leaf discoloration present, while phosphite treatment in plants without pathogen present had no effect (Fig. 5). For *R. minus*, however, phosphite treatment only reduced leaf discoloration proportion when the pathogen was not present (Fig. 5). The high susceptibility of *R. minus* to root rot is further supported by its high mortality rate when the pathogen was introduced (Liu et al. 2023), as well as its heightened proportion of leaf discoloration compared to the other species in this study (Fig. 5). Phosphite's usefulness in counteracting root rot is consistent with the literature, and may directly reduce pathogen abundance and boost the plant immune system, lowering the proportion of leaf discoloration (e.g. Havlin and Schlegel 2021), especially if discoloration is indicative of damage to the plant.

(2) *Does leaf discoloration correlate with plant survival?*

To test the assumption that leaf discoloration correlates with damage to the plant, rather than indicating a stress response (Adams et al. 2004), we tested whether leaf discoloration correlated with plant survival outcomes at the following census. At the time of the leaf discoloration census in August, 467 of the total 576 plants in the experiment were alive. Of these, 438 showed at least some leaf discoloration. Of those plants with no leaf discoloration, 90% survived to the following 6 October survival census. Of those plants with at least some leaf discoloration, 67% survived to the 6 October census, a statistically significant difference ($DF = 1$, Deviance $= 7.82$, Residual $DF = 465$, Residual Deviance $= 209$, $P = 0.005$). In other words, plants with discolored leaves died at a 3.3 × higher proportion than plants without discolored leaves. In addition, plants with larger proportion of leaf discoloration were more likely to die than plants with a lower proportion of leaf discoloration (estimate $= -4.03$, $DF = 1$, Residual $DF = 317$, Deviance $= 54.65$, Residual Deviance $= 125.55$, $P < 0.0001$), again consistent with the prediction

that leaf discoloration is a measure of plant health in this experimental context.

Implications for horticulture. *Phytophthora cinnamomi* is an oomycete pathogen that can infect roughly 5,000 different woody plant species, suggesting that a quick and efficient method to quantify leaf discoloration could be useful in many contexts. This pathogen causes root rot disease in Rhododendron. In this experiment, we have demonstrated that leaf discoloration presence and proportion corresponded to an experimental pathogen treatment. We have also shown phosphite, a known root rot treatment, reduces leaf discoloration. The correspondence between leaf discoloration and subsequent mortality suggests that our measure of leaf discoloration was an indicator of plant health. Overall, this method of quantifying leaf discoloration is inexpensive, simple, and quick, providing potential advantages in some contexts over more complex color analyses. However, this technique would only be useful, or necessary, for plants with high survival. Species such as *R. minus*, with high disease susceptibility, die quickly, making this technique unnecessary, as well as limiting statistical power in our analyses. Thus, use of this leaf discoloration technique should also include survival analyses, to capture effects of pathogens accurately. Calibration of this technique with multi-spectral and hyperspectral techniques could be a valuable addition to this work and might generation mechanistic insights. Citizen science projects or applications in horticulture might benefit from this inexpensive quantification of leaf discoloration, using a cell phone camera and the free software ImageJ (Pride et al. 2020).

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