Experimental Evidence That Bark Responses Shape Insect-Fungus Interactions in A Widespread Tree Disease Complex

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Introduction

- Beech bark disease (BBD) is a chronic disease of American beech (*Fagus grandifolia*) caused by an exotic scale insect, the beech scale (*Cryptococcus fagisuga*), and two pathogenic fungi (*Neonectria* spp.). For the fungi to infect the tree, the tree must first be colonized and fed upon by the scale insect.
- Although the precise mechanism is unknown, the scale insect is generally thought to be predisposing beech to BBD by creating infection courts from feeding activity that facilitate the annual reinfection of *Neonectria*.
- Beech hosts have been responding to chronic beech bark disease infections in New Hampshire for half a century and many trees have developed extensive areas of rhytidome (Fig 3 – Fig 5).
- Forest trees respond to bark infections and injuries by producing barriers around injured or infected bark areas. The infected dead tissue remains in place and functions as a wound-induced rhytidome as new replacement tissues are formed under a new necrophylactic periderm (wound-induced periderm) (Fig 4).
- Rhytidome offers effective protection against scale insect feeding, but in contrast, wound periderms are highly susceptible (Fig 4).
- I hypothesize that rhytidome developing from prior BBD-related *Neonectria* infection on American beech has an impact on future beech scale colonization rates.
- I predict trees with rhytidome (rough) will have lower beech scale establishment than trees without rhytidome (smooth).
- If this prediction is true, the potential for a feedback loop between BBD, rhytidome development, and wound-induced periderm exposure should be investigated in future studies.

Methodology

To test if trees with BBD-induced rhytidome (rough) and trees without (smooth) differ in beech scale establishment rates, I set up a field experiment that involved the artificial infestation of beech scale on designated areas of bark over 80 American beech trees. Trees were evenly stratified by bark type and two diameter classes (10-20 cm & 20-30 cm dbh).

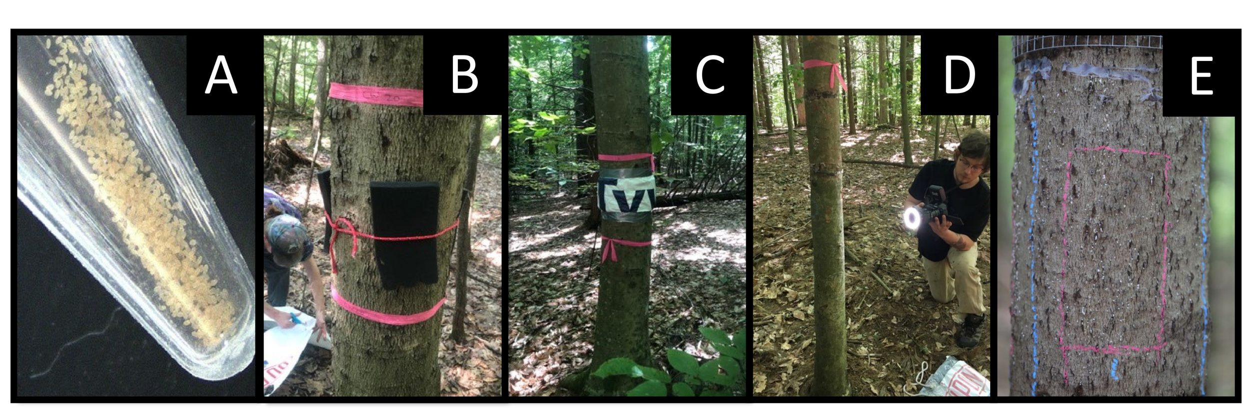

Figure 1 (left to right): a) Partitioned density of beech scale eggs, b) foam pads used to place eggs on tree, c) vapor-permeable house wrap to keep foam dry, d) imaging results e) establishment 1 year later

Before artificially challenging trees, beech scale eggs were collected, mixed, and partitioned volumetrically into two different densities within 2.5 ml microcentrifuge tubes. The egg count for the low-density volume was found to be ~1050 and the high-density was ~1320. Note that eggs are ~150x250 µm in size (the size of a grain of sand). Originally there were three partitioned egg volumes, but the two lowest volumes were found to be no different in the density of eggs upon counting (see sample sizes in Table 2). I then applied the two density treatments to each tree at breast height. One year later, scale insect establishment was measured photometrically using the area of the white waxy material these insects produced as a proxy for establishment rate. Wax mass area is highly linearly correlated to establishment rate (Wieferich, 2013). I then converted the wax mass area (mm^2) to the percent cover of the challenge assay area (15 cm x 10 cm). Using percent cover as the response variable, I constructed a mixed model with the tree as the random effect to test the effects of bark type, tree size, and egg density on scale insect establishment rate.

Results

Scale insect establishment by bark type, dbh and egg density

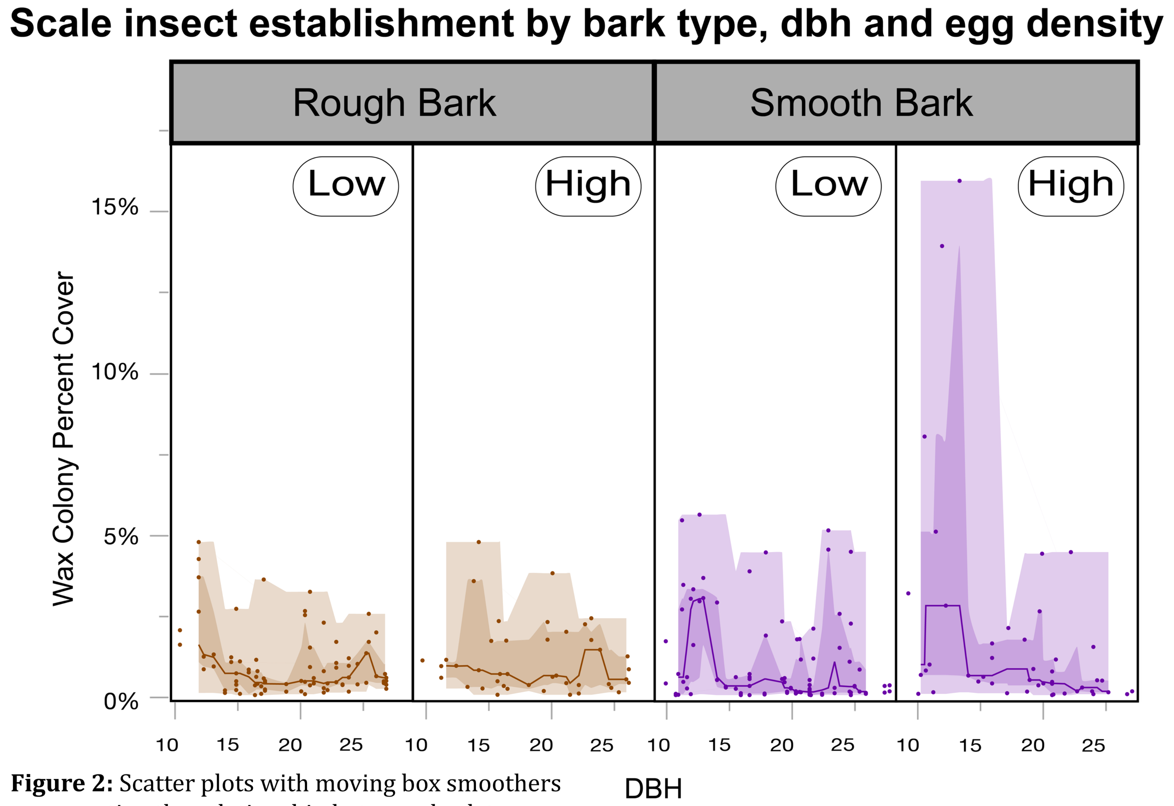

Figure 2: Scatter plots with moving box smoothers representing the relationship between bark type, insect egg density and tree diameter size.

Table 1: Summary statistics for significant main effects and interactions

Main & Interaction Effects	F	*df*	p value
Egg Density	3.8	1, 87.4	0.0225
Size Class	5.12	1, 87.38	0.0261
Size Class * Bark Type	4.8	1, 87.38	0.0311
		Model fit: $R^2 = 0.67$	

Table 2: Mean percent wax cover & standard error (SE) by bark type, egg density and size class. Size classes: 1 = 10–20 cm dbh, 2 = 20–30 cm dbh

Bark Type	Egg Density	Size Class	Ave. Wax Cover	SE	n
rough	high	1	1.14%	0.28	20
rough	high	2	1.07%	0.22	22
rough	low	1	1.06%	0.2	40
rough	low	2	0.87%	0.12	44
smooth	high	1	3.07%	1.04	20
smooth	high	2	0.79%	0.25	25
smooth	low	1	1.48%	0.27	40
smooth	low	2	0.71%	0.17	51

Conclusions

- Overall, scale insect establishment was lower than expected in all categories.
- Highest establishment on the smallest smooth bark trees indicates there is a new cohort of American beech in NH aftermath forests that are highly susceptible to the beech scale. These trees may develop rhytidome over time as BBD progresses on these hosts.
- Lowest establishment on large smooth bark trees indicates there are beech trees in our forests that are genetically resistant to the beech scale.
- The significant interaction effect of bark type * size class indicates that rhytidome reduces beech scale establishment on American beech compared to susceptible smaller beech without rhytidome.
- The varying intermediate establishment rates on rough bark trees indicate that although rhytidome is reducing establishment, wound-induced periderm may be contributing to the variation in establishment among rough bark trees. This also demonstrates that although rhytidome appears to offer varying levels of tolerance to BBD, genetic resistance offers the greatest level of protection (Fig 6).
- In conclusion, the presence of rhytidome can reduce scale insect establishment. Therefore, fungal-induced responses in beech can impact future beech scale establishment rates.
- It will be important for future studies to ascertain whether the scale insect provides the sole pathway for *Neonectria* reinfection. If the beech scale is the sole pathway or primary pathway of reinfection, these results support the possibility of a long-term BBD feedback loop, where rhytidome development decreases BBD severity, and subsequent wound-induced periderm exposure increases BBD severity.

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Bark Response Observations and Concepts in Relation to BBD

Key to Bark Response Figures:

Wound Rhytidome Square brackets: ⊓

Wound Periderm Rounded brackets: ⌒

- **DT** — **Dead tissue:** A mix of necrotic phloem parenchyma and stone cells
- **G** — **Gall:** Scale insect induced proliferation of ligno-suberized periderm tissue
- **HP** — **Healthy Original Periderm:** Original periderm associated with smooth bark beech, functioning as normal
- **I** — **Immature tissue:** Young phloem, typically lacking the physiochemical characteristics of mature phloem. Highly susceptible to scale insect feeding.
- **M** — **Mature tissue:** Containing all the physiochemical characteristics evolution has to offer
- **PC** — **Phloem parenchyma cells:** Containing all the physiochemical characteristics evolution has to offer
- **SC** — **Phloem sclerenchyma cells:** Containing all the physiochemical characteristics evolution has to offer
- **1P** — **Dead original periderm:** The first periderm produced by the tree that has been infected and killed by disease

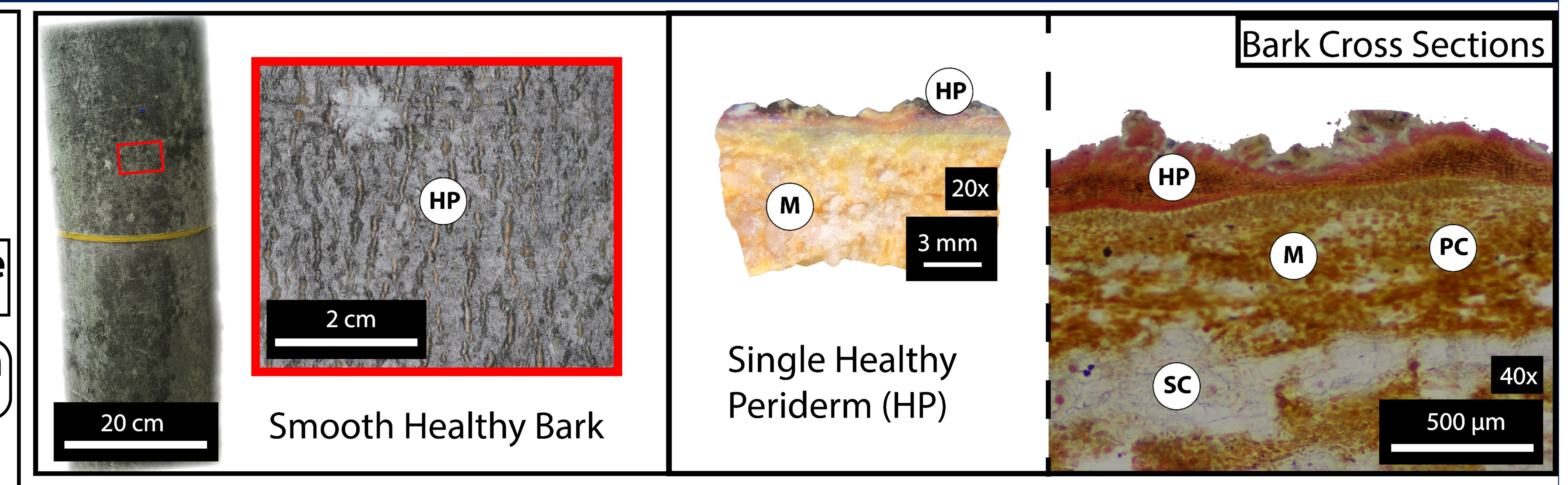

Figure 3: Smooth, healthy, American beech bark. Histoloy image: (far right) cross section, the periderm is stained red with Sudan IV, notice the variation in periderm thickness, where it is only a few cells thick in some areas.

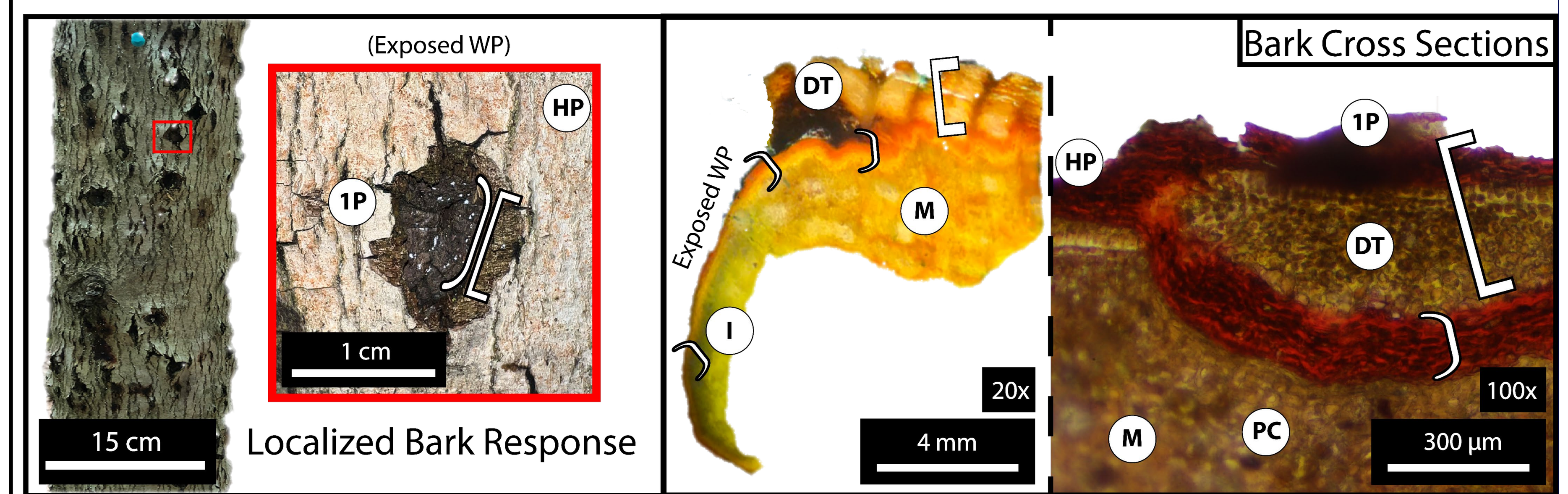

Figure 4: Wound/infection-induced secondary periderm development on American beech. Notice the immature, unprotected new growth (I) in the 20x cross-section of bark. This type of underdeveloped tissue is defenseless to scale insects. Histology image (far right): Sudan IV dye binds to suberin, it highlights periderm tissues in red (both the dead original periderm and wound periderm).

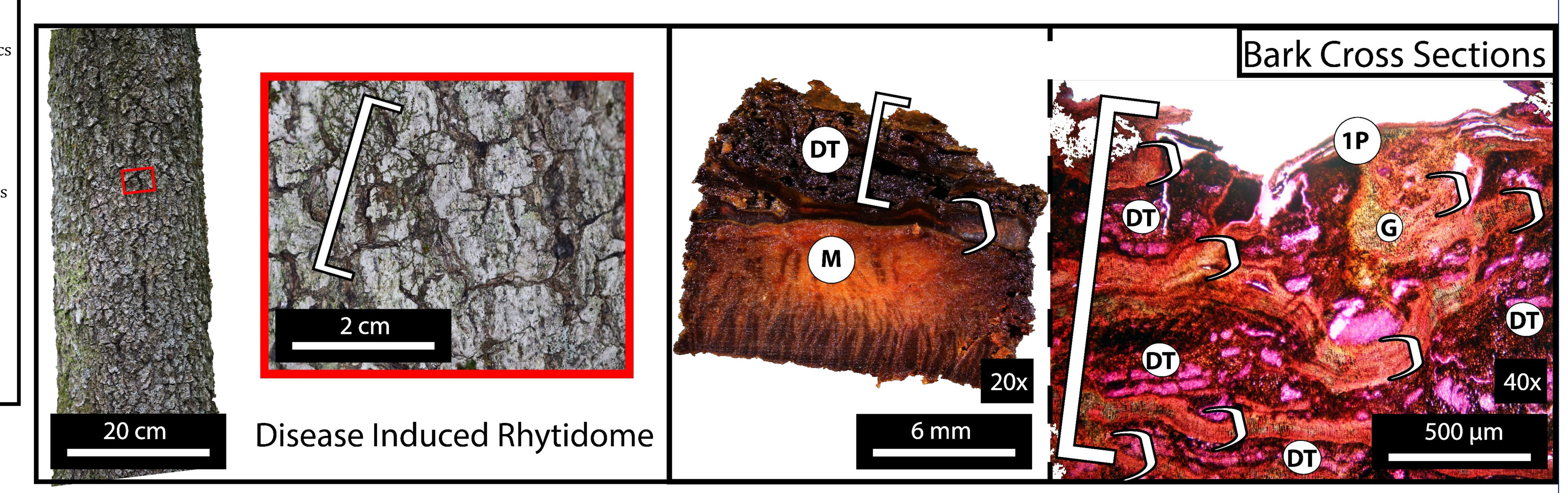

Figure 5: Disease-induced rhytidome development on American beech. Several inner periderms will develop in response to sequential superficial infection on highly tolerant hosts. Notice the thick physical barrier separating the outer bark surface from the phloem tissue in the 20x cross-section. Histology image (far right): Rhodamine 6g fluorescent dye turning sequential periderm layers greenish/orange. This cross-section highlights the results of many years of reinfection. Each DT layer and wound periderm layer corresponds to another year of infection, astonishingly the functional phloem is doing just fine! Note the proliferation of periderm tissue associated with scale insect feeding (G).

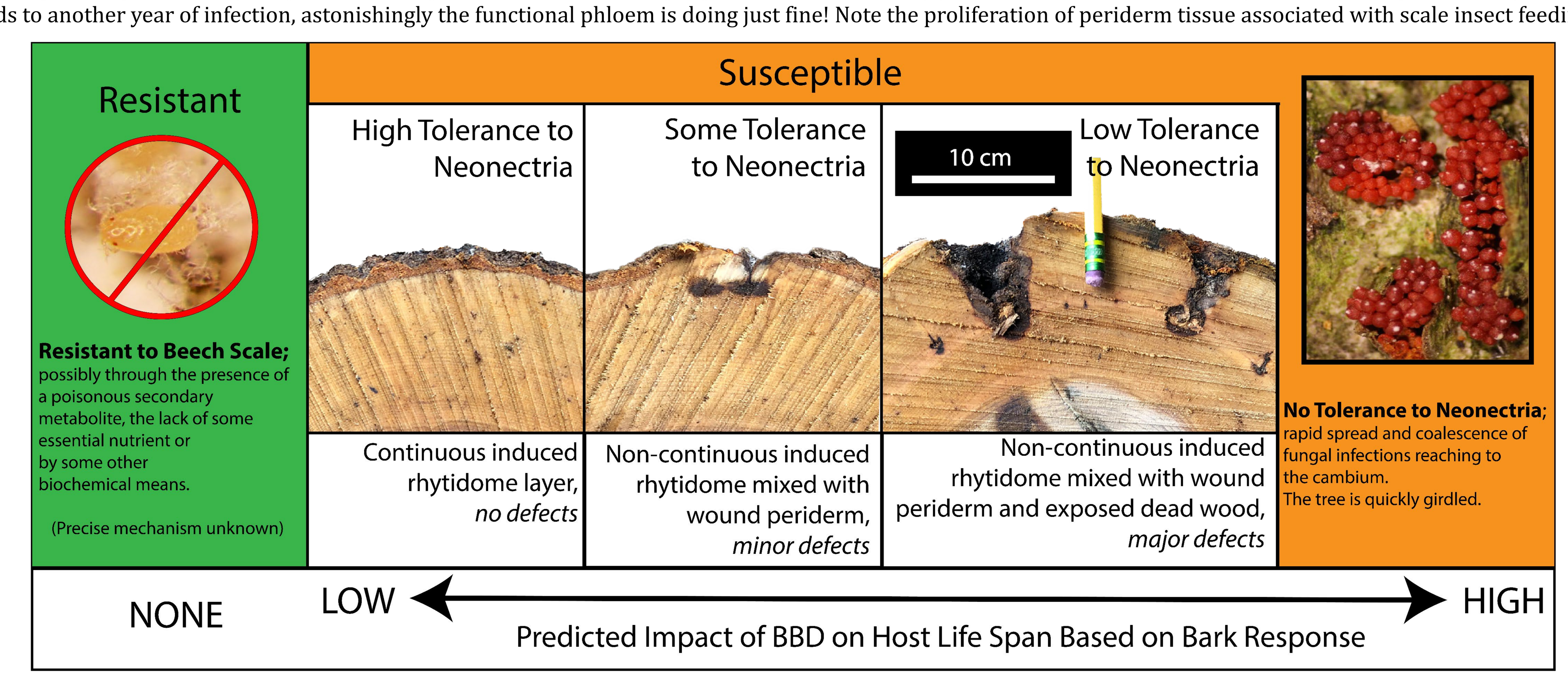

Figure 6: Depiction of the spectrum of tolerance to *Neonectria* infection observed in American beech. Tolerance correlates to lifespan and to the ratio of exposed wound periderm versus wound rhytidome. The less tolerant the host, the more wound periderm tends to be present (unless the host is completely intolerant and doesn't respond at all). This figure is a conceptual figure that builds from the work of Burns and Houston, 1987.