

Natural Fluorescence of Planetree Extracts

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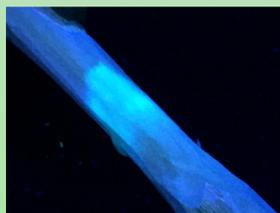
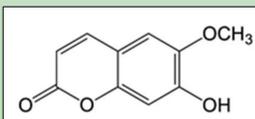
Introduction

Natural wood fluorescence has been an area of interest for the Muyskens lab group for many years. Recently, this interest has focused in on Planetrees. Easily identifiable by their exfoliating bark, which forms patchy, camouflage-like patterns on the trunk and branches, the platanus family is common across much of the United States.

The characteristic of planetrees that interests our lab group is their fluorescence. When stirred in water, planetree wood shavings under a blacklight emit a strong blue light.

Two summers ago, the compound responsible for this phenomenon was identified by the Muyskens lab as scopoletin, a coumarin. This summer's research was focused on learning more about scopoletin production in planetrees, and on sharing those (and previous) findings with the scientific community.

Scopoletin



Above: our fluorescence activity. The left shows the extract with an addition of base, the right shows the extract with added acid.
Left: Planetree wood is fluorescent under the blacklight. Adding base causes a yellow fluorescence.

Objectives

Primary Objective: Write a scientific paper describing a classroom demonstration and activity which explores the fluorescence of planetrees.

Secondary Questions:

- ❖ What are the different species of planetree?
- ❖ Which is best: wood, bark, or leaves?
- ❖ When do samples become strongly fluorescent?
- ❖ Is scopoletin the primary fluorescent compound?
- ❖ Does fluorescence degrade over time?

American Sycamore Platanus occidentalis



Two common USA planetree species:

Platanus occidentalis:

- Native
- Wetlands/creeks in Midwestern / Eastern USA
- Thick brown bark on lower trunk
- Large leaves with shallow sinuses

Platanus acerifolia:

- European Hybrid
- Ornamental street tree across USA
- Mottled bark from roots to branches
- Smaller leaves, deeper sinuses

Both work for activity

London Planetree Platanus acerifolia



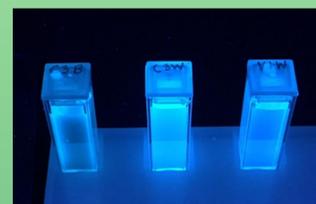
Methods

The primary method of experimentation was the extraction. This technique was developed by Dr. Muyskens and previous students last summer.

1. Shave 1.00 g wood with a knife
2. Add to beaker with 50 mL deionized water
3. Add 200 μ L dilute ammonia (a base)
4. Stir on stir plate for 20 minutes
5. Filter through funnel/filter paper

This technique allowed me to extract scopoletin from the wood and into solution. I could then measure how much light was absorbed and emitted by the solution, and calculate a relative potency of the extract fluorescence. I used a Cary 50 UV-Visible Spectrophotometer to measure absorbance, then diluted the sample in pH 9.5 buffer to an absorbance of .10, and ran the sample through a FluoroMax-4 spectrofluorometer to get emission and excitation spectra. We calculated potency (relative fluorescence) as follows:

$$\text{Potency} = (\text{Dilution Factor} * \text{emission}) / 1,000,000$$

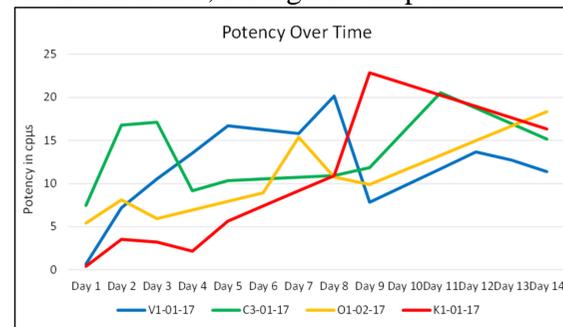


- ❖ Bark, on the left, has a strong color in solution which causes cloudiness in the fluorescence.
- ❖ Leaves are not potent enough to fluoresce well.
- ❖ Wood, at center, has a strong, clear fluorescence ideal for the demonstration.

Results

Fluorescence Over Time

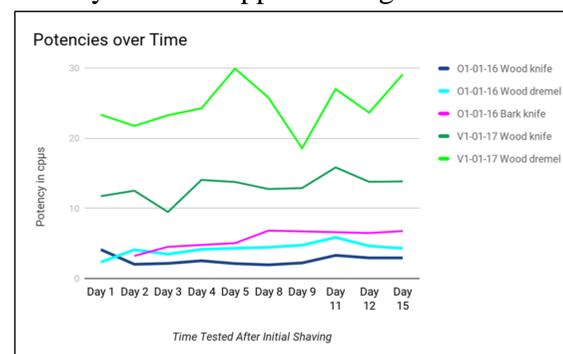
- Fresh samples are barely fluorescent, but year-old samples are strongly fluorescent
- When does the change occur?
- Tested 5 woods (4 shown) over 2 weeks
- Strong fluorescence within 2-5 days.
- Lots of variation; biological samples!



Why? Scopoletin is produced in response to wounding and abiotic stress. Cutting the sample branch = wound + dehydration stress, which leads to increased scopoletin production.

Potency Degradation?

- We may need to send shaving samples to reviewers for the paper
- Two woods, one bark, two shaving methods
- Shavings left out in weigh boats at room temperature (~22°C)
- Tested periodically over two weeks
- Potency does not appear to degrade!

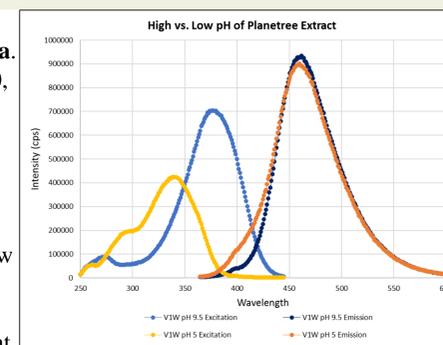


Primary Fluorescent Compound

This summer we determined that scopoletin is the primary fluorescent compound in planetree wood. The emission and excitation spectra of the extract match those of scopoletin, as does the pH dependence of the extract.

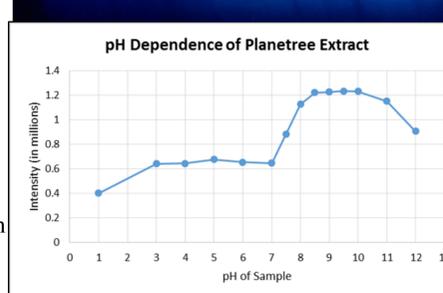
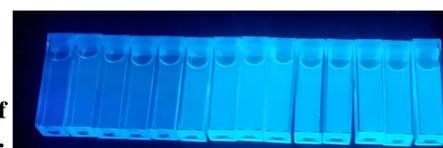
Emission/Excitation Spectra.

Blue lines = pH 10, Orange lines = pH 5. The excitation peaks (left) show that scopoletin is twice as absorbing at high pH as at low pH. However, the emission (right) remains the same at low vs. high pH. This agrees with pure scopoletin.



pH Dependence of Planetree Extract.

This graph shows the extract in buffers of pH 1 through 12. The intensity of the excitation nearly doubles in magnitude between pH 7 and 9, just like scopoletin.



Conclusions

- ❖ There are two common species of planetree— either can work for the activity
- ❖ Wood is best for the activity
- ❖ Samples become potent 2-5 days after harvest because scopoletin is produced as a stress response
- ❖ Scopoletin is the primary fluorescent compound in planetree wood
 - ❖ Emission/Excitation spectra and the pH spectrum of the extract match those of pure scopoletin.
- ❖ We can send shaving samples to reviewers without fear of potency degradation
- ❖ Our paper is ready for submission!

References

Dr. Mark Muyskens
Isaac Izaguirre and previous summer lab students
Calvin College Chemistry & Biochemistry Department
Rich Huisman and Dave Ross
Dr. Thomas J. Hoogboom Memorial Research Fellowship in Chemistry and Biochemistry