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Fluorometry of natural latex from Maclura pomifera

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Abstract
Maclura pomifera is a small tree that produces rot-resistant wood and a strange fruit called an Osage orange which is bright green. The fruits contain a high content of latex with strong fluorescence in UV light, similar in color to the green-yellow fluorescence of uranyl glass. Measurements were made using a computer-assisted microscope with grating mono chromators in both excitation and emission pathways. A photomultiplier was calibrated using a tungsten illuminator, then a mercury illuminator was used to measure the fluorescence emission spectrum: the peak was at 530 nm (green-yellow). A xenon illuminator was used to measure the fluorescence excitation spectrum: the peak was at 380 nm (UV).

Keywords: *Maclura pomifera*, Osage orange, Fluorescence, Latex

Introduction
The Osage orange is the bright green fruit of *Maclura pomifera*, in the mulberry family, Moraceae (Figure 1). It has a strong smell from isoflavones and, botanically speaking, is a syncarp of fused drupes with an internal structure like a pineapple [1].

Figure 1: An Osage orange

The generic name remembers William Maclure (1763-1840), a very successful merchant in Scotland who used his fortune to become a respected scientist in the USA. Without any formal training as a geologist, but with an astounding itinerary in pioneer travelling, in 1809 he published a geological map of Eastern North America [2]. The species name *pomifera* means apple-bearing. The common name relates to the Osage Nation who valued the hard, springy wood of the tree for making bows to shoot arrows. Ontario is at the northern range of this small tree, which has sharp spines and a geological heritage.

When North and South America first became connected by plate tectonics and volcanism, some animals went south, but others came north, like the giant ground sloth, *Megatherium americanum*. Possibly they and other extinct large mammals fed on Osage oranges which may have originated in South America [3, 4]. This is hard to prove, but the idea seems reasonable-because there is little evidence that other large herbivores eat Osage oranges to spread their seeds [5, 6]. So why are the trees still here without a herbivore to spread their seeds? The rot-resistant wood makes excellent fence posts and the trees were widely planted to combat dust-bowl disasters in the USA [7]. Those planted near water mills on the Niagara Escarpment of Ontario may have provided superior wood for the mill.

The wood resists rot-but so do the oranges. So how do they resist rotting? Cutting an Osage orange open with a sharp knife immediately gives the answer-they are filled with sticky latex. Fluorescent latex microspheres are widely used in many histochemical methods but the fluorescence of natural latex has been largely ignored except in the rubber industry [8]. When tested under a black-light, the interior of an Osage orange glowed with a green-yellow fluorescence (Figure 2). The objective of the research reported here was to measure emission and excitation spectra at the cellular level.

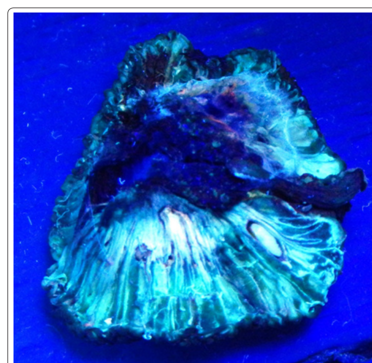

Figure 2: Green-yellow fluorescence from the interior of an Osage orange illuminated by a black-light. Blue areas are not fluorescent but are reflecting wavelengths from the upper edge of the band

pass of the excitation filter-pseudo fluorescence. This is a persistent problem at all levels of fluorometry-the excitation is very intense while the fluorescence is much weaker.

Materials and Method

Osage oranges were harvested as fallen fruits from trees on the Niagara Escarpment and in the University of Guelph arboretum. Samples were shattered to produce fragments mounted in glycerol for microscopic analysis.

The microscope was a Zeiss Universal mounted in an optical bench with grating monochromators for fluorescence excitation (Figure 3, C) and emission pathways (Figure 3, J) [9]. Each monochromator required a filter wheel to remove stray-light (unwanted high orders from the monochromator, Figure 3, D and K). Shutters were needed to block illuminators during standardization (Figure 3, B, F and L).

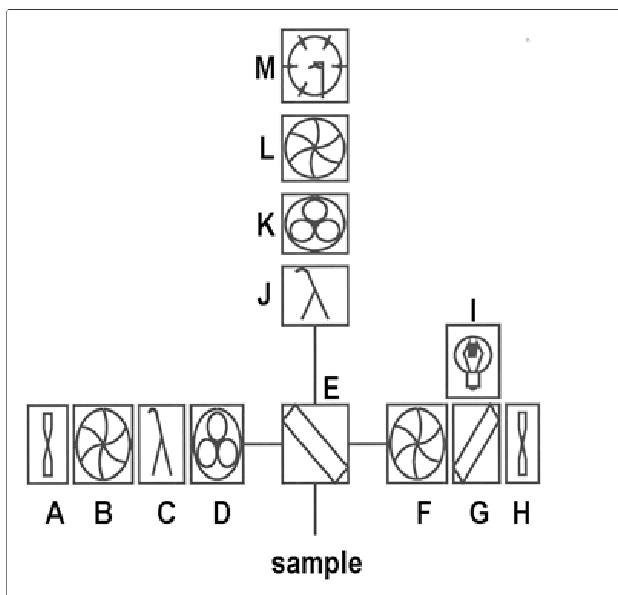

Figure 3: Key parts of the microscope. Xenon arc, A; shutter, B; grating monochromator, C; stray-light filters, D; hand operated half-silvered mirror, E; shutter, F; hand-operated full-silvered mirror, G; mercury arc, H; tungsten lamp, I; grating monochromator, J; stray-light filters, K; shutter, L; and photomultiplier, M. This configuration is for epifluorescence (illuminating the sample from above)

The key point about fluorometry is that it is more difficult than simple transmittance or reflectance spectrophotometry, where the properties of the sample may be compared to a blank space to one side of the sample. The answer required is then primarily a simple ratio-outgoing light as a fraction of ingoing light, and the ingoing light is part of the measurement. But fluorometry measures outgoing light from an unknown system-the fluorescent sample. Thus, a reflectance standard was required to calibrate the microscope photometer and, at each wavelength, the photometer response was weighted by the light intensity given by the manufacturer's data sheet.

In practical terms, three illuminators were needed, switched one at a time into the optical axis of the microscope by mirror rotation. The first was a tungsten illuminator (Figure 3, I) to calibrate the photometer using a smooth emission spectrum without peaks, with the operating amperage matched to manufacturer's data and modulated by changes in the diameter of the measuring aperture or insertion of neutral density filters. A mercury source with a strong UV peak (Figure 3, H) simulated the UV black-light that first showed fluorescence in an Osage orange (Figure 2). But is 365 nm from a mercury source the optimum wavelength to excite fluorescence in an Osage orange? To answer this question required a xenon illuminator with a smooth emission spectrum in the range of interest (Figure 3, A).

A key component of the microscope was a mirror in the vertical illuminator (Fig. 3, E). Most commercial fluorescence microscopes now have a UV source directed through a heat filter, an excitation filter, and then reflected from a dichroic mirror to excite fluorescence in the sample, which then passes back on a different optical pathway, through the dichroic mirror to the observer. For a dichroic mirror in a typical fluorescence microscope, there is a cutoff-low wavelengths are reflected (to the sample) and long wavelengths are transmitted (to the observer). This is a good system for observing fluorescence, but the microscope used here had a half-silvered mirror (Figure 3, E) rather than a dichroic mirror. This was to avoid distorting measurements by the cut-off in a dichroic mirror. When the apparatus was tested with transmitted light passing through a dichroic mirror the light was not white, thus indicating a half-silvered mirror would be more reliable.

All the optics in illumination pathways were quartz glass (high UV transmittance) and the microscope objective was a Zeiss Ultrafluar (× 6.3, NA 0.2). Pseudofluorescence, as explained in Fig. 2, was cancelled by subtracting from all spectra the reflectance of fresh charcoal burned to remove keratin dust. This corrected for any fluorescent dust that might have been in the microscope optics.

Checking the system

Many things need to be optimized in programming a fluorescence microscope, so it is essential to measure a standard to check on the system [9]. Zeiss uranyl glass has peak UV fluorescence emission at 530 nm. Fig. 4 shows correct detection. Other fluorescence standards were also checked.

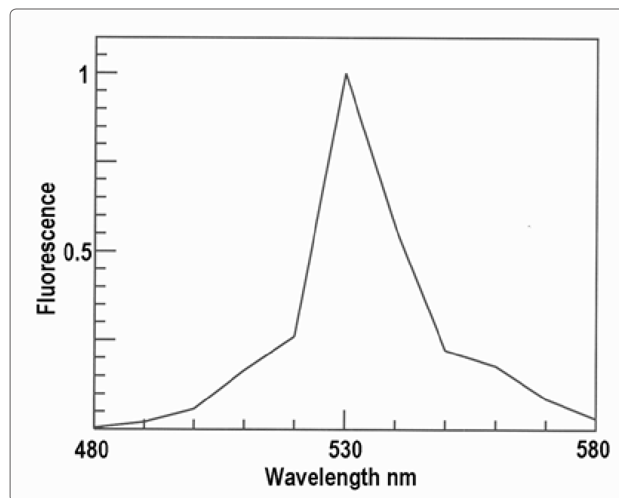

Figure 4: Fluorescence emission spectrum of a Zeiss uranyl glass standard illuminated by the 365 nm peak from a mercury source passing through a heat filter and a Zeiss UG5 excitation filter. An average of 5 measurements.

Results

The latex of the Osage orange had a similar fluorescence emission spectrum to uranyl glass with a peak at 530 nm (Figure 5). Both appeared to be green-yellow, so this seemed reasonable.

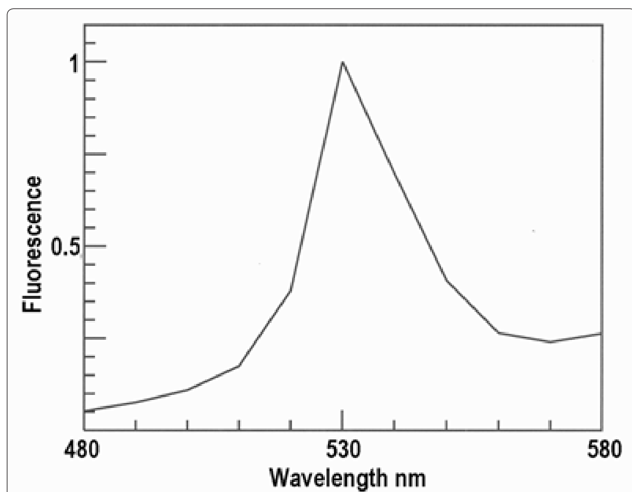

Figure 5: Fluorescence emission spectrum of drupes in the interior of an Osage orange illuminated by the 365 nm peak from a mercury source passing through a heat filter and a Zeiss UG5 excitation filter. An average of 5 measurements

But does a black-light with a peak intensity at 365 nm obtain the maximum fluorescence emission? To answer this question, the illuminator was changed to the weaker UV of a xenon source (weaker but lacking the peaks of a mercury source). As seen in Fig. 6, the 365 nm mercury peak was not the optimum wavelength to excite fluorescence in an Osage orange. But with a black-light or a mercury peak passing through a UG5 excitation filter, the band pass is probably quite wide and will include light at 380 nm.

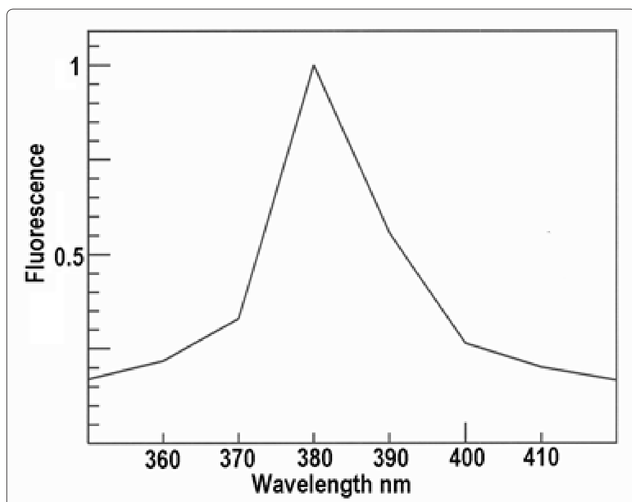

Figure 6: Fluorescence excitation spectrum of drupes in the interior of an Osage orange illuminated from a xenon source. An average of 5 measurements.

Discussion

The results prove that something in an Osage orange is fluorescent and, with synthetic latex microspheres being a common tool in fluorescence histochemistry, it seems highly likely that natural latex in the Osage orange is also fluorescent. However, a little caution is required. Osage oranges have yielded many unusual chemical compounds and it is possible that some of these are contributing to Osage orange fluorescence [10-14]. Testing the fluorescence of newly identified chemical compounds from the Osage orange might help to resolve this doubt.

Osage orange fluorescence is strongest in the outer part of the fruit, under the green exterior, so it is possible that an underlying fluorescence contributes to the strange bright green appearance of the fruit. Work is in progress to test this idea, and also to correlate fluorescence with the strongly reflective birefringence detected in the outer part of the fruit.

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