Spectral Characteristics of Natural and Laboratory-Induced Leaf Senescence in Four Common North American Tree Species

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Abstract

Two laboratory techniques (air drying and oven drying) were implemented to compare the spectral reflectance differences between laboratory-induced and natural leaf senescence. The spectral signatures of four common North American tree species were recorded over the 2011 summer-autumn season. Natural 'on-tree' senescence was measured throughout the senescence period and simultaneously with the two laboratory methods. Two substantial differences were observed between the natural senescence and laboratory techniques: the persistence of the 'green peak' for the laboratory methods; and the much higher reflectance values throughout the middle infrared region for the laboratory methods. These results suggested that a more sophisticated laboratory method including the ability to export leaf constituents during senescence is required to accurately reproduce autumn leaf senescence in a laboratory setting. If achieved, this could greatly improve analyst's ability to reproduce the spectral changes associated with vegetation health and leaf senescence.

Keywords

Senescence; Leaf spectra; Chlorophyll; Dehydration; Laboratory

Introduction

Senescence, the final stage of leaf development, is critical to a plant's overall fitness. Its primary purpose to relocate nutrients from the leaf to other organs of the plant [16] has important implications for agriculture and forestry, specifically, in impacting crop yield and acting as an indicator of tree health [18][20][11]. Optical remote sensing has proven to be a valuable tool to measure plant health. Water and leaf biochemical content can be predicted and provide us with information on plant productivity, rate of litter decomposition, and availability of nutrients in space and time [7][19][21][8][1][4]. The ability to predict concentrations of these leaf constituents, coupled with an analysis of plant senescence, has the potential to produce an even more comprehensive understanding of plant health. However, natural senescence is not always a process that analysts can predict. In addition to this, if the purpose of the study is to determine the processes that induce senescence so that they can be mediated, the point at which natural senescence begins may be too late to save the plant from destruction. The ability to re-create this natural phenomenon in the laboratory would enable analysts to measure and understand plant health in a more complete and controlled setting. This study is the first step in developing techniques to re-create natural senescence in the laboratory, which includes an analysis on the differences between natural 'on tree' senescence, and two easily applied techniques for laboratory-induced senescence.

Background

Senescence refers to changes in the biology of an organism as it crosses into old age. These deterioration processes can happen on the cellular, tissue, organ, or organismal level, and can occur both naturally or prematurely depending on the conditions which the organism experiences. Leaf senescence classifies as organ level senescence. This biological phenomenon, which is ultimately governed by a process known as programmed cell death (PDC), is considered to be an altruistic process [16]. Though the life of the leaf may come to an end, the process of its degeneration is beneficial to the organism as a whole.

Senescence can be cued both naturally and prematurely by internal endogenous factors and external environmental factors. Natural senescence refers to the timely death of the organ, for example when leaves turn colour in fall. Premature senescence is the product of an unexpected change in the organism's environment, such as the onset of drought.

Further examples of environmental factors that may induce both premature and/or natural senescence include the shortening of the photoperiod, nutrient limitation, extreme temperature, oxidative stress by ultraviolet-B (shortwave) irradiation, pathogen infection, or shading by other plants. Examples of endogenous factors that may induce both premature and/or natural senescence include high sugar concentration, the correlation of senescence with other organs within the plant, or correlation with the lifespan of the plant[16].

Once a change factor is present, whether environmental (exogenous) or endogenous, the leaf commences PCD is mediated and controlled by many active genetic programs [4]. The purpose of PCD is primarily to remobilize the nutrients within the leaf to other organs in the plant [16], such as to the developing seeds during reproduction or to the stem for winter storage and survival. Within PCD, there are both structural and biochemical changes that occur within the leaf. The first change is a reduction in anabolic activity of the leaf or the "building up" of organs and tissues. The cellular content of polysomes and ribosomes decreases, resulting in reduced synthesis of rRNA and tRNA [16]. The next step, which marks the beginning of leaf senescence, is the disintegration of the chloroplast and the degradation of the chlorophyll pigments within the chloroplast [16]. Here chlorophyll molecules are broken down so that the nitrogen within them can be released for exportation from the leaf. The nucleus and mitochondria of the cells remain intact so they are able to carry out the remaining tasks of senescence such as gene expression and energy production [16]. This step can be visibly detected by a change in color of the leaves, which is an expression of the different concentrations of certain pigments within the leaves. Following chloroplast disintegration, the plant cell undergoes controlled vacuolar collapse, chromatic condensation and DNA laddering. Proteases located in the vacuole further break down the proteins and other macromolecules of the cell for the exportation of nutrients [16]. Once the integrity of the plasma membrane has been lost, cellular homeostasis is disrupted and the cell is considered to be dead; senescence is complete.

The three main pigments present in leaves are chlorophylls (65%), xanthophylls (29%), and carotenoids (6%) (Gates et al., 1965; [11]. The colour that a leaf turns as it senesces is dependent on the concentrations and combinations of these three pigments. Carotenoids, such as β-carotene,

preferentially absorbed in the blue wavelength region, have higher reflectance (less absorbance) in the longer green and red regions, resulting in a general reflectance of the colour yellow. Chlorophylls, such as chlorophylls a and b which are responsible for photosynthesis, preferentially absorbed in the blue and red regions , have higher reflectance in the green, leading to a green colouration when leaves are photosynthesizing. Anthocyanins preferentially absorb light in the blue-green wavelengths and scatter in the red, producing a brilliant red colour. They account for not only the red color of senescing leaves, but also the brown to red colors observed in many fruits and foliage. They function in protecting plants against photoinhibition and permanent damage by acting as antioxidants [15][17].

There are many biochemical changes that accompany leaf senescence, as mentioned above. The ability to detect the onset of senescence, particularly prior to the onset of visually obvious senescence, would be useful for many applications such as in forestry and agriculture. The first biochemical change associated with senescence is the disintegration of the chloroplast [18][20][11][12]. Since this change is visually detectable, using spectroscopy to detect the onset of senescence seems redundant. As an alternative, rather than looking for the biochemical changes associated with senescence, it may be possible to look for the biochemical components that are associated with precursors of senescence. An example would be the endogenous factor of high sugar concentrations [1]. Curran et al. [8] found a correlation, albeit low, between derivative reflectance and the concentration of sugar and protein in fresh whole leaves (1992). As there are many factors that cue the onset of senescence, the potential exists to find the biochemical spectral signatures associated with those factors leading to senescence.

Methods

Data Collection and Study Area

Leaf samples from four separate tree species on the University of Winnipeg campus were collected throughout fall season from September to November, 2011. The four tree species selected for the study include: Ulmus americana, (American elm); Betula papyrifera, (paper birch); Acer negundo, (Manitoba maple); and Fraxinus nigra, (black ash). The selected trees, all classified as deciduous hardwood species, were chosen for a number of reasons: (1) they are

employed in a large range of commercial applications including city landscaping, construction, and the building of furniture, flooring, and other wooden objects; (2) they are relatively abundant throughout the mid-eastern regions of North America [14] and (3) U. americana is of particular interest as it has experienced massive devastation in recent years from the insect-spread Dutch Elm Disease.

One tree from each of the above mentioned species was selected for sampling. All were located in various green-spaces throughout the University of Winnipeg campus grounds. During the sample period, three 'fresh' leaf sample sets (chosen randomly) were picked from three separate marked branches of each of the four tree species. Depending on the progression of senescence, the leaf samples were picked either once or twice a week throughout the sampling period. Sampling dates and intervals are displayed in Table 1.

TABLE 1 SAMPLING AND SHOOTING DATES OF THE FRESH LEAVES AND THE VARIOUS LAB TECHNIQUES THAT WERE SHOT IN CONJUNCTION WITH THOSE DATES

Sample Date	Day	Spectra acquired			Fallen
		Fresh	Air Dried	Oven Dried	
09/21/11	1	X	X	X	
09/23/11	3		X	X	
09/27/11	7	X	X		
09/30/11	10	X	X		
10/04/11	14	X	X		
10/07/11	17	X	X		
10/11/11	21	X	X		
10/14/11	24	X	X		X
10/18/11	28	X	X		X
10/25/11	35	X	X		X
11/01/11	42	X			
11/04/11	45	X			X

Once picked, the three leaf sample sets were placed in air-tight bags to prevent moisture loss during transport, then immediately brought to the lab, placed in an ASD Leaf Clip (Figure 1), and shot by a Plant Probe attached to an ASD Field Spec Pro HR Spectrometer (Figure 2); spectra were acquired within 30 minutes of collection. The Plant Probe uses a low intensity 6.5 Watt halogen light source to minimize leaf dehydration during spectral data acquisition. The three leaf sample sets were stacked on top of each other to minimize transmission of light within the Leaf Clip.

300 spectra were collected and then averaged. Spectral data were measured between 350 and 2500 nm with a resolution of 2-7 nm and 1.4 nm sampling interval. A calibrated Spectralon disk was used to derive absolute reflectance. The instrument has a 10 mm field of view. Photographs were taken of all the samples on every sample date to capture the visible change in colour during senescence. Sampling of on-tree leaves continued until the last leaf had fallen from each of the marked branches.

FIG. 1 LEAVES WERE PLACED IN A LEAF CLIP

FIG. 2 PLANT PROBE AND ASD FIELD SPEC PRO HR SPECTROMETER

After the initial leaf samples were picked and spectrally characterized, the fresh leaves were placed within the air dry and oven dry experiments where they remained throughout the sampling period. To prevent leaf curl during the dehydration of these experiments, and to enable proper flat-surface shooting of the spectra, forms made of plastic sheets and clamps were created to hold the three leaf sample sets flat. The centre of these forms was kept open to the air on one side so that spectra could be shot on the bare upper surface of the leaf samples. The oven dry samples were placed in the drying oven at a temperature of 70°C [10][2][9] and the air dry samples were left out at room temperature, ~23°C. Weights of the fresh leaf samples were also taken to record moisture loss throughout the experiment. For the oven-dried leaves, spectra were shot after 24 and 48 hours. Beyond this time frame, no spectral changes or further weight losses were observed. For the air-dried experiment, spectra were shot in conjunction with every new sampling of freshly picked leaves (Table 1).

Weight measurements of all samples were taken any time when new spectra were acquired in order to monitor water loss. At the end of the sampling period (Day 24 for U. americana, Day 28 for F. nigra, Day 42 for A. negundo, and Day 45 for B. papyrifera), leaves that had fallen to the ground naturally from each of the four tree species were also collected and spectrally characterized.

Data Analysis

Once the collection of spectral data was complete, absolute reflectance values from the three sample sets from each of the four tree species were averaged. The averaged data was used to observe the spectral changes in absolute reflectance for all four tree species and each of the senescence techniques (natural, oven dried, and air dried) over time (see Figures 3-6).

Based on previous work conducted by Curran and Wessman [7][21], different biochemical absorption bands were observed within the data that could be related to specific leaf components. These include, the chlorophyll-a bands at 430 and 660 nm, chlorophyll-b bands at 450 and 650 nm, carbon-hydrogen bands at 1780 and 2300 nm, and water bands at 970,1190, 1450, and1940 nm (Table 2). Change in band depth (ΔD) over time was calculated for the four tree species and three senescence techniques by dividing the absolute reflectance at the base of the band (R_b) by means of the absolute reflectance at the top of the band (R_c) (Equation 32 of [6]).

$$\Delta D = 1 - \frac{R_b}{R_c} \qquad (1)$$

Changes in selected band depths over time are displayed in Figures 7-9.

Results and Discussion

In general, more spectral variation was observed between senescence techniques than that between the four tree species. For the oven-dried technique, the slope break between the red and near-infrared regions or 'red edge' (around 700 nm) of U. americana and F. nigra experienced a more dramatic shift to longer wavelengths than that of A. negundo and B. papyrifera. For the natural 'on-tree' technique, there was a larger increase in reflectance in the visible region for U. americana, B. payprifera, and F. nigra than that for A. negundo. Apart from these differences, each species had the same general response to the different senescence techniques. For this reason, the following section will deal with the species U. americana when compared to the different techniques

of senescence.

TABLE 2 WAVELENGTHS USED TO CALCULATE BAND DEPTHS FOR THE VARIOUS TYPES

Band Type	Wavelengths (nm)	
	Selected Minimum Wavelength (R_b)	Selected Continuum Wavelength (R_c)
chlorophyll - a	430	555
	660	750
chlorophyll - b	450	555
	650	750
carbon-hydrogen	1780	1660
	2300	2220
water	970	1100
	1190	1295
	1450	1300
	1940	1850

Natural 'On-Tree' Senescence

In the visible region (Figure 3-a), absolute reflectance has increased gradually for the first 10 days. On day 14, this region experienced a substantial increase in reflectance. Figure 7-a shows the drop in absorption band depth for chlorophyll-a at 660 nm from 93 to 47% from day 10 to day 14. A further increase in reflectance and decrease in band depth occurred between day 14 and day 17 at which point no further significant changes in band depth or visible region reflectance were noted. In conjunction with this increase in visible region reflectance, the 'red edge' experienced a shift towards shorter wavelengths with a substantial shift at day 14. This shift corresponds to the first observed incidence of colour change within the leaves and chlorophyll pigments disintegrated to reveal spectral signatures of the carotenes and other yellow pigments which then became dominant [13]. In the near-infrared (NIR) region from 700 – 1300 nm, there was an increase in reflectance from day 1 to day 7which was attributed to structural changes of the air-cell interface within the spongy mesophyll of the leaf as it got matured (Gates et al., 1965; [21][2]. During the later stages of senescence, the reflectance began to decrease as further changes were experienced within the internal leaf cell structure [3]. In the middle infrared (MIR) region from 1300 – 2500 nm, there was virtually no change in reflectance value until the last day (Day 24) when there was a drop in reflectance across the region. This lack of change in the MIR region has demonstrated that the leaves were under little water stress for the duration of their senescence on the tree as this wavelength region was dominated by water absorption bands near 1450, 1900, and 2700 nm.

The spectral signature of the leaves that had fallen to the ground (fully senesced) showed large differences compared to the leaves that were still senescing on the trees. Extending across the visible and NIR regions of the fallen leaves, there is complete disappearance of the 'green peak' and a flattening of the 'red edge'. This marks the disintegration of chlorophyll molecules within the leaves. In addition, overall reflectance in the MIR region is higher in comparasom with that for the leaves on the tree, which suggests that the fallen leaves have undergone higher levels of dehydration than the leaves still on the tree.

Air Dried Senescence

For the air dried technique (Figure 1-b), the visible region only experienced a minor increase in overall reflectance from day 1 to day 2. Following this, there was little increase in the visible region overall reflectance. The 'red edge' experienced virtually no change in location for the duration of the study period. These subtle changes within the visible region were consistent with our visual observations: little if any colour change was observed with the leaves in the air dried experiment. In the NIR region, steady increases in reflectance were observed for the first week of the experiment, after which little further change occurred. This steady state coincides with the point at which the leaves reached maximum moisture loss, based on the weight loss measurements. Changes in reflectance were also the largest towards the longer wavelengths of this region. For the MIR, steady increases in reflectance, but with larger absolute values, were also only observed during the first week of the experiment, after which there was little further change. Water bands located at 970, 1190, 1450, and 1940 nm were all observed to have substantial reductions in band depth as the leaves became dehydrated. In response to this decrease in water-band depth, C-H bands located at 1780 and 2300 nm became more evident and were observed to increase in depth. This demonstrated how water bands tended to dominate the spectral signatures of hydrated leaves, masking the spectral signatures of other biochemical components.

Oven Dried Senescence

The oven drying experiment (Figure 1-c) only lasted three days as maximum dehydration was reached at this time according to the weight loss measurements. For the visible wavelengths, there was no region-wide increase in reflectance, rather, the 'green peak' shifted towards longer wavelengths. This was also noticed

visually as the leaves turned brown after the first day of oven drying. The 'red edge' at 700 nm became shallower with oven drying. An increase in detail in the 600-700 nm region was also noticed, with two distinct bands appearing after oven drying. These might be explained by differences in pigment metabolism induced by the agent of water stress, but it seemed probable that they resulted from variables such as interspecific differences in pigmentation [5]. Like the air dried technique, the NIR experienced an increase in reflectance with the greater differences existing towards the long wavelengths. The MIR for the oven drying experiment behaved much in the same way that it did for the air drying experiment except that it happened at a much faster rate (in three days rather than one week). The water bands within this region, like the air drying technique, were also observed to decrease in band depth. Again, this resulted in an increase of band depth for the C-H bands as the water lessened its spectral dominance over this region. As mentioned, the other tree species (Figures 4, 5, and 6) showed similar spectral behavior.

Comparing Senescence Techniques

The laboratory senescence techniques in this experiment have demonstrated two major differences from the 'on-tree' natural senescence. For the visible region, the laboratory techniques did not show the same general increase in reflectance that the 'on-tree' method did. Figure 7-a demonstrates the change of band depth for chlorophyll-a at 660 nm for all three techniques. The natural 'on-tree' technique experienced a larger decrease in band depth than the two laboratory techniques. This was also evident in the visual record of the experiments, and the leaves only turned their natural yellow when on the trees. This difference is most likely due to the lab techniques' inability to allow for degeneration and exportation of the chlorophyll molecules and subsequent disclosure of the other leaf pigments, which would have caused the colour change. Further evidence for this is that a sharp 'red edge' only occurred for the natural senescence. The second major difference between the laboratory and natural techniques included a higher reflectance of the MIR region for the two laboratory techniques. This difference indicates that natural fall senescence does not experience the same levels of dehydration as the two laboratory techniques tested in this study. Figure 7-b demonstrates the difference in 1940 nm water-band depth as it has changed throughout the experiments. Though water stress may

be a factor that could induce senescence, it was clearly not the case for the natural autumn senescence experienced in this study. The forced dehydration conducted in the laboratory was not an accurate reproduction of natural autumn senescence and therefore it should not be used to study this type of senescence. However, this reduction in water-band depth allowing for some of the different biochemical band signatures became more evident than that in the natural senescence (such as the C-H bands at 1780, 2100 and 2300 nm). Therefore, the lab dehydration technique confirmed that by dehydrating leaves one can better detect various biochemical components within them [19][9].

There are also many differences found within the final spectral signatures of the laboratory techniques and the natural fallen leaves. In addition, within the laboratory techniques the presence of chlorophyll is observed throughout the study, as the 'green peak' and adjacent absorption bands do not disappear. Nearly complete disappearance of the 'green peak' was, however, observed in the spectral signature of the natural fallen leaves. Reflectance values in the MIR region for the natural fallen leaves are also not as high as the values found within the MIR regions of the laboratory techniques. This confirms that the levels of dehydration were much higher for the laboratory techniques than that in the natural senescence. Once again, it was noted that the other tree species showed similar spectral behavior (Figures 8, 9, and 10).

Conclusions

There are many factors that contribute to the understanding of plant health. The ability to mimic and study leaf senescence using reflectance spectroscopy in a laboratory setting has the potential to substantially deepen this understanding. In this study, laboratory induced senescence techniques have been designed to simulate what happens naturally when a leaf begins to die. Spectral signatures of the leaves recorded over time have been compared to test the validity of these techniques. The overall reflectance of the visible region and the disappearance of the 'green peak' for the two lab-induced techniques was much lower than that for the natural 'on-tree' analysis. In addition to this, the reflectance of the MIR region was much higher for the laboratory induced techniques than that for the 'on-tree' analysis. As such, these differences have indicated that the particular laboratory techniques used in this study are not adequate to properly explore spectral changes accompanying natural autumn senescence.

There are two recommendations for future studies: (1) that laboratory techniques require some method to control chlorophyll exportation from the leaves (as in natural senescence); and (2) that laboratory techniques employ a different factor to induce senescence, such as temperature, photoperiod, or nutrient deprivation (for example). Using dehydration (by removing leaves from trees) as a means to induce senescence has demonstrated markedly different characteristics compared to the observed, natural autumn senescence. Future experiments will examine whether the removal of entire branches from trees, and the application to air and oven-drying temperature excursions, variations in photoperiod, and nutrient and water deprivation are better to reproduce the spectral changes accompanying natural fall senescence.

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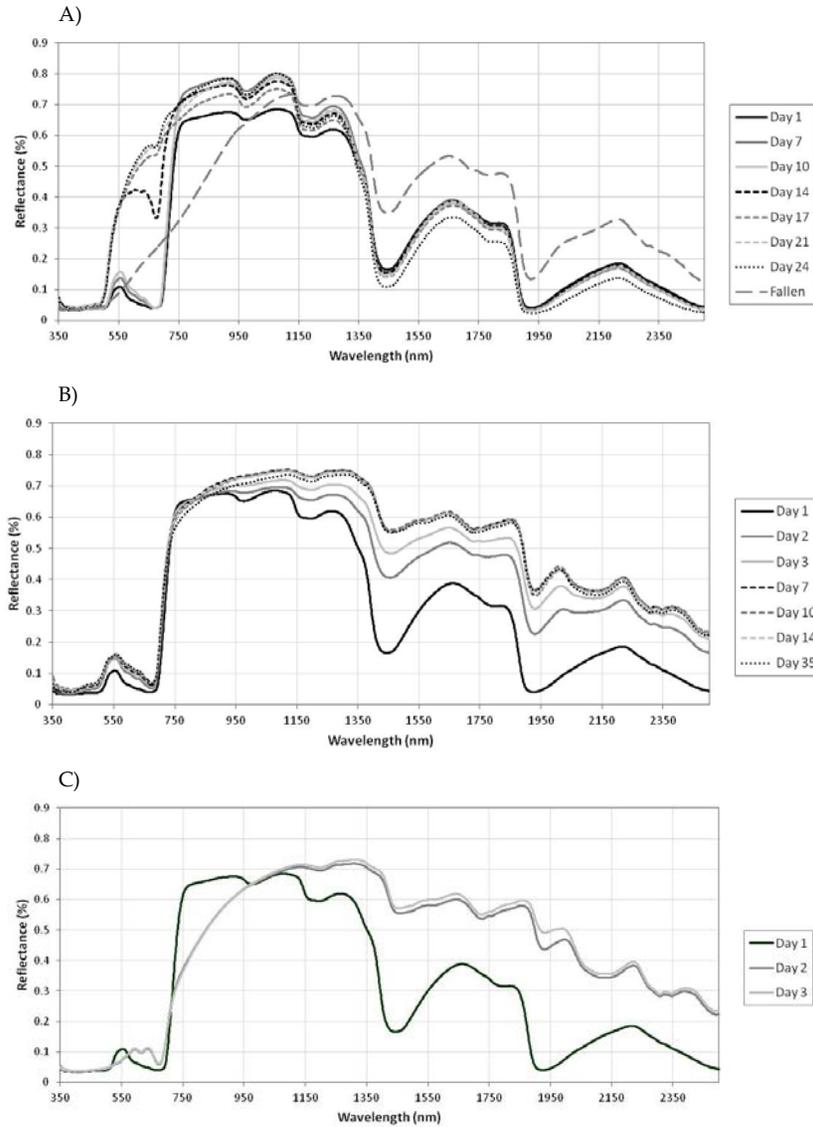
FIG. 3 REFLECTANCE SPECTRA OF *ULMUS* AMERICANA OVER THE DURATION OF THE STUDY PERIOD FOR THE A) NATURAL 'ON TREE' SENESCENCE, B) AIR DRIED SENESCENCE, AND C) OVEN DRIED SENESCENCE
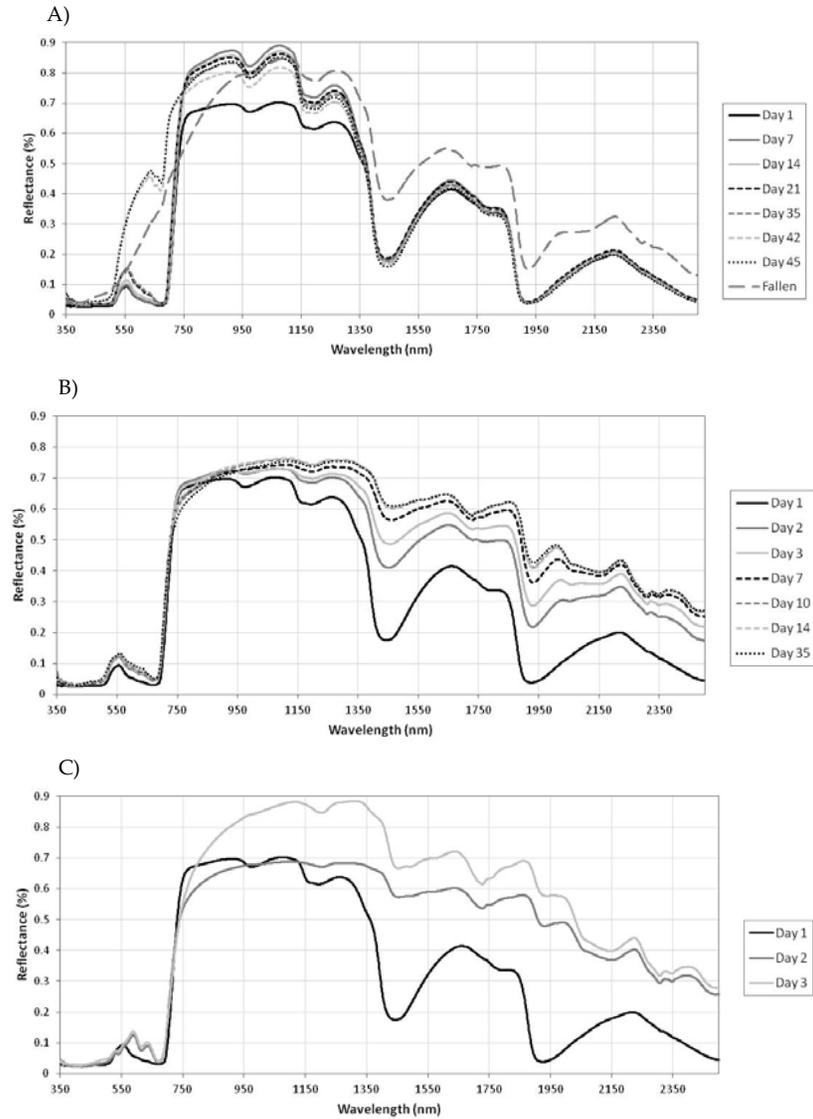
FIG. 4 REFLECTANCE SPECTRA OF *BETULA PAPYRIFERA* OVER THE DURATION OF THE STUDY PERIOD FOR THE A) NATURAL 'ON TREE' SENESCENCE, B) AIR DRIED SENESCENCE, AND C) OVEN DRIED SENESCENCE
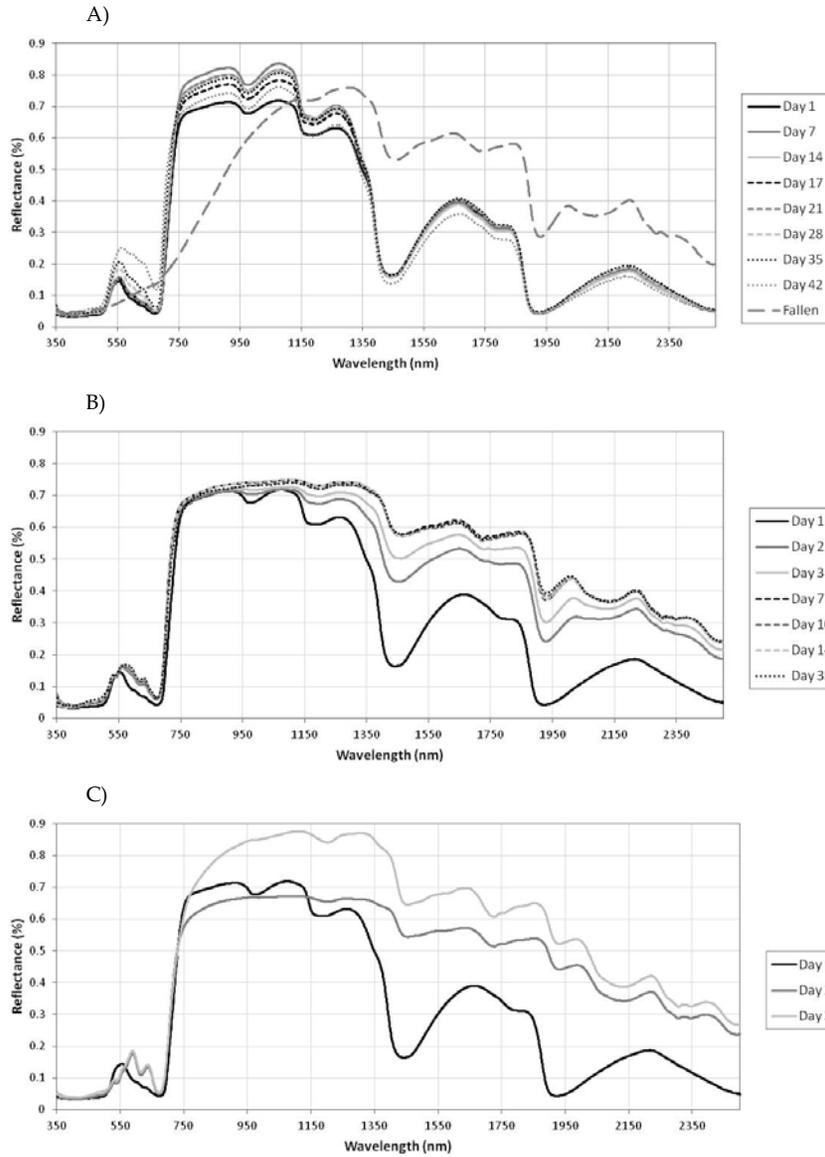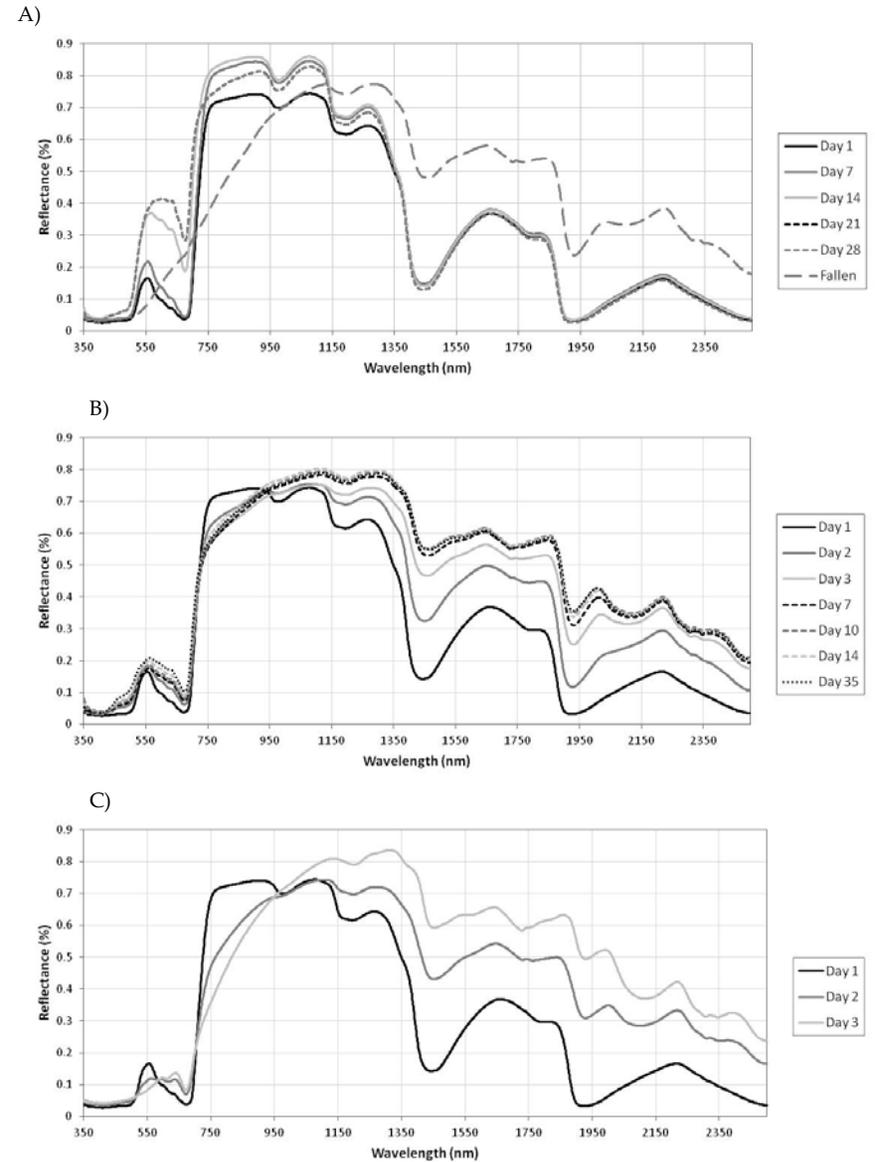
A)

B)

C)

A)

B)

C)

FIG. 5 REFLECTANCE SPECTRA OF *ACER NEGUNDO* OVER THE DURATION OF THE STUDY PERIOD FOR THE A) NATURAL 'ON TREE' SENESCENCE, B) AIR DRIED SENESCENCE, AND C) OVEN DRIED SENESCENCE
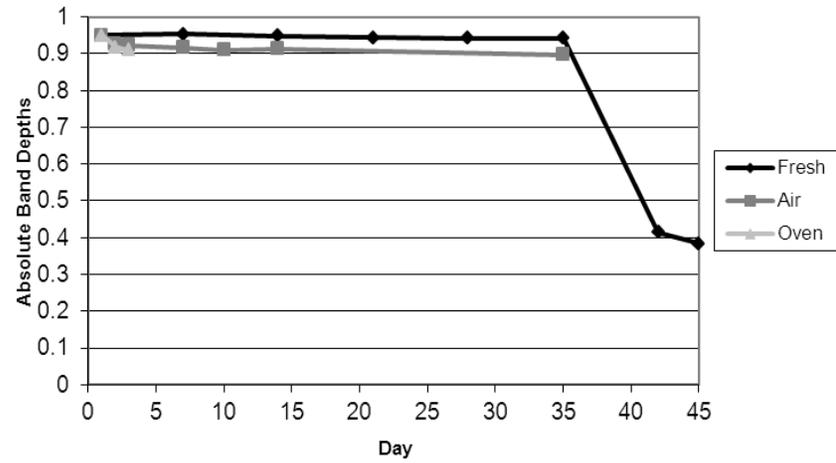
FIG. 6 REFLECTANCE SPECTRA OF *FRAXINUS NIGRA* OVER THE DURATION OF THE STUDY PERIOD FOR THE A) NATURAL 'ON TREE' SENESCENCE, B) AIR DRIED SENESCENCE, AND C) OVEN DRIED SENESCENCE
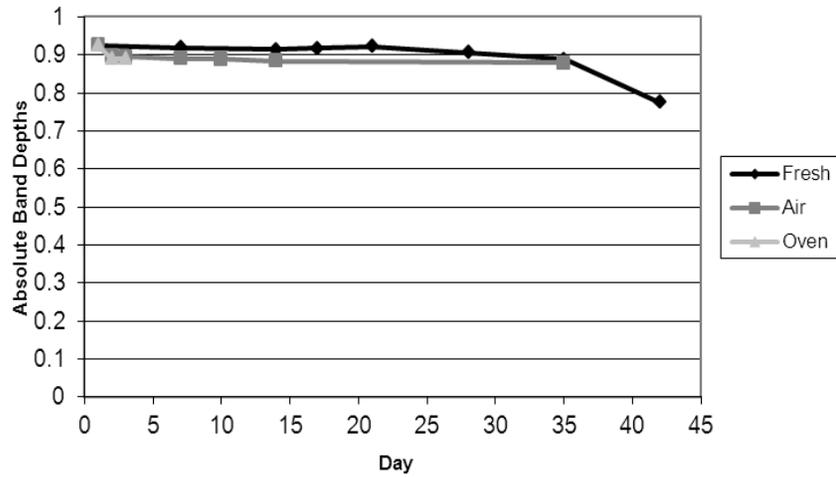
A)

Chlorophyll a: U. americana (660 nm)

A)
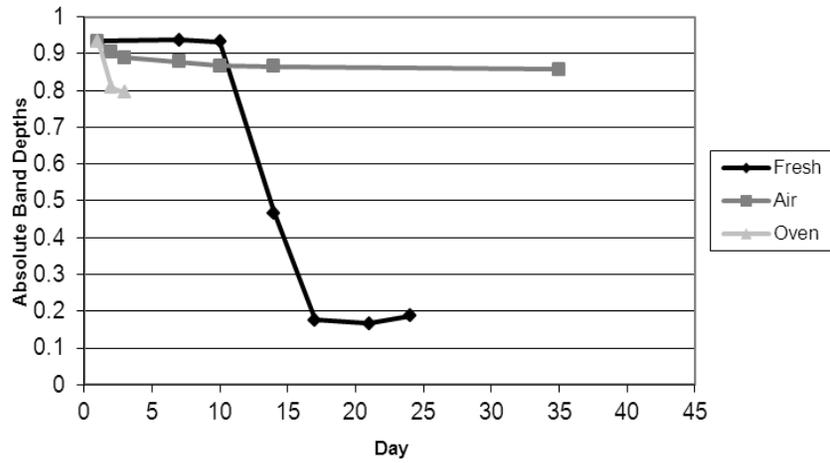
Chlorophyll a: B. papyrifera (660 nm)

B)

Water Band: U. americana (1940 nm)

B)

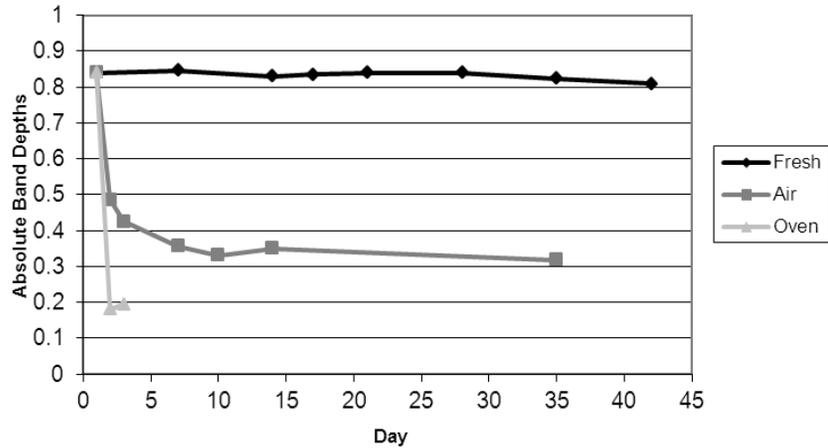Water Band: B. papyrifera (1940 nm)

FIG. 7 ABOSOLUTE BAND DEPTH OF *ULMUS* AMERICANA FOR THE A) CHLOROPHYLL-A BAND AT 660 NM AND THE B) WATER-BAND AT 1940 NM FOR ALL THREE SENESCENCE TECHNIQUES

FIG. 8 ABOSOLUTE BAND DEPTH OF *BETULA PAPYRIFERA* FOR THE A) CHLOROPHYLL-A BAND AT 660 NM AND THE B) WATER-BAND AT 1940 NM FOR ALL THREE SENESCENCE TECHNIQUES
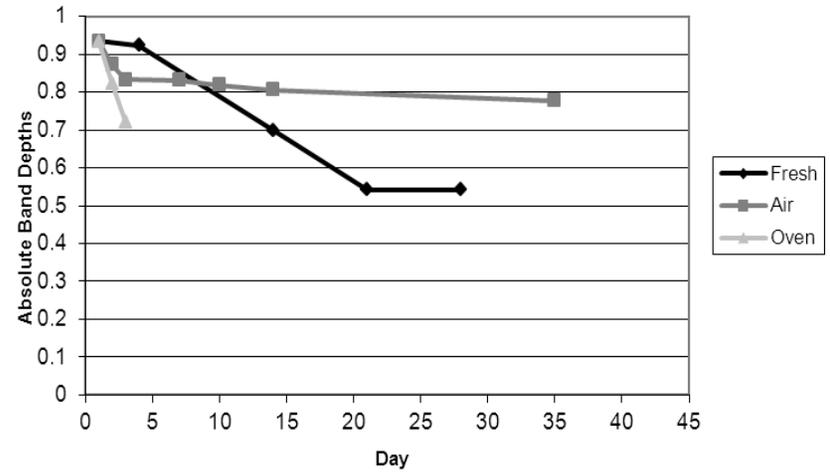
A)

A)

FIG. 9 ABOSOLUTE BAND DEPTH OF *ACER NEGUNDO* FOR THE A) CHLOROPHYLL-A BAND AT 660 NM AND THE B) WATER-BAND AT 1940 NM FOR ALL THREE SENESCENCE TECHNIQUES
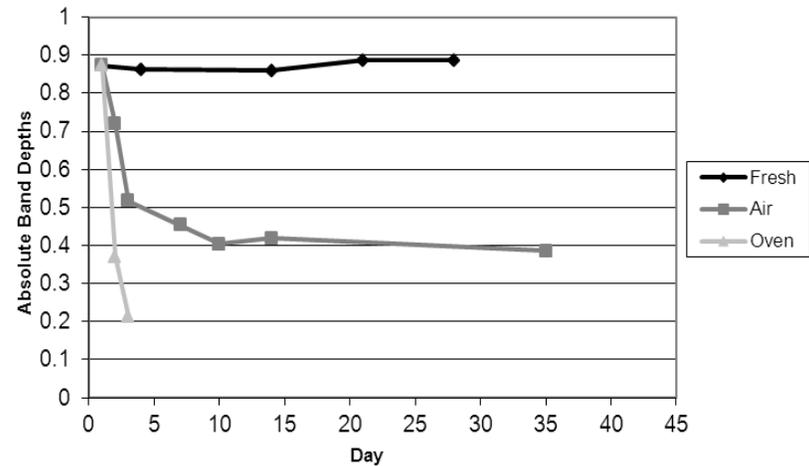
FIG. 10 ABOSOLUTE BAND DEPTH OF *FRAXINUS NIGRA* FOR THE A) CHLOROPHYLL-A BAND AT 660 NM AND THE B) WATER-BAND AT 1940 NM FOR ALL THREE SENESCENCE TECHNIQUES