\* E . 7 V2

1995

## Melvin B. Satterwhite

U.S. Army Topographic Engineering Center 7701 Telegraph Road Alexandria, VA 22315-3864

# ABSTRACT

The objective of this study was to describe the excitation-emission spectra of seed pubescence, pollen and spores, and senesced plant materials that could be carried in the air column. Reference samples were a mature green-colored corn leaf, green-, yellow- and brow-colored soybean leaves, cellulose, commercial grade cotton batting and a soil. Spectral luminescence signatures were collected over the 300 to 800 nanometer region using a scanning spectrofluorometer. The excitation-emission spectra were broadband emission centroids in the 400-nm to 600-nm spectrum. Emission maxima were associated with the 440-nm, 470-nm and 370-nm excitation bands and the 455-nm to 590-nm emission bands.

The coma of milkweed, silkvine, cotton (raw), cottonwood seeds and yellow-colored pollen and spores were higly fluorescent. The pappus of thistles, dandelion and goat's beard seeds and newly senesced grass leaves and glumes had moderate to high fluorescence. Dark brown-colored mushroom spores and weathered, senesced plant materials had low fluorescence. The emission spectra resembled that of reagent, microcrystalline cellulose although impurities incorporated within the plant materials altered their emission intensities from that of cellulose. Moderate to low emissions were from tan- to dark brown-colored materials, whereas the white-colored or light, tan-colored materials had high emissions.

Keywords: Fluorescence, luminescence, vegetation, pollen, pubescence, leaves,

# INTRODUCTION

Spectral luminescence has good potential as an analytical remote sensing tool for assessing plant vigor, plant stress<sup>1,2</sup>, or environmental contaminants. Luminescence remote sensing has been used for detecting and monitoring crude oil upon marine waters<sup>3</sup>, detecting fluorescent minerals<sup>4</sup>, and terrestrial vegetation fluorescence studies<sup>5</sup>. Emerging active luminescence systems with ultraviolet lasers have some flexibility for emission imaging band selection. Specific excitation and emission band combinations presently limited by available electro-optical technologies, should be optimize for excitation and emission band selection for material detection. As laser-induced, fluorescence-imaging (LIFI) sensor technologies evolve into complex multi-band imaging and multi-band lasing systems, spectral data requirements will evolve for sensor system design and imagery evaluation. These spectra must include both the object surface as well as airborne particulates, that might occur in the optical path and alter the object's emission signature. This study describes the excitation-emission spectra of selected plant materials carried in the air column.

## APPROACH

Three classes of airborne plant particulates were collected for measurement; seed pubescence structures, pollens and spores, and senesced plant parts (Table 1). Reagent grade microcrystalline cellulose, a turgid, green-colored corn leaf (*Zea mays*, L) and green-, yellow- and brown-colored soybean leaves (*Glycine max*, L), and an air-dry agricultural soil were included as comparative reference materials. Spectral luminescence measurements were made using an SLM Instruments scanning spectrofluorometer, model 8000C<sup>-</sup>. Solid samples were positioned in the

\* Product names and models are included for conviencance and do not imply product endorsement.

DISTRIBUTION STATEMENT A: Approved for Public Release -Distribution Unlimited 19981026 087

solid sample holder and were measured in the front surface mode at room temperature. Powder and granular samples were air-dried, sieved, and placed in Perkin-Elmer powder sample holders equipped with a fused silica window.

The excitation monochromator was stepped at 10-nm intervals over the 250 nm to 770 nm spectrum, at a 4-nm bandwidth. For each excitation interval, the emission monochromator was stepped at 5 nm intervals over the 300 nm to 800 nm spectrum. The emission monochromator bandwidth was 1.0 nm. Integration time was 0.2 seconds for each sample point. The system was operated in the photon-counting mode and the PMT was water cooled. All sample spectra were acquired at steady-state fluorescence (F, level). Emission spectra were adjusted by subtracting a PMT dark current taken at the beginning of each excitation-emission spectrum (EES). Long-pass glass filters mounted normal to the emission light path, remove the order effects. Each EES was corrected for filter transmission to a 100 percent basis. The spectra were adjusted for the PMT response function and the xenon lamp's radiance. Each EES was resampled into a 10 x 10 nm regularly spaced XYZ data array using commercial graphics software. The new Z-coordinate value was determined using an inverse distance algorithm and the 10 nearest data points to the X-Y grid coordinate. The emission maximum was determined for each EES which located the emission centroid within the scanned array.

## **RESULTS and DISCUSSION**

These samples had broad band emission centroids in the blue-green spectrum; 450-nm to 590-nm. Most samples had emission maxima associated with the Ex400-nm band. These samples were seed pubescence, alder pollen, and senesced leaf and stems of wheat, corn and soybean; and reagent cellulose and soil. Pollen and turgid, green-colored corn and soybean leaves, and some wheat leaf and culm samples had emission maxima associated with the Ex470-nm band. Samples maxima at the Ex370-nm band were the cattail pubescence and newly senesced corn leaf and glumes samples. For the Ex400-nm band, the emission bands ranged from 475-nm to 520-nm. This narrow range provided some material type discrimination, but is limited to emission intensity differences. Most samples, 60 of 67 samples had emissions greater than 1x10<sup>4</sup>DN; 6 samples had emissions greater than 3x10<sup>5</sup>DN, and 7 samples had emissions less than 1x10<sup>4</sup>DN. The green-colored corn and soybean leaves was at 685-nm which was substantially different from the emission maxima of other sample (Figure 1).

Pubescence, coma, and pappus are seed attachments that help seed extraction from the seed pods or flower receptacles. Once in the air column, the pubescence facilitates seeds dispersal through the air column. Because seed production can be enormously large to very large in species such as cattail, milkweed, thistle, or dandelion, these materials can be at significant concentration in those environments where these species grow. Mean emissions for a 70-nm wide band centered at Em495-nm shows some sample discrimination based on emission intensity (Figure 2). The emission spectra at Ex400-nm had similar shapes and resemble that the spectra of reagent cellulose. The pubescence materials were mostly cellulose, but contain impurities that brought about these differences between their emission amplitudes (Figure 3, 4, and 5). The seed pubescence emission intensities from highest to lowest were: coma > pubescence > pappus. The long, soft, white-colored coma of cattail, cottonwood, cotton (raw), silkvine, and milkweed were highly fluorescent, >1x10<sup>5</sup> DN. Dandelion and goat's beard pubescence have emission maxima ranging from 2.5-7x10<sup>4</sup> DN. The canadian thistle, bull thistle, and goldenrod pappus samples had moderate emissions, which ranged from 0.25-2x10<sup>4</sup> DN. These emissions varied with pubescence hue color. The lightest tan-colored samples were more fluorescent than the darker tan-colored samples.

Pollen can be disbursed in volume from most plants that rely upon wind for pollination. The pines, fir, spruce and emission maxima associated with Ex470-nm and Em565-nm, whereas the alder pollen had emission maximum at Ex400-nm/Em495-nm. Mature yellow-colored pollens were moderately to highly fluorescent, 1.3-30.5x10<sup>4</sup>DN. The cattail pollen samples had red pine, spruce, fir, and cattail pollen samples had higher emissions than the white pine and loblolly pine pollens (Figure 6).

Spore samples were taken from a club moss, shaggy mushroom and mature "yellow" slime mold. The yellow-colored club moss pollen was highly fluorescent, i.e, emissions were greater than 2x10<sup>5</sup>DN. The dark brown-colored spores from a shaggy mushroom and those of the mature yellow-colored slime mold were non-fluorescent, i.e., emissions were less than 1x10<sup>3</sup>DN. A reddish, brown-colored spore sample from a shaggy mushroom was moderately fluorescent. The non-fluorescent nature of dark-toned spores and pollens was observed for other plant species<sup>7</sup> (Figure 2).

Leaf, glume, and culm samples were taken from bromegrass, reed canary grass, wild oat grass, corn, wheat and soybean. The brittle nature of the air-dry, senesced materials can permit small fragments to be picked up from the ground surface or from standing stalks or stems by moderate to strong winds. Plant debris from recently harvested crops could be easily picked-up by the wind moving over recently harvested wheat, corn and soybean fields. Mechanical harvesting produces mixtures of finely fractured seed structures, leaves and stems of which the medium tan-colored wheat chaff sample is an example. This chaff sample was moderately fluorescent. Samples of recently senesced plants and plant debris, that had "aged" 6 to 12 months in open fields had emission maxima at Ex400-nm and emission bands ranging from 480-nm to 520-nm. Several newly senesced corn glume, wheat glume samples and wheat leaf samples had emission peaks at Ex370-nm/Em470-nm and Ex470-nm/Em565-nm (Figure 7 and 8)

Mean emissions for a 70-nm band centered at 495-nm and Ex400-nm were calculated for glume, leaf and culm samples (Figure 2). Highest emissions were from newly senesced corn glumes and lowest were from 'age' vegetation samples. Fluorescence apparently decreases rather rapidly as the plant materials 'weathered' in the out-of-doors environment over a short period of time. Newly senesced corn leaf had higher fluorescence than the older, weathered leaf materials. The new corn leaf was a bright, very light tan color, without blemish or discoloration. The older leaf samples were a grayish tan color with some leaf areas containing a dark-toned fungi growth (Figure 9 and 10)

Soil, reagent microcrystalline cellulose, and commercial grade cotton batting were measured for comparison purposes. The soil was very weakly fluorescent whereas the cellulose and cotton batting samples were highly fluorescent (Figure 2). Cellulose, a major structural plant material, would be present along with other residual materials, following the breakdown or the translocation of leaf pigments. The commercial, medical grade, processed cotton was pure cellulose. Its emission spectrum was very similar to the microcrystalline cellulose.

The fluorescence of a healthy green-colored leaves were distinctly different from with the senesced leaves (Figures 10 and 11). The turgid green-colored corn leaf had two characteristic broad emission centroids; a major centroid in the red-near infrared spectrum and a minor centroid in the blue spectrum. The soybean leaf has a major emission centroid in the red-far-red region, but a low emission in the blue spectrum. The major red-near-infrared fluorescence makes the green leaves spectrally separable from the senesced corn leaf and glume materials because they have no significant emissions in this spectral region. The significant blue fluorescence can differentiates the turgid, green-colored corn from other samples. Confusion can occur between weathered plant materials, or dark tan-colored materials and the green-colored soybean leaf samples which have similar blue fluorescence intensities. The fluorescence of senesced leaf materials were not related to wet chemistry biochemical interactions, as would be found in green, photosynthetically active corn leaf. These emissions are probably associated with large molecules, i.e., cellulose or lignin or constituents in the air-dry, senesced leaf<sup>6</sup>.

## CONCLUSIONS

Broad band fluorescence in the 455-nm to 535-nm spectrum at Ex400-nm werefound form many plant materials that can be carried in the air-column, seed pubescence, spores, and senesced plant leaves, stems and glumes. The broad band emissions of pollen samples have their emission maxima at Ex470-nm. Most materials had low to moderate fluorescence for other uv-visible excitation bands.

Sample fluorescences in the 400-600 nm region, were categorized in the following groups:

low emissions:	mushroom spores, soil
low-moderate emissions:	goat's beard, dandelion, old senesced corn lcaf, green-colored corn and soybean leaf
moderate emissions:	thistle pappus, some pollens
high emissions:	grass glumes, some pollens, corn glume
very high emissions:	seed coma, cellulose, grass glumes, newly senesced corn leaf

Pollen, leaf, and pubescence sample emission spectra resembled that of reagent, microcrystalline cellulose. Impurities incorporated these plant structureal materials altered their emissions from that of cellulose. Low emissions were found for the dark tan-colored materials, whereas the very light tan-colored materials had higher emissions.

## REFERENCES

- E.W. Chappell, F.M. Wood, jr., J.E. McMurtry, III, and W.W. Newcomb. "Laser-induced fluorescence of green plants. 1. A technique for the remote detection of plant stress and species differentiation", *Applied Optics* 23, pp. 134-138. 1984.
- C.S.T. Daughtry, J.E. McMurtrey, III, E.W. Chappelle, W.P. Dulaney, J.R. Irons, and M.B. Satterwhite. "Potential for discriminating crop residues from soil by reflectance and fluorescence", *Agronomy Journ.* 87. pp. 165-171. 1995.
- 3. F.E. Hoge and R.N. Swift. "Airborne simultaneous spectroscopic detection of laser-induced water raman backscatter and fluorescence from chlorophyll *a* and other naturally occurring pigments", *Applied Optics* 20, pp. 3197-3205. 1981
- 4. W.R. Hempill, R.M. Tyson, and A.F. Theisen. "Spectral luminescence properties of natural specimens in the scheelite-powellite series and an assessment of their detectivity with an airborne Fraunhofer line discriminator", *Economic Geology* 83, pp. 637-646. 1988.
- 5. H.K. Lichenthalter, "Laser-induced chlorophyll fluorescence and blue fluorescence of plants", 10th Annual Internat. Geoscience and Remote Sensing Symposium. Ch2825-8, pp. 1913-1918. 1990
- 6. J.E. McMurtrey, III, E.W. Chappell, C.S.T. Daughtry, and M.S. Kim. "Fluorescence and reflectance of crop residue and Soil", *Journ. Soil and Water Conserv.* 48-3, pp. 207-213. 1993.
- 7. M.B. Satterwhite, "Spectral luminescence of plant pollen", IEEE, Proc. Int. Geosci. Remote Sensing Symp., New York, pp. 1945-1948. 1990

# Table 1 Listing of Plant and Soil Samples

### Sample Name

## Specific Name

#### Pubescence/Pappus/Coma

Cattail, pubescence & seeds Cotton, coma, raw Milkweed, coma Silkvine, coma Cottonwood, coma Rubber Rabbit Brush, pappus Canadian Thistle, pappus Bull Thistle, pappus Goldenrod, pappus Dandeline pubescence Goat's Beard, pubescence

Pollen White Pine, pollen Loblolly Pine, pollen Red Pine, pollen Pine, pollen Lodge Pole Pine, pollen Subalpine Fir, pollen Blue Spruce, pollen Alder, pollen Cattail, pollen

Spores Clubmoss, spores Mushroom, mature spores Yellow slime mode, spores

#### Leave/Glumes/Culm/Stems

Brome Grass, glumes, senesced Reed Grass, glumes, senesced Wild Oat Grass, glumes, senesced Corn, glumes, senesced, new Corn, leaf, green Corn, leaf, senseced, new Corn, leaf, senesced, aged Corn, stalk, aged Corn, stalk, aged Wheat, glumes Wheat, culms, new Wheat, culms, aged Wheat, chaff, new Wheat, leaf, new Soybean, leaf, senesced, new Soybean, leaf, senesced, aged. Soybean, stalk, senesced, aged. Soybean, leaf, green, turgid Soybean, leaf, yellow, turgid Soybean, leaf, brown, wilted

<u>Reference Samples</u> Cellulose, microcrystalline Cotton, commercial medical Soil, air dry, <210um Typha latifolia, L. Gossypium sp., L. Asclepias sp., L. Periploca graeca, L. Populus deltoides, Marsh Chrysothamnus nauseosus, Britt. Cirsium arvense, (L) Scop. Cirsium vulgare, (Savi) Tenore Solidago sp., L. Taraxacum officinale, Wiggers. Tragopogon dubois, Scop.

Pinus strobus, L Pinus taeda, L. Pinus virginiana, Mill Pinus spp., Mill Pinus contorta, Mill Abies lasiocarpa, Nutt. Picea pungens, Engelm. Alnus sp. L. Typha latifolia, L.

Lycopodium sp., L. Coprinus comatus, L.

Bromus inermis, Leyss. Phalaris arundinacea, L. Avena fatua, L. Zea mays, L. Triticum aestivum, L. Triticum aestivum, L. Triticum aestivum, L. Triticum aestivum, L. Triticum aestivum. L. Glycine max, L.







Center at Ex400-nm/Em49







-----



.



Emission Spectra for Ex400-nm.







----

