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Leaf Optical Properties EXperiment 93 (LOPEX93)

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Abstract

An experiment was organized in the Joint Research Centre at Ispra during the summer of 1993 in which a data set associating visible / infrared spectra of vegetation elements (leaves, conifer needles, stems, etc) with physical measurements and biochemical analyses was constructed. This document describes how the experiment was performed and how the main results have been classified and archived.

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1. Introduction

The estimation of leaf biochemistry and leaf water status with remote sensing data is a challenge for the years to come. It also has an important potential in agriculture to follow crop development and yield predictions. The biochemical constituents of interest in this experiment were lignin, proteins (nitrogen), cellulose and starch, as well as chlorophyll and foliar water. The major processes involved in the terrestrial ecosystem such as photosynthesis, primary production, or foliar decomposition can be related to these constituents. As leaves are the most important surfaces of a plant canopy, relating their optical properties to these constituents is a priority (Jacquemoud et al., 1994).
The overall objective of the experiment was to investigate the use of high resolution visible and near infrared reflectance spectroscopy for the retrieval of chlorophylls, water, protein, cellulose, lignin, and starch both on fresh and dry material, on individual leaves and on optically thick samples (stacked leaves + needles or powders).

2. The Experiment

In order to have a wide range of variation of leaf internal structure, pigmentation, water content and biochemical components, plant species with different types of leaves were collected during two separate periods during the summer of 1993. About 70 leaf samples representative of more than 50 species were obtained from trees, crops and plants in the area of the JRC (Tables 1 & 2). In addition, various substances such as powdered starch or proteins and vegetative material such as stems or bark were also included in the data set to increase its variability.

About 800g of leaves were required for each sample which normally yielded about 80g of dry material.

3. Spectral measurements

A Perkin Elmer Lambda 19 double-beam spectrophotometer (Fig.4) equipped with a BaSo4 integrating sphere was used for the measurement of the reflectance (R) and transmittance (T) of the upper faces of leaves. In addition, the reflectance of optically thick samples ($R\infty$) was measured by stacking leaves in order to magnify the radiometric signal and minimize the leaf to leaf variability or, in the case of needles or powders, by placing them in a quartz cuvette.

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Spectra were scanned over the 400–2500 nm wavelength interval with 1 nm step starting at 2500 nm and ending at 400 nm. The spectral resolution varied from 1 to 2 nm in the visible / near infrared (400–1000 nm) and from 4 to 5 nm in the middle infrared (1000–2500 nm). The calibration of the instrument was performed using Spectralon™ reflectance and wavelength calibration standards. For each sample, measurements were made on 5 different areas in order to quantify the small but not negligible leaf to leaf variability. The scan time required for each sample was about 4 minutes. In the case of needles and powdered material, the quartz cuvette was positioned vertically against the side of the BaSo4 integrating sphere. The reflectance spectra made in this mode have been corrected for the effect of the quartz plate in front of the sample taking into account the reflectance and transmittance of a single quartz plate of the same thickness, as described below.
All the above procedure was repeated some days later on dried leaves and needles to analyse the influence of water which is known to obscure the biochemical information in the middle infrared region.

3.1 Instrumental corrections

The integrating sphere is 60 mm in diameter with a ratio aperture/internal surface of 8 %. In the VIS/NIR, the detector is a photomultiplier; in the IR region , a PbS detector is used. The transition between the two detectors occurs at 860.8 nm.
First the full scale was set by running the instrument with two white diffusing reflectors positioned on the sample and reference ports of the sphere. The instrument stores this measurement and uses it to automatically correct the following measurements. The diffusing reflectors should be calibrated standards; however, at the time of the experiment, these were not available and two uncalibrated spectralon samples (sample A on the sample port, sample B on the reference port) were used. The reflectance of these two samples was later measured with reference to a SR99 diffuse reflectance standard. Let r_B be the measured ratio of sample B to SR99 standard reflectances ($r_B=R_B/R_{SR99}$). A background measurement was also performed by positioning a light trap (reflectance < 10^{-4}) on the sample port. The apparent measured background reflectance (r_0) has its origin in the small fraction of the sample beam not incident on the light trap but on the surface of the sphere.
The reflectance measurements were then performed by placing the sample on the sample port, leaving the diffuse reflector B on the reference port.
Transmittance measurements were performed with diffuse reflector B positioned on the sample port while the sample itself intercepted the sample beam at its entrance in the sphere. Diffuse reflector B was always used on the reference port.

If r_S and t_S denote the raw reflectance and transmittance measurements, the absolute reflectance (R_S) and transmittance (T_S) can be approximated with the following formulæ:

$$R_s = \frac{(r_s - r_0).r_B.R_{SR99}}{(1.-r_0)} \qquad (1)$$

$$T_s = \frac{t_s.r_B.R_{SR99}}{(1.-r_0)} \qquad (2)$$

where R_{SR99} is provided by the certified calibration of the standard.

In the transmittance formula, the background r_0 is not subtracted, as the fraction of the sample beam not incident on the sample port is part of the signal (being transmitted through the sample). The denominator of the formulæ takes into account the effect of r_0 on the instrumental full scale value.
These correction formulæ were tested in various ways.

(i) Results on diffuse reflectance standards

Grey standard diffusers (reflectance of 80, 60, 40 ,20 ,10, 5 and 2%) were measured and their corrected reflectance was found to lie within the calibration specifications (std. dev. ± 0.005). The same was done for a number of coloured standard diffusers.

(ii) Results on transmittance samples

Two diffusing transmittance samples were measured both for reflectance and transmittance (SDM-200-DU and SDM-200-DM). These samples are made of a film (~300 μm) of Spectralon™ and the sum of their reflectance and transmittance should be very close to 1 (almost negligible absorption). Figure 6. shows that by summing the raw r and t measurements, the result is >1. After correction, however, the result is acceptable.

(iii) Example on a leaf

Figure 7 shows the corrections on the spectra of a leaf of *Lactuca Sativa*, fresh and dried.

3.2 Correction for samples measured in a cuvette.

Since the spectrophotometer does not allow to position the sample horizontally, some material (needles, uncompressed powders) had to be contained in a glass cuvette. The reflectance (R_g) and transmittance (T_g) of the cuvette wall was measured and the reflectance of the studied material (R_S) retrieved using the following formula:

$$R_s = \frac{R_{s+c} - R_g}{R_g.(R_{s+c} - R_g) + T_g^2} \qquad (3)$$

where R_{S+C} is the corrected (with formula (1)) reflectance measurement on the sample in the cuvette. The formula takes into account the multiple reflections.
The validity of this correction was checked by measuring a black painted aluminium plate both inside and outside the cuvette. The results are shown in figure 8. and are satisfactory.

In most of the spectra, a small disturbance can be observed at the 860 nm point due to the automatic change from Pbs detector to photomultiplier. In the case of some optically thick samples such as stalks, this disturbance may increase noticeably since the instrument slit width also changes at this point and thus the geometry of the target surface observed may be altered.
Technical specifications of the spectrophotometer are given in Table 8 and the configuration of the instrument during the experiment is given in Table 9.

4. Auxiliary measurements

In parallel with the spectral measurements, many physical and biological measurements were performed on the samples. Leaf blade thickness was measured with a calliper rule (5 measurements per leaf). The fresh weight of a 4.10 cm^2 disk taken on each leaf using a cork borer was then immediately measured. The disk was then placed in a drying oven at 85°C for 48 hours and reweighed to determine the water content (WC = water mass over fresh mass), the equivalent water thickness or water depth (EWT = water mass per unit leaf area), and the specific leaf area (SLA = dry weight per unit leaf area).

With regard to the other **biochemical constituents**, about 250 g of fresh material were partially dried in an oven and then sent to two independent and specialized laboratories in France and Belgium which performed the measurements of total proteins, cellulose, lignin, and starch using standard wet chemical analyses. The comparison between the concentration values (g/g) provided by the two laboratories gives an idea of the precision of these analyses: protein and cellulose measurements were quite consistent while lignin and starch measurements differed significantly. These discrepancies are probably mainly due to the different methods of chemical extraction.

Extraction methods: Protein: Kjeldahl
Cellulose: Weende (B) / Van Soest (F)
Lignin : Van Soest
Starch : Ewerts (B)

A total of 120 samples was sent to each laboratory in 2 batches. The first batch, collected in July, contained 70 samples and the second batch , collected in September, contained 50 samples. Each batch contained a number of double samples which allows an estimation to be made of the repeatability of the chemical analyses. Furthermore, some of the vegetation types contained in batch 1 were repeated in batch 2 in order to be able to assess the natural variation of the biochemical concentrations during the period of maximum phenological activity of the vegetation.

Part of the remaining leaf samples was frozen for later biochemical analysis: the photometric determination of **photosynthetic pigments** (chlorophyll a, b and total carotenoids) was performed with a UV-2001 PC spectrophotometer in 100% acetone using the equations of Lichtenthaler (1987) at the University of Karlsruhe (Botanical Institute II).

1. Chlorophyll a : $c[\text{chl a}] = 11.24 * A_{661.6} - 2.04 * A_{644.8}$

2. Chlorophyll b : $c[\text{chl b}] = 20.13 * A_{644.8} - 4.19 * A_{661.6}$

3. Total chlorophylls a + b : $c[\text{chl a}] = 7.05 * A_{661.6} + 18.09 * A_{644.8}$

4. Total carotenoids : $c[x+c] = (1000 * A_{470} - 1.9 * c[\text{Chl a}] - 63.14\ [\text{Chl b}]\ ) / 214$

where A = absorption coefficient

Another part of the remaining samples was ground to a fine powder using a Retsch ZM1 grinder equipped with a 10µm filter. Part of the **powders** was then compressed under a pressure of 20 tons and formed into pastilles in aluminium and plastic cups (∅ = 30 mm). Figure 5 shows a selection of these pastilles.
The **pastilles** were then dried in an oven for one week at 40°C before their spectral characteristics were measured again in the Lambda 19 spectrophotometer. Each spectral measurement was made on three different points of the pastille. In all, 94 pastilles were measured in this way. (See data files OPEX2---)

A small part of the powders (~ 8g.) was put aside for analysis of the elemental composition of the samples.

The elemental analyses were made at the bioclimatology laboratory of INRA Clermont Ferrand (F) using a microanalyser ERBA. The elements of interest were Carbon (C), Hydrogen (H), Nitrogen (N) and Oxygen (O). The elements were not analysed simultaneously. The composition in C, H, and N was

estimated using the Dumas and Pregl method. Samples and standards are weighted into tin containers and sealed. The sample is dropped into the combustion furnace. A fixed volume of oxygen is flushed in by the heluim gas carrier. The tin oxydizes immediately and temperature rises to 1800° C. Combustion gases pass on a first catalyst (CR2O3) to produce CO2, H2O, SO2/SO3 and NOx and on a second catalyst (pure copper) to reduce NOx, sulphur and residual oxygen. Gases are then separated in a chromatographic column and quantified using a thermal conductivity detector.
The composition in oxygen was determined using the Unterzaucher method. The method is similar to the Dumas and Pregl method except that the catalyst is nickel and combustion gases are transformed in NO. Similary, gases are separated in a chromatographic column and quantified in the same way.
Results are expressed in % of dry matter. 2 or 3 repetitions were made for each sample analysis.

5. Classification of the experimental results

The experimental results have been classified and archived for future use in a series of ASCII files in the main directory **lopex93.** The overall structure of the classification system is shown in Fig.1.
The bulk of the data files is constituted by the reflectance and transmittance spectra. A total of 1938 files has been generated with the root name **OPEX** contained in the sub-directory **spectra**. Each file has been radiometrically corrected and is expressed in terms of absolute reflectance (as a fraction of 1). The corresponding wavelengths which are identical for all spectra are contained in the file **OPEX.WVL** and are expressed in nanometres (integer values ranging from 400 to 2500). Examples of reflectance spectra are shown in Figures 2 and 3.

All auxiliary measurements are contained in a separate sub-directory (**auxmeas**).
The complete list of samples is given in Latin (where possible) and English in Tables 1 and 2 respectively. These names are also contained in the files **SAM_LNAM** and **SAM_ENAM.LST**.

A key element in this classification is the association between the **spectrum number** and the relative **auxiliary measurements.** This is the file **SPEC_AUX.DAT**. An explanation of the code employed in this file is given in Table 3.

The association between the **sample number** and the relative **biochemical analyses** is contained in the file **SAM_BIO.DAT**. This file also contains the code indicating the type of sample in question (ie. monocotyledon, dicotyledon etc). An explanation of the code employed is given in Table 4.

The association between the **sample number** and the relative **spectra** is contained in the file **SAM_SPEC.DAT**. An explanation of the code employed in this file is given in Table 5.

The association between the sample number and the spectrum number can thus be obtained in 2 ways:
1. Indirectly, by means of the spectrum block number in the SAM_BIO and SPEC_AUX files
2. Directly, by means of the SAM_SPEC.DAT file

The results of the chlorophyll and total carotenoids analyses can be found in the file **SAM_PIG.DAT**. An explanation of the code employed in this file is given in Table 6.

The results of the elemental analyses can be found in the file **SAM_ELE.DAT**. An explanation of the code employed in this file is given in Table 7.

6. Conclusion

An important and valuable data set has been put together with these measurements. The preparation of the leaf samples was particularly time-consuming especially in the case of plants with small leaves. The spectral measurements were made with the best equipment available and can be considered to be very precise. The fact that the samples were also powdered and compressed means that they will also be available in the future for further measurements or comparison. Preliminary analyses of the data show many promising results but there are many other analyses of correlation which still remain to be made at the time of writing. The authors hope that these data can be used by other researchers in this field and that the results will contribute to a better understanding of the relationship between the spectral characteristics of vegetation and its biochemical components for application in Remote Sensing.

For further information regarding the availability of the data set please contact G.Schmuck :
Tel. (39)-332-785313 / Fax (39)-332-785469/ email: guido.schmuck@cen.jrc.it

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- *M. Lang of the University of Karlsruhe (D) for the pigment analyses*
- *The Institute for the Environment at the JRC Ispra for the use of their equipment in the preparation of the powdered material*

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8. References

Jacquemoud, S., Verdebout, J., Schmuck, G., Andreoli, G., Hosgood, B., (1994), Investigation of leaf biochemistry by statistics, *Remote Sens. Environ.*, forthcoming

Lichtenthaler, H.K. (1987), Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods Enzymol.*, 148:350-382.

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Fig. 1 Key Elements in LOPEX93

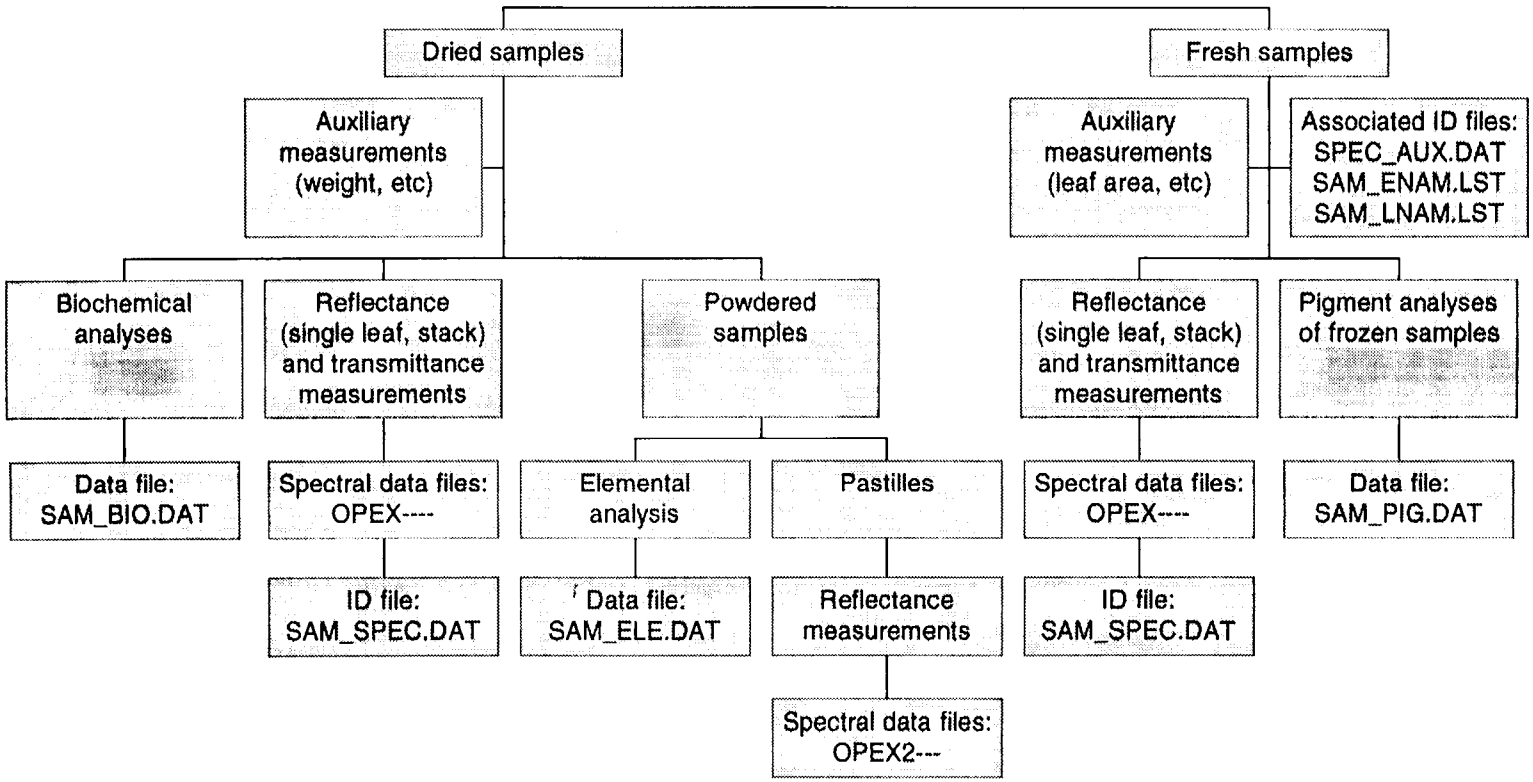


Fig.2 Example of leaf reflectance spectra

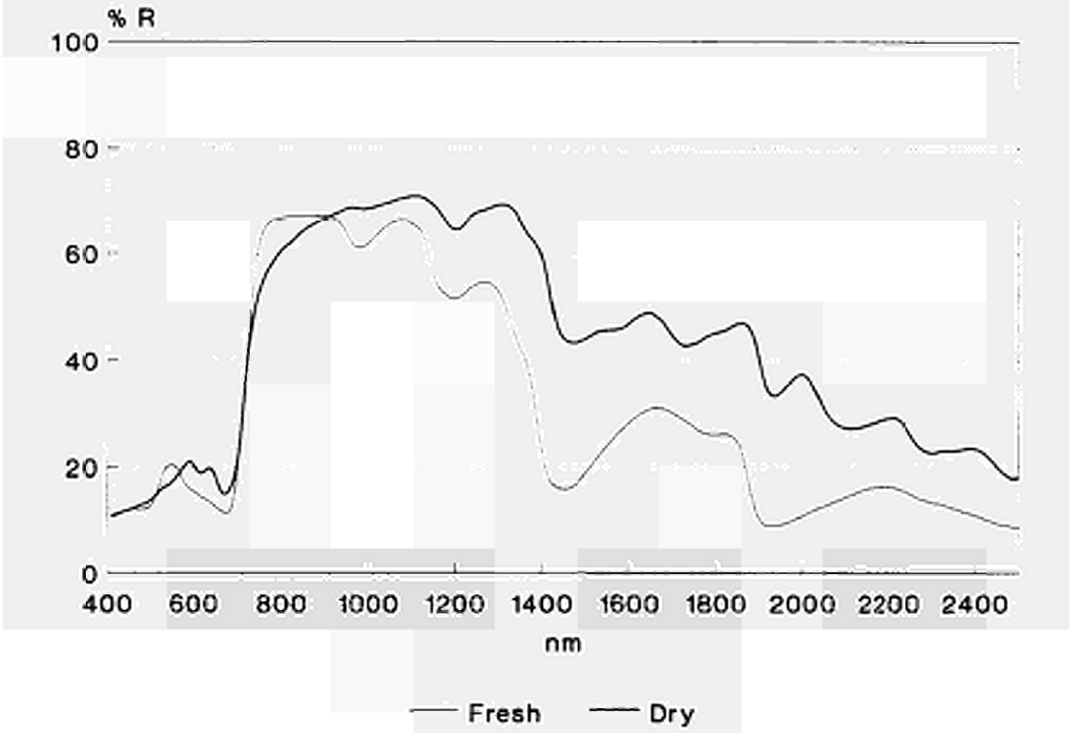
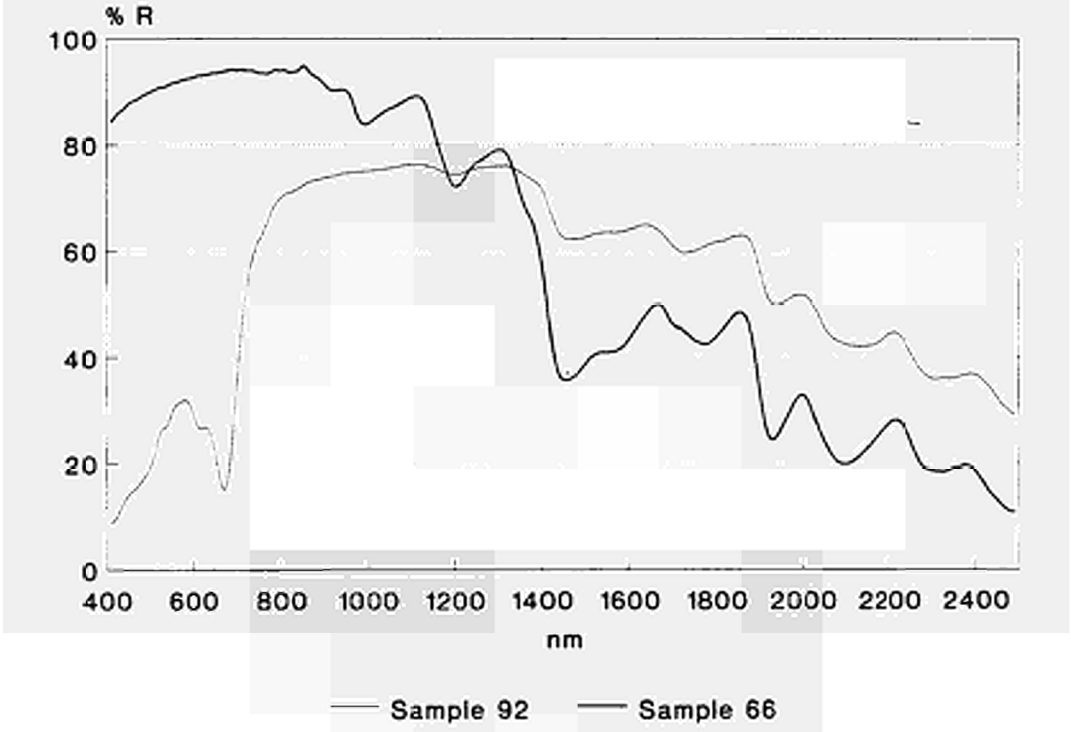


Fig. 3 Example of pastille reflectance spectra



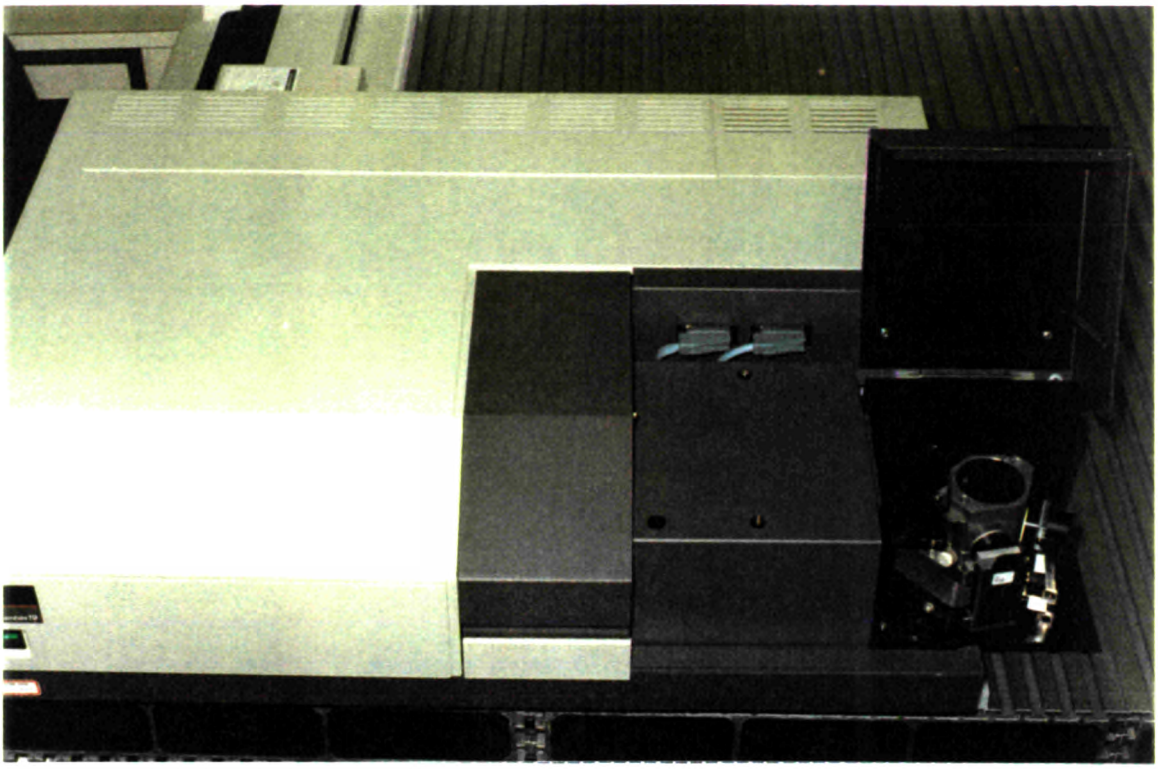


Fig. 4 The λ19 spectrophotometer

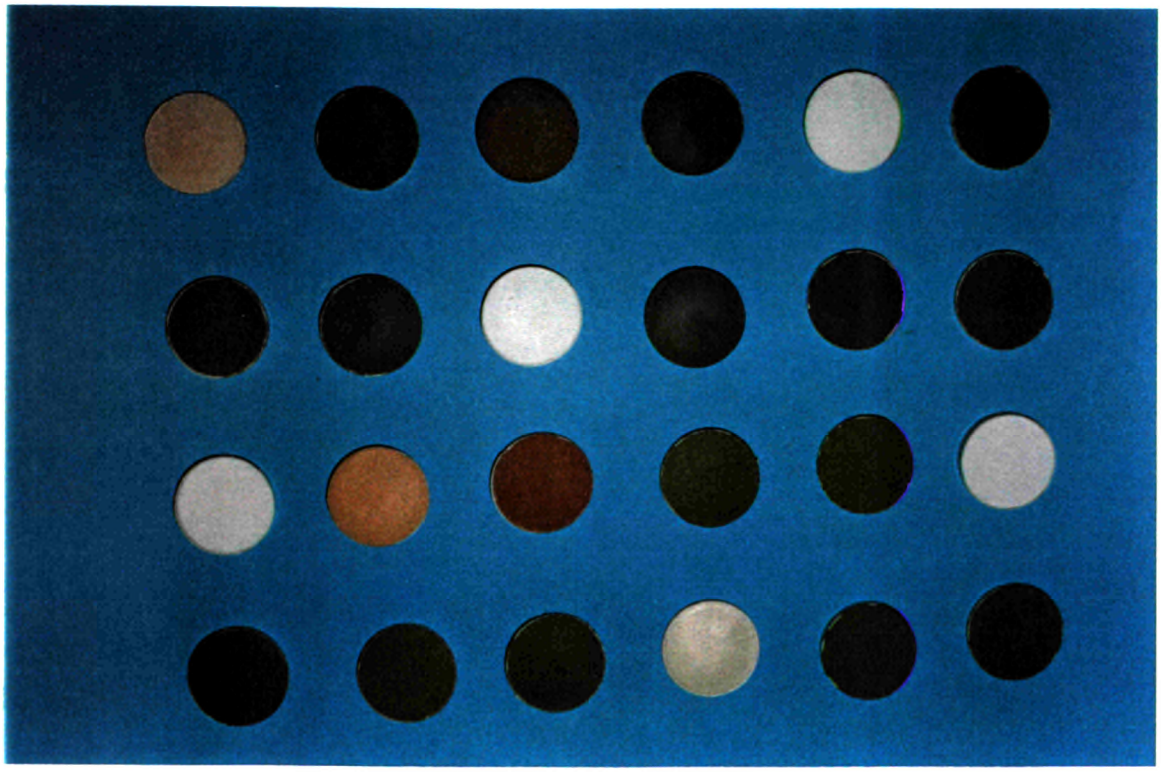


Fig. 5 A selection of pastilles

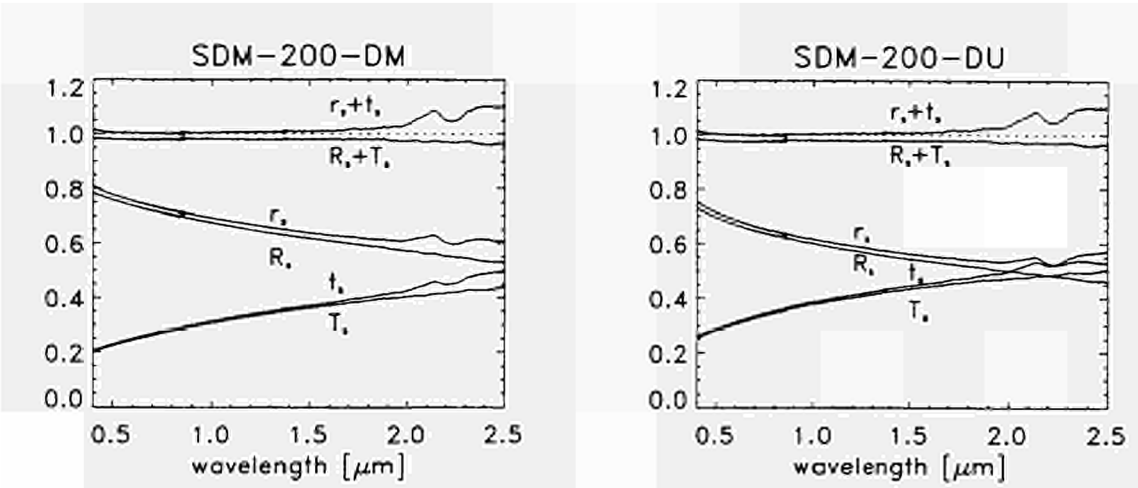


Fig. 6 Raw (r_S and t_S) and corrected (R_S and T_S) reflectance and transmittance of two films of spectralon.

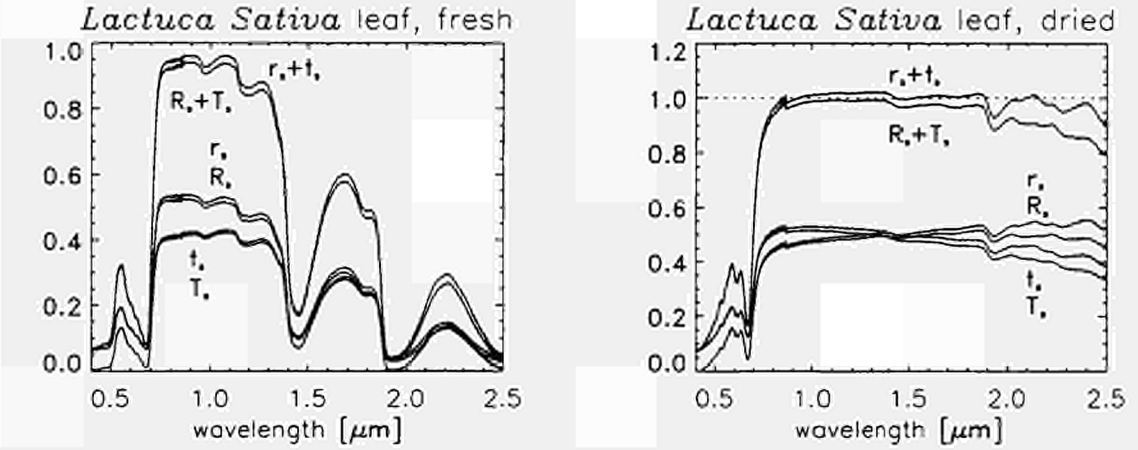


Fig. 7 Raw (r_S and t_S) and corrected (R_S and T_S) reflectance and transmittance of a *Lactuca Sativa* leaf, fresh and dried.

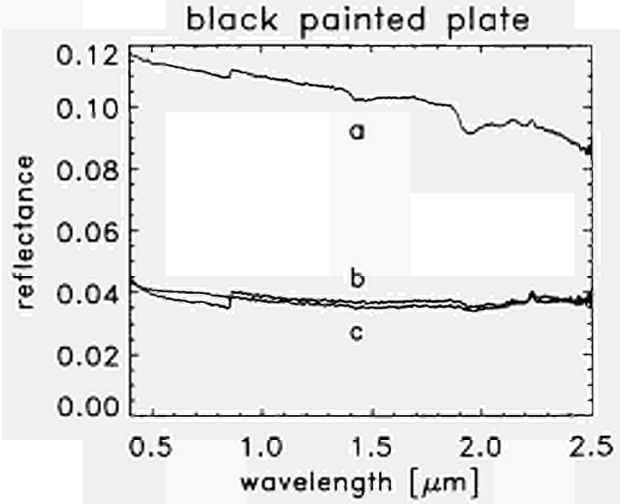


Fig. 8 Correction for the cuvette contribution (a black painted plate is used as a test sample): a. reflectance measured in the cuvette; b. retrieved reflectance after correction; c. reflectance measured without the cuvette .

Appendix

01	*Trifolium pratense L.*	61	.....
02	*Sorghum halepense*	62	*Corylus avellana L.*
03	*Picea abies*	63	.....
04	*Vitis silvestris*	64	.....
05	*Fraxinus excelsior L.*	65	*Lanugo*
06	*Lactuca sativa*	66	*Amylum solanaceum*
07	*Pseudotsuga menziesii*	67	*Amylum ex oryza*
08	*Prunus laurocerasus*	68	*Amylum ex mays*
09	*Picea abies*	69	*Amylum triticeum*
10	*Populus canadensis*	70	*Furfures triticei*
11	*Medicago sativa L.*	71	*Tilia platyphyllos*
12	*Zea mays L.*	72	*Pinus contorta*
13	*Solanum tuberosum L.*	73	*Populus tremula L.*
14	*Vitis silvestris*	74	*Pseudotsuga menziesii*
15	*Fraxinus excelsior L.*	75	*Quercus pubescens*
16	*Zea mays L.*	76	*Alnus glutinosa*
17	*Pinus contorta*	77	*Zea mays L.*
18	*Psalliota hortensis*	78	*Zea mays L.*
19	*Prunus laurocerasus*	79	*Quercus rubra*
20	*Fagus sylvatica L.*	80	*Zea mays L.*
21	*Laurus nobilis L.*	81	*Zea mays L.*
22	*Robinia pseudoacacia L.*	82	*Quercus rubra*
23	*Quercus pubescens*	83	*Corylus avellana L.*
24	*Helianthus annuus L.*	84	*Castanea sativa*
25	*Tilia platyphyllos*	85	*Acer pseudoplatanus L.*
26	*Zea mays L.*	86	*Salvia officinalis L.*
27	*Juglans regia L.*	87	*Ficus carica L.*
28	*Juglans regia L.*	88	*Bambusa acundinacea*
29	*Populus canadensis*	89	*Chamaerops humilis*
30	*Fagus sylvatica L.*	90	*Phragmites communis*
31	*Laurus nobilis L.*	91	*Bambusa acundinacea*
32	*Robinia pseudoacacia L.*	92	*Armeniaca vulgaris*
33	*Quercus pubescens*	93	*Ulmus glabra*
34	*Zea mays L.*	94	*Hedera helix L.*
35	*Medicago sativa L.*	95	*Zea mays L.*
36	*Beta vulgaris L.*	96	*Picea abies*
37	*Urtica dioica L.*	97	*Robinia pseudoacacia L.*
38	*Picea abies*	98	*Prunus serotina*
39	*Populus canadensis*	99	*Fraxinus excelsior L.*
40	*Oryza sativa*	100	*Brassica oleracea L.*
41	*Phleum pratense L.*	101	*Pinus wallichiana*
42	*Secale cereale*	102	*Iris germanica L.*
43	*Triticum*	103	*Vitis vinifera L.*
44	*Triticum*	104	*Morus alba L.*
45	*Soja hispida*	105	*Salix alba L.*
46	*Beta vulgaris L.*	106	*Vitis vinifera L.*
47	*Triticum*	107	*Musa ensete*
48	*Triticum*	108	*Picea abies*
49	*Secale cereale*	109	*Medicago sativa L.*
50	*Oryza sativa*	110	*Oryza sativa*
51	*Acer pseudoplatanus L.*	111	*Castanea sativa*
52	*Acer pseudoplatanus L.*	112	*Betula alba L.*
53	*Helianthus annuus L.*	113	*Medicago sativa L.*
54	*Armeniaca vulgaris*	114	*Lycopersicum esculentum*
55	*Morus nigra*	115	*Soja hispida*
56	*Platanus acerifolia*	116	*Oryza (foliis siccis)*
57	*Morus nigra*	117	*Oryza (integra-cum glumis)*
58	*Zea mays L.*	118	*Oryza (glumae)*
59	*Castanea sativa*	119	*Oryza (integra)*
60	*Corylus avellana L.*	120	*Oryza (.....)*

Table 1. Latin names of samples

01	Clover	61	Wood shavings
02	*Sorghum halepense*	62	Hazel (2/2)
03	Norway spruce (91)	63	Soy Lecithin
04	Wild vines (1/2)	64	Ecofoam ® (maize)
05	Ash (1/2)	65	Cotton wool
06	Lettuce	66	Potato starch
07	Douglas fir (93)	67	Rice starch
08	Laurel (*ceraso*) old	68	Maize starch
09	Norway spruce (92)	69	Wheat starch
10	Poplar (1/3)	70	Bran
11	Alfalfa	71	Linden
12	Maize (1)	72	Contorta Pine
13	Potato	73	Poplar
14	Wild vines (2/2)	74	Douglas Fir
15	Ash (2/2)	75	Oak
16	Maize 3 (1/2)	76	Alder
17	Contorta Pine	77	Maize (1/2)
18	*Psalliota Hortensis*	78	Maize (dry)
19	Laurel (*ceraso*) young	79	Red oak (1/2)
20	Beech (1/2)	80	Maize (2/2)
21	Laurel (*nobilis*) old (1/2)	81	Maize (half dry)
22	*Pseudo Acacia* (1/2)	82	Red oak (2/2)
23	Oak (1/2)	83	Hazel (2)
24	Sunflower	84	Chestnut (dry)
25	Linden	85	Maple (2)
26	Maize 3 (2/2)	86	Sage
27	Walnut (no stem)	87	Fig
28	Walnut	88	Bamboo (1)
29	Poplar (2/3)	89	Palm
30	Beech (2/2)	90	Lake reeds
31	Laurel (*nobilis*) old (2/2)	91	Bamboo (2)
32	*Pseudo Acacia* (2/2)	92	Apricot (2)
33	Oak (2/2)	93	Elm
34	Maize (stalks)	94	Ivy
35	Alfalfa (stalks)	95	Maize (stalks) (2)
36	Sugar beet (1/2)	96	Norway spruce (93)
37	Nettles	97	*Pseudo Acacia* 2
38	Norway Spruce (93)	98	*Prunus serotina*
39	Poplar (3/3)	99	Ash (2)
40	Rice (1/2)	100	Cabbage
41	*Phleum pratense*	101	Bhutan pine
42	Rye (1/2)	102	Iris
43	Wheat (*salmone*) (1/2)	103	Vine (white)
44	Wheat (*pandas*) (1/2)	104	Mulberry (2)
45	Soy	105	Willow
46	Sugar beet (2/2)	106	Vine (american)
47	Wheat (*pandas*) (2/2)	107	Bananna
48	Wheat (*salmone*) 2/2	108	Norway Spruce (92)
49	Rye (2/2)	109	Alfalfa (stalks) (2)
50	Rice (2/2)	110	Rice (stalks)
51	Maple (1/2)	111	Chestnut (2)
52	Maple (2/2)	112	Birch
53	Sunflower (stalks)	113	Alfalfa (2)
54	Apricot	114	Tomato
55	Mulberry (1/2)	115	Soy (2)
56	Plane (bark)	116	Rice (dry leaves)
57	Mulberry (2/2)	117	Rice (whole grain)
58	Maize (2)	118	Rice (husks)
59	Chestnut	119	Rice (whole grain)
60	Hazel (1/2)	120	Rice (parboiled)

Table 2. English names of samples

[1] : Spectrum number: 0001 - 2307
[2] : Spectrum type : 1 = reflectance 2 = transmittance
[3] : State of sample: 0 = fresh 1 = dry
[4] : Type of sample : 1 = single leaf
2 = stack of leaves (eg. 50 leaves)
3 = material in quartz cuvette (eg. needles)
4 = stalks
5 = optically dense material (eg. bark)
6 = pastilles (compressed powder))
[5] : Spectrum block number: 001 - 103
[6] : Average leaf thickness (microns)
or average of averages in the case of leaf stacks
[7] : Fresh weight (grammes)
[8] : Dry weight (grammes)
[9] : Leaf area used in weighing (cm^2)

-1 = Measurement not made or not applicable

Extract from data file: SPEC_AUX.DAT

```
0400 1 1 1 004 208.0 -1.0000 -1.0000 -1.00
0401 2 1 1 004 208.0 -1.0000 -1.0000 -1.00
0402 1 1 2 004 208.0 -1.0000 -1.0000 -1.00
0403 1 0 5 033  -1.0  8.5752  6.8440 -1.00
0404 1 0 5 033  -1.0  8.5752  6.8440 -1.00
0405 1 0 5 033  -1.0  8.5752  6.8440 -1.00
0406 1 0 5 033  -1.0  8.5752  6.8440 -1.00
0407 1 0 5 033  -1.0  8.5752  6.8440 -1.00
0410 1 0 1 034 122.0   .0429   .0147  4.10
0411 2 0 1 034 122.0   .0429   .0147  4.10
0412 1 0 1 034 118.0   .0397   .0119  4.10
0413 2 0 1 034 118.0   .0397   .0119  4.10
0414 1 0 1 034 134.0   .0480   .0157  4.10
0415 2 0 1 034 134.0   .0480   .0157  4.10
0416 1 0 1 034  82.0   .0315   .0079  4.10
0417 2 0 1 034  82.0   .0315   .0079  4.10
0418 1 0 1 034 134.0   .0394   .0149  4.10
0419 2 0 1 034 134.0   .0394   .0149  4.10
0420 1 0 2 034 118.0   .4263   .1223 41.00
0421 1 1 1 002  72.0 -1.0000 -1.0000 -1.00
0422 2 1 1 002  72.0 -1.0000 -1.0000 -1.00
0423 1 1 1 002  90.0 -1.0000 -1.0000 -1.00
0424 2 1 1 002  90.0 -1.0000 -1.0000 -1.00
0425 1 1 1 002 104.0 -1.0000 -1.0000 -1.00
```

Table 3. Explanation of code used in Spec. / Aux. meas. file (SPEC_AUX.DAT)

[01] = sample number (001-120)
[02] = type of sample 1: Monocotyledon
2: Dicotyledon
3: Gymnosperm
0: Other
[03] = sample status 1: Single sample
2: Double sample (first occurence)
3: Triple sample (first occurence)
[04] = associated spectrum block number (SPEC_AUX.DAT)
[05] = Nitrogen % dry weight (France)
[06] = Nitrogen % dry weight (Belgium)
[07] = Cellulose % dry weight (France)
[08] = Cellulose % dry weight (Belgium)
[09] = Lignin % dry weight (France)
[10] = Lignin % dry weight (Belgium)
[11] = Starch % dry weight (France)
[12] = Starch % dry weight (Belgium)

-1.00 = No analysis or not applicable

Extract from SAM_BIO.DAT

001	2	1	026	31.69	31.35	12.10	15.78	3.04	2.16	0.00	2.43
002	1	1	015	24.21	23.69	24.90	30.01	3.45	3.58	0.00	0.40
003	3	1	009	6.26	7.11	25.20	25.49	12.51	12.29	0.00	2.95
004	2	1	038	10.89	11.86	9.10	11.55	4.28	21.29	9.25	5.13
005	2	1	029	20.64	20.41	11.10	14.79	9.25	22.80	0.35	3.89
006	2	1	024	35.52	35.58	12.40	16.82	3.93	1.60	2.74	2.25
007	3	1	012	7.63	7.94	23.50	27.13	10.68	16.44	0.00	0.00
008	2	1	006	7.37	7.42	14.30	16.66	11.92	22.53	0.00	7.28
009	3	1	010	6.06	7.28	25.10	26.76	12.35	14.46	0.00	0.00
010	2	1	019	18.19	17.69	13.90	15.98	9.82	11.34	0.00	1.61
011	2	1	014	33.05	32.66	2.10	11.34	2.68	3.43	3.02	9.99
012	1	1	013	25.31	26.55	21.80	26.60	2.19	3.03	9.42	0.40
013	2	1	032	31.93	30.33	11.00	14.50	2.62	1.09	1.43	3.66
014	2	2	038	13.70	11.96	8.69	10.61	3.49	17.82	8.67	6.17
015	2	2	029	20.66	19.43	11.50	14.98	6.92	19.12	0.94	4.11
016	1	1	039	25.65	24.09	22.60	25.89	2.39	2.75	0.34	0.00
017	3	1	011	7.90	8.58	29.80	32.51	11.34	13.31	0.00	1.63
018	0	1	042	41.07	40.83	10.90	14.15	10.32	6.82	2.17	6.00
019	2	1	005	9.13	9.83	16.80	19.40	13.17	26.22	3.38	4.01
020	2	1	031	16.99	17.01	22.60	25.56	15.56	16.59	0.70	4.86
021	2	1	006	10.48	11.82	21.90	26.81	20.09	16.80	6.73	2.03
022	2	1	002	25.86	25.13	15.30	18.27	17.36	16.73	2.34	6.52
023	2	1	001	17.02	16.17	23.20	26.29	23.31	18.13	0.12	3.64
024	2	1	017	35.75	34.89	8.30	9.06	3.28	12.49	0.00	0.83

Table 4. Explanation of code used in Sample / Biochemical file (SAM_BIO.DAT)

[1] = sample number (001 - 120)
[2 - 6] = reflectance spectrum number of fresh single leaf (eg. OPEX0306)
[7 - 11] = transmittance spectrum number of fresh single leaf (eg. OPEX0307)
[12] = reflectance spectrum number of fresh leaf stack (eg. OPEX0316)
[13 - 17] = reflectance spectrum number of fresh optically thick material
[18 - 22] = reflectance spectrum number of dry single leaf (eg. OPEX0489)
[23 - 27] = transmittance spectrum number of dry single leaf (eg. OPEX490)
[28] = reflectance spectrum number of dry leaf stack (eg. OPEX0499)
[29 - 33] = reflectance spectrum number of dry optically thick material
[34 - 36] = reflectance spectrum number of pastilles (eg. OPEX2005)

-1 = measurement not made or not applicable

Extract from SAM_SPEC.DAT

---

001 0306 0308 0310 0312 0314 0307 0309 0311 0313 0315 0316 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 2003 2004 2005

002 0163 0165 0167 0169 0171 0164 0166 0168 0170 0172 0173 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 2006 2007 2008

003 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0111 0112 0113 0114 0115 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0437 0438 0439 0440 0441 -1 -1 -1

004 0522 0524 0526 0528 0530 0523 0525 0527 0529 0531 0532 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0768 0770 0772 0774 0776 0769 0771 0773 0775 0777 0778 -1 -1 -1 -1 -1 2013 2014 2015

005 0335 0337 0339 0341 0343 0336 0338 0340 0342 0344 0345 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 2016 2017 2018

006 0288 0290 0292 0294 0296 0289 0291 0293 0295 0297 0298 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0489 0491 0493 0495 0497 0490 0492 0494 0496 0498 0499 -1 -1 -1 -1 -1 -1 -1 -1 -1

007 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0134 0135 0136 0137 0138 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0745 0746 0747 0748 0749 2019 2020 2021

008 0073 0075 0077 0079 0081 0074 0076 0078 0080 0082 0084 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0454 0456 0458 0460 0462 0455 0457 0459 0461 0463 0464 -1 -1 -1 -1 -1 2022 2023 2024

009 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0117 0118 0119 0120 0121 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 0442 0443 0444 0445 0446 2025 2026 2027

Table 5. Explanation of code used in Sample / Spectrum file (SAM_SPEC.DAT)

Note: Special case is sample no.56 (plane bark)
[2-6] = reflectance of inner side of fresh bark
[13-17] = reflectance of outer side of fresh bark
[29-33] = reflectance of outer side of dry bark

[1] = sample number
[2] = type of sample 1: fresh leaf (flmr / flmt / flmri --> 66 spectra)
2: dry leaf (dlmr / dlmt / dlmri --> 60 spectra)
3: fresh needle (fnmr --> 10 spectra)
4: dry needle (dnmr --> 10 spectra)
5: fresh stalk (fsmr --> 12 spectra)
6: dry stalk (dsmr --> 7 spectra)
7: powder (pwmr --> 11 spectra)
[3] = type of plant (1: Monocotyledon 2: Dicotyledon 3: Gymnosperm)
[4-5] = Chlorophyll a content per fresh weight (mg / g)
[6] = average value
[7-8] = Chlorophyll b content per fresh weight (mg / g)
[9] = average value
[10-11] = Carotenoids content per fresh weight (mg / g)
[12] = average value
[13-14] = Chlorophyll a content per dry weight (mg / g)
[15] = average value
[16-17] = Chlorophyll b content per dry weight (mg / g)
[18] = average value
[19-20] = Carotenoids content per dry weight (mg / g)
[21] = average value

-1 = measurement not made or not applicable

Extract from SAM_PIG..DAT

001 1 1 2 2.61 2.97 2.79 0.94 1.00 0.97 0.51 0.56 0.54 10.01 11.40 10.71
3.61 3.86 3.74 1.94 2.15 2.05
002 1 2 1 2.42 2.32 2.37 0.55 0.52 0.54 0.68 0.69 0.69 9.59 9.20 9.40
2.19 2.05 2.12 2.72 2.74 2.73
003 3 1 3 0.67 0.64 0.66 0.27 0.23 0.25 0.22 0.21 0.22 0.82 0.77 0.80
0.32 0.29 0.31 0.26 0.25 0.26
003 4 1 3 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1 -1
-1 -1 -1 -1 -1
004 1 3 2 0.88 0.89 0.89 0.24 0.25 0.25 0.34 0.35 0.35 2.95 2.97 2.96
0.81 0.84 0.83 1.14 1.16 1.15
004 2 1 2 1.34 1.41 1.38 0.22 0.18 0.20 0.19 0.21 0.20 3.67 3.87 3.77
0.61 0.48 0.54 0.53 0.58 0.55
005 1 4 2 3.35 3.31 3.33 1.05 1.03 1.04 0.83 0.83 0.83 8.72 8.61 8.66
2.74 2.69 2.72 2.16 2.15 2.16
006 1 5 2 1.17 1.02 1.10 0.40 0.36 0.38 0.36 0.31 0.34 11.50 10.04 10.77
3.92 3.54 3.73 3.51 3.05 3.28
006 2 2 2 3.12 3.20 3.16 0.44 0.40 0.42 0.35 0.37 0.36 3.47 3.56 3.52
0.48 0.45 0.47 0.39 0.41 0.40

Table 6. Explanation of code used in Sample / Pigments file (SAM_PIG.DAT)

[1] = sample number
[2–4] = Carbon (% dry matter)
[5] = Carbon (average value)
[6–8] = Hydrogen (% dry matter)
[9] = Hydrogen (average value)
[10–12] = Oxygen (% dry matter)
[13] = Oxygen (average value)
[14–16] = Nitrogen (% dry matter)
[17] = Nitrogen (average value)

-1 = measurement not made or not applicable

Extract from SAM_ELE.DAT

---

001 46.30 46.96 -1.00 46.63 6.13 6.23 -1.00 6.18 36.82 37.96 -1.00 37.39
5.22 5.06 -1.00 5.14
002 47.32 46.50 47.26 47.03 6.19 6.13 6.63 6.31 36.76 40.71 39.57 39.02 3.32
3.30 3.30 3.31
003 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00
-1.00 -1.00 -1.00 -1.00
004 46.14 45.82 46.02 45.99 5.56 5.85 5.56 5.65 44.09 43.68 -1.00 43.88
2.00 1.87 -1.00 1.94
005 45.82 46.08 45.95 45.96 5.94 -1.00 -1.00 5.95 37.83 39.83 39.30 38.99
3.32 3.16 -1.00 3.24
006 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00
-1.00 -1.00 -1.00 -1.00
007 50.46 50.73 50.57 50.59 6.61 6.83 7.04 6.83 40.60 41.28 -1.00 40.94
1.43 1.22 1.10 1.25
008 49.07 48.67 47.75 48.50 6.26 6.23 5.99 6.16 39.66 40.32 -1.00 39.99
1.41 1.17 -1.00 1.29
009 51.93 51.83 51.76 51.84 7.51 7.53 7.00 7.35 38.91 40.92 39.34 39.72 1.66
1.28 -1.00 1.47
010 47.32 46.77 47.01 47.03 6.93 6.89 5.69 6.50 38.54 37.02 -1.00 37.78
2.94 2.72 -1.00 2.83
011 46.21 46.86 46.96 46.68 6.13 6.43 -1.00 6.28 36.52 37.33 -1.00 36.93
5.09 5.26 5.11 5.15
012 46.96 47.06 47.08 47.03 7.35 6.43 6.11 6.63 37.32 37.66 -1.00 37.49
4.31 4.41 -1.00 4.36
013 42.82 43.66 43.83 43.44 5.68 5.26 5.89 5.61 37.53 39.03 -1.00 38.28
4.88 4.98 -1.00 4.93
014 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00
-1.00 -1.00 -1.00 -1.00
015 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00
-1.00 -1.00 -1.00 -1.00
016 46.22 46.78 45.97 46.32 7.18 5.84 6.00 6.34 38.37 38.31 -1.00 38.34
3.74 3.56 -1.00 3.65
017 50.20 50.06 49.49 49.92 7.89 6.14 6.74 6.92 42.21 42.93 -1.00 42.57
1.27 1.51 -1.00 1.39
018 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00 -1.00
-1.00 -1.00 -1.00 -1.00

Table 7. Explanation of code used in Sample / Elements file (SAM_ELE.DAT)

Principle of operation	Double-beam, double-monochromator spectrometer
Spectral range	UV / Vis / NIR (175 - 3200 nm)
Instrument control	External PC (COMPAQ 386 Deskpro)
Optics	2 monochromators in series, each with 2 gratings
Gratings	UV/Vis: Holographic grating with 1440 lines/mm NIR: Ruled grating with 360 lines/mm Automatic grating change during monochromator slewing
Filters	Programmed optical filters with automatic filter change during monochromator slewing
Light sources	UV: Deuterium lamp Vis/NIR: Tungsten-halogen lamp Automatic source change during monochromator slewing
Beam incidence angle	8°
Detectors	UV/Vis : Side window photomultiplier NIR: PbS Automatic detector change during monochromator slewing
Dimensions	845 * 250 * 610 mm
λ accuracy	UV/Vis: ± 0.15 nm NIR: ± 0.6 nm
λ repeatability	UV/Vis: better than 0.02 nm NIR: better than 0.08 nm
λ resolution	UV/Vis: 0.05 to 5.0 nm NIR: 0.2 to 20 nm
Stray radiation	< 0.00008% at 220, 340 and 370 nm < 0.002% at 1690 nm
Photometric accuracy	± 0.08% T at 1A ± 0.05% T at 0.05A
Baseline flatness	UV/Vis: ± 0.001 A NIR: ± 0.002 A
Scan speed	0.9 - 960 nm/min.
Integrating sphere	BaSo4 coating

Table 8. Technical specifications of the Perkin Elmer λ19 spectrophotometer

Ordinate limits / mode	0 - 100 / reflectance
Abscissa range (170 - 3200 nm)	400 - 2500 nm
Data interval (0.01 - 100 nm)	1.00 nm
Slit width UV/Vis (0.05 - 5 nm)	2.00 nm (fixed)
NIR servo (1-8)	3
Lamps	D2 off / Tungsten (W) on
Detector	Auto (detector change at 860.8 nm)
Instrument speed	480 nm/min
Smoothing	2 nm
Cycles / Time	1 / Auto

Table 9. Configuration of the Perkin Elmer λ19 spectrophotometer during LOPEX93