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FIELD SPECTROSCOPY IN THE FOOD PRODUCTION CHAIN

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C A N A D A

Abstract

This paper reviews the application of field spectroscopy in the food production chain, particularly how field spectroscopy aids research and production of agricultural crops, and helps to understand the process of converting these crops into food.

The review discusses the nondestructive application of field spectroscopy such as ultraviolet, reflective, visible, near infrared and fluorescence techniques using conventional or laser light sources, to determine qualitative and quantitative properties of agricultural crops and produce.

Field spectroscopy can provide data on soil constituents and moisture, crop maturity, crop vigor, crop health, variety, variety within crops, and protein and moisture content in a standing crop. It provides data on gas particulates in cold storage, and growth rooms (CO_2 and O_2).

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Introduction

The term of "Field Spectroscopy" implies that spectroscopic measurements are performed in the field without disturbing the object to be measured, be they growing crops, soil, harvested produce or processed food, in other words a nondestructive method.

Spectroscopy measures the electromagnetic spectra (EMS) generated by the interaction of incident radiant energy (solar or artificial) with the material to be measured or analyzed. The EMS produced by this interaction produces two types of spectra: a) emission; b) absorption. In spectroradiometric measurements these spectra are characteristics of transitions whose energy requirements are related to specific wavelengths where transition energies can be measured by the amount of energy absorbed or reflected. In the field spectroscopy domain this is confined to the ultraviolet (UV), visible and infrared (IR) spectral ranges.

In the food production chain, the application of field spectroscopy is diverse, and requires spectroradiometers of different kinds both in principle and technical arrangement.

The theory of spectroscopy is well recorded in the literature [2,4,13,19,21,22,23,25,27,34,35,41,42,43,52,53]. Several practical applications are described by authors in scientific and technical papers of various aspects of spectroscopy applied to soils, crops and produce. This paper will review these applications along with the work accomplished at the Engineering and Statistical Research Institute in cooperation with various Research Stations of Agriculture Canada and Universities.

The objective of this paper is to focus the attention of food scientists on field spectroscopy since it is gaining importance in the evaluation of agricultural crops by remote sensers.

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Fundamental Concepts

In the investigation of the role of radiant energy in biological systems, one must consider both the complexity of photochemical processes in biological systems and the proper selection and application of appropriate techniques of radiation production, control, and measurement.

Radiant energy is propagated through space as electromagnetic waves. On interaction with matter, this energy behaves as "quanta" or "photons". In field spectroscopy we utilize that region of the spectrum in which the photons have sufficient energy to alter the outer electronic energy levels of atoms, i.e. the UV, visible and IR regions, but not the regions of very high energy which result in complete ionization.

The visible spectrum extends from about 380 nm in the violet to 720 nm in the red, as determined by the limits of the spectral sensitivity of the average human eye. The ultraviolet region, useful in field spectroscopy as a fluorosensor range, extends from 250 nm to 380 nm.

The infrared range extends from 720 nm to thousands of micrometers. The region of principal biological interest, especially for field spectroscopy, is between 720 nm and 2.4μm. Radiant energy is characterized quantitatively by wavelength (λcm) or frequency (νHz), and expressed as:

$$c = \nu\lambda, \text{ or } \lambda = c/\nu, \qquad (1)$$

where c = velocity of electromagnetic waves in vacuo,

$$2.998 \times 10^{10} \text{ cm/sec};$$

In addition to wavelength and frequency, radiant energy may be characterized by the energy of its smallest element, ε the quantum, or photon and is expressed as:

$$\varepsilon = h\upsilon = hc/\lambda \qquad (2)$$

Equation 2 indicates that the photon energy is proportional to frequency and inversely proportional to wavelength, and expresses the energy of one molecule. A more practical unit introduced is the Einstein (E):

$$E = Nhc/\lambda \qquad (3)$$

where $N = 6.02.10^{23}$ molecules/gram-molecule.

Equation 3 indicates that the number of molecules photochemically activated must equal the number of photons absorbed. The quantum or photon yield (Φ) provided by the radiant energy is calculated from[47]:

$$\Phi = \frac{N_m}{N_q} \qquad (4)$$

where N_m = the number of molecules reacting and

N_q = the number of quanta absorbed

Growing plants or plant materials are composed of carbohydrates, fats, protein, etc. with each having various quantum yields at various wavelengths[1,14,17,56]. The reflectance of the leaves of a plant is related to its cellular structure. Therefore, its absorption, reflection and transmission (optical) properties can be interpreted for various plant stresses, growth stage and maturity detection.

Relationship Between Reflectance and Leaf Structure

Plants depend upon radiant energy to carry on photosynthesis and other physiological processes[51]. The leaf of a plant is the primary photosynthesizing organ as they interact with electromagnetic radiation. The optical characteristics of leaves (transmission, absorbance, reflectance, refraction, scatter) are a function of the wavelength of the incident energy.

Figure 1 shows micrographs (Dr. Gausman, USDA, personal communication) of various transactions of corn, wheat, cotton and avocado leaves.

The literature on optical properties of leaves is extensive[18,28,29,30,55,59,60] and as Figure 1 indicates, it depends on the age of the leaves which is given for the agricultural crops by Gausman[29] as 0.2 mm. Generally, the reflectance increases as the thickness of the leaves increases. A thin leaf transmits a larger amount of the incident energy than a thick leaf.

The reflectance spectra of leaves over the 380 nm to 2500 nm are divided into three wavelength bands. The visible wavelength (380-720 nm) is highly absorbed by plant pigments especially chlorophylls and carotenoids present in chloroplasts which are integral parts of palisade cells. The mesophyll scatters the near infrared and affects the 750-1350nm wavelength band, whereas the 1350-2500 nm band is affected by the concentration and distribution of the leaf water content. Later on, the spectra of lettuce and wheat cultivars are shown, which indicate a strong water absorption band at 1450 nm. The wheat spectra also indicate that maturity greatly affects the reflectance of the leaves, because more mature plants demonstrate higher infrared reflectance, this is related to structural changes in the leaves as indicated in the micrographs (Fig. 1).

Measurement

The food production chain starts by cultivating the soil, in which the crops for food grow. For healthy crop production, the mineral, moisture and nutrient content of the soil are of great importance and can be analyzed with field spectroscopy techniques. For mineral content in soil the line depth (FLD) measurement of the Fraunhofer lines (FL)

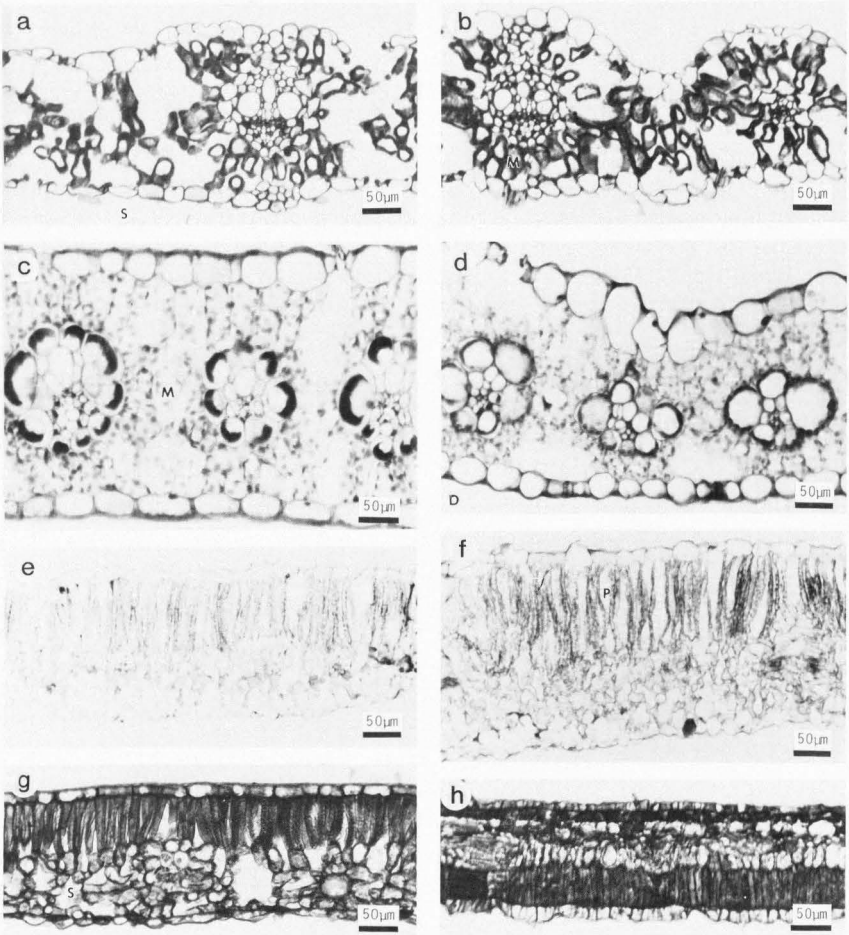


Figure 1. Transections of leaves; a, wheat cultivar Penjame (S stomata); b, wheat cultivar Milam (M mesophill); c, corn seedling (M mesophill); d, mature corn (D dorsal side); e, cotton seedling; f, mature cotton (P palisade cells); g, young avocado; h, mature avocado. (courtesy of Dr. H.W. Gausman, U.S.D.A.)

were used successfully[46,57,58]. This technique indirectly measures the soil mineral content by measuring the growing plants.

Fraunhofer lines are sharp dark lines in the solar spectrum caused by the selective absorption of light by gases in the upper part

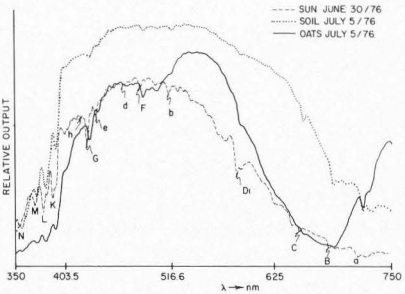


Figure 2. Fraunhofer lines appear on spectra curves of sun, soil, and oat crops. Hydrogen (H) lines; C,F, h; Oxygen (O_2) lines a and B; sodium (Na) lines D_1; Iron (Fe) lines d, e and G, L, M, N; Magnesium (Mg) line b; calcium (Ca) line K.

of the solar atmosphere. Line widths range from 0.01 nm to several tenths of a nanometer and are most numerous in the uv, visible and near-ir regions of the electromagnetic spectrum. Line depth measurement of the Fraunhofer lines involves observing a selected FL from the solar incident radiation and measuring the ratio of the central intensity of the line to a definable point on the continuum a few tenths of a nanometer distant. The solar ratio is then compared with that observed from a conjugate spectrum of the experimental material or crop. Both ratios are normally identical, however, luminescence (L) is indicated where the material ratio exceeds that of the solar ratio.

The luminescence component is given by[32,48]:

$$L = (c - a \cdot d/b)/(b - a) \qquad (5)$$

where:

a is the central line intensity FL (observed directly from the reference)
b is the nearby component of the continuum level (observed directly from the reference)

c is the central-line intensity at the same FL, and (from the plate or crop)
d is the nearby component of the continuum intensity (from the soil)

In 1973, Watson et al.[57] demonstrated that luminescence is an indicator of geochemical stress produced by metal toxicity. Fraunhofer line depths were obtained from a helicopter and an aircraft to measure the luminescence of stressed and nonstressed trees, both on a diurnal and seasonal basis. Plascyk[45] discussed the feasibility of performing luminescence measurements from a spacecraft with an FLD imaging system.

Strong FL's (Figure 2) appear in soils and crop measurements at 589.5 nm and 588.9 nm (Na) which are sodium lines, iron (Fe) lines show up at 526.5, 516.8, 516.7, 495.7, 438.3 and 430.7 nm. Magnesium (Mg) lines appear at 518.3, 517.2 and 516.7 nm and calcium (Ca) lines appear at 430.7, 422.6, 396.84 and 393.6 nm. FLD can be measured with a high resolution spectroradiometer which measures the incoming and reflected radiant energies simultaneously, and can be related to iron or magnesium deficiencies on crops.

Laser fluorescence techniques are also used[6,36,37], to determine soil types or characteristic differences among soils. Using a 20 mWatt Cd Ne laser in which energy is focused by a lens system on to the slit input of a monochromator, soil was made to fluoresce at a wavelength and energy level usually not attainable with a conventional filtered light source. Using this fluorosensor, soil materials such as clay, chlorites and sands exhibited pronounced fluorescence over a range of 380 to

Fig. 3

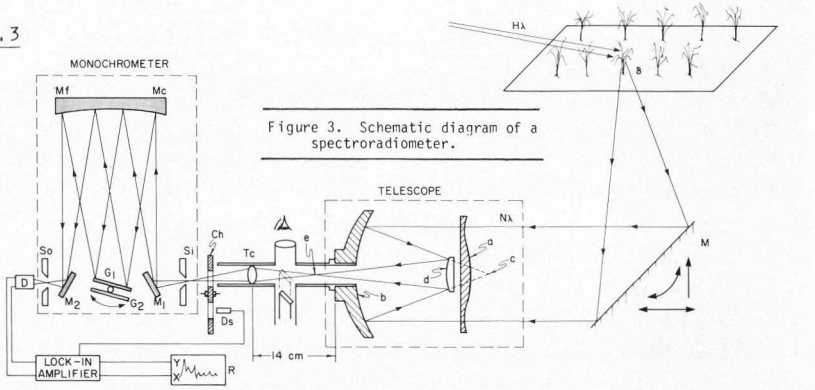


Figure 3. Schematic diagram of a spectroradiometer.

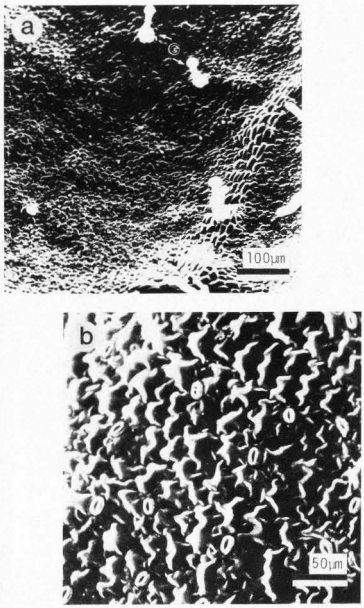


Figure 4. Morphology of lettuce leaf surface a. Surface covered with both capitate-stalked glandular trichomes (G) and septate trichomes. b. Surface relatively free of trichomes.

660 nm. Several clearly discernible peaks occurred within this range, particularly between 384 and 454 nm. Kaolinite, montmorillonite, Ottawa washed sand, Fe-rich chlorite and two soils (Uplands LS and St. Rosalie HC) had response peaks at 384 and 396 nm, whereas glassbeads (29 µm) and Mg-rich chlorite showed little response. Both groups exhibited similar peaks at 403, 408 nm, 430 and 438 nm. Overall fluorescence yield was higher with decreased grain size. Using chemically similar crystaline materials (Mg-rich and Fe-rich chlorites) separated into several grain-sized fractions, the yield of fluorescence was in the following order: (under 2µm) > (5 to 20 µm) >(50 to 100 µm) grain sizes.

Overall intensity was considerably higher for all grain-sized fractions of Mg-rich chlorite than for Fe-rich chlorite. Increase in clay concentration (>2 µm clay) above 10% raised fluorescence yield. However, in materials of over 63% sand, small amounts of clay (<10%) appeared to produce a higher yield than expected from the regression equations for relation of fluorescent yield and clay content. The results show that granular materials associated with soils exhibit measurable fluorescent spectra which may affect spectra obtained from laser-fluorosensing of airborne remote sensing missions over open vegetated and non-vegetated areas.

To use field spectroscopic techniques for crop measurements, it must be recognized that during the growing season, the reflected energy from a field varies with changing crop cover, rate of plant development, concentration of plant pigments and degree of water stress. The spectral characteristics are influenced greatly by physiological and biochemical changes in the plants.

Various spectroradiometers used to measure plant optical characteristics and the results obtained from them, have been reported in the literature[20,31,33,50]. At Engineering and Statistical Research Institute laboratories, several application oriented spectroradiometers have been developed[34-36]. The basic concept of a spectroradiometer is indicated in Figure 3, where the reflected radiance N_λ from the plant into the spectroradiometer is given by:

$$N_\lambda(\text{Watt.cm}^{-1}.\mu^{-1}.\text{sterradian}^{-1}) =$$

$$= \frac{1}{\pi} (E_\lambda \text{Cos}\theta_S \rho) \qquad (6)$$

The irradiance E_λ from the sun falls on the plant at an angle of θ_S and the radiance from the plants is collected by a Schmidt-Cassegrain telescope via a flat folding mirror M with 4 degrees of freedom. The detector (D) converts the radiance into electrical signals, which are measured by a photon quantum meter or lock-in amplifier, displayed on a recorder (R), and recorded by a paper tape punch for computer processing. The monochromator can be replaced by interference filters, the telescope by various lens arrangements. The detector can be a photomultiplier or silicon detector for the visible spectrum, and lead sulphide (PbS), lead selenide (PbSe), gallium arsenide (GaAs) or indium antimonide (InSb) for the infrared spectrum. Each spectroradiometer is constructed to suit its application.

Lettuce Maturity Detection: Two kinds of experiments have been tried to detect lettuce maturity: optical, and laser fluorescence spectroscopy[8,12,44]. The objective of these experiments was to determine the correlation between lettuce reflection or fluorescence and its stage of maturity. Anatomical studies with a scanning electron microscope showed that the surfaces of young head leaves were covered initially with capitated-stalked glandular trichomes (Fig. 4a) which at later stages were broken and appeared as septate trichomes. At maturity the surface of the leaf appeared free of trichomes (Fig. 4b).

Cross sections of the first head leaf for all four cultivars showed a rather dense and uniform cell arrangement (Fig. 5). Well defined, spongy and palisade tissues were apparent only in the exterior leaves and cotyledons (Fig. 6). In the young (44-day-old) heads the cells were closely packed with few,

small intercellular spaces (Fig. 7, micrographs a and b). In the mature heads the number and volume of the intercellular spaces, especially those immediately below the epidermis were larger (Fig. 7, micrographs c and d). As a consequence of cell enlargement, the subcellular organelles, especially chloroplasts, were more dispersed in old than in young tissues.

The absence or presence of trichomes on the leaf surface (or anatomical structure of the leaf) may cause variation of reflectance, cellular enlargement may have a twofold consequence, a change in light: 1) scattering; 2) reflection.

Field experiments were conducted with the spectroradiometers housed in a mobile laboratory. The incoming radiant energy (vertical sighting) falling on the lettuce was measured and at each wavelength point the reflected energy (oblique measurement) was normalized by dividing it by the incoming energy to eliminate cloud effects. Visible and infrared incoming and reflected spectral curves of lettuce are shown for 41 days after planting (Fig. 8a) and the spectral curves of the same plot, at 61 days after planting (Fig. 8b). While the incoming radiant energy did not differ greatly, the reflected energy in the visible as well as in the infrared wavelength range showed a marked difference between the two dates which is due to cell structural changes. Plant leaves reflect incident radiation due to its pigmented cells containing water solutions. As indicated in Figure 8a, the UV blue and red reflectance are low and the green high, due to chlorophyll content. The water absorption bands (Figure 8a, 8b) at 900, 1100 and 1450 nm are due to liquid water in the leaf. The spectral curves were taken at approximately the same time of day (11:32 vs 11:13 hours).

Experiments using field spectroscopy techniques were conducted on various grain crops (wheat, barley, oat, corn, soybean) to determine agronomic conditions from their spectral (optical) properties [7,9,31,38,39,49,54].

The experimental set up and instrumentation was similar to those used in the lettuce maturity measurements. The spectral data collection and analysis done in 1976 is presented where spectral data were collected from experimental field plots of various crops and varieties. The crop spectra were obtained at wavelengths from 350 to 1840 nm with a spectroradiometer having a grating resolution in the order of 0.1 nm. The data were recorded and digitized onto magnetic tape for computer processing at a reduced accuracy of 1 nm. Calibration was performed regularly by measuring the incident solar intensity and checking for the

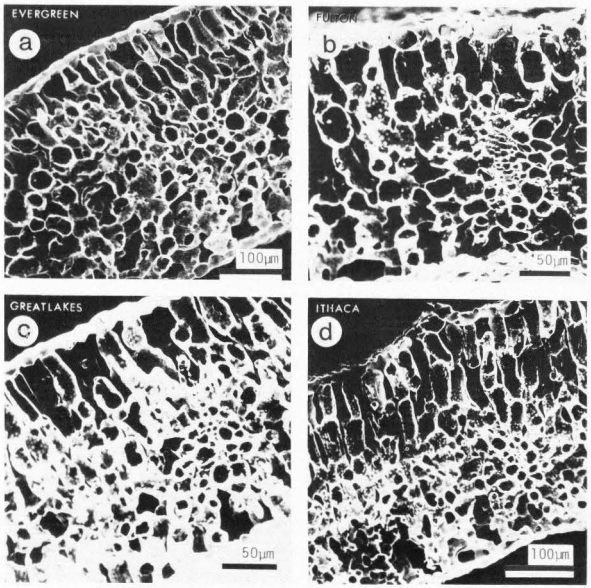
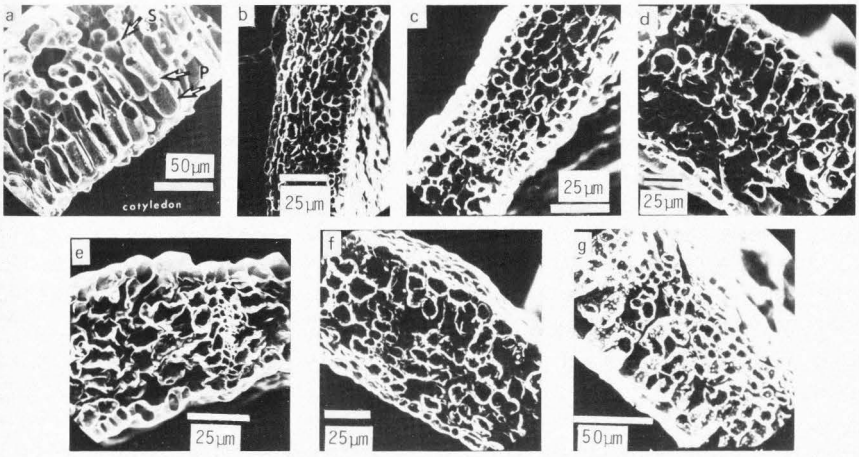
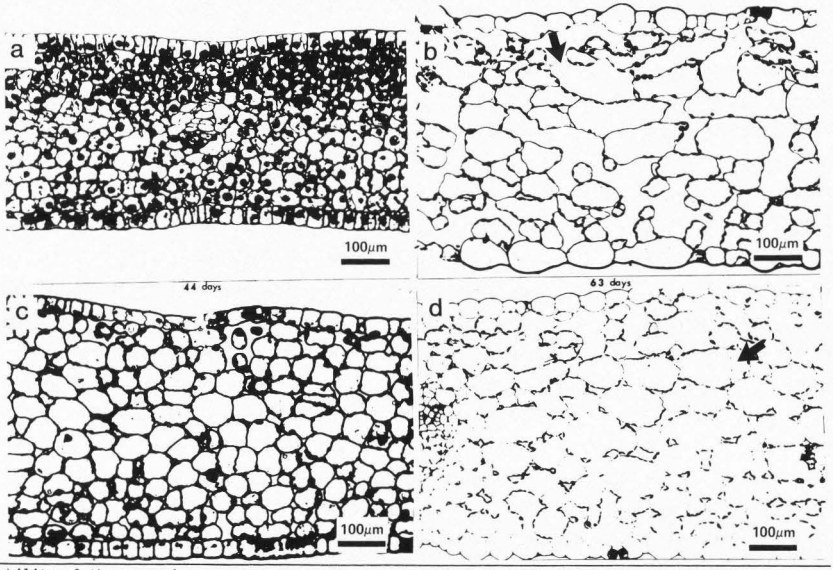


Figure 5. (to the left). Cross-section view of the first head leaves of different lettuce cultivars at the same stage of maturity. a. Evergreen; b. Fulton; c. Great Lakes; d. Ithaca. No defined palisade or spongy mesophyll cells are apparent.

Figure 6 (facing page - top). Cross-sectional view of the cotyledon and leaves of lettuce (cv. Fulton) at the sixth-leaf stage. a. Cotyledon; b. Sixth leaf; c. Fifth leaf; d. Fourth leaf; e. Third leaf; f. Second leaf; g. First leaf. Palisade (P) and spongy mesophyll (S) cells are apparent in the cotyledon and leaves up to the fourth leaf.

Figure 7 (facing page - bottom). Cross-section view of the first head leaf of two lettuce cultivars. a,c. Ithaca and Great Lakes, 44-day-old plants. b,d. Ithaca and Great Lakes, 63-day-old plants. Compactness of leaf structure decreases with maturity and chloroplasts are more dispersed in older leaves (b,d). Arrow indicates airspace between cells.





stability of the measuring system. Preceding the measurement of the crop spectra, reflected radiation was obtained from a Kodak standard gray card for the visible region and from an aluminum plate for the near infrared region. Correction for the viewing geometry and atmospheric conditions were applied using atmospheric models. By averaging the 600 spectra according to seeding dates (D_1, D_2) in two different replicates, the complexity in the interpretation was minimized. An error less than 20% was estimated near the

750 nm region but it was less than 5% within the spectral regions of 350-750 and 750-1840 nm.

Principal-component analysis was conducted to obtain information at 10 nm band-widths which would be independent of daily standardization and atmospheric variations. The computations were done for all 10 nm bands-widths from 350 to 1840 nm (150 10 nm bands) using 35 spectra available from the crops and cultivars within the period from early August to late September, 1976. The analysis was based on the determination of the covariance matrix that was calculated from the crop spectra. As a measure of the amount of information within a specific band, the variance of the reflectances of a number of spectra was computed. There were six to eight spectra available for each of the crops covering the growth stages V (early heading) to X (ripe). Crops seeded May 27, 1976 (D_1) were more advanced in their growth stages than those seeded June 23, 1976 (D_2); thus, there was overlap in growth stages for part of the period. The analyses for the D_1 and D_2 crops were carried out separately.

Certain spectral bands may be differently absorbed and reflected by certain crops features. For example, near the growth stages V to VI, the chlorophyl of green plants absorbs red radiation (640 to 690 nm) but turgid leaves greatly reflect the near-infrared (e.g. near 740 nm). Later both bands may reflect similarly. Thus, certain pairs of bands, having a negative correlation, may be more useful for discriminating crops and separating crop features than individual bands.

The changes in the reflectance spectra for wheat (cult. Neepawa) over the infrared spectral range of 750 to 1840 nm are shown for four growth stages from the boot stage to full maturity (Figure 9). The spectral bands of water absoption are clearly shown by the minima near 950, 1150 and 1450 nm. The absorption bands near 950 and 1150 nm regions disappear as the maturity of the wheat plant reaches the growth stage X.

For each cultivar, the spectral values at each nm interval were averaged for the different measuring dates. The standard deviations of the periodic measurements were calculated and coefficient of variation (CV) was obtained and plotted.

$$CV = \sigma/\mu \qquad (7)$$

σ = the standard deviations
μ = the average spectral value for each 1 nm bandwidth

Figure 10a shows that the soil has a smoother spectrum than the sod except in the water absorption regions. The CV of sod varies mainly in the 450-700 nm region which corresponds to chlorophyll and carotenoids whereas the CV of soil lies in the 1350-1600 nm region due to moisture.

The CV varied among the wheat cultivars throughout the entire spectrum (Figure 10b). Among the wheat varieties (Sinton, Neepawa, Hercules, and Marquis SR6), Sinton (D2) had the largest CV (60 percent) while Marquis SR6 (D1) had the smallest (15 percent to 20 percent). The general pattern of the CV throughout the spectral range 350 to 1850 nm was somewhat similar. Large CV's occurred near 500 to 550 nm, 650 to 700 nm, and 1250 to 1350 nm, which correspond physiologically to the wavelength regions of minimal radiation absorption by plants, chlorophyll and plant water, respectively. Among the barley cultivars (Figure 10c) Fergus (D1) showed the maximum CV of 45 percent and Conquest (D2) the lowest CV of 10 percent. The pattern for Fergus was distinctly different from the others, having a number of broad plateaus with low indentations near 750, 925, 1125 and 1350 nm. Among the oat varieties (Figure 10d) Garry showed a high CV, >40 percent, partiularly in the range of 550 to 650 nm, whereas Terra (D1) was low, <40 percent. It is worthwhile to note that the diseased Garry variety had a markedly larger CV than the healthy variety. This technique indicates that with field spectroscopy the identification of crop variety, cultivar within a crop and crop maturity is possible. It also indicates that a diseased (stressed) crop can be detected.

Protein and moisture analysis on ground grain products have been established [5, 40], and commercial laboratory protein moisture analyzers of grain products are available. However, little is reported in the scientific literature on protein analysis of standing grain crops. In 1980 and 1981, protein measurements on standing crops were attempted [3] with field spectroscopic techniques. In this experiment, absolute relationships between reflectance and grain protein content was not conclusively acheived but a number of important points were noted.

1) The highest correlation between near infrared reflectance and protein content was at the 2.07 to 2.11 μm wavelength band, with a secondary absorption band at 2.15 to 2.17 μm. 2) The relationship between near infrared reflectance and protein content was found to be negative, that is, the protein content increased as the reflectance of the plant decreased. 3) The growth stages from the beginning of heading to maturity yielded better results than the earlier preheading stages. 4) The growth stage which yielded the best results was growth stage 6 (beginning to head) where the correlation coefficient for the regression of protein content on reflectance was equal to -0.72 at 2.11 μm (Table 1). 5) The correlation between reflectance and protein content was not high enough to develop a model for predicting protein content on the basis of near infrared reflectance.

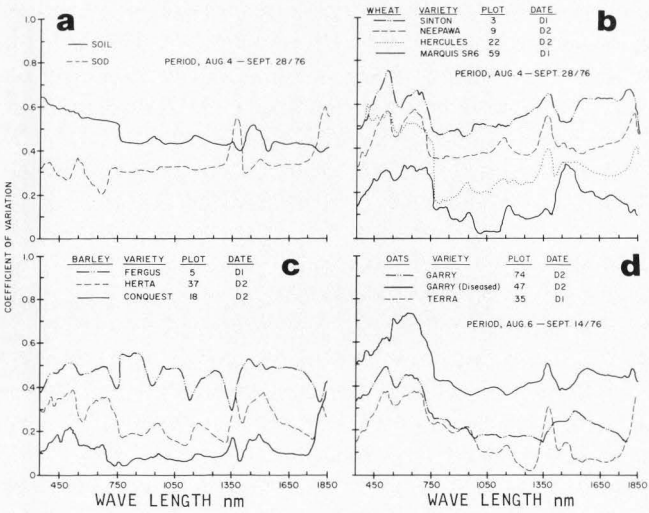


Figure 10. Coefficient of variation of the spectral values for selected crops and soil around the mean value for each 1.0 nm bandwidth.

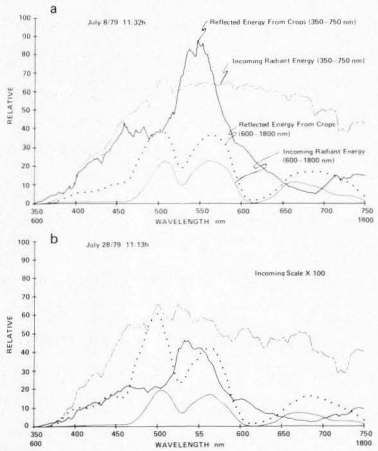


Figure 8. Visible and infrared spectral distribution of incoming and reflected radiation energies from lettuce: a) 41 days after planting; b) 61 days after planting. Incoming energy scale is multiplied by 100.

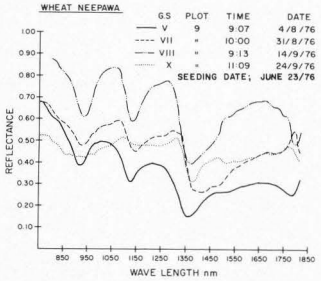


Figure 9. Reflectance spectra of a wheat cultivar (normalized infra-red) from 750 to 1840 nm at four growth stages from early heading to maturity.

Table 1. Summarized results of the analytical procedure of correlation between protein content and wavelength band for all of the growth stages of hard red spring wheat, Triticum aestiuum L., CV. Neepawa.

Growth stage	Wavelength band of highest correlation (μm)	Highest correlation value (r)
4	2.34 - 2.36	-0.40
5	2.23 - 2.25	-0.22
6	2.08 - 2.11	-0.72
7.5	2.06 - 2.09 2.15 - 2.17	-0.44 -0.42
8	2.06 - 2.08	-0.42
9	2.00 - 2.02	-0.50

TABLE 2. Holographic identification of variety of species (B,O,R,T,W) between species (OC, OL, OS...) and age (0, 1, 2) by the value of slope angle "α"

Variety		Number of Fringes	α
Barley	(BVO)	2	25.82
Oat	(OCO)	2	19.98
Rye	(RSO)	2	38.65
Wheat	(WFO)	2	36.86
Barley	(BHO)	3	53.13
Oat	(OLO)	3	25.01
Rye	(RPO)	3	26.56
Barley	(BH1)	4	38.33
Oat	(OS1)	4	30.35
Triticale	(TS1)	4	26.56
Wheat	(WF1)	4	42.27
Oat	(OF1)	5	56.30
Wheat	(WR1)	5	22.38
Oat	(OC2)	5	53.74
Rye	(RS2)	5	45.00
Triticale	(TS2)	5	20.55
Barley	(BHO)	6	21.80
Oat	(OLO)	6	27.89
Rye	(RPO)	6	30.96

Further research is ongoing to refine the technique of measuring protein, also moisture and fat in standing crops during their growth.

In breeding experiments, it is useful to monitor the growth of plants and the rates of the growth of the plant which occur in response to applied stimuli or changes in the environment.

Fox and Puffer[26] reported in 1976 on a holographic method to measure motions in mature plants. Briers in 1973[15] and in 1976[16] discussed a holographic technique to visualize plant growth. Brach and Fejer[10] discussed in 1980, the merits of holographic interferometry to measure changes in the morphology of various cereal crops.

A fast and reliable method for the determination of differences in the morphology of plant species, and between cultivars of the same species at different ages, would be welcomed by plant scientists.

Figure 11 presents reconstructed holograms of plant leaves which are characterized by the shape and slope of the fringes, the distance between fringes and the number of veins in the hologram. The measurement of the slope angle "α" is indicated by the inset drawings of Figure 11.

The number of fringes in the hologram is determined by the setting of the subject lens or its magnification factor. Therefore, one must always compare holograms with the same number of fringes. Larger numbers of fringes shown in the hologram indicate a larger cross section of plant leaf. Figure 11 as well as Table 2, indicates that in any age group and with the same number of fringes, species can be identified by the slope angle α.

Plant CO_2 Uptake as a Measure of Crop Growth:

Under the influence of light energy, plants perform the overall reaction:

$$6\,CO_2 + 6\,H_2O \xrightarrow{nh\nu} C_6H_{12}O_6 + 6\,O_2 \qquad (7)$$

where $C_6H_{12}O_6$ stands for carbohydrate in general. Equation 7 indicates when the plant is stimulated by the photon $h\nu$, it converts CO_2 using available H_2O to organic matter ($C_6H_{12}O_6$) and oxygen O_2. The carbon C content C_C of $C_6H_{12}O_6$ is:

$$C_C = \frac{C_6}{C_6H_{12}O_6} = \frac{72.066}{72.066+12.084+95.94} = 39.79 \approx 40\%$$

The ratio of the molecular weight of CO_2 to carbon is:

$$(CO_2) = \frac{C + O_2}{C} = \frac{12.011 + 31.998}{12.011} = 3.6$$

The amount of CO_2 assimilated (T_{CO_2}) by the plant is:

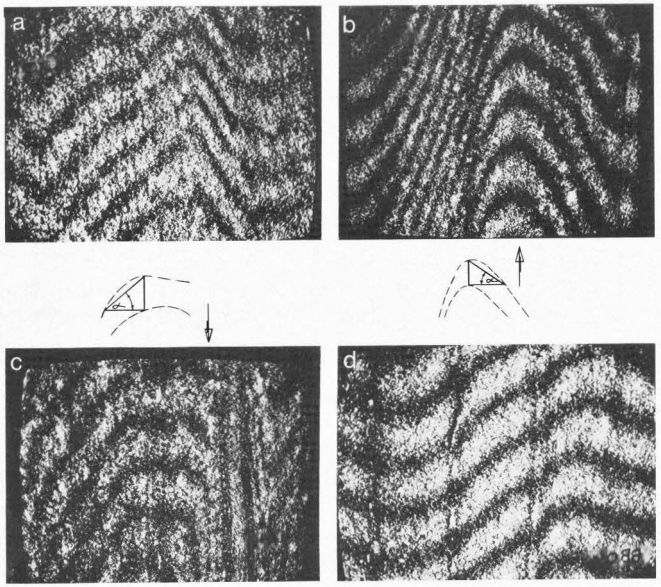


Figure 11. Reconstructed holograms of: a) young oat leaf variety C.I. 4492; b) old oat leaf variety Foothill; c) triticale leaf variety Rosener; d) old barley leaf variety Betzes.

$T_{CO_2} = W.C_C.(CO_2)_C$
where W is the growth rate of the plant gDM/day

DM = dry matter

If W = 100 gDM m^{-2} day^{-1} then,

T_{CO_2} = 100 x 0·4x 3·6
= 146·56 g CO_2 m^{-2} day^{-1}

By measuring the assimilation of CO_2 by a crop, one can then measure its rate of growth and measure its retardation due to environmental stresses. For that purpose, an open path CO_2 analyzer was developed [11]. The analyzer is based on the differential infrared absorption by CO_2 at 3·7 and 4·3 µm over a 1.5 m measuring path. It measures the CO_2 concentration with 0.3 ppm sensitivity by volume with a time constant of 0.075 s. The normal operating range is between 240 to 400 ppm. The concentration CO_2 in the atmosphere is 330 ppm, therefore measuring the difference in CO_2 concentration between the air moving upward and downward above a crop canopy, estimates the CO_2 assimilated by the crop.

Desjardins et al. [24] mounted this analyzer on an aircraft and measured the exchange of CO_2 above a cornfield, a forest and a lake at midday. Repeated test flights over these ecosystems were conducted. Mean CO_2 absorption values obtained from these measurements were 3400, 1200, and 100 mg $m^{-2}hour^{-1}$ for corn, forest and water, respectively (Table 3).

The CO_2 analyzer was also adapted to control the CO_2 content in plant growth rooms and greenhouses. Horticultural and ornamental crops grow faster with a greater yield at a high CO_2 content in the atmosphere (800 -1800 ppm) thus shortening the period for producing the crops and increasing the productivity. The open path of the CO_2 analyzer was reduced to 50 cm, since there is no need for more than a 10 ppm sensitivity. A digital controller was added to regulate the CO_2 supply into the greenhouse. If too much CO_2 is in the room (e.g. in the early morning, due to CO_2 release by the crop) the controller opens inlets admitting fresh air. The required upper and lower limits can be preset on the analyzer.

Adapting the analyzer to monitor and control the CO_2 content of atmospherically controlled storage for apples is underway.

TABLE 3. Carbon dioxide and heat flux densities for passes over corn, forest, and water around midday on August 28, 1980.

Altitude (m)	CO_2 flux density ($mg.m^{-2}.h^{-1} \times 10^2$)	Heat flux density ($W.m^{-2}$)
Corn		
23	-14	14
24	-34	42
25	-35	70
33	-36	70
33	-47	84
35	-36	63
Average	-34	57
Forest		
33	-8	56
34	-10	28
47	-12	42
48	-11	42
60	-14	42
61	-18	56
Average	-12	44
Water		
28	3	21
29	-4	14
31	-3	28
Average	-1	21

Conclusion

Field spectroscopy and its usefulness to research and production in the agricultural industry was presented. The technology of field spectroscopy and its application is relatively new and therefore not fully explored. As more researchers in agricultural research and production get acquainted with the technology, additional applications will be discovered, besides detecting stress in plants, identifying varieties, measuring protein and estimating biomass. Concurrently, the potential for developing equipment is being enhanced by rapid developments and cost reductions in computer technology.

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Discussion with Reviewers

Reviewer III: The techniques as discussed in the paper may be related to microstructural components of food or plant related systems, but the exact relationships and interrelationships are not made parallel to one another in terms of macroscopic or macrostructural versus microscopic or microstructural parts of the systems being evaluated. The techniques at best measure overall protein, fat, moisture, etc. and are not specific analysis (even at specified wavelengths). Please comment.

Are plants being treated as optical systems or as objects containing atoms and molecules of specific types that interact with radiation of specific wavelengths, therefore, characterized by the vibrational spectral transitions and atomic electronic spectral transitions?

Do you believe the molecular and atomic spectral differences are averaged measurements of a great number of different (but perhaps related molecules) which absorb, emit and reflect radiation at specific wavelength positions?

Author: I agree with your comment, but in field spectroscopy we don't intend to study the specific atom and molecule of the standing crop, we rather observe the spectral changes on the surface of the leaves caused by molecular changes within the plants.

Reviewer IV: If "the visible wavelength (380-720 nm) is highly absorbed by plant pigments..." then they would probably be black and not green. Fig. 8 shows high 90% reflectance at 550 nm! Please comment.

Author: Figure 8a and 8b are indicating relative values of reflectance, and not absolute values. A young plant with a high chlorophyll content will highly absorb in the blue and red wavelength bands but less in the green, giving to the eye a green perception.