THE FEASIBILITY OF DETECTING THE PROTEIN CONTENT OF A STANDING WHEAT CROP BY NEAR INFRARED REFLECTANCE SPECTROSCOPY

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Raymond Gilbert Berard

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Berard, Raymond Gilbert. M.Sc., The University of Manitoba, May, 1982. The Feasibility of Detecting the Protein Content of a Standing Wheat Crop by Near Infrared Reflectance Spectroscopy. Major Professor; William C. Bell.

The near infrared reflectance spectra (1.4 - 2.4 μm) of wheat (Triticum aestivum L., cv. Neepawa) plots grown in a greenhouse under different levels of available nitrogen were analyzed to determine whether the spectral data could be used to differentiate between different protein levels in the plants. The spectral measurements were carried out at several developmental stages of the crop using a mobile field spectroscopy laboratory.

The highest correlation between near infrared reflectance and protein content was found at the 2.07 to 2.11 μm wavelength band. This relationship was found to be negative, that is, the reflectance increased as the protein content decreased.

The results from the beginning of heading to maturity were more consistent than the earlier growth stages in terms of the wavelengths associated with absorption of radiation by protein. The best correlation between near infrared reflectance and protein content was obtained at the beginning of heading where the highest coefficient of correlation was equal to -0.72 at the 2.11 μm wavelength.

The ability to differentiate between different protein levels was also better at these later growth stages. In particular, at the beginning of heading stage, a mean protein content difference of 1% produced a corresponding significant difference in the mean reflectance at the 2.07 to 2.11 μm wavelength band.

The correlation between reflectance and protein content was not high enough to develop a model for predicting, with reasonable accuracy, the protein content on the basis of near infrared reflectance.

INTRODUCTION

"As our grain reserves become depleted and world population and demand for food increase, the need to improve the quality of world crop production information becomes ever more critical" (Bauer, 1975). This statement finds ample support from the benefits of improved crop information: accurate estimates result in price stability; timely and accurate forecasts of production allow governments to plan domestic and foreign policies and actions; and accurate forecasts enable optimal utilization of storage, transportation, and processing facilities. The detection of diseased or physiologically stressed crops is another important aspect of agriculture in that it permits corrective action to be taken and yield predictions to be adjusted.

During the past twenty years, considerable evidence has shown that remote sensing from aerospace platforms can provide valuable information on agricultural resources on a worldwide basis. It has the potential to revolutionize the detection and characterization of many agricultural phenomena. Remote sensing techniques can be used in the visible, infrared, and microwave regions of the electromagnetic spectrum to collect measurements of reflectance and emittance of plants, soils, water, and other materials. With a minimum amount of ground sampling, remote sensing data can permit identification and area measurements of crops, assessment of crop stress, yield forecasts, range surveys, and mapping of major soil boundaries, as well as many non-

agricultural applications.

Remote sensing is a relatively new term, used only since about 1960, and refers to the acquisition and interpretation of spectral measurements made at a distant location to obtain information about the earth's surface. It is an outgrowth of aerial photography which has been developing for more than a hundred years. Since 1960 remote sensing has been rapidly evolving and expanding as new sensors and interpretation techniques become available and new uses for the technology are developed.

Remote sensing of agricultural resources involves the detection of electromagnetic energy that is reflected or emitted from the crops. The data obtained can be meaningfully interpreted and processed only if we have a fundamental understanding of these energy-matter interactions that account for variations in the quantity and quality of radiation recorded by air and space-borne sensors (Knipling, 1970). Fundamental studies in leaf and canopy reflectance as well as applied research in field spectroscopy provide necessary knowledge as to which portions of the electromagnetic spectrum are important in applications such as species and cultivar identification, detection of physiological stresses such as disease, moisture and nutrient stress.

The objective of this research was to assess the potential of remote sensing for detecting the nitrogen status of a crop as expressed by the protein content of the vegetation. In particular, the research was an attempt to apply to a standing crop the principle of near infrared reflectance spectroscopy (NIRS) developed by Norris (1978) for measuring the protein content of grain. The specific objectives of the research were:

(1) To determine the wavelength bands of importance in the absorption of near infrared radiation by protein.

(2) To determine which developmental stage of the crop would yield the best correlation between protein content and near infrared reflectance.

(3) To determine whether different levels of protein content in the plants could be detected by near infrared reflectance.

LITERATURE REVIEW

Physical and Physiological Basis for Remote Sensing

Leaf Reflectance

General Energy-Matter Interactions of Leaves. The reflectance spectrum of leaves can be divided into three rather distinct regions. The visible wavelengths (400 - 700 nm) have received considerable attention because they are highly absorbed by plant pigments, especially chlorophylls and carotenoids, and thus are of primary importance in plant photosynthesis. Due to the high absorption, these wavelengths are poorly reflected by leaves, having a reflectance of about 10% with a peak at about 550 nm in the green region (Knipling, 1970). The near infrared wavelengths (700 - 1300 nm) in contrast, are essentially unabsorbed by foliage. The reflectances in this region are usually around 50% with the other 50% being transmitted. While some studies have found light absorption in this region (Woolley, 1971), the amount is usually less than 4%. The far infrared wavelengths (1300 - 2600 nm) have moderate to high absorption, primarily due to water present in leaves. Bands of very high water absorption occur at 1430, 1950 and about 2600 nm, resulting in low reflectance.

The absorptive characteristics of plants are perfect examples of their adaptation to the radiation environment. Plants absorb very efficiently in the visible regions of the spectrum where the energy is

required for photosynthesis. However, the absorption drops to a very low value in the near infrared (700 - 1300 nm) where the direct sunlight incident on the plant has the bulk of its energy. If plants absorbed the energy with the same efficiency as they do in the visible they would frequently become too warm and the proteins would be irreversibly denatured. At wavelengths greater than 2500 nm, plants become nearly black bodies absorbing the far infrared very efficiently. At these longer wavelengths there is not sufficient solar energy remaining in the spectrum to influence substantially the plant temperature. However, plants are very efficient radiators of these long wavelengths which permits them to cool themselves substantially by reradiation. Gates and Benedict (1963) have shown that of the total energy absorbed by plants approximately 75% is reradiated and 25% is dissipated by convection and transpiration.

Mechanisms of Leaf Reflectance. Most of the work done on leaf reflectance has involved the concept of the internal reflectance mechanism. Willstatter and Stoll (1913) were the first to recognize the importance of this internal reflectance mechanism. They hypothesized that leaf reflectance had to occur at interfaces within the leaves where total or critical reflectance was possible. The two basic requirements for critical reflectance are: (a) that the radiation must pass from a material with a high index of refraction to a material with a low index of refraction, and (b) that the angle of incidence must be sufficiently large. They suggested that the spongy mesophyll of leaves was most favorable to meet these requirements since this tissue contained large intercellular spaces and cells with very irregular structure having

cell walls oriented at virtually all angles.

The evidence for the internal reflection mechanism is quite strong. Knipling (1970) provided a very convincing piece of evidence in favour of this mechanism by demonstrating a drastic reduction in the near infrared reflectance of a leaf infiltrated with water. The water fills the air cavities and forms a continuous liquid phase medium throughout the leaf. The elimination of many of the refractive index differences within the leaf increases the direct transmittance at the expense of scattering. However, Knipling felt that the Willstatter and Stoll theory placed too much emphasis on the role of the spongy mesophyll and its large air cavities in relation to that of other interior parts of a leaf. He suggested that the important parameter in determining the level of reflectance is the number or total area of the air-wall interfaces and not the volume of air space. In this regard he noted that the palisade mesophyll of a leaf was probably as important as the spongy mesophyll in the internal scattering of radiation. Many small air cavities exist between adjacent palisade cells, and the area of exposed cell walls in this region is as large and perhaps even larger in some cases as in the spongy mesophyll which generally has larger air cavities and fewer cells.

Gates et al. (1965) published a comprehensive review on the spectral properties of plants. They stated that the materials of the leaf which are important in light interactions are: cellulose of the cell walls, water containing solutes (ions, small and large molecules such as protein and nucleic acid) within the cells, and intercellular air spaces and pigments within the chloroplasts. Noting that the grana within the chloroplasts may be 0.5 μm in length and 0.05 μm in diameter

which is the dimension of a wavelength of light, they stated that these may produce a considerable scattering of light entering the chloroplasts. Scattering would also be caused by structures such as mitochondria, ribosomes, nuclei, starch grains, and other plastids which also are of the dimension of a wavelength of light. They suggested that whatever scattering which does exist is probably more of the Mie type (particles of the dimension of a wavelength of light) than the Rayleigh type (particles relatively smaller than a wavelength of light) because spectral reflectance and transmittance curves showed that the scattering phenomenon was not strongly wavelength dependent, which would be the case in Rayleigh scattering.

Mestre (1935) did not feel that small particles in the cell would cause the scattering. He considered such particles to be relatively smaller than the wavelengths of the radiation and should thus produce scattering according to Rayleigh's equation. Since results of previous experimenters showed no tendency for increased reflectance at the shorter wavelengths as predicted by Rayleigh scattering, he concluded that such particles had little influence on internal scattering. Mestre recognized that reflectance of solar radiation occurred at both the leaf surface and within the internal structure. He hypothesized that the incident flux could be reflected at the surface by either specular or diffuse reflectance. Specular reflectance would occur from leaves with extreme glossy cuticles and would obey Fresnel's law while diffuse reflectance would result from leaves with tomentose surfaces and would obey Lambert's cosine law. Light not reflected at the surface would travel into the mesophyll of the leaf and would be transformed into a diffuse flux to the extent that the leaf material was dif-

fusive which he called the "scattering power of the tissue". He reasoned that the greater the scattering power of the mesophyll the larger would be the potential reflectance of the leaf. He therefore believed that the internal reflections proposed by the Willstatter and Stoll theory must be the major source of the scattering power of the leaves.

Sinclair et al. (1965) carried out an extensive investigation of the spectral characteristics of leaves from several species involving measurements of reflectance and transmittance of energy in both visible and reflective infrared wavelengths. The purpose was to better understand the interrelationships between the existing theories of energy-matter interactions in both the visible and reflective infrared wavelengths and to better understand the relationship between reflectance and leaf structure. They studied three aspects of histological differences in leaves: (1) dorsiventral structural differences, using leaves from both dicotyledonous and monocotyledonous species, (2) differences in the depth of the palisade layers using sunlit and shade leaves from a single species, and (3) differences in histology due to variations in moisture content.

To examine dorsiventral structure effects, measurements of reflectance and transmission were made from the dorsal and ventral sides of soybean (Glycine max. L.) leaves and corn (Zea mays L.). The corn was used as a representative of monocotyledonous leaves having a non-differentiated mesophyll and the soybean leaves were used to represent dicotyledonous leaves with their distinct dorsiventral structure. The palisade tissue on the ventral side is comprised of several layers of densely arranged cylindrical cells, whereas the

spongy mesophyll on the dorsal side has large intercellular air spaces among parenchyma cells of irregular shape. The reflectance of both sides of the corn leaves did not differ significantly in the shorter infrared wavelengths, as would be predicted by the Willstatter and Stoll theory, since the radiation encounters the same internal structure on either side. However, in the soybean leaf, the reflectance in the near infrared wavelengths was higher from the ventral side than from the dorsal side, which is contrary to the Willstatter and Stoll theory which would have predicted a higher reflectance from the dorsal side of the leaf where the spongy mesophyll is first encountered. They attributed this difference in prediction capability of the Willstatter and Stoll theory to the fact that their theory was developed on the basis of results obtained only from the visible wavelengths, rather than from both visible and reflective infrared wavelengths.

In studying the effect of the depth of the palisade layers on reflectance, both shaded and sunlit apple leaves were used. While the shaded leaves were similar in structure to the soybean leaves, the sun leaves were thicker, having palisade tissue three or four layers in depth, where the cells were more densely arranged, and the spongy mesophyll was more highly developed. As was the case with the soybean leaves, the ventral sides were more reflective than the dorsal sides of both the sunlit and shaded leaves in the near infrared wavelengths. However, comparison between the sunlit and shaded leaves showed that the sunlit leaves were significantly more reflective and less transmissive than the shaded leaves. These results contradict the concept which stresses the importance of lacunose mesophyll for high reflectance, since the spongy mesophyll was more compact in the sunlit leaves

where the reflectance was higher.

Finally, reflectance measurements were made on corn leaves having different degrees of turgidity. Differences in reflectance were expected to occur due to drastic changes in leaf structure which occur upon dehydration, especially in dry, dead leaves where the cell walls collapsed into a mat-like series of layers lying parallel to the epidermis. The Willstatter and Stoll theory would have predicted a reduction in reflectance as dehydration progressed since their theory depends upon cell walls oriented at angles greater than the critical angle. However, the reflectance at 1.04 to 1.07 μm increased as the moisture content of the leaves decreased.

In order to explain their results as well as the apparent anomalies of the Willstatter and Stoll theory, Sinclair et al. (1965) proposed a diffuse reflectance hypothesis based on the scattering and reflective characteristics of cell walls. They suggested that the microfibrillar structure of cell walls might induce a high degree of radiation scattering within the walls. If the theory were to explain leaf reflectance, one would predict that for the shorter infrared wavelengths, the greater the number of layers of cellular material the incident radiation encounters at a perpendicular angle, the greater would be the reflectance and the lower the transmission. The diffuse reflectance hypothesis satisfactorily explained the higher levels of reflectance observed from the ventral side of dorsiventral leaves in the near infrared. It could also explain the higher levels of reflectance observed in dead and dried leaves where the cell walls had collapsed into a single mat. Most importantly the hypothesis explained observed reflectance phenomena both in the visible and reflective infrared wave-

lengths.

Sinclair et al. (1971) also carried out an analysis of the reflectance characteristics of the leaves of six different crops, namely soybeans, corn, wheat, oats, sorghum, and sudan grass in order to relate these characteristics to changes in leaf internal structure and water content. They concluded that maturation and senescence caused the greatest reflectance changes in both visible and infrared wavelengths between 500 nm and 2600 nm. Of the species studied, senescent wheat leaves showed the greatest difference from the reflectance observed for green leaves. Increased reflectance for senescing leaves of all species was caused primarily by loss of chlorophyll in the 500 to 700 nm portion of the spectrum and leaf dehydration in the 1300 to 2600 nm region. The 700 to 1300 nm wavelength band was concluded to be primarily affected by many changes in internal leaf structure as well as the loss of moisture content.

Gausman (1974) studying near infrared reflectance (750 - 1350 nm) of leaves concluded that the most important source of leaf reflectance is the internal scattering caused by refractive index discontinuities, primarily the cell wall/air-space interfaces. He stated that this could be shown by the vacuum infiltration of leaves with water as was previously reported by Knipling (1970), or by considering the effect of maturity on leaf reflectance. Using young and old citrus leaves he showed that the young leaves which are compact with few air spaces in the mesophyll had lower reflectance in the near and far infrared (750 - 2600 nm) than the older leaves which have many air spaces in the mesophyll. He generalized by stating that the reflectances of leaves with porous compared with compact mesophylls were highest because light

passed more often from hydrated cell walls to air spaces. This change in density or refractive index caused light scattering and subsequently reflectance was increased. He also concluded that the contribution of subcellular particles in leaves to reflectance of near infrared light is small compared with infrared light reflectance caused by cell wall-air interfaces in leaf mesophylls.

There have been conflicting hypotheses concerning the importance of the leaf epidermes and cuticle in leaf reflectance. Woolley (1971) idealized a soybean leaf as a diffusing and pigmented structure (mesophyll) having transparent plates (epidermes) on both surfaces. The back plate is essentially separated from the mesophyll by an air space so that both the inner and outer surfaces of this back epidermis can reflect and refract light. The front epidermis, however, is attached to the mesophyll over most of its surface, so that light, once past the outer epidermal surface, can easily pass into the center of the leaf. Since light entering or leaving the back of the leaf must pass through two semiplanar interfaces, while light entering or leaving the face passes through only one such interface, he proposed that this selective effect of the two leaf surfaces would result in higher reflectance from the ventral side where the internal diffused light could escape much easier.

Gates and Tantraporn (1952) separated the epidermes from the parenchyma of a Bryophyllum plant leaf and then determined their respective transmissivity and reflectivity in order to isolate the boundaries at which the reflection takes place. They concluded from their results that 80% or more of the total reflectivity of the leaf takes place at the outer epidermal surface to radiations in the infrared beyond 1.0 μm.

They also concluded that the layer of waxy cuticle on the leaf surface greatly enhances the reflectivity at the outermost surface. A thick cutin producing a smooth surface over the bead-like protrusions of the epidermal cells will result in a higher reflectance than if the cuticle is thin or entirely lacking, giving a rough surface due to the exposed contours of the epidermal cell walls.

Knipling (1970) did not attach the same importance to the cuticle as did Gates and Tantraporn (1952). In fact, he stated that the cuticular wax on a leaf is nearly transparent to visible and infrared radiation, and that very little of the solar energy incident on a leaf is reflected directly from its outer surface. The radiation is diffused and scattered through the cuticle and epidermis to the mesophyll cells and air cavities in the interior of the leaf.

Obatan (1941) went further in refuting Gates and Tantraporn by concluding that neither the cuticle nor the epidermis were particularly involved in the reflectance of near infrared radiation. He studied the coefficient of reflection for leaves in the near infrared and found these wavelengths to be highly reflected.

Gates (1970) also seemed to question the importance he had given to the cuticle and epidermis in some earlier work. He concluded that a small amount of light is reflected from the leaf cuticle, whereas much is transmitted into the spongy mesophyll. There the rays have frequent encounters with cell walls and are critically reflected if the angles of incidence become sufficiently large. He stated that because of numerous cell walls, nearly as many rays are reflected back toward the source as are transmitted through the leaf, depending on the thickness of the leaf.

Nutrient deficiencies in plants may affect the color, moisture content, and internal structure of leaves, and as a result their reflecting power will also change.

Myers (1970), working with sweet pepper, reported that the reflectance in the visible region of the spectrum (0.38 - 0.7 μm) increased as the nitrogen deficiency symptoms became more pronounced. He associated this with a lower chlorophyll content of the nitrogen deficient leaves. In the infrared region (0.7 - 1.0 μm) the reflectance increased as the leaves became more nitrogen deficient. He explained that this was due to the deficient leaves being thicker and that reflectance increases as leaf thickness increases. In the far infrared region (1.3 - 2.5 μm) the reflectance decreased with increasing nitrogen deficiency. Since the shape of the reflectance curve in this wavelength interval is due mainly to water, he suggested that the lower reflectance for the very deficient leaves was due to the moisture content being higher than in the mildly deficient leaves.

Thomas et al. (1966) also studied the effect of nitrogen deficiency on the visible and near infrared reflectance of cotton leaves. Increasing the nitrogen concentration in the nutrient solution from 28 to 196 ppm resulted in a decrease in reflectance in the visible region of the spectrum and an increase in the near infrared wavelengths.

Canopy Reflectance

Single Leaves vs. Canopy. While the reflectance characteristics of single leaves are basic to understanding the reflectivity of an entire plant or vegetative canopy in a field situation, single leaf data cannot be applied directly without modifications (Knipling, 1970). There are

both quantitative and qualitative differences between leaf reflectance and canopy reflectance. The reflectance from a canopy is considerably less than that from a single leaf. The visible and near infrared reflectance from a nearly continuous broad-leaved canopy typically might be about 3 to 5% and 35%, respectively (Steiner and Gutermann, 1966), whereas the corresponding values for a single leaf are about 10% and 50%. The relatively smaller reduction in infrared reflectance is due to the fact that much of the incident infrared energy transmitted through the uppermost leaves is reflected from lower leaves and re-transmitted up through the upper leaves to enhance their reflectance. This is the so-called infrared enhancement effect.

Factors Affecting Canopy Reflectance. Other parameters besides hemispherical leaf reflectance which may be very important in determining the reflectance of a vegetation canopy include: (a) hemispherical transmittance of leaves, (b) amount and arrangement of leaves, (c) characteristics of other components of the vegetative canopy (stalks, trunks, limbs), (d) characteristics of the background (soil reflectance, amount of leaf litter), (e) solar zenith angle, (f) look angle, and (g) azimuth angle (Colwell, 1974).

Most vegetation canopies are mixtures of different components which are oriented at many different angles with respect to the source of incident radiation. In addition, the projected area of each component illuminated and viewed, depends on the solar zenith angle and the look and azimuth angles. The use of bidirectional reflectance has been found very useful in determining the relative tone (reflectance) of a canopy in the visible and infrared spectrum (0.3 - 3.0 μm).

Bidirectional reflectance is defined as π times the ratio of radiance from a canopy at a particular polar look angle and the irradiance on a horizontal datum from a source at a particular polar zenith angle and azimuth angle.

Two of the most important factors affecting canopy reflectance - leaf area and percent ground cover - were studied by Bauer and Cipra (Bauer, 1975). They found the strongest relationships between leaf area index (LAI) and reflectance in the near infrared region. Reflectance increased linearly between LAI's of 0.5 and about 3; further increases in LAI had relatively little influence in reflectance.

Colwell (1973) also showed by analytical modelling and by emperical measurement that a decrease in leaf area index can also cause canopy reflectance to decrease in the near IR and increase in the red without any change occurring in the hemispherical reflectance of the individual leaves.

Bauer (1975) noted that disease, damage, and physiological stresses in plants also change the geometry and density of foliage as well as the infrared reflectance of the individual leaves. These changes are manifested in the visible as well as the infrared portion of the spectrum.

Background reflectance may be quite important in affecting canopy reflectance, especially at low values of percent vegetation cover. For example, Colwell (1974) found that a grass canopy with 37% vegetation cover and a light-toned soil background had a canopy reflectance of 9.0% in the red spectral region (650 nm), whereas a canopy with equivalent percent cover and with dark-toned soil background had a reflectance of 3.2%.

The amount of shadow in a vegetative canopy is very important in affecting canopy reflectance. Vinogradov (1969) found a negative correlation between reflectance and percent vegetation cover for grasses in the visible spectral region, which he attributed to an increasing amount of shadow in the canopy as the percent vegetation cover increases.

Changes in near-IR hemispherical reflectance of individual leaves caused by changes in leaf age, stress, and the like have been found to be negatively correlated with hemispherical transmittance (Colwell, 1974). This negative correlation may mean that a significant difference in near-IR hemispherical reflectance of leaves of one genus (or species) with respect to the leaves of another genus (or species) may not result in a significant difference in vegetative canopy reflectance, all other parameters being equal. Unless the canopies are different in structural configuration or some other important parameter, they may be indistinguishable on the basis of their near-IR canopy reflectance.

Allen and Richardson (1968) and Allen et al. (1970) applied the theory of Kubelka and Munk (1931) for attenuation of light in a diffusing medium to a crop of constant depth and random leaf orientation. They showed that spectral reflectance and transmittance of a plant canopy are functions of total leaf area, an absorption coefficient, a scattering coefficient, and background reflectivity. The coefficients are related to the geometry of the canopy and optical properties of individual leaves.

Suits (1972) extended the model of Allen et al. (1970) to include multiple layers having different biological components. He calculated

the directional reflectance rather than assuming that the canopies are Lambertian reflectors. He showed that the near-IR reflectance of a canopy may decrease and the red reflectance may increase when some of the leaves change from a predominantly horizontal to a predominantly vertical orientation.

Near Infrared Reflectance Spectroscopy (NIRS)

Basis for the NIRS Technique

Near infrared reflectance spectroscopy (NIRS) has recently been developed for rapid prediction of the composition of grains and oilseeds. The technique was originally developed for moisture analysis (Norris and Hart, 1965) but has since then been expanded to measure protein, starch, oil, sugar and fiber content. It is also being used in assessing forage quality (Norris et al., 1976).

The basis of the NIRS technique lies in the near-IR absorption bands present in the components of the material to be analysed. For example, water has absorption peaks at 1.45 and 1.94 μm, oil has major peaks at 2.31 and 2.33 μm, while starch and protein have several peaks with the most prominent ones at 2.10 and 2.18 μm, respectively. Instruments have been developed which can measure the reflectance from samples at the wavelengths corresponding to absorption maxima of the different chemical components (Rotolo, 1979). The use of multiple regression techniques coupled with different mathematical data treatments give very high correlations between near-IR reflectance and protein, oil, water and carbohydrate content.

The greatest source of variability with the NIRS technique results from sample preparation. The samples must be ground to a uniform powder and compacted into small test cells designed for the instruments. The reproducibility of the sample preparation is extremely important since large reflectance changes occur due to particle size differences. Another major source of variability is in the calibration. The instrument must be calibrated by correlation of readings with values from some standardized chemical method (e.g. Kjeldahl method for protein determination) using a range of samples. The choice of the samples used for calibration can greatly influence the performance (Norris, 1978).

Evaluation of the NIRS Technique

The performance of the near infrared reflectance instruments has been reported by a number of workers with the evaluation by Williams (1975) being the most extensive. He obtained an accuracy, as measured by the standard error of estimate, of about ± 0.22% protein and ± 0.16% moisture in the case of red spring wheat, with a coefficient of variability of 1.5% in each case. Accuracy of analyses of other cereals, oilseeds, and legumes for oil, protein, and moisture varied, but coefficients of variability were usually between 1 to 5%. Williams concluded that the most important factor influencing the accuracy and precision of analysis with infrared reflectance equipment is mean particle size of the ground sample, which is, in turn, influenced mainly by method of grinding.

Other collaborative studies conducted by Hunt et al. (1977) and by Miller et al. (1978) have demonstrated the interlaboratory and

intralaboratory reproducibility of protein determination using the near infrared reflectance instruments. The results from both studies were in very close agreement with each other. The pooled within-laboratory variance component for protein determined by NIRS was 0.029 for the study by Hunt et al. and 0.028 for the other study. However, the between-laboratory variance component for the NIRS method was less in the Hunt et al. study (0.102) than in the Miller et al. study (0.148). This was expected since the instruments used in the Miller study were not calibrated against results from a single Kjeldahl laboratory as was the case for the Hunt study.

Summary

The reflectance spectrum of leaves in the visible and reflective infrared wavelengths (350 - 2600 nm) is largely a function of morphological and anatomical features of leaves (leaf thickness, cell size, shape, and distribution, etc.) which affect the internal reflectance of the radiation, and also a function of leaf composition (chlorophyll, water, proteins, etc.) which result in selective absorption of the radiation at certain wavelengths. These factors affecting leaf reflectance are the basis upon which remotely sensed spectral data can be used to differentiate between plant species (or cultivars) or detect water and nutrient deficiencies.

A practical remote sensing system must be designed to measure whole plant communities as opposed to single leaves and therefore one must consider all of the factors (plant morphology, background, leaf area and orientation, etc.) affecting plant canopy reflectance. Application of mathematical models which incorporate these factors can

be very useful in normalizing the crop spectral curves prior to further analysis.

Near infrared reflectance spectroscopy (NIRS) is a revolutionary technique as a rapid and non-destructive method of analysing grain and forage quality. The use of the NIRS principle in assessing water and nutrient status in standing crops would be a significant step towards a remote sensing crop evaluation system. More research is necessary to study the feasibility of utilizing near infrared reflectance spectroscopy for assessing the nutritional status of standing crops.

The objective of the present research was to see whether the NIRS concept could be applied to a standing crop for detecting its protein status. While the basic principles of leaf and canopy reflectance are important in understanding the relationship between crop reflectance and protein content, the research does not attempt to develop any theories relating these principles to the measurement of protein content by the near infrared reflectance of crop canopies. The research was more practically oriented towards applying the NIRS concept to a standing crop. In particular, it was an attempt to determine which wavelengths were associated with absorption of near infrared radiation by proteins, which growth stage was better in terms of protein detection, and to what degree the technique was successful in detecting differences in protein levels.

While the literature on leaf and canopy reflectance usually refers to the near infrared region as that portion of the spectrum from 700 to 1300 nm and the next segment from 1300 to 2600 nm as the far infrared region, the literature relating to NIRS refers to the near infrared region as the region from 1400 to 2400 nm. In order to be consistent

with the NIRS literature, the latter designation for the near infrared region will be used in this study.

MATERIALS AND METHODS

Because this was a first attempt at applying the NIRS technique to a standing crop, it was recognized that the control of as many variables as possible would be desirable if not essential. Hopefully, these controlled conditions, as outlined below, would reduce some of the variability which would be encountered in a field situation and would thus produce more meaningful results.

Experimental Material

The research was carried out on one cultivar of hard red spring wheat, Triticum aestivum L., cv. Neepawa. The wheat was grown in wooden boxes 70 cm x 70 cm and 20 cm high. These were filled to 2 cm from the top with a soil obtained from a local commercial soils company (Cheetham Soils Ltd.). The soil originated from the Carman area and was classified as a very fine sandy loam by the Manitoba Provincial Soil Testing Laboratory. Results from the soil testing laboratory on the soil fertility prior to seeding were: pH - 7.3, nitrate nitrogen - 16.3 ppm (40.8 kg/ha), available phosphorous - 7.2 ppm (18 kg/ha), available potassium - 51 ppm (127.5 kg/ha) and sulphate sulphur - 13.2 ppm (33 kg/ha).

The plots (boxes) were seeded on December 22, 1980, using a constant seeding density of 121 seeds per plot (equivalent to about 84 kg/ha) which is typical of the seeding rates used in commercial wheat production. During the entire experiment the boxes were kept in a

plastic greenhouse equipped with high intensity metal halide and high pressure sodium vapour lamps which provided a good intensity and distribution of light energy for the plants. The lighting regime was set at 16 hr. day/8 hr. night with a temperature regime of 20 - 25°C day/ 15 - 20°C night. The boxes were randomized periodically in order to avoid growth differences due to any non-uniformity in temperature and lighting conditions.

The soil moisture regime was the same for all plots. It consisted of watering to field capacity (approximately 20% water content on a dry weight basis) and then rewatering when the soil moisture content was approximately 10%. The period between successive waterings varied from 4 to 7 days depending on the growth stage. Soil moisture monitoring was made possible with the use of a specially designed fork lift which was used to move the boxes (weighing up to 100 kg) onto a large capacity scale.

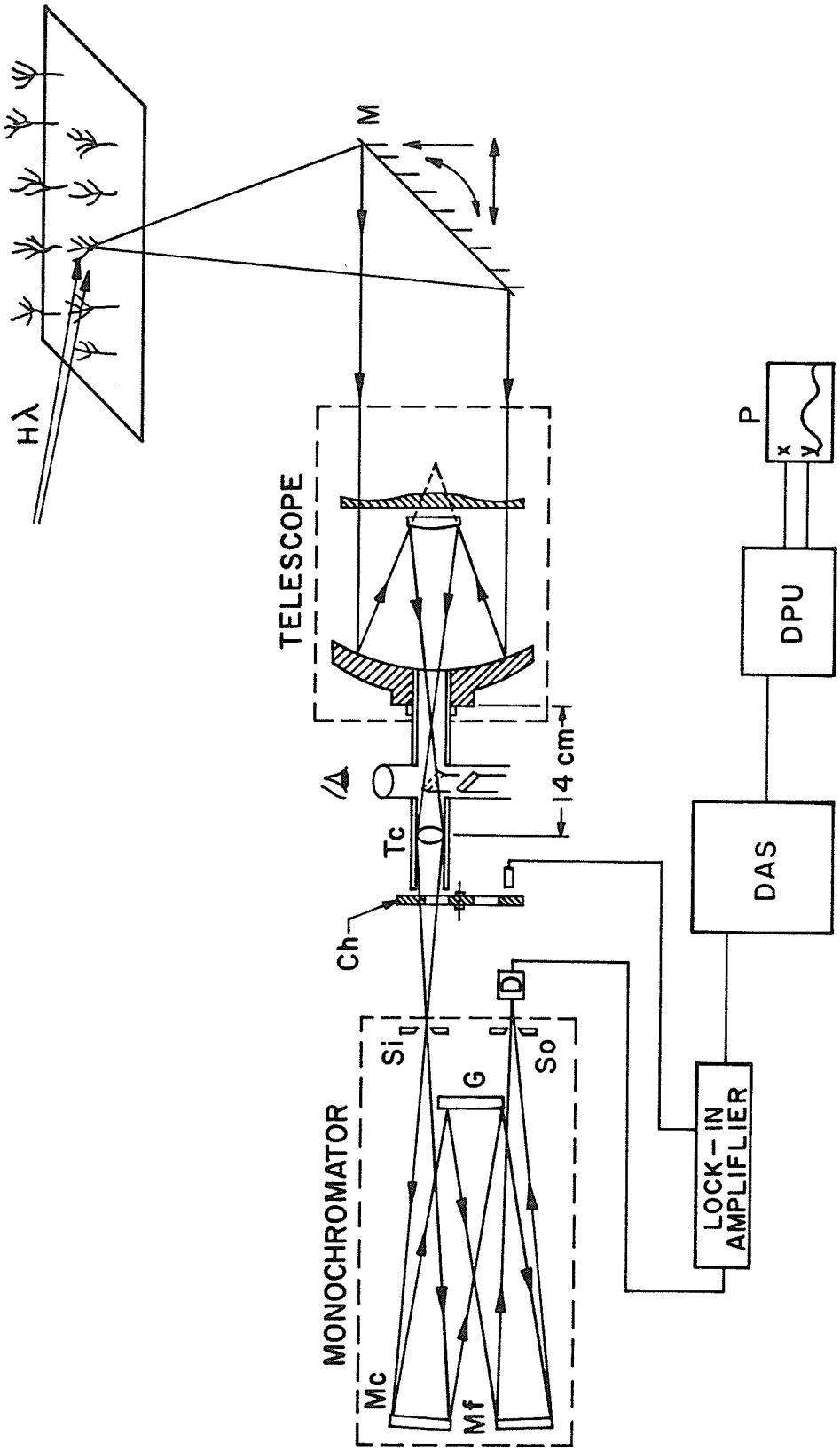
The fertilizer treatments consisted of 5 different levels of ammonium nitrate (NH_4NO_3) fertilizer (34 - 0 - 0) applied with the water in 6 separate applications throughout the growing period. The rates applied were equivalent to 0, 62.5, 125, 187.5 and 250 kg/ha actual nitrogen and each treatment was replicated 4 times for a total of 20 experimental plots. Phosphate was also applied in the form of 10 - 52 - 10 soluble fertilizer in a single application (applied with the first N application) at a rate equivalent to 30 kg/ha P_2O_5 for all of the plots.

Instrumentation

The mobile field spectroscopy laboratory developed by the Engineering and Statistical Research Institute, Agriculture Canada in Ottawa was used for the spectral measurements (Brach et al., 1977). This instrument was also used for cereal crop discrimination (Tinker et al., 1979; Glick et al., 1979).

The instrumentation was modified for this project by converting the visible system to an infrared detecting system with a very high resolution capacity (Fig. 1). The reflected energy from the plants is collected by the folding mirror (M) which directs the energy to the Cassegrain-Schmidt telescope. The image of the telescope is focused by a telecompressor lens (Tc) onto the monochromator entrance slit (Si) after being chopped by a 30 Hz signal chopper (Ch). The energy then passes to the collimating mirror (Mc) where it is collimated and reflected onto the grating (G). This grating is blazed at the wavelength of 1.85 μm, making it very efficient in diffracting light energy between 1.4 and 2.4 μm. The diffracted energy from the grating is reflected to the focusing mirror (Mf) which focuses the reflected energy onto the exit slit (So) of the monochromator. The energy is then detected by a lead sulfide detector (D) which has excellent sensitivity in the range of 1.0 to 3.0 μm. After the signal from the detector is amplified by the lock-in amplifier, it is received by the Data Acquisition System (DAS) and then displayed on an X-Y plotter. The data is also recorded in digital form on magnetic tape cassettes via a data processing unit (DPU). This unit was later interfaced with the Ambdahl computer system of the University of Manitoba in order to transfer the data from the cassettes into computer storage for subsequent manipulation and analysis.

Figure 1. Schematic diagram of the infrared spectral system. Hλ, incident energy; M, folding mirror; Telescope (Celestron, f.o.v. 0.2834°/cm); Tc, telecompressor; Ch, 30 Hz chopper; Monochromator (McPherson model 2051, Acton, Mass.); Si, entrance slit; So, exit slit; Mc, collimating mirror; Mf, focusing mirror; G, grating; D, lead-sulfide detector; DAS, Data Acquisition System (Hewlett-Packard model 3052A); DPU, Data Processing Unit (Hewlett-Packard, model 9825A); P, Plotter (Hewlett-Packard, model 9872A).


Hλ
M
TELESCOPE
14 cm
Tc
Ch
D
Si
So
G
Mc
Mf
MONOCHROMATOR
LOCK-IN AMPLIFLIER
DAS
DPU
P
x
y

Experimental Procedure

Layout

The mobile laboratory was placed at the south end of the greenhouse and a wooden enclosure was built between them to create a closed system (Fig. 2). The boxes were moved to a stationary viewing platform (VP) for all of the measurements. The distance between the instrument and the viewing platform was 6.92 m which is near the focal limit of the telescope and resulted in a viewed surface area well within the plot area.

The readings were taken at night using an artificial light source to irradiate the plots. This was done to avoid problems with changing azimuth and zenith sun angles, light quality and intensity due to the plastic covering of the greenhouse, and also shadowing from the structural components of the greenhouse. The light source consisted of two 1000 watt quartz halogen tungsten filament studio lamps (with reflectors) set side by side in front of the plots. The position of the lamps was constant throughout all of the readings for a particular growth stage and was adjusted from one growth stage to the next to compensate for the increasing height of the crop.

Spectral Measurements

The spectral measurements were carried out on the entire set of plots (20 plots) at 7 different growth stages from the tillering stage up to maturity (stages 4 to 9 with 2 sets of measurements within growth stage 7 - see Table 1). A measurement consisted of a scan of the relative reflectance of a plot from 1400 to 2400 nm (1.4 - 2.4 μm) with a recorded value at intervals of 2.5 nm. This resulted in 401

Figure 2. Schematic diagram of experimental layout. VP, viewing platform; LS, light source.

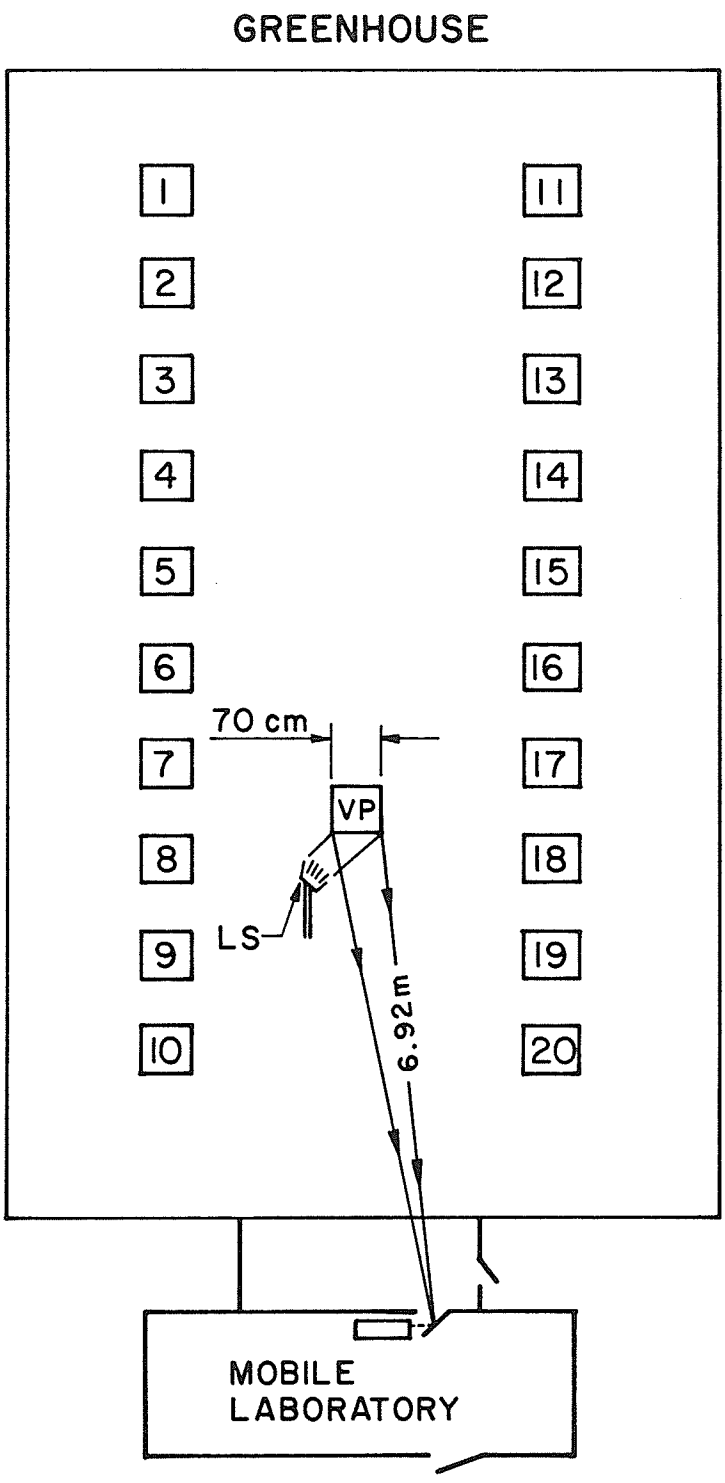

GREENHOUSE
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
70 cm
VP
LS
6.92m
MOBILE
LABORATORY

TABLE 1. LACIE[1] growth stage classes.

Code No.	Description
1.0	Not planted
2.0	Planted, no emergence
3.0	Emergence - one to three leaves
4.0	Tillering, preboot, prebud
5.0	Booted or budded
6.0	Beginning to head or flower
7.0	Fully headed or flowered
8.0	Beginning to ripen
9.0	Ripe - mature
10.0	Harvested
11.0	Does not apply - fallow, sod, pasture

[1]Large Area Crop Inventory Experiment, NASA.

data points for each measurement.

At each growth stage repeated measurements were made of the reflectance from a standard aluminum plate which was placed in a vertical position at the same location and height as the crop. This provided a relative measure of the spectral irradiance to the crop and would later be useful in making corrections to the total reflectance of the crops.

The absolute measure of the energy incident to the crop throughout the 1400 to 2400 nm region was not possible since the standard aluminum plate was not calibrated for this wavelength region of the study. Therefore, the term "reflectance" which normally refers to the ratio of reflected to incident energy was used in this study to denote the relative amount of light reflected from the crop. Since the position and intensity of the radiant source was constant within each growth stage, this amount of light reflected from the crop would be directly proportional to the ratio of reflected to incident energy. It was therefore valid to use this modified "reflectance" term to compare between the reflective characteristics of each plot within a particular growth stage. However, the coefficient of proportionality between the amount of energy reflected and the ratio of reflected to incident energy would not be constant from one growth stage to the next if there were any changes in incident energy from one growth stage to the next. Therefore, comparisons between different growth stages would not be valid using this modified "reflectance" term.

Protein Measurements

Concurrent with each spectral measurement, samples were collected (4 plants/sample) for moisture content determination and protein analy-

sis using the Kjeldahl method. For the preheading stages of growth (stages 4, 5 and 6) the total above ground vegetative matter was used for the protein analysis. However, for the post heading stages, protein determinations were made on both vegetative matter minus the heads and on the head material minus the seed (chaff). The protein content of the head material was of primary interest since the oblique viewing angle was such that only the heads could be seen at those stages. The grain protein content for the last two growth stages was also determined.

Analysis

Preliminary Data Manipulation

Reduction of Data Points. Before any analysis was carried out in the data, the number of data points per spectral curve was reduced from 401 to 101 (equivalent to a value for each 10 nm band) by averaging every four consecutive values. The benefits of this procedure were: (a) to have a smoothing effect on the curves thereby eliminating some of the noise, (b) to allow curves to be plotted on standard 132 character computer output, and (c) to reduce the computational time involved in the analysis.

Corrections to Crop Spectral Data. Important corrections were made on the original spectral data. The first involved the subtraction of the specular component from the total reflectance; the second involved a correction due to differences in amount of plant matter.

The first correction is based on the fact that the spectral

reflectance has two main components: specular and diffuse reflectance. As explained by Rotolo (1979) the specular component consists of light which has been reflected from the surface without penetrating the sample and thus contains no information about the composition of the sample. On the other hand, the diffuse component consists of light which has penetrated the sample, has undergone multiple reflections within the leaves and absorption by such components as water and proteins before emerging as diffuse light. This diffuse component contains all the information about the composition of the sample. It is therefore desirable to eliminate from the total reflectance that component which is due to specular reflectance so that we are left with the diffuse component which carries all the information. This can be expressed in the following form:

$$DR = TR - SR$$

where DR = diffuse reflectance (component of interest)

SR = specular reflectance (derived from standard plate reflectance)

TR = total reflectance (obtained from crop reflectance measurements)

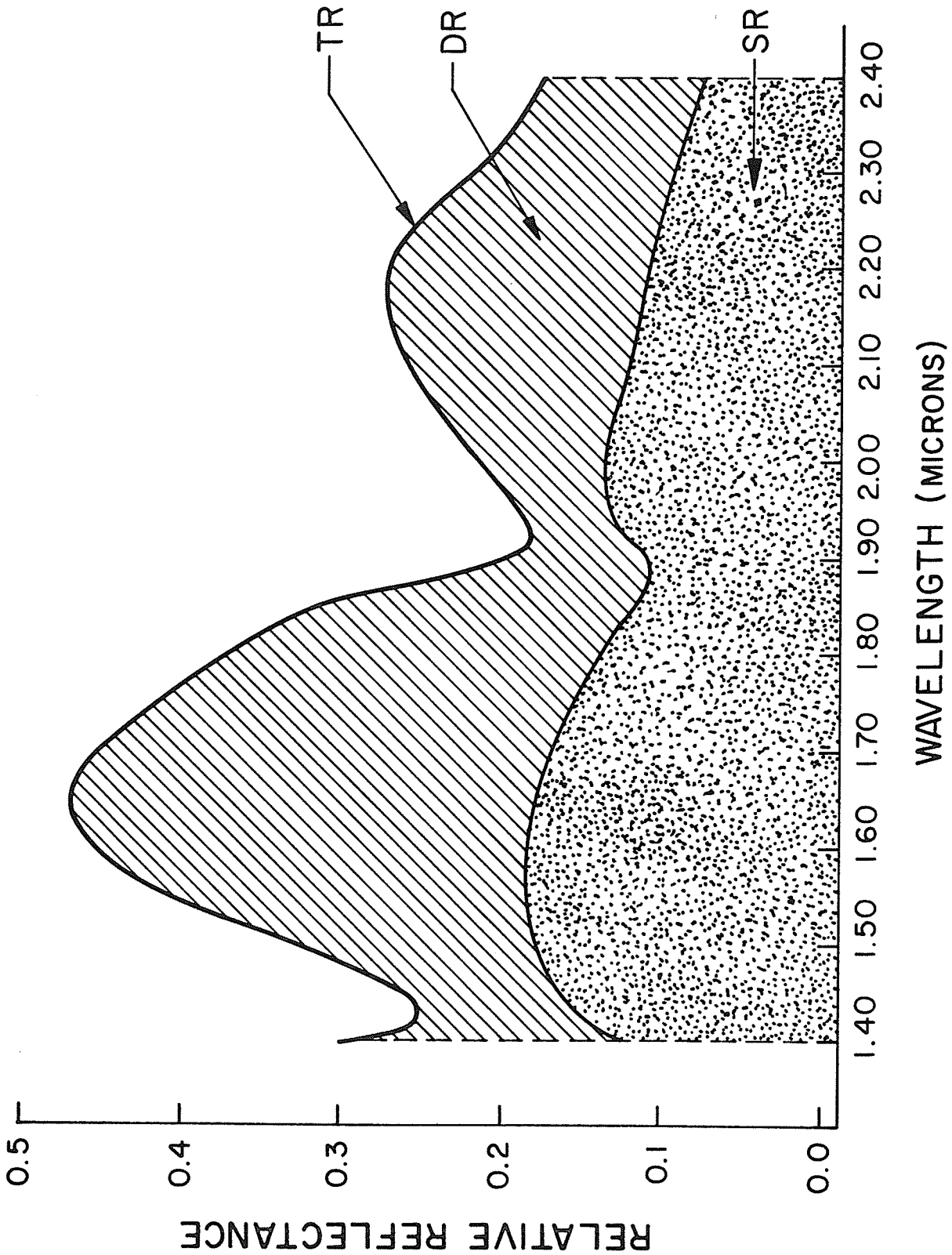
If we were to assume that the specular reflectance of the plants was mirror-like, that is, reflecting equally at all wavelengths, then the specular component would be identical to the spectrum of the light source irradiating the plants. This would seem to be an unrealistic assumption considering that the reflectance of plants in the far infrared (1.4 - 2.4 μm) varies between 10 and 50%. However, the reflectance

from the standard aluminum plate is a better approximation to leaf specular reflectance since the plate has a reflectance between 20 and 30% in the visible and between 25 and 50% in the infrared region. The standard plate curves were therefore assumed to be similar to the specular reflectance component of the plants and were used in making the corrections. Before subtracting the plate reflectance curves (specular component) from the crop reflectance curves (total reflectance) to yield a diffuse component, the plate reflectance curves were scaled down by an appropriate factor so that the subtraction would not result in negative reflectance values at any wavelength. A typical crop reflectance curve (total reflectance) with its corresponding specular and diffuse components is shown in Figure 3. All subsequent data manipulation and analysis were carried out on the diffuse reflectance component.

Once the diffuse reflectance component was obtained, a second correction was made to account for plant matter differences. It was recognized that the variability between plots in the amount of plant matter being viewed by the instrument would be one of the greatest sources of variability (error). The more plant material present, the more light reflected back to the instrument, and therefore the higher the reflectance values obtained, regardless of protein content.

Assuming that the two greatest sources of variability in infrared reflectance between the plots was due to plant matter and protein content, it was stipulated that the variability at a wavelength with no protein absorption was mainly due to the amount of plant matter. We could then normalize all of the plot reflectance curves within each growth stage on the basis of plant matter by standardizing the curves

Figure 3. Typical crop reflectance curve showing corresponding spectral components. TR, total reflectance; DR, diffuse reflectance; SR, specular reflectance.


TR
DR
SR
0.5
0.4
0.3
0.2
0.1
0.0
RELATIVE REFLECTANCE
1.40
1.50
1.60
1.70
1.80
1.90
2.00
2.10
2.20
2.30
2.40
WAVELENGTH (MICRONS)

to a constant value at that particular wavelength. To find the wavelength of least protein absorption, the mean reflectance value at every wavelength for the 20 plots of each growth stage was determined along with the standard deviation. The wavelength with the lowest amount of variability (lowest coefficient of variability) was taken to be the one where no protein absorption occurred. For growth stages 4, 5, and 6 the wavelength was found to be 2.26 μm, while growth stage 7.0 was 2.23 μm and growth stages 7.5, 8, and 9 was 2.25 μm.

Analysis on Corrected Data

Once the spectral data were corrected, the first analytical procedure carried out was a simple linear regression analysis between the protein values for each plot and their corresponding reflectance value. This was done at each individual wavelength throughout the whole spectrum (i.e. 101 regression analyses for each growth stage). The resulting correlation coefficients from each of these regressions were plotted against wavelength in order to find the wavelength of best correlation.

The next step was to carry out an analysis of variance on the reflectance data at the wavelengths of best correlation in order to test for significant effects due to the different protein levels. The reflectance data from at least three consecutive wavelengths (referred to as a wavelength band) were used in this analysis. The different protein levels constituted the main factor while the wavelengths were used as a blocking variable.

Finally, a comparison test was made to test for significant differences in reflectance between protein levels. The Duncan's multiple

range test was used for this purpose.

In summary, the corrections made to the original data were based on assumptions about the reflective characteristics of the plants and the factors contributing to the variability of the reflectance. While these corrections may not have removed all of the variability due to other extraneous factors involved, they were necessary to extract some meaningful information from the raw data. As for the analysis on the corrected data, the procedures were fairly straightforward and statistically valid. The computerized statistical facility known as SAS (Statistical Analysis System, SAS Institute Inc., Cary, North Carolina) was used for this analysis.

RESULTS AND DISCUSSION

I will first examine the protein analysis results to assess the effectiveness of the fertilizer treatments in inducing different levels of protein content. The results from the reflectance data analysis will then be examined to see whether these different levels of protein content could be detected from the near infrared reflectance spectra.

Protein Analysis Results

Fertilizer Treatment Differences

The results of the protein determinations on all the samples collected throughout the experiment are given in Tables 1 to 7 of the Appendix. The results have been summarized for each growth stage and are given in Tables 2 and 3. In general, the protein content increased with additional nitrate fertilizer up to a level of 125 kg/ha after which the protein content levelled off with increased fertilizer.

At the early growth stages (growth stages 4 and 5) the lack of significant differences between most of the treatments was probably due to the initial levels of nitrogen in the soil (40.8 kg/ha) prior to application of nitrate. These levels were sufficiently high to maintain high levels of protein in the plants. Any additional nitrate fertilizer added at these stages was probably superfluous and therefore did not produce any significant increase in protein content.

TABLE 2. Summarized results of protein analysis for the first three growth stages (pre-heading stages).

Growth stage	Fertilizer treatment (kg N/ha)	Protein content[1]	
4.0	0	31.65[2]	ab
	62.5	32.48	bc
	125	32.85	c
	187.5	32.88	c
	250	32.80	c
5.0	0	22.60	a
	62.5	22.75	a
	125	28.53	b
	187.5	28.34	b
	250	29.38	b
6.0	0	12.93	a
	62.5	16.96	b
	125	21.96	cd
	187.5	21.30	d
	250	22.86	c

[1]Protein values represent the means of four replicates. Protein content = % N x 6.25 on a dry wt. basis.

[2]Values within each growth stage with the same letter are not significantly different at the 5% level.

TABLE 3. Summarized results of protein analysis for the last four growth stages (post-heading stages).

Growth stage	Fertilizer treatment (kg N/ha)	Vegetative protein content[1]		Head protein content		Grain protein content[2]	
7.0	0	8.29[3]	a	12.25	a		
	62.5	13.79	b	15.30	b		
	125	15.76	cd	15.10	b		
	187.5	16.65	de	15.55	b		
	250	17.39	e	15.95	b		
7.5	0	6.45	a	12.40	a		
	62.5	11.79	b	16.05	b		
	125	14.13	cd	14.65	b		
	187.5	13.43	d	15.65	b		
	250	14.56	c	15.70	b		
8.0	0	4.36	a	6.25	a	12.15	a
	62.5	7.10	b	9.98	b	16.75	b
	125	9.44	c	10.95	b	16.77	b
	187.5	9.20	c	10.58	b	17.94	b
	250	9.57	c	10.35	b	18.05	b
9.0	0	1.94	a	3.68	a	13.22	a
	62.5	3.66	b	7.48	b	18.39	b
	125	5.46	c	8.83	b	18.24	b
	187.5	5.71	c	8.35	b	18.75	b
	250	6.99	d	9.45	b	18.61	b

[1]Vegetative and head protein = % N x 6.25 on a dry wt. basis.

[2]Grain protein = % N x 5.70 on a 14% moisture basis.

[3]Values within each growth stage with the same letter are not significantly different at the 5% level. Each value represents a mean of four replicates.

As the plants reached the heading stage and beyond, the increased demand for nitrogen by the plants coupled with a steady depletion from the soil resulted in a situation where significant differences in protein content were observed even with the high levels of application (Table 3). This is evident in growth stages 6, 7.0 and 7.5 (0, 12, and 20 days after heading, respectively) where significant differences in vegetative protein content occurred between the first three treatments.

However, as was mentioned previously, the plant component of interest at the post heading stages (stages 7.0, 7.5, 8 and 9) was the heads, and as can be seen in Table 3, there were no significant differences in head protein content between the four highest fertilizer treatments at those growth stages.

Protein determinations were also made on the grain at growth stages 8 and 9. However, these protein values were not used in any subsequent analyses since the grain protein does not have any effect on the reflectance. It is interesting to note that very high levels of protein were obtained in the grain at the higher fertilizer treatments (Table 3). This may be due in part to the fact that there were six applications throughout the growing period and this may have resulted in a more efficient use of the available nitrogen by the plants.

Grouping of Protein Data

The main purpose of the experiment was to see whether the near infrared reflectance technique could be used to differentiate between different levels of protein content, as opposed to different levels of fertilizer applications (treatments). Consequently, the protein values corresponding to the 20 plots at each growth stage were partitioned

into protein groups of specified protein ranges. The number of groups formed at each growth stage depended on the significance of the differences between the mean protein content of each group. For example, in growth stage 5, three groups were formed which were significantly different from each other while at growth stage 6, there was a wide range of protein values which could be separated into 5 separate groups, the mean of each group being significantly different from one another (Table 4).

Once these groups were formed, the analysis of the reflectance data was directed towards determining whether these differences in protein content between groups could be detected by the near infrared reflectance spectra of the crops.

Results from Reflectance Data Analysis

As was mentioned previously, the first step was to find the wavelength band which yielded the best correlation between protein content and near infrared reflectance. Using the reflectance data from this particular band, the next step involved an analysis of variance to see whether the protein groups had a significant effect on the reflectance at these wavelengths. Finally, a comparison test was used to determine whether there were significant differences between the mean reflectance values of these groups. The results of these analytical procedures are now presented for each individual growth stage.

Growth Stage 4 - Tillering

The correlation coefficients for the regression of reflectance on protein content were very low for most of the wavelengths at this growth stage (Fig. 4). Most of the values are negative which implies that we

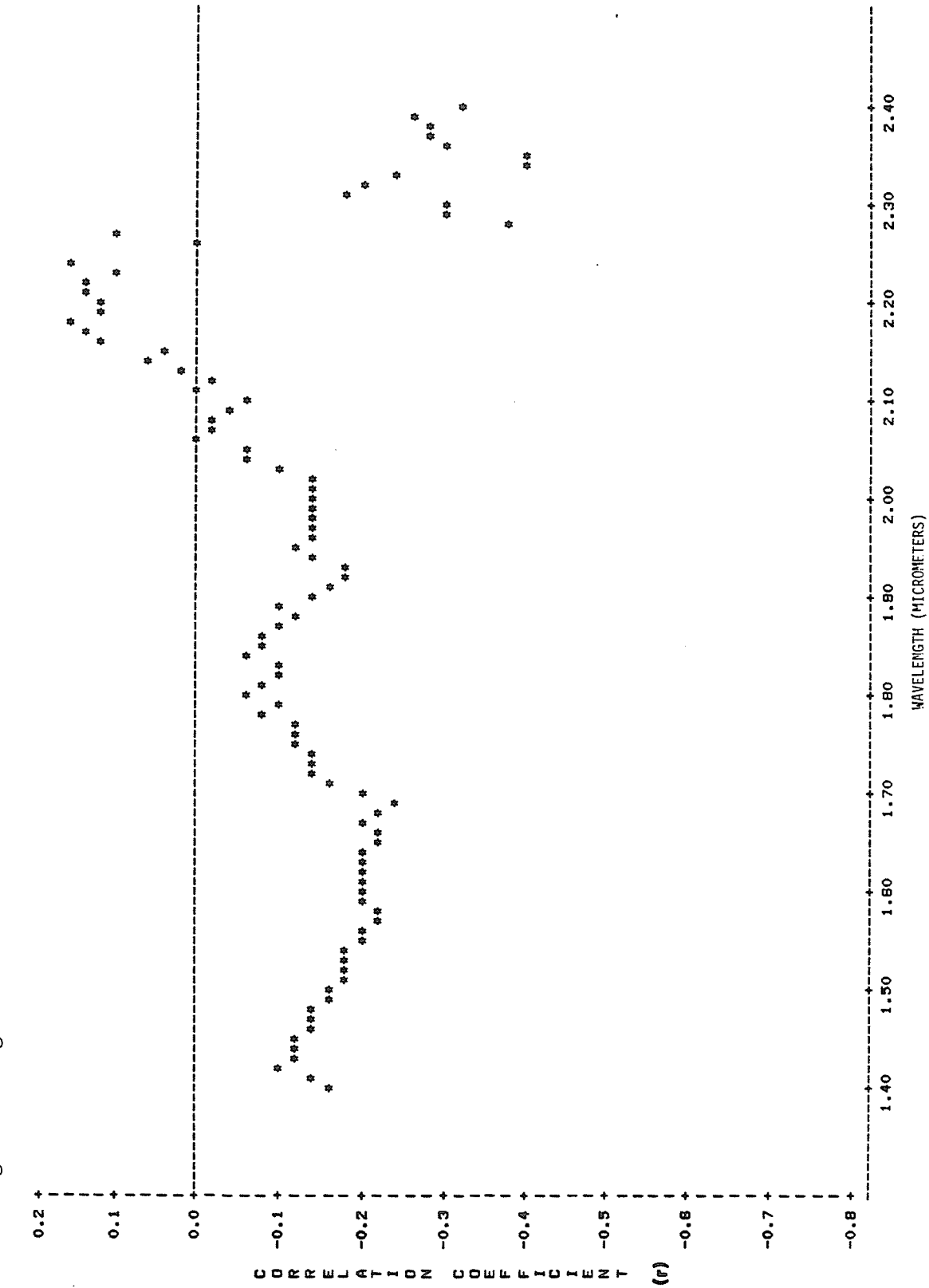
TABLE 4. Protein groups and their mean protein contents for all growth stages.

Growth stage	Protein group no.	No. of plots	Mean protein content[1]
4.0	3	6	33.48[2]
	2	10	32.39
	1	4	31.45
5.0	3	7	29.39
	2	5	27.15
	1	7	22.19
6.0	5	4	23.10
	4	4	22.02
	3	4	21.00
	2	4	16.96
	1	4	12.93
7.0	3	10	16.04
	2	6	14.53
	1	4	12.25
7.5	3	7	16.29
	2	8	15.38
	1	4	12.40
8.0	3	7	11.26
	2	9	9.84
	1	4	6.25
9.0	3	9	9.37
	2	6	7.23
	1	3	3.77

[1]Growth stages 4.0 to 6.0: vegetative protein; growth stages 7.0 to 9.0: head protein. Protein content = % N x 6.25 on a dry wt. basis.

[2]All values within each growth stage are significantly different at the 5% level.

Figure 4. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 4.



are, in fact, dealing with a negative relationship. That is, the higher the protein content of the plants, the more absorption of the radiant energy and therefore the lower the reflectance.

The wavelengths of best correlation were 2.34 and 2.35 μm with a correlation coefficient of 0.40. The reflectance data between 2.34 and 2.36 μm were used for the analysis of variance. The level of protein content as expressed in the groups significantly affected the reflectance (Table 5a). Also, the Duncan's multiple range test showed a significant difference between the mean reflectance of groups 1 and 3 but groups 1 and 2 or groups 2 and 3 were indistinguishable (Table 5b).

Although the differentiation between protein groups at this growth stage was not very successful, the results did show that the mean reflectance between 2.34 and 2.36 μm decreased as the protein content increased.

Growth Stage 5 - Boot Stage

The results from this growth stage were somewhat more difficult to interpret. The correlation coefficients were also very low throughout most of the wavelengths (Fig. 5). There were two major peaks in the correlation curve with the most significant correlation occurring between 1.41 and 1.43 μm which happens to coincide with a water absorption band.

The difficulty arises in deciding whether the correlation is due to protein absorption or whether it is due to water absorption. That protein content and infrared reflectance were correlated at the water absorption wavelengths could possibly be due to the fact that protein content and water content may have been correlated to some extent.

TABLE 5a. Analysis of variance table for reflectance at 2.34 to 2.36 μm for growth stage 4.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	2.413	1.207	3.49	0.0375
Wavelengths	2	12.493	6.247	18.06	0.0001
Error	55	19.027	0.346		
Total	59	33.933			

TABLE 5b. Duncan's multiple range test on protein content and reflectance (at 2.34 - 2.36 μm) of protein groups at growth stage 4.

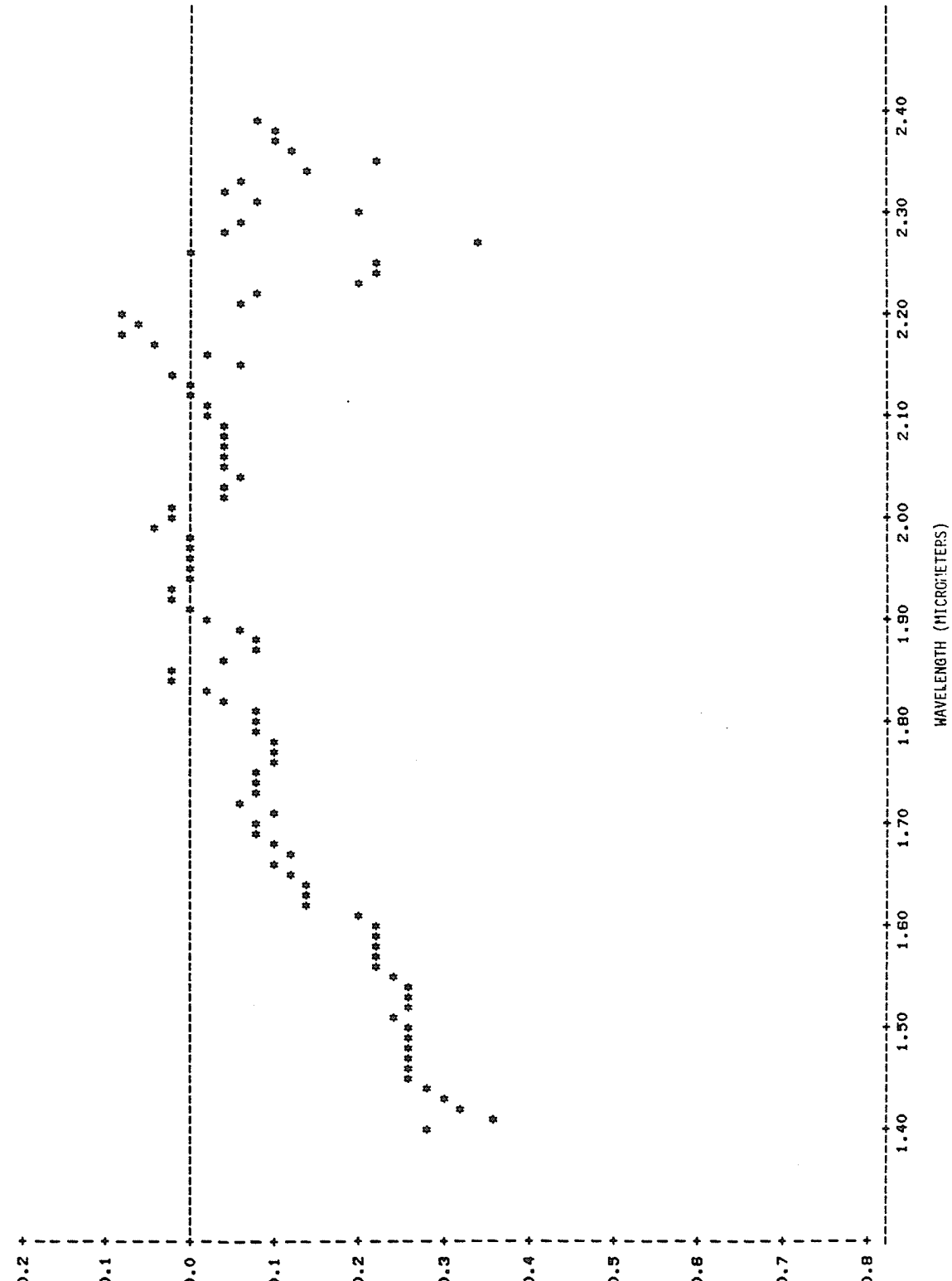
Protein group	Protein content[1]	Reflectance[2]	
3	33.48	14.43	a[3]
2	32.39	14.77	ab
1	31.45	14.96	b

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

Figure 5. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 5.



This would result in an indirect relationship between protein content and infrared reflectance at these wavelengths.

A linear regression analysis between protein content and moisture content was carried out to test this hypothesis. The correlation coefficient was found to be 0.78. This relatively good correlation gives some support to the hypothesis that the reflectance in the wavelength band between 1.41 and 1.43 μm is primarily influenced by water and may give an indirect assessment of protein content because of the correlation between moisture and protein content. The word "may" should be stressed here since some workers have found that protein content is inversely correlated with water content (Thomas et al., 1966; Myers, 1970; Thomas and Oerther, 1972; Al-Abbas et al., 1974). Therefore, if the protein content had not been correlated with water content, as may well be the case under different growing conditions, the reflectance at the water absorption band would probably not be related, directly or indirectly, to protein content.

For this reason, the wavelengths at the second peak of the correlation curve (2.23 - 2.25 μm) were used for the analysis of variance. The effect due to protein groups was found to be significant at the 1% level (Table 6a). Protein groups 1 and 2 were not significantly different using Duncan's multiple range test but these two groups were significantly different from group 3 (Table 6b). This is inconsistent with the actual protein contents of groups 1, 2, and 3 (22.19, 27.15, and 29.39, respectively), since I would have expected groups 2 and 3 to be indistinguishable and group 1 to be different from these two groups based on their protein contents. Also, group 2 yielded a higher reflectance than group 1 which is not consistent with a negative

TABLE 6a. Analysis of variance table for reflectance at 2.23 to 2.25 μm for growth stage 5.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	0.263	0.132	6.07	0.004
Wavelengths	2	28.218	14.109	650.56	0.0001
Error	52	1.128	0.022		
Total	56	29.609			

TABLE 6b. Duncan's multiple range test on protein content and reflectance (at 2.23 - 2.25 μm) of protein groups at growth stage 5.

Protein group	Protein content[1]	Reflectance[2]	
3	29.39	30.55	a[3]
2	27.15	30.71	b
1	22.19	30.67	b

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

correlation between protein content and infrared reflectance. Therefore, it appears there was no significant improvement over the last growth stage in the ability to differentiate between protein groups.

Growth Stage 6 - Beginning to Head

The best results of the experiment were obtained with this growth stage. There was a relatively good correlation between reflectance and protein content at the wavelengths between 2.08 and 2.11 μm, where the r value varied between -0.68 and -0.72 (Fig. 6). As in the previous growth stage, there was also evidence of some correlation between protein content and reflectance at the water absorption band between 1.40 and 1.50 μm (r = -0.58). This is probably an indirect effect due to water absorption as was discussed for the previous growth stage since the correlation between moisture and protein content was also found to be relatively high (0.79). Nevertheless, the analysis of variance was carried out using the reflectance data between 2.08 and 2.11 μm.

There was a highly significant effect due to the protein groups as indicated by a F value of 22.69 (Table 7a). This high significance is clearly expressed in the results of the Duncan's test (Table 7b). At this particular growth stage the protein values had been separated into five groups whose mean protein contents were all significantly different from each other (Table 4). The Duncan's test on the reflectance data showed that the mean reflectance values for each group were also significantly different from each other except for groups 2 and 3. Also, the order of magnitude for the five reflectance means was the exact inverse of that of the five protein means, which is consistent with a negative correlation between protein content and infrared

Figure 6. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 6.

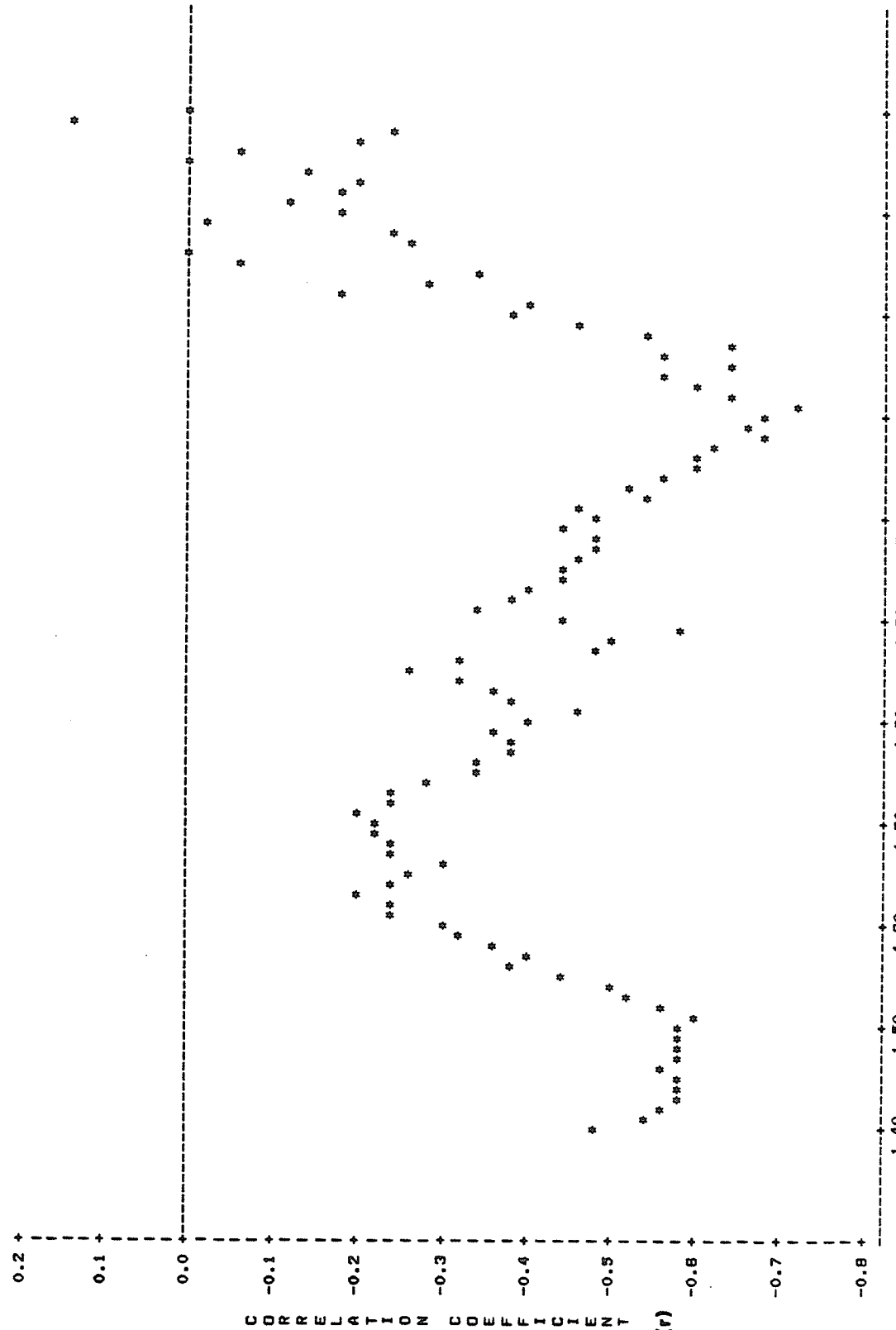


TABLE 7a. Analysis of variance table for reflectance at 2.08 to 2.11 μm for growth stage 6.

Source	DF	S	MS	F value	Prob > F
Protein groups	4	19.827	4.957	22.69	0.0001
Wavelengths	3	22.300	7.433	34.02	0.0001
Error	72	15.729	0.218		
Total	79	57.856			

TABLE 7b. Duncan's multiple range test on protein content on reflectance (at 2.08 - 2.11 μm) of protein groups at growth stage 6.

Protein group	Protein content[1]	Reflectance[2]	
5	23.10	12.66	a[3]
4	22.02	13.04	b
3	21.00	13.42	c
2	16.96	13.74	c
1	12.93	14.07	d

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

reflectance.

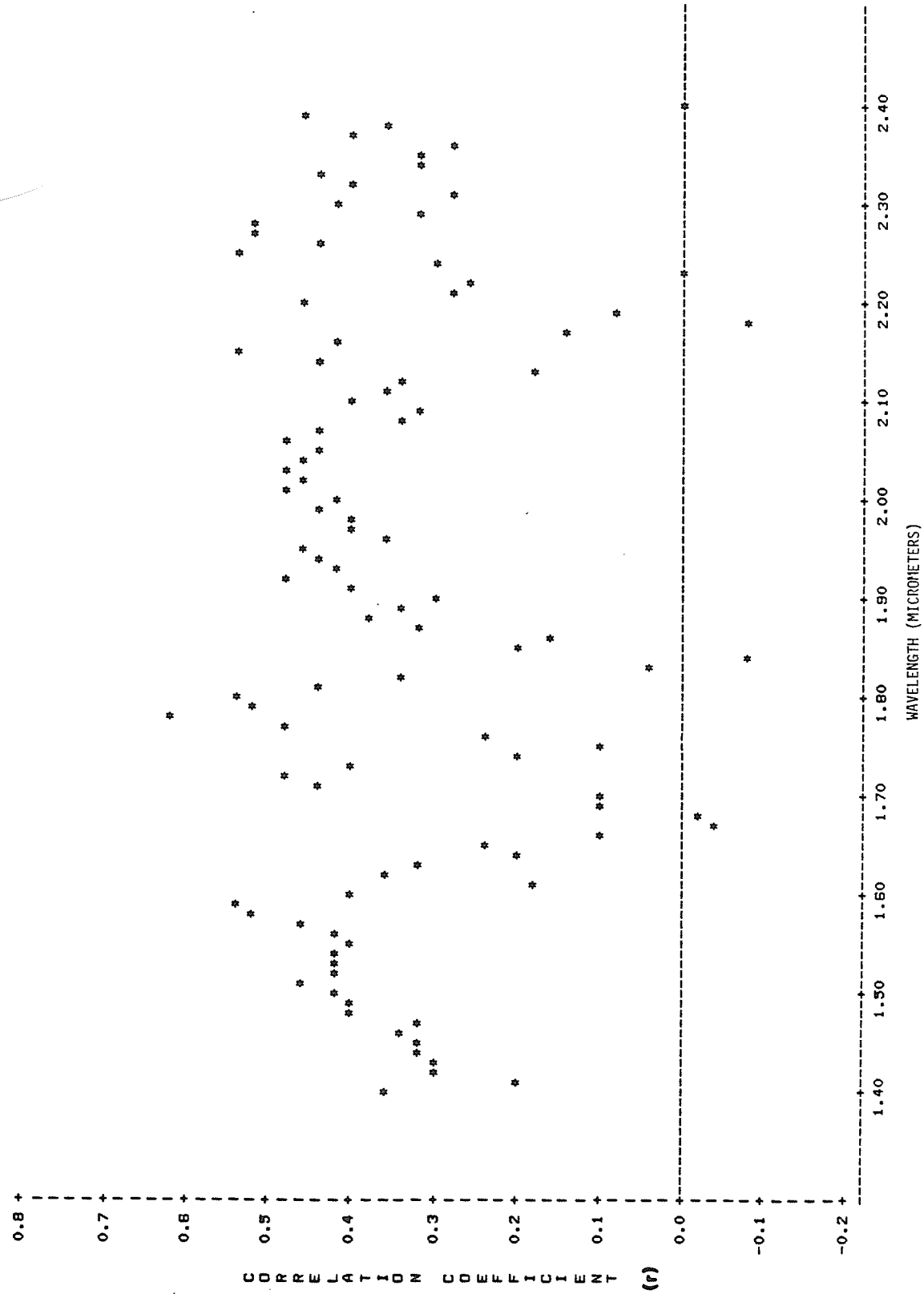
These results represent a substantial improvement from the previous growth stage where only one group was found to be significantly different from the other two. However, the maximum correlation coefficient ($r = -0.72$) is still too low to be confident in developing a regression equation for predicting protein content on the basis of infrared reflectance. The difficulty in obtaining a very high correlation between protein content and reflectance may be due to other extraneous factors such as plant morphology, leaf area, background reflectance, etc. which can add to the variability of the crop reflectance. In any event, the results from this growth stage indicate that the wavelength band between 2.08 and 2.11 μm may well be a primary absorption band for protein.

Growth Stage 7.0 - 12 Days after Heading

The results from this particular growth stage were found to be completely inconsistent with all of the other growth stages. As can be seen in Figure 7, most of the correlation coefficients were positive. This is contrary to all other growth stages in which a negative relationship between reflectance and protein content occurred. Also, the region of best positive correlation was found between 1.78 and 1.80 μm which does not coincide at all with the wavelength region of best correlation for the other growth stages.

An explanation for these anomalous results is difficult to find. It seems appropriate to assume that the positive correlation found at this growth stage does not reflect the true relationship between reflectance and protein content since we would not expect such a

Figure 7. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 7.0.



drastic change to occur at one point in the development of the crop, followed by a reversal back to the negative relationship shortly thereafter. One would immediately suspect that an error in the data manipulation prior to analysis could have produced such unexpected results. However, a thorough examination of the data manipulation procedures for this growth stage did not reveal any discrepancies in these procedures. In fact, the same computer programs were utilized for all of the growth stages.

Due to this unexplained anomaly, there was no further analysis carried out for this particular growth stage. I will, therefore, not refer to this growth stage in further discussions of the experimental results. More replication of this research will be necessary in determining whether this anomaly persists, and if so, which factors are responsible for it.

Growth Stage 7.5 - 20 Days after Heading

At this particular growth stage the best correlation between reflectance and protein content was found at the 2.09 μm wavelength where r = -0.44 (Fig. 8). This wavelength coincides with the wavelength band of best correlation for growth stage 6 (2.08 - 2.11 μm). There was another peak in the correlation curve just next to the 2.09 μm peak at 2.15 and 2.16 μm. Both peaks were assumed to represent separate absorption bands and a separate analysis of variance was carried out for the reflectance data at both wavelength bands.

Both wavelength bands showed a significant effect due to protein groups (Tables 8a and 9a). A significant difference was found at both wavelength bands between groups 1 and 2 and between groups 1 and 3, but

Figure 8. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 7.5.

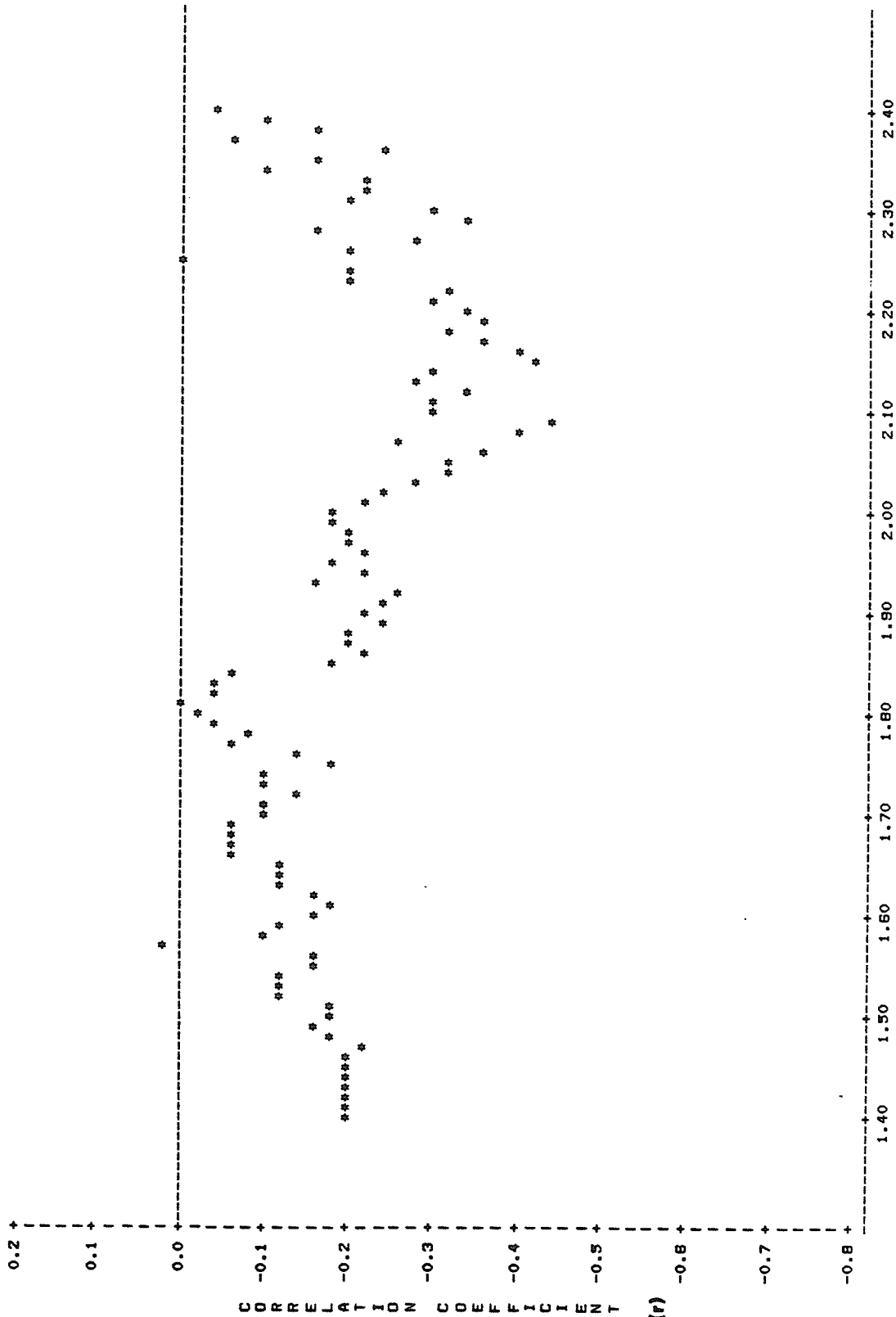


TABLE 8a. Analysis of variance table for reflectance at 2.06, 2.08, and 2.09 μm for growth stage 7.5.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	16.949	8.475	6.09	0.0043
Wavelengths	2	3.612	1.806	1.30	0.2825
Error	49	68.204	1.392		
Total	53	88.765			

TABLE 8b. Duncan's multiple range test on protein content and reflectance (at 2.06, 2.08 and 2.09 μm) of protein groups at growth stage 7.5.

Protein group	Protein content[1]	Reflectance[2]	
3	16.29	10.15	a[3]
2	15.38	10.02	a
1	12.40	11.43	b

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 1% level.

TABLE 9a. Analysis of variance table for reflectance at 2.15 to 2.17 μm for growth stage 7.5.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	7.750	3.875	7.73	0.0012
Wavelengths	2	1.486	0.743	1.48	0.2370
Error	49	24.547	0.501		
Total	53	33.782			

TABLE 9b. Duncan's multiple range test on protein content and reflectance (at 2.15 - 2.17 μm) of protein groups at growth stage 7.5.

Protein group	Protein content[1]	Reflectance[2]	
3	16.29	12.16	a[3]
2	15.38	11.84	a
1	12.40	12.84	b

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

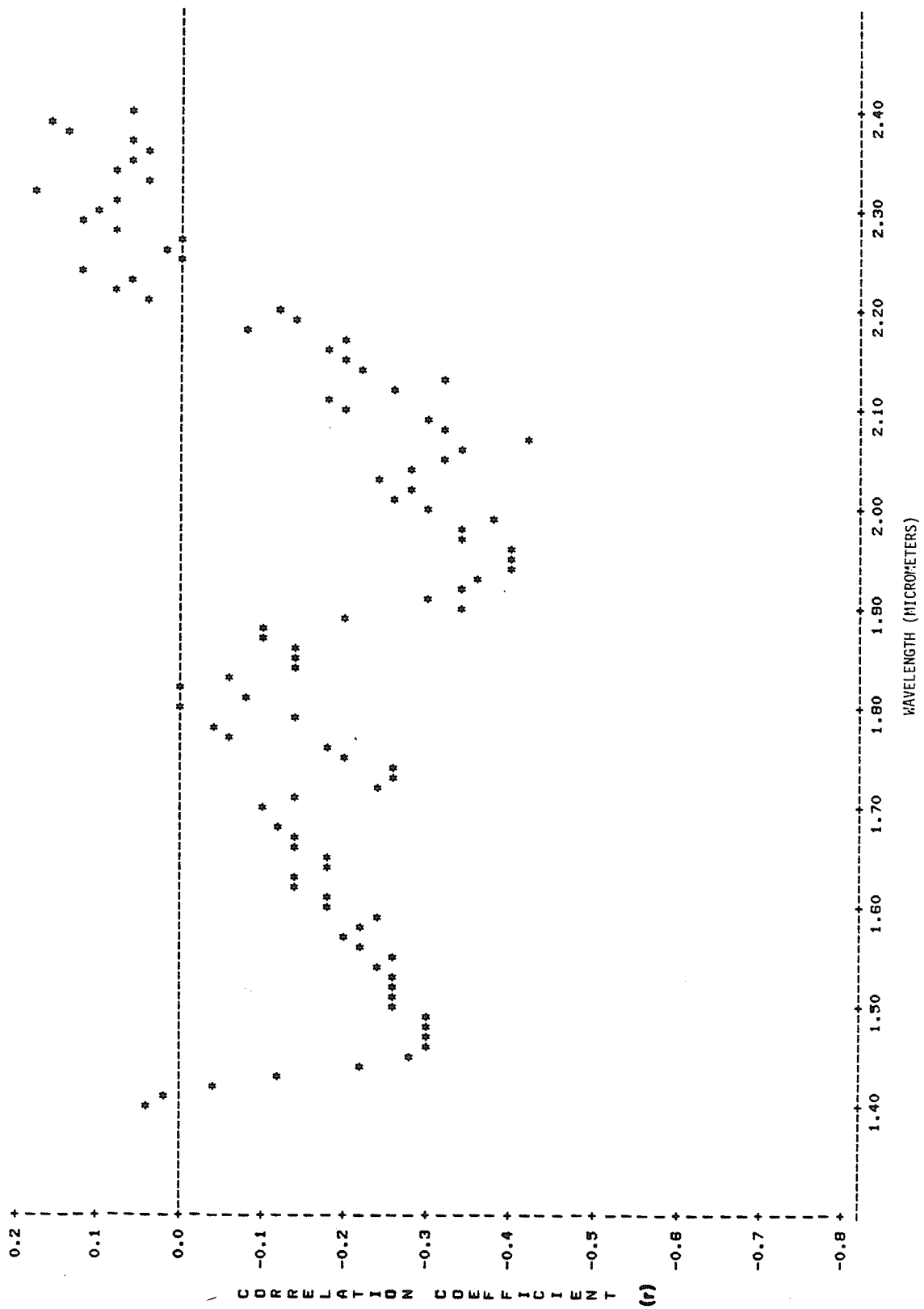
not between groups 2 and 3 (Tables 8b and 9b). The significance was greater for the 2.09 μm band ($\alpha = 0.01$) than for the other band ($\alpha = 0.05$).

A better appreciation of the significance of these results can be gained if one considers the actual mean protein contents of the three groups. The difficulty in differentiating between the mean reflectance of groups 2 and 3 may be due in part to the fact that the protein contents were very similar (less than 1% difference). On the other hand, the mean protein content of group 1 was different from groups 2 and 3 by 2.98 and 3.88%, respectively, and these differences were enough to produce a corresponding significant difference in the mean reflectances. The fact that both wavelength bands used in the analysis gave similar results might be interpreted as indicating the presence of two different absorption bands due to protein. The evidence for this is not conclusive in this study. Nevertheless, the higher correlation coefficient at 2.09 μm and the higher significance of the Duncan's test coupled with the fact that this wavelength coincides with the wavelength band of best correlation for growth stage 6 gives preference to the possibility of this wavelength band as a primary absorption band for protein.

Growth Stage 8 - Beginning to Ripen

The correlation coefficients were relatively low for this growth stage (Fig. 9). Whereas the single wavelength of best correlation was at 2.07 μm with $r = -0.42$, the wavelength band (at least 3 consecutive wavelengths) of best correlation was found between 1.94 and 1.96 μm with $r = -0.40$. This coincides with the major water absorption band

Figure 9. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 8.



centered at 1.95 μm and we have a situation similar to growth stage 5 where the best correlation was found within a water absorption band at 1.45 μm. However, at growth stage 8, the correlation between protein content and water content was very low ($r = -0.20$). It is, therefore, possible that the correlation between protein content and reflectance at the 1.94 to 1.96 μm band might not be an indirect effect due to water absorption but may in fact be a direct effect due to absorption by protein at those wavelengths.

The analysis of variance showed a significant effect due to protein groups (Table 10a). However, as was the case in growth stage 7.5, there was no significant difference between protein groups 2 and 3, while these two groups were significantly different from group 1 (Table 10b).

In order to verify whether similar results would be obtained at the 2.07 μm wavelength, an analysis of variance was carried out using the reflectance data between 2.06 and 2.08 μm (Table 11a). The effect of the protein groups was highly significant, yielding an F value of 17.88 as opposed to 5.59 for the 1.94 to 1.96 μm band. The mean reflectance of all three groups was found to be significantly different from one another, but while the lowest protein group had the highest reflectance as expected, the mean reflectance of the highest protein group (group 3) was greater than that of protein group 2 (Table 11b). This is inconsistent with a negative relationship between protein content and reflectance since the reflectance should decrease as the protein content increases. It appears that some extraneous factor is interacting with the protein effect to yield inconsistent reflectance values at those wavelengths.

TABLE 10a. Analysis of variance table for reflectance at 1.94 to 1.96 μm for growth stage 8.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	4.903	2.452	5.59	0.0062
Wavelengths	2	2.014	1.007	2.30	0.1102
Error	55	24.112	0.438		
Total	59	31.029			

TABLE 10b. Duncan's multiple range test on protein content and reflectance (at 1.94 - 1.96 μm) of protein groups at growth stage 8.

Protein group	Protein content[1]	Reflectance[2]	
3	11.26	5.89	a[3]
2	9.84	5.99	a
1	6.25	6.65	b

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

TABLE 11a. Analysis of variance table for reflectance at 2.06 to 2.08 μm for growth stage 8.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	7.511	3.756	17.88	0.0001
Wavelengths	2	0.070	0.035	0.17	0.8423
Error	55	11.152	0.203		
Total	59	18.473			

TABLE 11b. Duncan's multiple range test on protein content and reflectance (at 2.06 - 2.08 μm) of protein groups at growth stage 8.

Protein group	Protein content[1]	Reflectance[2]	
3	11.26	7.79	a[3]
2	9.84	7.37	b
1	6.25	8.28	c

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

Despite this anomaly, the highly significant protein group effect coupled with the fact that this wavelength band (2.06 - 2.08 μm) coincides with those of the two previous growth stages gives preference to this wavelength band as a primary absorption band for protein. While the results from the 1.94 to 1.96 μm band were more consistent with a negative relationship between protein and reflectance, the fact that this band coincides with a water absorption band does create suspicion as to whether the relationship between protein and reflectance may be indirectly influenced by water absorption.

Growth Stage 9 - Maturity

The results from this growth stage were quite comparable to those of the previous two growth stages in finding significant group differences. However, in this case, the wavelength of best correlation was at 2.00 μm (Fig. 10). The analysis of variance using the reflectance data between 2.00 and 2.02 μm showed significance due to protein groups (Table 12a). The lowest protein group (group 1) was found to be significantly different from the other two groups but groups 2 and 3 were not significantly different from each other (Table 12b). The mean reflectance decreased as the protein content increased.

Summary

The experimental results of each growth stage have shown that there were differences and similarities between the growth stages, either in the wavelength bands of best correlation or in the ability to find significant differences between protein groups. These results are summarized in Table 13. A general discussion of these results is certainly in order to propose some explanations for these differences

Figure 10. Plot of correlation coefficients between reflectance and protein content vs. wavelength for growth stage 9.

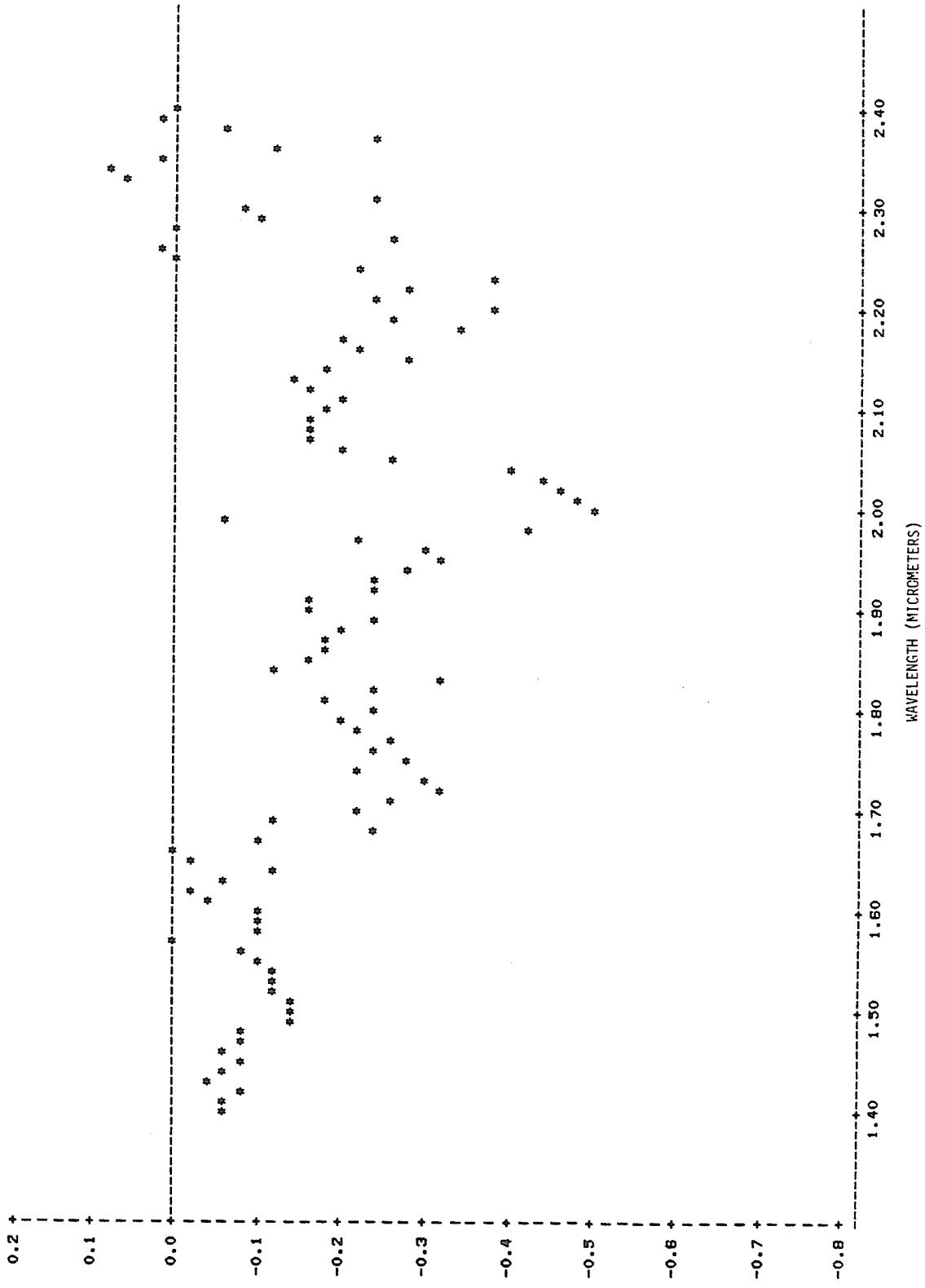


TABLE 12a. Analysis of variance table for reflectance at 2.00 to 2.02 μm for growth stage 9.

Source	DF	SS	MS	F value	Prob > F
Protein groups	2	4.923	2.462	7.24	0.002
Wavelengths	2	1.043	0.522	1.53	0.227
Error	43	14.625	0.340		
Total	47	20.591			

TABLE 12b. Duncan's multiple range test on protein content and reflectance (at 2.00 - 2.02 μm) of protein groups at growth stage 9.

Protein group	Protein content[1]	Reflectance[2]	
3	9.37	10.13	a[3]
2	7.23	10.29	a
1	3.77	10.98	b

[1]Values represent the mean protein content of all plots in each group.

[2]Values represent the mean reflectance of all plots in each group.

[3]Reflectance values with the same letter are not significantly different at the 5% level.

TABLE 13. Summarized results of the analytical procedures for all of the growth stages.

Growth stage	Wavelength band of highest correlation (μm)	Highest correlation value (r)	Differentiation between groups[1]
4	2.34 - 2.36	-0.40	1 2 3
5	2.23 - 2.25	-0.22	2 1 3
6	2.08 - 2.11	-0.72	1 2 3 4 5
7.5	2.06 - 2.09	-0.44	1 3 2
	2.15 - 2.17	-0.42	1 3 2
8	2.06 - 2.08	-0.42	1 3 2
9	2.00 - 2.02	-0.50	1 2 3

[1]Groups which are joined with a bar are not significantly different at the 5% level.

and similarities. Only then can the proper conclusions be drawn concerning the implications of these results on the feasibility of detecting protein content by near infrared reflectance spectroscopy.

GENERAL DISCUSSION

As a general assessment of the experimental results, it is suggested that the growth stages from the beginning of heading to maturity (growth stages 6 - 9) yielded better results than the earlier growth stages (4 - 5). While growth stage 6 yielded the best results, the last three growth stages were also consistent in that the low protein group 1 was found to have a significantly higher mean reflectance than the higher protein groups 2 and 3. These two higher protein groups were not significantly different from each other. These results are consistent with the fact that the difference in protein content between groups 1 and 2 for these three growth stages was between 1.5 to 3 times greater than the difference between groups 2 and 3 (Table 14). Therefore, we would expect more difficulty in finding significant differences between groups 2 and 3.

On the other hand, the results from growth stage 5 were not consistent in that no significant difference was found in the mean reflectance between groups 1 and 2 when, in fact, these two protein groups differed in mean protein content by 4.96%. The difference in mean reflectance between groups 1 and 2 at growth stage 4 was also found to be non-significant but this would be expected since the mean protein content of these two groups differed by only 0.94%.

While the performance of the near infrared reflectance technique in detecting values that were significantly different between protein

TABLE 14. Differences in mean protein content between protein groups 1, 2, and 3 at all growth stages.

Growth stage	Protein group contrast		
	1 - 2	2 - 3	1 - 3
4	0.94[1]	1.09	2.03
5	4.96	2.24	7.20
6	4.03	4.04	8.07
7.5	2.98	0.91	3.89
8	3.59	1.42	5.01
9	3.46	2.14	5.60

[1]Values represent the difference in mean protein content between the two specified groups.

groups was quite different between the preheading and post heading stages, there were also differences in the wavelength bands (presumed to be protein absorption bands) between these two growth periods, as well as within each growth period. The wavelength bands for the post heading stages (beginning to head to maturity) were concentrated within a narrow segment of the spectrum between 2.00 and 2.17 μm with some overlap between the bands, while the bands for the two preheading stages were completely apart from this portion of the spectrum (2.23 - 2.36 μm).

Other researchers have found the 2.00 to 2.17 μm segment of the infrared spectrum to be important in protein determination by near infrared reflectance. Norris et al. (1976) analyzed forage samples for crude protein determination and found that low protein samples were characterized by a broad absorption band at 2.1 μm. In multiple regression analyses using up to eight different wavelengths, he found that the two most important wavelengths for protein determination were 2.08 and 2.16 μm. Norris (1978) also studied the reflectance spectra of soybean protein (96% protein) as a dry powder and found absorption bands at 1.19, 1.51, 1.70, 1.75, 1.98, 2.06, 2.18 and 2.33 μm. He examined the absorption spectra of proteins from different sources and found that they all had these bands in common, although the magnitudes of the different bands varied slightly from one protein to the other. Klepper and Wilhelmi (1979) analyzed vegetative and head samples of wheat with a near infrared reflectance instrument manufactured by the Dickey John Corporation which uses six wavelengths (1.68, 1.94, 2.10, 2.18, 2.23 and 2.31 μm). They found that the two most important wavelengths for protein determination were at 2.10 and 2.18 μm.

Therefore, there is evidence to suggest that the wavelength bands obtained between 2.00 and 2.17 μm are not just artifactual but may, in fact, result from the absorption of infrared radiation by protein at those wavelengths. As for the wavelength bands at growth stages 4 and 5, there is little evidence, based on the difficulty in finding significant differences in protein groups, that the wavelength bands at 2.34 to 2.36 μm and 2.23 to 2.25 μm represent protein absorption bands. In particular, growth stage 5 had very low correlation coefficients ($r = -0.22$) at the 2.23 to 2.25 μm wavelength band.

That the growth stages from the beginning of heading to maturity yielded better results than the preheading growth stages might be explained on the basis of the reflective characteristics of the vegetative leaf material as opposed to that of the head material. As was explained in a previous chapter, the crop spectral curves were corrected by subtracting the specular component from the total reflectance curve to yield a diffuse component. The specular component was obtained from the reflectance plate with the assumption that the reflectance characteristics of the plate were similar to those of the crop. This assumption may be more appropriate for the post heading stages where the object viewed consists mainly of wheat heads than for the preheading stages where we are dealing mostly with leaf material. We would expect the wheat heads which have a rough, irregularly shaped surface to be more diffusive (and thus resemble the reflectance plate which is a Lambertian reflector) than the leaf material, which has smooth plane surfaces and thus is more specular than diffusive in nature. Also, since we can expect a greater specular component from the leaf material than from the head material, we would also expect any error resulting

from the subtraction of the specular component to be greater for the growth stages where the leaf material is the object of view.

While the results from the post heading growth stages indicate that there was absorption of infrared radiation by protein between 2.00 and 2.17 μm with a major peak between 2.08 and 2.11 μm for growth stage 6, there is no clear indication as to why different absorption bands were obtained at different growth stages. Assuming that the nature of the protein is constant from one growth stage to the next, we would have expected the same protein absorption bands in all of the growth stages. It would appear that there are extraneous factors other than protein absorption, such as anatomical changes in cellular structure and changes in configuration of the plant material which interact differently at different growth stages to result in maximum absorption of infrared radiation at different wavelengths.

Another possible source of variation in the absorption bands may be the normalization procedure with respect to the amount of plant material which was carried out prior to analysis of the data. As was explained in the experimental methods, the normalization involved finding the wavelength of least variability (c.v.) for each growth stage and then, assuming that the variability at that wavelength was only due to plant matter differences (i.e. no protein absorption at that wavelength), the reflectance curves were normalized so that their relative reflectance was equal at that particular wavelength. In assuming that the wavelength of lowest variability in reflectance corresponds to a wavelength where there is no absorption by protein, we have assumed that the effect due to protein absorption and the effect due to plant matter differences are additive. Therefore, since the plant matter

differences are expected to affect the reflectance equally at all wavelengths, we concluded that the wavelength of least variability represents a wavelength of minimal or no absorption by protein. If these basic assumptions were not completely valid, it is possible that some of the wavelengths chosen for the normalization process were not wavelengths of zero absorption by protein. The normalization would thus be based on incorrect wavelengths and this would certainly have an effect on the subsequent analysis as to which wavelength band would yield the best correlation between protein content and reflectance.

The results obtained suggest that there were probably two absorption bands by protein within the 2.00 to 2.17 μm range. A primary band occurred between 2.07 and 2.11 μm which coincides with the absorption bands from three of the last four growth stages. A secondary absorption band occurred between 2.15 and 2.17 μm which corresponds to one of the absorption bands at growth stage 7.5. These two protein bands coincide with those found to be important in protein analysis by other workers previously mentioned (Norris et al., 1976; Norris, 1978; Klepper and Wilhelmi, 1979). The fact that the wavelength band for protein absorption at growth stage 9 was found at 2.00 to 2.02 μm might be the result of the normalization based on the incorrect wavelength.

CONCLUSION

Contributions to Research

While the attempt to apply the NIRS technique to a standing crop was not successful in pinpointing the exact wavelengths of protein absorption (as opposed to wavelength bands) or in establishing any absolute relationships between reflectance and protein content, a number of important points can be concluded from the research.

(1) The highest correlation between near infrared reflectance and protein content was found 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 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.

(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.

(6) At the growth stages from the beginning of heading to maturity, the mean reflectance of the lowest protein group (group 1) was always found to be significantly greater than the higher protein groups.

Recommendations for Further Research

The research project has provided some insight as to which wavelengths and which growth stages were important in the application of the NIRS technique to a standing wheat crop. However, the accuracy of the technique for determining protein content was relatively poor. Also, some of the inconsistencies in the results, such as different absorption bands for different growth stages or sudden changes in the nature of the relationship between protein content and reflectance remain essentially unanswered. Further research will be necessary to provide answers to these questions and to improve on the technique. The following recommendations may be useful in achieving this purpose:

(1) Different crop species and cultivars should be used to see whether or not similar results would be obtained.

(2) Since fertilizer application rates above 125 kg/ha did not result in significantly higher levels of protein, a greater number of fertilizer rates between 0 and 125 kg/ha should be used to induce a more even distribution in protein contents.

(3) Spectral measurements should be taken more often throughout the growing period so that we can gain more information as to how or why the performance of the technique changes with the growth stage of the crop.

(4) Measurements taken with the light source at various azimuth and zenith angles could be useful in determining whether significant differences in the performance of the NIRS technique would result and whether normalization procedures could be applied in these cases.

(5) Some basic work on the relationship between protein content and near infrared reflectance of single plant parts (leaves, influorescent parts, etc.) would be invaluable in determining the wavelengths important in protein absorption.

(6) While a field experiment would be exposed to environmental variables as opposed to the controlled greenhouse experiment, there would be some definite advantages:

   (a) The greater irradiation of the crop by the sun and diffuse sky light would require less amplification of the signal from the detector and thus would increase the signal/noise ratio resulting in smoother and more precise spectral curves.

   (b) The variability due to such factors as leaf orientation and amount of plant material viewed would be reduced considerably in a field situation where the viewed area is usually much greater than in the greenhouse experiment. The larger crop surface would have a buffering effect on the individual components and would also provide more uniformity in the amount of plant matter viewed from one plot to the next.

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APPENDIX

APPENDIX TABLE 1. Protein and moisture content data from all replicates at growth stage 4.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Moisture content[2]
0	1	31.30	85.86
	2	31.00	86.09
	3	32.40	85.40
	4	31.90	85.18
62.5	1	33.30	86.25
	2	32.40	84.34
	3	32.40	86.61
	4	31.80	85.33
125	1	33.90	87.29
	2	33.30	85.42
	3	32.50	85.75
	4	31.70	85.26
187.5	1	32.50	86.55
	2	33.90	85.60
	3	32.30	86.11
	4	32.80	85.83
250	1	32.00	85.71
	2	32.70	86.00
	3	33.50	85.84
	4	33.00	85.47

[1]Each value represents 1 protein determination on a combined sample of 4 plants. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Moisture determinations were carried out on total above ground material from a sample of 4 plants. Moisture content = % fresh wt. basis.

APPENDIX TABLE 2. Protein and moisture content data from all replicates at growth stage 5.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Moisture content[2]
0	1	22.80	84.02
	2	22.55	85.01
	3	21.70	84.46
	4	23.35	84.60
62.5	1	26.05	85.52
	2	23.65	84.76
	3	20.95	84.13
	4	20.35	82.73
125	1	25.95	84.88
	2	29.85	85.62
	3	29.35	85.55
	4	28.95	85.70
187.5	1	27.90	85.04
	2	28.05	85.15
	3	27.80	84.63
	4	29.60	86.52
250	1	29.65	85.59
	2	28.75	85.01
	3	29.50	85.27
	4	29.60	84.90

[1]Each value represents the mean of 2 separate determinations, each carried out on a combined sample of 2 plants. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Moisture determinations were carried out on total above ground material from a sample of 4 plants. Moisture content = % fresh wt. basis.

APPENDIX TABLE 3. Protein and moisture content data from all replicates at growth stage 6.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Moisture content[2]
0	1	12.55	80.61
	2	13.05	79.32
	3	13.10	78.74
	4	13.00	78.90
62.5	1	17.60	80.57
	2	15.50	79.97
	3	16.35	81.11
	4	18.39	80.66
125	1	21.16	82.04
	2	23.09	83.45
	3	22.25	81.87
	4	21.35	81.83
187.5	1	20.53	78.94
	2	21.95	82.75
	3	21.75	82.76
	4	20.95	82.13
250	1	22.13	81.84
	2	23.15	82.44
	3	22.70	80.88
	4	23.45	82.51

[1]Each value represents the mean of 4 separate determinations run on single plant samples. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Moisture determinations were carried out on total above ground material from a sample of 4 plants. Moisture content = % fresh wt. basis.

APPENDIX TABLE 4. Protein and moisture content data from all replicates at growth stage 7.0.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Head protein[2]	Moisture content[3]
0	1	7.80	11.80	68.57
	2	8.20	11.60	69.40
	3	8.65	12.60	70.58
	4	8.50	13.00	69.67
62.5	1	13.10	13.80	72.78
	2	13.70	15.00	72.99
	3	13.25	16.00	73.03
	4	15.10	16.40	72.50
125	1	16.95	17.00	73.88
	2	15.30	13.60	74.96
	3	15.00	15.40	71.96
	4	15.80	14.40	73.53
187.5	1	17.90	16.20	74.60
	2	15.55	15.20	73.40
	3	17.00	15.60	75.28
	4	16.15	15.20	74.36
250	1	18.00	16.60	78.16
	2	17.25	15.80	75.29
	3	16.65	15.40	72.39
	4	17.65	16.00	75.88

[1]Each value represents the mean of 4 separate determinations run on single plant samples. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Each value represents a single protein determination on the combined head material from 4 plants. Head protein = % N x 6.25 on a dry wt. basis.

[3]Moisture determinations were carried out on total above ground material from a sample of 4 plants. Moisture content = % fresh wt. basis.

APPENDIX TABLE 5. Protein and moisture content data from all replicates at growth stage 7.5.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Head protein[2]	Moisture content[3]
0	1	6.70	12.80	64.59
	2	6.15	12.60	65.45
	3	5.45	11.20	65.00
	4	7.50	13.00	66.07
62.5	1	11.30	15.60	68.00
	2	11.90	15.40	67.76
	3	12.35	16.20	68.13
	4	11.60	17.00	65.83
125	1	13.70	16.60	69.34
	2	14.10	11.20	69.22
	3	14.40	15.00	69.69
	4	14.30	15.80	69.91
187.5	1	13.70	16.60	67.09
	2	12.70	15.20	68.25
	3	13.25	15.80	68.17
	4	14.05	15.00	69.38
250	1	14.20	15.60	68.68
	2	14.85	15.60	69.45
	3	15.25	16.00	70.63
	4	13.95	15.60	67.25

[1]Each value represents the mean of 4 separate determinations run on single plant samples. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Each value represents a single protein determination on the combined head material from 4 plants. Head protein = % N x 6.25 on a dry wt. basis.

[3]Moisture determinations were carried out on total above ground material from a sample of 4 plants. Moisture content = % fresh wt. basis.

APPENDIX TABLE 6. Protein and moisture content data from all replicates at growth stage 8.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Head protein[2]	Grain protein	Moisture content[3]
0	1	3.95	6.70	12.99	60.69
	2	4.05	6.80	11.84	59.63
	3	4.35	5.30	11.69	58.56
	4	5.08	6.20	12.08	57.44
62.5	1	8.50	10.90	15.06	58.42
	2	6.30	9.90	17.18	49.86
	3	5.88	9.80	18.12	47.85
	4	7.72	9.30	16.63	54.73
125	1	7.15	10.10	17.10	54.46
	2	10.95	12.20	16.63	63.02
	3	9.95	11.10	17.02	61.09
	4	9.70	10.40	16.63	59.86
187.5	1	8.75	10.70	18.35	51.50
	2	9.60	11.40	16.63	55.31
	3	9.15	9.30	18.51	55.31
	4	9.28	10.90	18.27	52.88
250	1	9.90	10.40	18.33	50.60
	2	9.63	11.60	19.22	54.15
	3	9.45	9.80	16.78	56.96
	4	9.30	9.60	17.88	54.11

[1]Each value represents the mean of 4 separate determinations run on single plant samples. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Each value for head and grain protein represents a single determination on the combined material from 4 plants. Head protein = % N x 6.25 on a dry wt. basis; grain protein = % N x 5.70 on a 14% moisture basis.

[3]Moisture determinations were carried out on total above ground material from a sample of 4 plants. Moisture content = % fresh wt. basis.

APPENDIX TABLE 7. Protein content data from all replicates at growth stage 9.

Fertilizer treatment (kg N/ha)	Replicate	Vegetative protein[1]	Head protein[2]	Grain protein
0	1	1.85	3.40	13.57
	2	2.20	4.60	13.80
	3	1.70	2.50	12.23
	4	2.00	4.20	13.26
62.5	1	3.95	7.60	18.35
	2	3.50	7.80	18.27
	3	3.70	8.30	18.35
	4	3.50	6.20	18.59
125	1	3.90	7.20	18.67
	2	5.75	8.10	19.22
	3	5.50	10.40	20.47
	4	6.70	9.60	14.59
187.5	1	5.25	7.40	18.67
	2	6.00	10.10	19.06
	3	6.35	8.70	18.75
	4	5.25	7.20	18.51
250	1	7.10	9.10	18.20
	2	7.00	10.10	19.37
	3	7.15	10.00	18.43
	4	6.70	8.60	18.43

[1]Each value represents the mean of 2 separate determinations run on a combined sample of 4 plants. Vegetative protein = % N x 6.25 on a dry wt. basis.

[2]Each value for head and grain protein represents a single determination on the combined material from 4 plants. Head protein = % N x 6.25 on a dry wt. basis; grain protein = % N x 5.70 on a 14% moisture basis.