A spectral study of nutrient and disease stress in barley and wheat leaves

Luis Nuno Quental
Katholieke Universiteit Leuven
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Summary

Spectral discrimination of leaves subject to different nutrient and disease treatments was assessed using a spectrograph in a controlled greenhouse environment.

A new data normalization method is proposed based on reflection and enlightenment correction. Averaging data provided a faster, simpler and almost as precise way of noise reduction than windowing.

Results have shown the feasibility of ground based spectrography to efficiently discriminate different treatments. The number of wavebands to take into consideration depends on the intended application but good classifications were often achieved using only 5 wavebands.

Though the most discriminating wavebands varied with the combination of treatments considered, a robust model for disease distinction and recognition could be built. Constant wavebands were selected from a broad dataset comprising several treatments and then used in "sub-models" for pairwise comparisons. Robustness was higher using 15 variables than using only 5.

Further investigation under field conditions is essential before concrete applications can be produced.

Abbreviations and scientific names

Table 1 – Common and scientific names of plant and disease species referred throughout this work.

Common name	**Scientific name**
Barley	*Hordeum vulgare* L.
Bigleaf maple	*Acer macrophyllum* Pursh
Corn	*Zea mays* L.
Cucumber	*Cucumis sativus* L.
Field bean	*Vicia faba* L.
Gray leaf spot	*Pyricularia grisea* (Cooke) Sacc.
Greenbug	*Schizaphis graminum* Rondani
Horse chestnut	*Aesculus hippocastanum* L.
Loblolly pine	*Pinus taeda* L.
Mildew	*Erysiphe graminis* f. sp. *hordei*
Norway maple	*Acer platanoides* L.
Peanut	*Arachis hypogea* L.
Potato	*Solanum tuberosum* L.
Rapeseed	*Brassica napus* L.
Rhizoctonia blight	*Rhizoctonia solani* Kühn
Rice	*Oryza sativa* L.
Russian wheat aphid	*Diuraphis noxia* Mordvilko
Septoria leaf blotch	*Septoria tritici* Thüm.
Shortleaf pine	*Pinus echinata* Mill.
Slash pine	*Pinus elliottii* Engelm.
Southern pine beetle	*Dendroctonus frontalis* Zimm.
Soybean	*Glycine max* L. cv. 'Bragg'
Sunflower	*Helianthus annuus* L.
Tall fescue	*Festuca arundinacea* Schreb.
Tobacco	*Nicotiana tabacum* L.
Winter wheat	*Triticum aestivum* L.
Yellow rust, stripe rust	*Puccinia striiformis* West.

Table 2 – List of used abbreviations throughout this work. For vegetation indices please refer to Table 6.

Abbreviation	**Meaning**
λ_{re}	Wavelength position of the red edge
B	Blue wavelengths
Billion	10^3 million
dR_{re}	Amplitude of the red edge in the first derivative of reflectance spectra
FAO	Food and Agriculture Organization of the United Nations
G	Green wavelengths
GIS	Geographical information systems
GPS	Global positioning systems
ha	Hectare (= 0.01 km^2)
IPM	Integrated pest management
IR	Infrared wavelengths
LAI	Leaf area index
NIR	Near-infrared wavelengths
R	Red wavelengths
R_λ	Reflectance at the wavelength λ
RSA	Russian wheat aphid
SAIL	Scattering by Arbitrarily Inclined Leaves
TM bands	Landsat Thematic Mapper bands: B: 450–520, G: 520–600, R: 630–690, NIR: 760–900, 1550-1750, 2080-2350nm
UV	Ultraviolet radiation
VI	Vegetation index
WRI	World Resources Institute

1. Introduction

The "control of nature" is a phrase conceived in arrogance, born of the Neanderthal age of biology and philosophy, when it was supposed that nature exists only for the convenience of man. The concepts and practices of applied entomology for the most part date from that Stone Age of science. It is our alarming misfortune that so primitive a science has armed itself with the most modern and terrible weapons, and that in turning them against the insects it has also turned them against the earth.

Rachel Carson, *Silent Spring* (1962)

With these words Rachel Carson (1907-1964) described the use of pesticides to control insect plagues in her famous and reference work "Silent Spring". Carson is credited to be one the precursors of the environmentalist movement. Soon she became aware of the problems posed by the widespread use of chemicals such as eldrin, dieldrin and DDT in the United States during the 50's. As a scientist she dominated the mechanisms under the environmental and health effects, but she also had the ability to transform them in a clear language readable by the common person. She systematically showed the world the madness of indiscriminately spraying vast areas with biocides: the death of birds, mammals, beneficial insects and, ultimately, entire ecosystems. Millions of human beings also fell victims of acute poisoning or as the cancer rates soared. Ironically, Carson died with a cancer two years after the publication of "Silent Spring", the very disease she fought so intensely.

Terms involving *sustainable* have entered our daily lives and people have an intuitive idea of what they are about. Yet, the full meaning of such terms is seldom realized. The generally adopted definition for *sustainable development* was given by the Brundtland Commission in their 1987 report: "Sustainable development is development that meets the needs of the present without compromising the ability of future generations to meet their own needs." *Sustainable agriculture* is usually defined as the "successful management of resources for agriculture to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving natural resources". These statements incorporate an altruistic vision, for that they look into the future, the long term ecological conditions. The battle for a sustainable agriculture is therefore of major importance.

This work is related to precision agriculture. This is a recent form of agriculture system which acknowledges the hazard of pesticides and excessive fertilization. But before entering this subject it is important to consider a wide view on the evolution of agriculture and environment, specially in recent times and as a consequence of the so-called "green revolution".

1.1. Traditional agriculture

Traditional agriculture is the term given to the farming systems which are based on indigenous knowledge and practices, and have evolved over many generations. It still exists throughout the world, though because of population pressure or with emergence of fertilizers and pesticides many of them shifted to unsustainable practices with disastrous results.

Farming systems are in constant change, as experience is accumulated, populations increase or decrease, new opportunities and aspirations arise, and the natural resource base deteriorates or improves. The attempt is continuously made to adapt to the new conditions. Countless farming communities managed to survive throughout the times and, in some cases, to thrive by exploiting natural resource bases which their descendents have used for generations. Through a process of innovation and adaptation, indigenous farmers have developed numerous different farming systems, each of them finely tuned to its ecological, economic, sociocultural and political environment.

The indigenous knowledge of a farming population living in a specific area is derived from the local people's past farming experience, both that handed down from previous generations and that of the present generation. When a technology developed elsewhere has been incorporated by local farmers as an integral part of their agriculture, it is as much a part of their indigenous knowledge as self developed technologies. Farmers' practical knowledge about the local ecosystem – about the natural resources and how they interact – is reflected in their farming techniques and in their skill in using the natural resources to gain their livelihood. However, indigenous knowledge entails many insights, perceptions and intuitions related to the environment, often including lunar or solar cycles, astrology, and meteorological and geological conditions. Also, traditional methods of communication, e.g. through songs and proverbs, and traditional structures for social cooperation form part of the local knowledge system. Such rich knowledge, the product of thousands of years of evolution, is often regarded as backward and primitive by modern societies – and were, as well, by most of the colonial powers. This tremendous mistake is probably the main reason that accounts for its fast and irreversible destruction. Because it respects the environment and other humans, traditional knowledge is advanced, not primitive.

The traditional bush fallow system practiced by the farmers of Mampong Valley in Ghana worked effectively while there was still enough land to ensure that each plot left fallow had sufficient time to regain its fertility. However, increased pressure on the land as a result of population growth has led to shortening of the fallow period and, consequently, severe degradation of farm sites. The original semi-deciduous and evergreen forest has been reduced to bush and grassland. Soil is being depleted,

fuelwood has become scarce, animal fodder is lacking and important sources of local medicine from trees and plants have disappeared (Reijntjes *et al.*, 1992).

Even in ancient times, deforestation, overgrazing, waterlogging and salinization in Mesopotamia's fertile crescent were as much the immediate cause for the collapse of its societies as conquest by outside invaders. The Mesopotamians were able to bring more land into agricultural use, as well as boost the yields per acre, by developing an elaborate system of irrigation canals. But, by failing to provide adequate drainage systems, the build-up of toxic materials and salts in the soil gradually poisoned it for cropping. And by failing to arrest silting in the slow moving canals, the dependable supply of water was jeopardized. One of the main centres of Mayan civilization seems to have failed after centuries of expansion in Guatemala, because the demands of its growing population depleted the soil through erosion. The shifting sands of Libya and Algeria also testify to the failure of Roman agriculturists to husband the granary which supplied much of the Roman Empire with food (Reijntjes *et al.*, 1992).

1.2. The modernization of agriculture

The rapid growing of the world population specially in the last half of the twentieth century and of consumption in western countries was a strong incentive to the development of agricultural practices more productive and profitable. The population of the developing countries as a whole increased from 1.8 billion to 4.7 billion during that period. At the same time, according to figures from the Food and Agriculture Organization of the United Nations (FAO), cereal production rose from 0.5 to 0.65 tones *per capita* in developed countries and from 0.2 to 0.26 in developing countries. In some crops, namely wheat, corn and barley, yields doubled or tripled. This was a major growth but it hides periods of decreased productivity and famine, as during the 70's world food crisis. Net food imports are rising rapidly in developing countries, coupled with increasing exportations from developed countries (FAO, 2000). Resource distribution is more uneven than ever: from 1960 to 1994 the income ratio between the richest and poorest 20% increased from 30:1 to 78:1. Nowadays, these poorest 20% account for slightly more than 1% of the global income, while the richest 20% claim 86%. Thus, trends in production and malnutrition levels (which also decreased in the last fifty years) should be analyzed with suspicion. There are still more than 830 million people suffering from malnutrition, a number that should makes us think about the *real* concern of western countries upon tremendous tragedy.

The enormous increase in food production is attributable to (FAO, 2000):

- the spread in the developed countries of the modern agricultural revolution (involving motorization, large-scale mechanization, biological selection, use of chemicals, specialization) and its expansion into some sectors of the developing countries;

- the occurrence of the "green revolution" in the 60's, more noteworthy in the developing countries. The green revolution created an agriculture system highly dependent on inputs such as fertilizers, pesticides, irrigation and selected high yielding varieties, often developed through biotechnological improvement;
- the expansion of irrigated surfaces, from about 80 million ha (hectares) in 1950 to about 270 million today;
- the expansion of arable land and land under permanent crops, from some 1330 million ha to 1500 million ha since 1950;
- the development of mixed farming systems using high levels of available biomass (combining crops, arboriculture, livestock and, sometimes, fish farming) in the most densely populated areas that lack new land for clearing or irrigation.

However, two major misjudgments were made before the introduction of the green revolution (Reijntjes *et al.*, 1992):

- the price increase of chemical fertilizers and fuel, and the general decrease in international prices as a result of worldwide overproduction of grains, had not been foreseen. These changes led to higher consumer prices for food and lower farm-gate prices. The main beneficiaries have been the suppliers of the fertilizers and fuel;
- the ever increasing dependency on pesticides and fertilizers had not been foreseen. They have contaminated the streams and water tables with serious hazards for the population.

There are signs showing a stagnation in production increase (FAO, 2000). This raise serious doubts whether long-term productivity of such system as the modern agriculture is secure. Only a sustainable system will be able to meet the food needs of an additional 1.7 billion people over the next twenty years. Yet, environmental indicators point in the opposite direction.

Environmental hazards[1]

As stated above, environmental problems raise critical questions about the feasibility of modern agriculture.

Artificial fertilizers

The efficiency of fertilizers has proved to be lower than expected. Losses of 40 to 70% of applied nitrogen are common. Since nitrogen is easily leached, it will contaminate water supply (nitrogen poses a serious health risk) and contribute to the eutrophication of streams, killing fish and associated wildlife. Agriculture is the major cause of nonpoint source pollution. In soil, artificial fertilizers can disturb natural balances, increasing the

[1] - This chapter was based on Reijntjes *et al.* (1992) and on Poincelot (1986), except when otherwise stated.

rate of organic matter decomposition, eventually leading to degradation of soil structure and higher vulnerability to drought. Continuous use of only artificial NPK fertilizers leads to depletion of micronutrients upon which plant survival also depends. In the United States, average mineral fertilization rose from 20 to 120kg/ha between 1950 and late 90's (FAO, 2000).

Pesticides

Yearly, thousands of people are poisoned by pesticides, about half of them in the Third World. In 1983 there was a total of about 2 million human victims, 40 000 of them fatal. Over time, pests build up resistance to pesticides, which in turn must be used in ever increasing doses to have effect, killing not only undesirable organisms but also useful ones, such as natural enemies of pests. Moreover, only a small part of the chemical applied effectively reaches its target, the rest passing through soil, adversely affecting the soil community, and ending up in streams – eventually reaching toxic concentrations. Persistent pesticides enter the food chain, disrupting ecosystems and, ultimately, being assimilated by humans. Many types of cancer are associated with pesticide residues in food.

Improved seeds

In developing countries, new wheat and rice varieties cover approximately 24 and 45 million ha, respectively, or 50 and almost 60% of the total planted area of each crop (data from 1985). Modern varieties are essentially high-response varieties, bred to respond to high doses of fertilizers. If conditions of high nutrient and water supply, and pest control are not guaranteed, risks of yield losses may be higher than with local varieties. In these conditions, local varieties may outyield the modern ones. Perhaps an irreversible damage have been the continuous loss of genetic diversity accompanying the disappearance of many indigenous varieties. This spells disaster for farmers who have to produce their crops with low external inputs under highly variable and risk-prone conditions – and for all farmers who, for both economic and ecological reasons, will have to produce with less chemical inputs in the future. Indigenous varieties are adapted to local climatic conditions, such are drought periods, reducing the risk of low productivity, representing a step towards farmer autonomy. Instead, modern varieties have to be bought from seed companies every year to guarantee their purity, increasing farmer dependence on the exterior.

Irrigation

Water reserves throughout the world are diminishing as a result of increased irrigation. Consequently, many areas face severe salt intrusion which can render the soil completely unproductive. A large part of the irrigated water is lost by evaporation. As water costs are usually kept artificially low by government subsidies, there is little incentive to save on it. Immense sums have been spent by development agencies on large-scale irrigation projects and on construction of big dams. These projects create severe social problems, destroy unique ecosystems and, in developing countries,

exacerbate their foreign debt. According to the World Resources Institute (WRI), irrigated area grew by more than 70% over the past thirty years (WRI, 2000).

Ecosystem disruption and disease severity

Green revolution agriculture is often called "industrial agriculture" since it is based on large fields where plants usually share the same genetic traits. Unlike traditional farming which makes use of natural pest enemies to combat plagues, much as in a natural ecosystem, industrial agriculture disrupts the natural balance and stimulate this way disease propagation and its severity. This calls for ever increasing pesticide inputs. The wheat belt in North America, a "carpet" of wheat that extends from northern Mexico well into the prairie provinces of Canada, is a good example of this situation. Rust infection begins each year in northern Mexico and spores are blown northward, germinating and feeding on plant stems and leaves as they go. The rust cannot overwinter in the northern end of the greenbelt and must arrive each year from the south if wheat is to be rusted. In fact, rusts cannot oversummer in the southern part of the belt so spores must be blown southward to infect winter wheat and renew the spring migration. A gigantic epidemiological system was therefore created, in which several species of *Puccinia* migrate yearly over 4000km from a land where they cannot oversummer to a land where they cannot overwinter (Harlan, 1995). Harlan adds ironically: "The system must be included among the marvels of the biological world; it has been called the *Puccinia* path."

Soil erosion and desertification

Erosion of soil is not a phenomenon unique to agriculture, but a natural process that occurs even on land with permanent vegetation. What is unique to agriculture is the alarming rate of soil erosion. Topsoil is being lost ten times faster than its rate of formation (typically 1cm every 100 years). Topsoil is the richest in organic matter and nutrient content. Until now farmers have been trying to compensate the lost through the widespread use of fertilizers, but this cannot go forever. For soil is crucial to retain water and nutrients, anchor plants and sustain a community of organisms that co-operate with roots for nutrient uptake. Nearly 40% of the world's agricultural land is seriously degraded, which could undermine their long-term productive capacity (WRI, 2000). In Central America the figure rises up to 75%. Over the past half century, about 2 billion ha of agricultural land, permanent pastures, forests and woodlands have been degraded. Each year, about 5 to 10 million additional ha become too degraded to use (WRI, 1997). According to the International Food Policy Research Institute, soil degradation already had significant impacts on the productivity of about 16% of the globe's agricultural land. More than 250 million people are directly affected by desertification and nearly 1 billion are at risk (FAO, 2000).

Social inequity

Closely coupled with environmental problems are social inequities. Vandana Shiva, famous Indian eco-feminist, brought us a story of violence and destruction in the state of Punjab, India:

The tragedy of Punjab – of the thousands of innocent victims of violence over the past few years – has commonly been presented as an outcome of ethnic and communal conflict between two religious groups. This study presents a different aspect and interpretation of the Punjab tragedy. […] The Green Revolution has been heralded as a political and technological achievement, unprecedented in human history. It was designed as a techno-political strategy for peace, through the creation of abundance by breaking out of nature's limits and variabilities. Paradoxically, two decades of Green Revolution have left Punjab ravaged by violence and ecological scarcity. Instead of abundance, Punjab has been left with diseased soils, pest-infected crops, waterlogged deserts, and indebted and discontented farmers. Instead of peace, Punjab has inherited conflict and violence.

Vandana Shiva, *The Violence of the Green Revolution* (1993)

Farmers who are given easy access to credit may be tempted into high capital investment and modern production methods which demand that high levels of external inputs be maintained or increased. At the same time, as prices for petroleum-based fertilizers, herbicides, pesticides and fuels are increasing, prices of agricultural products are often being kept artificially low by national governments or by the flooding of local markets with subsidized imports of agricultural products from industrialized countries. Third World farmers are then in great danger of being trapped in a debt spiral.

Modern agriculture cannot cope with low productivity. The falling world prices of main crops (down by 43% for corn over the past three decades, for instance) considerably reduced the profit of small farmers. Large farmers who benefit from economies of scale, mechanization and improved seeds, on the other side, were able to increase production and profits. The global trend encourages the plantation of large unsustainable fields while disregarding small-scale systems, often held by poor farmers from the Third World. Thus, the gap between the rich and the poor is increased.

1.3. Organic agriculture

Organic agriculture has much in common with sustainable agriculture. The philosophical approach is not maximizing production anymore. Instead, organic farming emerges from an holistic vision of the ecosystem: tries to integrate agriculture in the cycles of Nature rather than opposing them, finds a natural balance within the field and acknowledges the complex relationships between the various organisms and plants.

Productivity can be lower than that achieved by modern agriculture, but the protection of soil and environment guarantee the sustainability of the system on the long term. Putting it into another way, present harvests are not made at the expense of future ones. Organic agriculture draws much of its inspiration from traditional farming techniques. A bottom-up approach which recognizes the importance of farmers' experience is coupled with the top-down ecological science knowledge of experts. Therefore, it is a system of learning with experience, sharing this knowledge and working in pair with scientific research.

Ecological principles[2]

The following section briefly describes the ecological principles that guide organic agriculture.

Sustaining the soil

Soil is often described as consisting of solid particles, water, gaseous elements, humus and raw organic matter. An extremely important aspect which is often forgotten is that soil is also the dwelling place for a large variety of living creatures. A layer of 10cm over 1ha with 1% of organic matter contains roughly 1500kg of soil fauna – the weight of 3 or 4 cows. These organisms play a major role in many soil processes and soil-plant interactions, such as soil formation, creating soil structure, build up of free nutrients and humus for plant growth, nitrogen fixation, phosphate solubilization and uptake of nutrients by plant roots. Humus – organic matter decomposed by soil life – plays a critical role in creating fertile soils. Sustaining the soil is thus fundamental.

The organic farmer uses a number of practices that are extremely effective in reducing soil erosion. Continuous cropping is replaced by rotations that include meadow, legumes and small grains along with row crops. Cover crops, green manure crops and reduced tillage help to conserve surface crop residues and to increase soil organic matter, water infiltration and its retention. Cover crops also help prevent soil losses to as much as 50% during the unproductive part of the growing season. These practices, therefore, decrease surface water runoff and wind blowoff, thus reducing soil erosion. Other conservation measures include contour planting and plowing, terraces, grassed waterways, using bushes as natural windbreaks, etc.

Water

Organic agriculture promotes water infiltration, making it available for plant uptake. This is possible through the use of surface crop residues obtained after plowing out or from reduced tillage practices. As stated above, soil organic matter also aids in water infiltration and retention.

[2] - This chapter was based on Reijntjes *et al.* (1992) and on Poincelot (1986), except when otherwise stated.

Aquifers and streams are less prone to pollution since artificial fertilization and use of pesticides are kept to a minimum or are even banned. Reduced soil erosion means that less sediments reach the rivers, contributing to clean waters where wildlife can flourish.

Natural fertilization

Organic farmers use little, if any, chemical fertilizers. Instead, they rely upon recycling of nutrients in their management approach. These nutrients are generally not susceptible to leaching because they become available slowly, providing a continuous natural fertilization of plants. They are usually contained in manure, compost, residues from other crops, etc. In addition, the alternation of crops that require high amounts of nitrogen (e.g., corn) with crops that require little amounts (e.g., soybeans and alfalfa) helps to lower the average nitrate available for leaching. Reduced losses of nitrates and phosphates of 300-600% were recorded with a corn-wheat-clover rotation comparing to continuous corn. It is important to note that improper use of manure and other natural fertilizers can also lead to environmental problems, though the risks are smaller than posed by modern agriculture.

Integrated pest management

The growing concerns about health and environmental risks associated with the use of pesticides encouraged the development of integrated pest management (IPM) techniques. In IPM, in the context of the farm's environment and the population dynamics of the pest species, all suitable techniques and methods (biological, genetic, mechanical and chemical) are used in the most compatible manner possible so as to maintain pest populations at levels below those causing economic injury. Every effort is made in order to avoid pest occurrence rather than in implementing curative measures.

Shifting cultivation not only conserves moisture, restores organic matter and nutrients to the soil, and prevents erosion and leaching, but it also controls weeds and reduces populations of insects, nematodes and various pathogens. The great diversity of crops grown is itself protective, as pests are seldom able build up to destructive proportions on the few isolated plants of each species. Farmers who observe pest life cycles can limit pests in crops and livestock by making use of the natural mechanisms that regulate the population dynamics of those organisms.

Maintaining diversity and flexibility

The sustainability of a farm system depends on its flexibility under changing circumstances. The availability of a wide diversity of genetic resources at the farm level contributes to it. Farmers can maintain biological diversity by using mixtures of different species, mixtures of different varieties of the same species, or varieties whose genetic composition is itself variable.

1.4. Precision agriculture

The extent and rate of development in information and communication technologies is opening a way for significant changes in crop management and agricultural decision making. It has led to cropping systems in which management operations are executed on a site-specific basis and with an increased temporal precision.

Precision agriculture has been defined as a management strategy that used information technology to bring data from multiple sources to bear on decisions associated with crop production. Therefore, it can increase the efficiency of applied fertilizers and maximize financial advantage while possibly operating within environmental constraints, improving agricultural practice in order to reduce the impact on the environment from agrochemical wastage. Adoption of technology of sufficient capability to provide fully supported precision farming is likely to be an evolutionary process, with farms gradually improving their information technology capability.

Geographical information systems

A geographical information system (GIS) is a software application that is designed to provide the tools to manipulate and display spatial data. It is effectively a way of computerizing maps. It goes further than that by being able to accept, organize, statistically analyze and display diverse types of spatial data that are digitally referenced to a common co-ordinate system. Each set of data is grouped together in an overlay and new data sets can be produced by combining a number of overlays.

Examples of overlays are soil type, topography, disease and nutrient distribution, stress and yield mapping, land ownership and crop cover. As all the data are stored digitally it can be modified, copied and reproduced as intended. If these datasets are then combined with agronomic models and decision support systems a powerful management tool can be constructed.

Farm management

It is crucial to be able to use the large amount of information gathered by the various sources. The farm manager can display a map of the farm on the computer and be able to recall both the field history (crop, treatment and application history), and the point, linear and geographical features (ponds, fences, hedgerows, soil type, etc.). Also, soil surveys are often conducted to give the spatial variability of soil characteristics (N, P, K, pH, Mg, moisture, compaction, etc.). The manager can then compare these field characteristics to the change in the yield profile to get a better understanding of why some areas of the field are more productive than others. The decisions made will be based on information from the GIS as well as traditional sources such as experience, advisers, suppliers, other farm records, etc.

Precision farming does not imply any particular management technique. What it does do is allow the manager a better understanding and a greater control over the treatments to the fields.

Financial requirements

There are different financial implications depending on the farming strategy. Generally, the costs of information technologies are very high. Therefore, for precision farming to achieve its goal, savings in fertilizer and pesticide use must surpass technology related expenditures. This is more likely to happen in large fields, where reductions can be considerable.

Possible benefits

Seeding

Hybrid seeds perform best when placed at spacing that allow the plants to obtain such benefits as maximum sunlight and moisture. This is best accomplished by varying the seeding rate according to the soil conditions such as texture, organic matter and available soil moisture. One would plant fewer seeds in sandy soil as compared to silt loam soils because of less available moisture. The lower seed population usually has larger heads (ears) of harvested seeds providing for a maximum yield. Since soils vary even across an individual farm field, the ability to change seeding rates as one goes across the field allows the farmer to maximize this seeding rate according to the soil conditions. A computerized soil map of a specific field on a computer fitted on the tractor along with a global positioning system (GPS) can tell farmers where they are in the field allowing the opportunity to adjust this seeding rate as they go across their fields.

Crop protection

The application of chemicals and fertilizers in proper proportions are of environmental and economic concern to the farmers. Environmental regulations are calling for the discontinuance of certain pesticide applications within a certain distance from a stream. Using a GPS along with a digital drainage map, the farmer is able to apply these pesticides in a safer manner. In fact, the spraying equipment can be pre-programmed to automatically turn off when it reaches the distance limitation or zone of the drainage feature. Additionally, farmers can pre-program the rate of pesticide or fertilizer to be applied so that only the amount needed determined by the soil condition is applied varying this rate from one area of the field to another. This saves money and allows for safer use of these materials.

Tillage

The ability to vary the depth of tillage along with soil conditions is very important to proper seedbed preparation, control of weeds and fuel consumption and therefore cost to the farmer. Most farmers are using conservation tillage which means leaving residues on the soil surface for erosion control. The use of GPS in making equipment adjustments as one goes across the different soil types would mean higher yields and safer production at lower cost. This part of precision farming is in its infancy. The equipment companies will be announcing tillage equipment with GPS and selected controls tailored to precision farming in the near future.

Harvesting

The proof in the use of variable rate technology (adjusting seed, pesticide, fertilizer and tillage) as one goes across the field is in knowing the precise yields. Combines and other harvesting equipment can be equipped with weighing devices that are coupled to a GPS. One literally measures yield on the go. With appropriate software, a yield map is produced showing the yield variation throughout the field. This allows farmers to inspect the precise location of the highest and the lowest yielding areas of the field and determine what caused the yield difference. It allows one to program cost and yield to determine the most profitable practices and rates that apply to each field location. In my opinion, the use of yield monitors is a good place to start if one wants to get started in precision farming.

1.5. Concluding remarks

Environmental problems in agricultural are compromising the ability of soil to cope with yield demands. World's growing population will certainly demand higher food production, but this increase must be sustainable. Otherwise, a new world food crisis could follow. Modern agricultural methods may be highly productive on the short run, but on the long run organic agriculture and traditional practices may be more suitable.

Precision farming can surely play a role in input reduction and minimize related environmental hazards – which is, itself, an invaluable contribution. However, because it is high capital demanding, precision agriculture will be primarily available to rich farmers cultivating extensive monoculture fields. The resulting system will probably be unsustainable. Thus, a deeper assessment of the benefits of precision farming should be made.

These conflicting trends – the very basis of modern agriculture and the need for more food – prevent the drawing of a definitive conclusion about the role of precision farming in agricultural sustainability. Instead, it is proposed to the reader to consider all its advantages and drawbacks so that he can reach its own, free, opinion.

2. Theoretical basis

In this chapter a theoretical approach to the relevant issues for this work is made. These include:

- plant nutritional requirements and physiological basis of stress responses;
- leaf and radiation interaction;
- remote detection of stress in plants;
- spectral analysis for stress detection (literature review).

As a large part of the topics is already scientifically well established, a literature review was only prepared for those over which subsist considerable uncertainties and that are most important to the project. As a result, the review dealt mainly with investigations on spectral changes with plant stress – like the most discriminating reflectance wavebands, sensitive vegetation indices, the "red edge" shift, etc. – specially in the visible part and involving leaf measurements. Techniques as thermography and fluorescence were not mentioned.

It is important to mention that research in this area, although with some decades of experience, is still little developed, with fundamental questions to answer. Moreover, results are sometimes contradictory or at least somehow variable. A careful analysis of them is, therefore, recommended. The review did not mean to be exhaustive, it only provides an overview of relevant papers on the subject.

2.1. Plant nutritional requirements and physiological basis of stress responses[3]

In order to thrive, a plant needs more than light and carbon dioxide. The complex mechanisms behind biosynthesis and responses to environmental conditions require a number of nutrients. Plant nutrition is still a field with much to discover, though the role of many elements is now well known. If we consider the diversity inside Plant kingdom, it is a remarkable advance. Probably the most astonishing fact in plants is a rather intuitive one – their ability to produce magnificent forms, colors, aromas and fruits that support whole ecosystems, from so simple sources.

Though they are simple, they are not always available. Stress is usually defined as an external factor that exerts a disadvantageous influence on the plant. In most cases, stress is measured in relation to growth or to primary assimilation processes (CO_2 and mineral uptake), which are related to overall growth. Under both natural and agricultural conditions, plants are constantly exposed to stress. Some environmental

[3] - This chapter was based on Taiz and Zeiger (1991).

factors (such as air temperature) can become stressful in just a few minutes, whereas others may take days to weeks (water availability) or even months (some mineral nutrients). These factors, which often occur simultaneously, severely condition plant growth and crop yields.

Essential elements and nutrient disorders

Mineral nutrients enter the biosphere at the plant's root system, where the large surface area of roots and their ability to absorb nutrients at low concentrations in the soil make mineral absorption a very effective process. Then they are translocated to the various parts of the plant where they are needed. However, not all nutrients are equally important. Some of them are said to be "essential", that is, in their absence a plant will demonstrate deficiency symptoms and die without completing its life cycle. Fortunately this is not usually the case, their seasonal or temporary shortage being more common.

Essential elements are usually classified as macronutrients or micronutrients, according to their relative concentration in plant tissue. However, this may not be a physiological justifiable classification since differences in content between some micro and macronutrients can be small and because nutrients with different roles would be grouped together. Instead, a classification according to their biochemical and physiological functions will be followed (Table 3).

Table 3 – Classification of plant nutrients according to biochemical function (from Lincoln and Zeiger, 1991).

Nutrient element	**Uptake mode**	**Biochemical functions**
C, H, O, N, S	H_2O. Ions from soil solution: HCO_3^-, NO_3^-, NH_4^+, SO_4^{2-}. Gases from the atmosphere: CO_2	Major constituent of all organic material. Assimilated by carboxylation and oxidation-reduction processes.
P, B, Si	In the form of phosphates, boric acid or borate. Silicate from the soil solution.	Esterification with alcohol groups in plants. Phosphate esters are involved in energy transfer reactions.
K, Na, Mg, Ca, Mn, Cl	Ions from the soil solution.	Nonspecific functions establishing osmotic potentials. Specific contributions to the structure and function of enzyme protein (cofactors). Balancing non-diffusible and diffusible anions.
Fe, Cu, Zn, Mo	In the form of ions or chelates from the soil solution.	Present predominantly in prosthetic groups. Permit electron transport by valence changes.

Inadequate supply of an essential nutrient results in a nutritional disorder manifested by characteristic deficiency symptoms which are the expression of related metabolic disorders. These disorders, in turn, are linked to the roles played by the essential elements in normal plant metabolism. In general, the essential elements function:

- as constituents of compounds;
- in the activation of enzymes;
- in contributing to the osmotic potential of plant cells.

When comparing deficiency symptoms with the role of an essential element, it is important to consider the extent to which it can be recycled from older to young leaves. Elements such as N, P and K are easily translocated, causing the symptoms to appear first in the older leaves. Other elements like B, Fe and Ca are relatively immobile and so younger leaves are affected first. Table 4 shows a partial listing of the roles and related disorders of some essential nutrients.

Table 4 – Roles of some essential nutrients and related disorders (based on Lincoln and Zeiger, 1991).

	Role	**Disorders**
N	Constituent of amino acids, amides, proteins, nucleic acids, nucleotides and coenzymes, hexosamines, etc.	Stunted growth, slender and woody stems. The woodiness comes from a buildup of excess carbohydrates which cannot be used in synthesis of amino acids and other N compounds, but can be in anthocyanin production. The accumulation of this pigment may lead to a purple coloration in leaves, petioles and stems of some plants. In many plants, the first symptom is chlorosis, especially in older leaves since N is a mobile element.
P	Component of sugar phosphates (respiration and photosynthesis), nucleic acids, nucleotides, coenzymes, phospholipids (cell membrane), phytic acid, etc. Has a key role in reactions involving ATP.	Stunted growth in young plants and a dark green coloration of leaves, which can be malformed and contain necrotic spots (small spots of dead tissue). As in N deficiency, anthocyanins may be formed in excess giving leaves a trace of purple coloration. However, unlike N deficiency, it not associated with chlorosis. Instead, a dark greenish-purple coloration of the leaves may occur. Also possible is the production of slender (but not woody) stems, death of older leaves and delay in maturation.
K	Required as a cofactor for 40 or more enzymes (respiration and photosynthesis). Has a role in stomatal movements. Maintains electroneutrality and osmotic pressure in plant cells.	Mottled or marginal chlorosis, which then develops into necrosis that occurs primarily at the leaf tips and margins and between veins. Because K can be readily mobilized to the younger leaves, these symptoms appear initially on the older, more mature leaves at the base of the plant. Curling and crinkling of leaves may also occur. Slender and weak stems, with abnormally short internodal regions.

S	Component of cysteine, cystine, methionine (and, thus, proteins). Constituent of lipoic acid, coenzyme A, thiamine pyrophosphate, glutathione, biotin, etc.	Similarly to what happens with N stress, it is often observed stunting of growth, chlorosis and anthocyanin accumulation. Usually, however, the symptoms appear first on younger leaves because, unlike N, S is not easily remobilized. In some species, though, chlorosis may occur all at once or even on the older leaves first.
Ca	Constituent of the middle lamella of cell walls. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a "second messenger" in metabolic regulation.	Necrosis of the tips and margins of young leaves, followed by necrosis of the terminal buds. These symptoms arise in the young meristematic regions of the plant, where cell division is occurring and new walls are forming. These symptoms are often preceded by a general chlorosis and downward hooking of the young leaves, which may also be deformed.
Mg	Required nonspecifically by a large number of enzymes involved phosphate transfer (respiration, photosynthesis and DNA and RNA synthesis). A constituent of the chlorophyll molecule.	Interveinal chlorosis that take place first in the older leaves due to the high mobility of Mg. This pattern occurs because chlorophyll in the vascular bundles remains unaffected for longer periods than that in the cells between the bundles. If deficiency is extensive, the leaves may become yellow or white. Premature leaf abscission is often observed as well.
Fe	Constituent of cytochromes and of nonheme iron proteins involved in photosynthesis, N_2 fixation and respiration.	Interveinal chlorosis that appear first on younger leaves. Under conditions of extreme or prolonged deficiency, the veins may become chlorotic so that the whole leaf takes on a white color.

The relationship between the growth or yield of a plant and the nutrient content of its tissues is not linear. Until a critical concentration is reached, an increase in nutrient content results in higher growth rates (deficiency zone). Then follows the adequate zone, where no correlation between growth rate and nutrient concentration is observed, and the toxic zone, where increased nutrient content is detrimental to plant growth or yield.

Water stress

Water stress is very common, specially in dry zones where rainfall is low. Extreme conditions such as droughts and floods can also severely influence plant growth during a period of time. If this period coincides with critic life cycle periods of crops, the losses can be enormous. An intelligent and sustainable way of minimizing the effects of these problems is to plant crops that are adapted to the local climatic conditions.

The earliest responses to stress appear to be mediated by biophysical events rather than by changes in chemical reactions due to dehydration. As the water content of a plant decreases, the cells shrink and the cell walls relax. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. The

smaller leaf area transpires less water, effectively conserving a limited supply in the soil for use over a longer period.

The total leaf area of a plant does not remain constant after all the leaves have maturated. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. This behavior improves the plant's fitness for a water limited environment.

When the onset of stress is more rapid or the plant has reached its full leaf area before initiation of stress, there are other responses that protect the plant from immediate desiccation. Under these conditions, the stomata close to reduce evaporation from existing leaf area. The extent of the response is highly dependent on the species considered and may depend on nutrient availability as well.

Other important responses to water stress include root expansion into deeper, moist soil, osmotic adjustment to maintain turgor, higher leaf temperatures as a result of reduced transpiration and increased wax deposition on the leaf surface. Photosynthetic rate diminishes, though it is less dependent on turgor than leaf expansion.

Protection against radiation

Ultraviolet radiation (UV) emitted from the sun is highly energetic and, despite the ozone layer in the upper atmosphere, a significant amount reaches the earth. It may cause harm to sensible cell structures and provoke potentially harmful mutations during DNA, RNA or protein synthesis.

Flavonoids are phenolic colored pigments of plants. Two major groups of flavonoids, flavones and flavonols, play a role in the protection again this radiation (though they have other important functions too). This property arises from their strong absorptance in the UV while letting the visible (photosynthetically active) wavelengths pass through uninterrupted. Exposure of plants to increased UV light has been demonstrated to increase the synthesis of flavones and flavonols.

2.2. Leaf and radiation interaction

Light reflectance[4] from plant leaves is the result of radiation interaction with various surfaces, structures, molecules and even air spaces. The detailed mechanisms explaining these interactions are beyond the scope of this work, as are the implications of leaf structure (monocots vs. dicots), pubescence, leaf stacks, etc.. Instead, an overview of the main factors that account for the observed leaf spectrum will be made.

[4] - Reflectance is defined as the ratio of the radiance reflected by a target to the radiance incident upon it.

Electromagnetic spectrum

Electromagnetic radiation spans many order of magnitude of wavelengths, from shorter than 10^{-13} m for the most energetic γ-rays to longer than 100km for very long radio waves (Figure 1). Therefore, it is convenient to divide the spectrum into several regions according to their uses or characteristics. In this work, only the visible and part of the near-infrared (NIR) wavelengths were used.

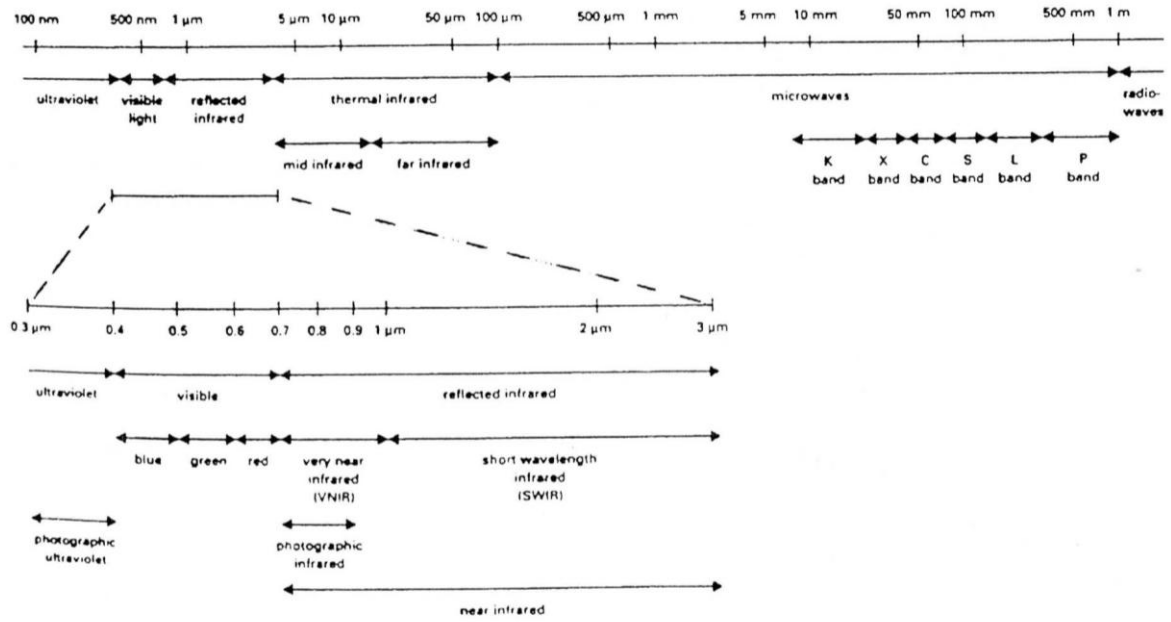

Figure 1 – Electromagnetic spectrum. Only the visible and very near infrared were used in this work.

Leaf spectrum

As the solar radiation comes into contact with the leaves, it interacts in various ways. A small amount is immediately reflected by the cuticular wax while the main part is transmitted into the inner portion of the leaf. Here, the radiation can be absorbed by leaf pigments or scattered by air spaces, cell walls or other constituents and transmitted out of the leaf.

A typical leaf spectrum is shown on Figure 2 and can be divided in three categories:

- below 400nm, in the UV, most of the radiation is absorbed (see chapter 2.1, "Protection against radiation") or reflected by cuticular wax or because of leaf orientation and geometry;
- the 400 to 750nm visible light absorptance region, specially by chlorophyll and other pigments (e.g., anthocyanin, carotenes and xanthophyll), producing a low reflectance. Chlorophyll has a particularly strong absorption in the blue (445nm) and red (645nm) portion of the visible spectrum. A peak in reflectance is found in the

green (520-550nm). Since 85-90% of the radiation between 400 and 700nm is absorbed by photosynthetic pigments, this region is also called "photosynthetic active radiation";

- the 750 to 1350nm NIR region, where reflectance is high (40 to 60%) and absorptance is low. This is because the cellular micro structure of the leaf is a very efficient medium for scattering radiation and because there is no strong absorption by any of its components. Thus, reflection in this region is mainly regulated by internal leaf structure;
- 1350 to 2500nm, a region dominated by the strong water absorption bands (at 1450 and 1950nm), and thus greatly affected by water concentration. Absorption by cellulose, lignin and some other plant materials also plays a role.

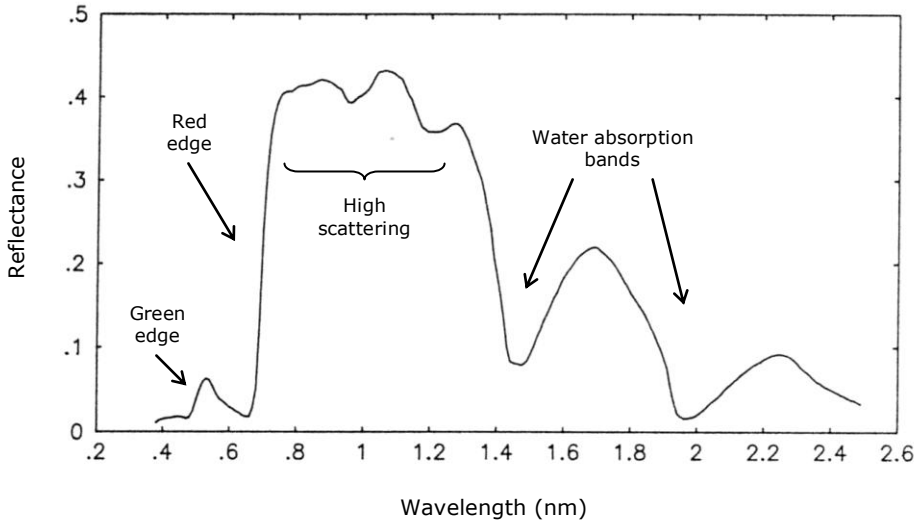

Figure 2 – A typical leaf spectrum.

Red edge

Horler *et al.* (1983) gave a big contribution to the study of the red edge, the sharp change in leaf reflectance between 680 and 750nm. In their own words, it "is a unique feature of green vegetation because it results from two special optical properties of plant tissue, high internal leaf scattering causing large NIR reflectance and chlorophyll absorption giving low red reflectance". Therefore it is suitable for green vegetation identification, and vegetation indices (VI) such as the NDVI (normalized differentiation vegetation index) take advantage of this characteristic. Their results support the idea that the red edge is mainly controlled by leaf chlorophyll content rather than by leaf structural properties, since λ_{re} (the wavelength position of the red edge) correlated well with R_{540} (r=0,842) and the relation was not significant with R_{800}.

The spectral behavior of the green edge around 520nm was found to be very similar to that of the red edge (Horler *et al.*, 1983). The difference, as stated by Gitelson and

Merzlyak (1996), is that the green edge is primarily determined by carotenoids, chlorophyll *a* and chlorophyll *b* absorption, while the red edge is governed mainly by chlorophyll *a*.

2.3. Remote detection of stress in plants

For years the most widespread method for stress detection has been visual survey. Experienced growers have the ability to detect subtle changes in plant color or a slight drop or curl of leaves. Irrigations are often scheduled when leaves feel warm to the touch. The limitations of these methods are that few people have either the experience or the insight to detect these signs and that fields are generally too large to be adequately surveyed by eye. Whether or not they are detectable by sight or touch, changes that take place as a result of stress affect the amount and direction of radiation reflected and emitted from plants. Remote sensing techniques offer the possibility of quantitatively assessing plant stress caused by biotic and abiotic factors.

Physiological and anatomical changes take place within plants as a result of stress. If transpiration is restricted from lack of water or a vascular disease, leaf temperatures will increase because of less cooling by transpired water as it evaporates from leaf surfaces. Leaf color may change as a result of a physiological changes caused by a water deficiency or a change in nutrient status. Plant pathogens may change leaf color by causing chemical changes within cells or by grouping on plant surfaces. Morphological changes such as leaf curl or droop may result from the action of any of several stress factors. A typical physiological effect of stress is a decrease in leaf chlorophyll concentration and an increase of the carotenoids to chlorophyll ratio (e.g., Peñuelas and Inoue, 1999). N deficiency is strongly coupled with this behavior since the majority of leaf N is contained in chlorophyll molecules (Daughtry *et al.*, 2000). Essential metal micronutrients Mn, Fe and Cu are directly involved in electron transport reactions critical for the biosynthesis of chlorophyll, though the effect of Cu on leaf chlorophyll concentration was not obvious (Adams *et al.*, 2000). More detailed information was given in chapter 2.1.

Visible reflectance responses are generally similar regardless of their cause (Carter, 1993), though they can differ in degree and depending on the species (Masoni *et al.*, 1996). This means that the prediction of the stress agent may be very difficult of perhaps even impossible if no other complementary information is available (Jackson, 1986). Green *et al.* (1998) could not differentiate epidemics of Rhizoctonia blight and gray leaf spot on the basis of any of the wavelengths measured.

Several remote sensing techniques are available for stress detection purposes. They include ground, air or satellite based spectroscopy, videography, infrared (IR) thermography, fluorescence, radar and microwave radiometry and nuclear magnetic resonance imaging, to name the most important. Considering the scope of this work,

only ground based spectroscopy will be accessed in some detail. Reviews prepared by Nilsson (1995), Jackson (1986) and Lichtenthaler *et al.* (1998), or even by Myers (1983) in the *Manual of Remote Sensing*, may provide a valuable overview of those techniques.

Ground based spectroscopy

Visible

As the visible part of the spectrum is essentially ruled by pigment absorption (specially chlorophyll *a*), the result is a higher reflectance on this entire band, since absorption is lower.

This was observed in six vascular plant species affected by eight stress agents and in mineral deficient crops by Carter (1993) and Masoni *et al.* (1996), respectively. The later highlighted, however, that the degree of spectral responses are dependent on the species and mineral deficiency involved. Schepers *et al.* (1996) and Serrano *et al.* (2000) observed lower N and chlorophyll concentration in corn leaves and wheat canopies, respectively, which were given a deficient supply of that nutrient. Moreover, plants with limited N usually develop higher concentrations of carotenoids relatively to chlorophyll *a* than abundantly supplied ones.

Yet, working with field bean leaves infected by the necrotrophic fungus *Botrytis fabae*, Malthus and Madeira (1993) found no significant change of leaf chlorophyll concentration and observed a "flattening" of reflectance response in the visible region (i.e., a lower reflectance) and a decrease in the NIR. This can be because of the way the fungal pathogens affect the crops (necrotic diseases vs. chlorotic diseases).

Near-infrared

Stress influence in the NIR region is governed mainly by two competing trends. On one hand, reflectance decreases as a result of leaf structure deterioration. This way reflectance decreases because part of the air spaces and cell walls previously responsible for reflection and scattering effects disappears. When dehydration is well in advance, though, its effect may overlap the one explained above. This happens because water strongly absorbs in the region 1350–2500nm and, as relative water content declines, reflection tends to increase.

Hinzman *et al.* (1986) observed the first effect measuring N-stressed wheat canopies and Peñuelas and Inoue (1999) the later through leaf desiccation studies in peanuts and wheat. The work of Schepers *et al.* (1996) with water or N-stressed corn leaves, and the research of Lorenzen and Jensen (1989) on barley inoculated with mildew, produced similar results.

Reflectance of P and Ca deficient corn leaves at 830, 940 and 1100nm was considerably higher than in normal plants (Al-Abbas *et al.*, 1974). Conversely, reflectance of the S, Mg, K and N deficient leaves was much lower than those of the control.

Riedell and Blackmer (1999) analysed the spectra of wheat leaves infected with Russian wheat aphids or greenbugs. In NIR, Russian wheat aphid damaged leaves had a higher reflectance, while the greenbug damaged showed a lower reflectance than the control. This is because leaves infected with Russian wheat aphids developed a lower relative water content and greenbug damaged have mostly disrupted leaf cellular structure.

Red edge

Until the leaf reaches its maturity chlorophyll concentration increases, noticeable by a shift of λ_{re} toward longer wavelengths. This happens because reflectance in the green tends to decrease. With senescence the inverse of maturation occurs – the usually called "blue shift" of red edge (Horler *et al.*, 1983; Lorenzen and Jensen, 1991).

In a study with phosphorus deficient soybeans Milton *et al.* (1991) observed a 7nm shift of the red edge (λ_{re}) towards shorter wavelengths, when compared to control plots. When only P deficient plants are considered, the usual shift to longer wavelengths with leaf maturation was not seen. Instead, authors refer to an "inhibition" of this behavior.

Water stress, simulated by Horler *et al.* (1983) trough desiccation, had the same effect as senescence. In fact, the reason behind this behavior is one and the same: change of leaf internal structure, in the later case by water.

Leaf damage caused by Russian wheat aphid and greenbug infections on winter wheat was accompanied by a shift of the wavelength at which the red-infrared transition region began (Riedell and Blackmer, 1999). In control leaves the transition started at 700nm compared to 680nm in aphid damaged leaves.

Leaf age

The change of spectra with leaf age can be divided in two parts: leaf maturation and senescence. While leaf maturates, chlorophyll concentration rises. When senescence begins, the opposite occurs until typical spectral features disappear. As a result, senescence and stress "symptoms" are very much alike. In principle, this could pose some problems to stress identification. The work of Adams *et al.* (1993) with soybeans concluded that effects arising from leaf age were generally smaller than those caused by manganese deficiency. Age and leaf nodal position effects were not so obvious in control plants. Al-Abbas *et al.* (1974) concluded that this factor did not influence the reflectance spectra from 750 to 2600nm of corn subject to several nutrient deficiencies (in separate treatments).

Leaf vs. canopy measurements

Canopy measurements are usually subject to more variability than leaf measurements (Yoder and Pettigrew-Crosby, 1995). Canopy reflectance may be influenced by any biotic or abiotic factor that changes its structure. Defoliation, disease progress, increased soil exposure, pubescence, pigment concentration and canopy architecture, among others, are all factors that tend to have more pronounced effects on canopy rather than on leaf spectra. This is especially true when plant stress symptoms are not the primary factor influencing canopy reflectance, such in early stages of nutrient deficit. Therefore, lower correlations to relevant variables as chlorophyll concentration or disease severity are often obtained using canopy data.

2.4. Spectral analysis for stress detection

Single wavebands

Single wavebands themselves can constitute good indicators of plant stress, specially, as would be expected, those where observed reflectance variation is more pronounced. However, single wavelengths are also subject to more variability caused by environmental factors such as enlightenment and solar angle. Table 5 summarizes important results obtained by several researchers.

Table 5 – Most stress or chlorophyll content discriminating wavelengths as obtained by various researchers through reflectance measurements.

Reference	Wavelength (nm)	R	Variability cause	Plant	Level
Anderson and Perry, 1996	550 770	-	Flooded soil	*Acer rubrum*	L
Blackmer *et al.*, 1994	550	0,95	N	Corn	L
Carter *et al.*, 1996	694 ± 3	-	Herbicide application	Loblolly pine and slash pine	C
Carter *et al.*, 1998	698 675	-	Southern pine beetle infection	Shortleaf pine	C
Carter, 1993	535 – 640 685 – 700 620 700	-	8 stress agents	6 higher plant species	L
Choubey and Choubey, 1999	450 – 520 760 – 900	0,91 0,81	Chlorophyll	Rice	C
Datt, 1999	710	0,80	Chlorophyll	21 *Eucalyptus* species	L
Daughtry *et al.*, 2000	715 550	0,90 0,87	Chlorophyll	Corn	L
Gitelson and	700	0,97	Chlorophyll	Norway maple and horse	L

Merzlyak, 1996	550	0,97		chestnut	
Gitelson and Merzlyak, 1997	700 530 – 630	0,98 -	Chlorophyll	9 species of higher plants	L
Green *et al.*, 1998	810 810	0,70[1] 0,75[1]	Rhizoctonia blight Gray leaf spot	Tall fescue	C
Lichtenthaler *et al.*, 1996	700 550 530 – 630	0,99 0,98	Chlorophyll	Tobacco	L
Lorenzen and Jensen, 1989	498 664	0,89 0,95	Mildew infection	Barley	L
Ma *et al.*, 1996	600 800	-	N	Corn	C
Masoni *et al.*, 1996	555 700	0,90 0,90	Fe, S, Mg, Mn	Barley, winter wheat, corn and sunflower	L
Merzlyak *et al.*, 1997	550 – 600 700	-	Chlorophyll	Norway maple, horse chestnut, corn and potato	L
Moran and Moran, 1998	636 692	-	Nutrient shortage	*Nepenthes rafflesiana*	L
Riedell and Blackmer, 1999	625 – 635 625 – 635 680 – 695 680 – 695	0,91 0,49 0,84 0,62	RSA infection Greenbug infection RSA infection Greenbug infection	Winter wheat	L
Schepers *et al.*, 1996	710 550	0,78 0,77	N	Corn	L
Yoder and Pettigrew-Crosby, 1995	550 722 2132	0,89 0,80 0,82	N	Bigleaf maple	L L C

R: correlation coefficient of reflectance at indicated wavelengths with leaf chlorophyll *a* concentration. **Variability cause**: experimental induced changes, usually treatments with different levels of the mentioned factor. **Level**: C – canopy, L – leaf. RSA – Russian wheat aphid. [1] – correlation with disease severity on plants treated with fungicide. "Minus" symbols represent intervals. Plant and disease scientific names can be found on Table 1.

Chlorophyll

Datt (1999) observed a higher sensitivity to chlorophyll concentration at 710 than at 550nm, as did Carter (1993) regarding the response to various stress agents – in contrast with Gitelson and Merzlyak (1996, 1997). In addition, the 710nm band was obtained from an improved spectrum insensitive to the effects of leaf scattering on reflectance. Datt (1999) points out that, instead of lying in the middle of the strong pigment absorption region stretching from 400 to 700nm, which may cause saturation of response (as happens to 550nm), the 710nm band is located in the "equilibrium region between chlorophyll *a* absorption and maximum NIR reflectance, and is further away from the absorption regions of other pigments". Since chlorophyll *a* absorptivity is low at 550 and 710nm, even small decreases in its content could result in significantly decreased absorption and increased reflectance (Carter, 1993). Gitelson and Merzlyak (1996) added that "the high variation of R_{700} with chlorophyll concentration is a result of

the shift of the red edge and it is caused by the same physical processes". They observed similar sensibility of the 550-560 and 700-710nm bands, and a strong correlation between them. Similarly, Daughtry *et al.* (2000) detected the highest sensibility of corn leaves on the broad band around 550 (r=0,87), 715 (r=0,90) and beyond 750nm. Lichtenthaler *et al.* (1996) found maximum sensitivity from 530 to 630 and near 700nm. The relationship between leaf chlorophyll content and reflectance is hyperbolic (e.g., Gitelson *et al.*, 1996).

Gitelson *et al.* (1996) and Carter (1993) found little variation of R_{675} and R_{NIR} with leaf chlorophyll concentration and in response to several stress agents, respectively. R_{675} stands in the high absorption peak of chlorophyll *a*. Lichtenthaler *et al.* (1996) reinforced these results with data from tobacco plants. Besides these wavelengths, the region between 400 and 500nm was also insensitive to the pigment content in nine higher plant species (Gitelson and Merzlyak, 1997). Carter (1993) found smaller differences in visible spectrum due to several stress agents at violet (380–424nm) and blue (424–491nm).

Nutrients

Ma *et al.* (1996) carried out canopy reflectance readings in corn and found that measurements at 600 and 800nm were the best to separate different N treatments. Reflectances at 550 and 680 can be inaccurate estimators of chlorophyll *a* content (Filella *et al.*, 1995). These researchers observed an asymptotic relationship showing a saturation in the reflectance response to that pigment. They worked with five different N treatments of wheat at canopy level. Contrary to these results, the analysis of spectra from corn leaves subject to similar treatments by Blackmer *et al.* (1994) revealed more distinct separation of them near 550nm. Measurements at 450 and 650 showed little or no effect on reflectance in any of the four hybrids used. R_{550} was still correlated to grain yield (r=0,95).

Hinzman *et al.* (1986) performed reflectance measurements of wheat canopies with different N levels over the six TM bands. Their results suggested that the NIR permits the greatest treatment discrimination, in spite of the significant decrease in leaf chlorophyll content that is usually better perceptible in the visible. Leaf area index (LAI) correlated well and linearly (r=0,89) with NIR.

Water

As a result of dehydration, a peak difference in spectra occurred in yellow at 584nm (Carter, 1993).

Diseases

Barley spectra infected with mildew revealed maximum response to the disease at 498 and 664nm, near the high absorption region of chlorophyll *a* (Lorenzen and Jensen, 1989). The NIR region was less reliable than visible for stress detection purposes, since

its changes were small and occurred several days later. The differences in reflectance in the blue and red between control and inoculated plants were highly correlated to the chlorophyll content of infected leaves (r=0,89 and 0,95, respectively).

Malthus and Madeira (1993) found maximum correlation between percentage infection (*Botrytis fabae*) in field bean leaves and reflectance at 720nm (r=0,9). No relationship was found between reflectance and leaf chlorophyll *a* concentration, which can account for the low correlation coefficient between infection and reflectance in the visible region.

Vegetation indices

Vegetation indices (VI) were developed to surpass the limitations of single wavebands. They are said to minimize external or environmental factors such as enlightenment conditions, view and solar angle, background reflection, etc. and so tend to correlate more closely with chlorophyll content (Carter *et al.*, 1996). This is because their computation uses a highly sensitive wavelength to this pigment and an insensitive one.

A number of VI have been developed. Nilsson (1995) presents an extensive list. Some of them are only seldom used while others are very popular and repeatedly assessed. NDVI is probably the most studied of ever. It makes use of the characteristic feature of plant spectra "red edge" as an indicator of their vigour or simply to recognition purposes (e.g., to eliminate the background influence). However, some authors point out that it is not a good indicator of stress as it is precise for chlorophyll determination only at fairly low levels of the pigment (Lichtenthaler, 1996). Instead, it is often correlated to green biomass (Nilson, 1995). The ratio NIR/Red correlates to the leaf area index (Serrano *et al.*, 2000). SIPI, NPCI and PSR were developed to assess pigment changes since they relate to the ratio between carotenoids and chlorophyll, which increases as a result of N-stress (Peñuelas and Inoue, 1999; Filella *et al.*, 1995). VI that combine NIR and red (R) as OSAVI and NIR/R minimize background interference but are little sensitive to chlorophyll concentration, while in others that combine NIR and other visible band, such as MCARI and NIR/Green, the opposite happens (Daughtry *et al.*, 2000). The yellowness index, developed by Adams *et al.* (1999), is an approximation of the second derivative of the spectrum, and so should be less affected by atmospheric or soil effects than NIR/R or NDVI. Thus, it should be primarily a function of changes in pigment absorption, independent of leaf structure and water content.

Table 6 shows the formulas of some of the most used VI.

Table 6 – Some VI and their formulas using reflectance measurements at the indicated wavelengths. Numbers in italic are not wavelengths.

Index	Name	Formula
Green NDVI	-	(NIR – G) / (NIR + G)
Greenness	-	[refer to Hinzman *et al.*, 1986]
MCARI	Modified chlorophyll absorption reflectance index	(700–670)–*0,2**(700–550)*(700/670)
NDVI	Normalized differentiation vegetation index	(NIR – R) / (NIR + R)
NIR/R	-	NIR / R
NPCI	Normalized difference pigment index	(680 – 430) / (680 + 430)
OSAVI	Optimized SAVI	(*1+0,16*)*(801-670)/(801+670+*0,16*)
PSR	Pigment simple ratio	430 / 680
SAVI	Soil-adjusted vegetation index	(*1+0,5*) * (801–670) / (801+670+*0,5*)
SIPI	Structural independent pigment index	(800 – 445) / (800 – 680)
WBI	Water band index	950 / 900
WI	Water index	900 / 970
Yellowness	-	[refer to Adams *et al.*, 1999]

Besides these VI, virtually any combination of sensitive/insensitive wavelengths is possible, and it is often a question of trial and error the achievement of good correlations to chlorophyll content or other relevant variables. Table 7 summarizes important results obtained by several researchers.

Table 7 – VI most suitable for stress or chlorophyll content discrimination as obtained by various researchers.

Reference	VI (nm)	R	Variability cause	Plant	Level
Adams *et al.*, 1999	Yellowness (624) 744 / 666 NDVI (744, 666)	0,98 0,98 0,98	Mn	Soybean	L
Adams *et al.*, 2000	750 / 550 750 / 650	-	Fe, Zn, Cu, Mn	Soybean	L
Carter *et al.*, 1996	694 / 760	-	Herbicide application	Loblolly pine and slash pine	C
Carter *et al.*, 1998	NDVI (840, 698)	-	Southern pine beetle infection	Shortleaf pine	C
Carter, 1994	695 / 420 695 / 760	0,88 0,87	8 stress agents	6 higher plant species	L
Datt, 1999	(850–710) / (850–680) 850 / 710	0,89 0,86	Chlorophyll	21 *Eucalyptus* species	L
Filella *et al.*, 1995	NPCI	-	N	Winter wheat	C
Gitelson and Merzlyak, 1996	750 / 550 750 / 700	0,97 0,97	Chlorophyll	Norway maple and horse chestnut	L
Gitelson and Merzlyak, 1997	Green NDVI 750 / 700 750 / 550	- 0,96 0,95	Chlorophyll	9 species of higher plants	L
Gitelson *et al.*, 1996	750 / 700	0,97	Chlorophyll	Norway maple and horse chestnut	L / C

Green *et al.*, 1998	NDVI (760, 710) NDVI (760, 710)	0,77[3] 0,72[3]	Rhizoctonia blight Gray leaf spot	Tall fescue	C
Lichtenthaler *et al.*, 1996	750 / 550 750 / 700	0,98 0,96	Chlorophyll	Tobacco	L
Ma *et al.*, 1996	NDVI (800, 600)	-	N	Corn	C
Merzlyak *et al.*, 1997	750 / 550	-	Chlorophyll	Norway maple, horse chestnut, corn and potato	L
Moran and Moran, 1998	692 / 420 692 / 760 636 / 420 636 / 760	-	Nutrient shortage	*Nepenthes rafflesiana*	L
Peñuelas and Inoue, 1999	WI WI WI / NDVI (900, 680)	0,92[1] 0,60[1] -	Progressive desiccation	Peanut Wheat Peanut and wheat	L
Peñuelas *et al.*, 1994	NPCI	0,637	N	Sunflower	L
Riedell and Blackmer	NPCI NPCI WBI WBI	0,84 0,52 0,92 ns	RSA infection Greenbug infection RSA infection Greenbug infection	Winter wheat	L
Schepers *et al.*, 1996	550 / 850 710 / 850	0,86 0,89	N	Corn	L
Serrano *et al.*, 2000	900 / 680 NDVI (900, 680)	0,93[2] 0,85[2]	N	Winter wheat	C

VI: wavelengths used in the vegetation indices. **R**: correlation coefficient of the indicated VI with leaf chlorophyll *a* concentration. **Variability cause**: experimental induced changes, usually treatments with different levels of the mentioned factor. **Level**: C – canopy, L – leaf. [1] - correlation with plant water concentration (i.e., chlorophyll content). [2] - correlation with LAI * Chlorophyll *a* concentration. [3] – correlation with disease severity on plants treated with fungicide. RSA – Russian wheat aphid. ns – not significant. Plant and disease scientific names can be found on Table 1.

Chlorophyll

Using a portable radiometer measuring four bands similar to the Landsat TM satellite (from blue to NIR) placed 1m over rice canopies, Indian researchers Choubey and Choubey (1999) found a relationship between both NDVI (r=0,65) and NIR/R (r=0,78) and chlorophyll concentration. NDVI was insensitive when chlorophyll concentration was about 7,0 mg/l. Lichtenthaler *et al.* (1996) proposed the index ratios R_{750} / R_{550} and R_{750} / R_{700} which proved to be sensitive indicators for a wide range of concentrations (r>0,96, linear relation). Similar results were reached by Gitelson and Merzlyak (1996, 1997). With these ratios they developed the following regressions:

$$\text{Chlorophyll } a + b \ (\mu g/cm^2) = -12,5 + 13,29 * R_{750} / R_{700} \qquad \text{Equation 1}$$

$$\text{Chlorophyll } a + b \ (\mu g/cm^2) = -12,7 + 13,7 * R_{750} / R_{550} \qquad \text{Equation 2}$$

Chlorophyll contents were then calculated using both equations. Predicted values were compared to the analytically measured ones and the result was a strong correlation (r=0,97), with an error in total chlorophyll prediction of less than 4,26μg/cm^2. The authors still tested Green NDVI and NDVI based on the band 690-710nm and found them to be about five times more sensitive to chlorophyll content than the usual NDVI based on red – with error estimations lower than 2μg/cm^2.

Carter (1994) indicated the ratios R_{695} / R_{420} and R_{695} / R_{760} were significantly affected by eight stress agents tested on six vascular plant species (mean correlation values: r=0,88 and r=0,87, respectively).

The development of the VI (R_{850} – R_{710}) / (R_{850} – R_{680}) by Datt (1999) was based on R_{710} as a sensitive indicator of chlorophyll concentration (r=0,89) and on R_{850} as an insensitive one. The ratio was obtained from an improved spectrum which effectively eliminates scattering effects, so that it is mainly dependent on pigment concentration. With its regression against chlorophyll concentration the following algorithms were constructed:

$$\text{Chlorophyll } a \ (\mu g/cm^2) = 1{,}22 * \exp [4{,}596 * (R_{850} - R_{710}) / (R_{850} - R_{680})] \qquad \text{Equation 3}$$

$$\text{Chlorophyll } a + b \ (\mu g/cm^2) = 1{,}88 * \exp [4{,}510 * (R_{850} - R_{710}) / (R_{850} - R_{680})] \qquad \text{Equation 4}$$

Variation caused by background and LAI confounded detection of subtle differences in canopy reflectance as an indicator of chlorophyll concentration (Daughtry *et al.*, 2000). These results were based on a canopy reflectance model (SAIL) that simulated a wide range of background reflectances, LAI and leaf chlorophyll concentrations. Pairs of spectral VI (responsive to both chlorophyll and background and insensitive to background) plotted together produced lines of constant leaf chlorophyll concentration, the slopes of which were linearly correlated to the later. The slope between NIR/Green and NIR/R vs. chlorophyll yielded r=0,99.

Because NIR seems to be less sensitive to stress, it could be measured in broader wavebands than is necessary in the visible region to yield a stress sensitive ratio (Carter, 1994).

Nutrients

The correlation between N content and reflectance measurements is highly dependent on water availability (Schepers *et al.*, 1996). The ratio R_{550} / R_{850} was sensitive to leaf N content in corn water control plants (r=0,99) and not correlated to stressed ones (r=0,14). Similarly, the relation of the ratio with chlorophyll concentration followed this pattern. Assessment of N and chlorophyll status through reflectance measurements must take into account this kind of interaction.

The greenness index was the best to discriminate canopy based treatments of wheat with different N supply (Hinzman *et al.*, 1986). This index takes into account all the six TM bands that were measured with the spectroradiometer. LAI and NDVI showed a strong exponential relation (r=0,96), as well as chlorophyll density and IR/R (r=0,95) and N and IR/R (r=0,91). Chlorophyll density (the product of leaf chlorophyll concentration and LAI) is usually better correlated to IR/R in canopy studies instead of simple chlorophyll concentrations, typical at leaf level.

Adams *et al.* (2000) induced Mn, Fe and Cu deficiencies separately in soybeans. The yellowness index (624, 580, 668nm) was sensitive to total chlorophyll concentrations lower than about 25$\mu g/cm^2$ but insensitive when they were greater than this value. NDVI was more sensitive than R_{750} / R_{650} at lower concentrations of the pigment, while the opposite took place at higher concentrations. R_{750} / R_{550} showed a strong linear correlation to chlorophyll in a wide range of concentrations. The same happened with R_{744} / R_{666} (AVIRIS bands) (Adams *et al.*, 1999).

Ma *et al.* (1996) used a SPAD-502 chlorophyll meter and a LI-3000A leaf area meter in corn with different N treatments. To the product of these variables they called "field greenness". NDVI (800, 600) had much smaller coefficient of variation values than R_{600} but showed stronger correlation with field greenness (r=0,88) than values at any single band and could usually differentiate treatments. Both NDVI and field greenness, measured preanthesis, were correlated with grain yield at harvest.

NPCI and PSR were able to distinguish treatments of wheat submitted to different levels of N fertilization (Filella *et al.*, 1995). The use of R_{430}, R_{550}, R_{680}, λ_{re}, dR_{re} (the amplitude of the red edge in the first derivative of reflectance spectra) and NPCI in a discriminant analysis allowed a clear separation of the N-deficient from the well-fertilized treatments. This multivariate technique integrates information provided by the various spectral indices and this way allowed a better classification than obtained through the use of any of them separately.

Water

The work of Peñuelas *et al.* (1997) with several Mediterranean species of trees and shrubs showed the WI to be a moderate indicator of plant water concentration (r=0,66) and the ratio WI / NDVI a better one (r=0,71). The WI, however, was only sensitive when the drying process of leaves was well in advance.

Diseases

Using a "distinction index" based on a Bayes decision classifier, Sasaki *et al.* (unpublished) found the ratio R_{660} / R_{400} the best to discriminate between healthy and diseased cucumber leaves (infected with the fungus *Colletotrichum orbiculare*).

Red edge and derivative spectra

Vegetation indices as the NDVI and also ratios of the form NIR/R make indirectly use of the so called "red edge". This feature, characteristic of plants, is better analysed through the first or second derivative spectra. This way it is possible to identify precisely the inflection point of the slope (usually called in literature "λ_{re}"). Also important can be the shape of the curve (on the first derivative spectra), its height and width (Boochs *et al.*, 1990). However, as a good analysis of the red edge involves the calculation of the derivative spectra, it can be an significant drawback and, perhaps, render impossible some remote sensing applications.

Some authors stress the advantages of the derivative spectra, which enhance variation in reflectance (Boochs *et al.*, 1990). On the other side, noise is also increased, possibly requiring additional computation such as averaging – diminishing the spectra resolution. It is important to note that, while "zero-order" spectrum changes smoothly, with little noise, first and second order ones vary abruptly (the higher the order is the more the spectrum changes). This way, a supposedly sensitive band can easily become insensitive, provided that the reflectance spectra has changed enough – which can be just a little. That is, results are less versatile and trustful. Hence, the gain is not so obvious and the disadvantages must be considered.

Gitelson *et al.* (1996), Lichtenthaler *et al.* (1996) and Gitelson and Merzlyak (1997) found a strong correlation between R_{700} and λ_{re}, which in turn were hyperbolically correlated to leaf chlorophyll concentration (r=0,99 and r=0,95 for both in the second and third papers, respectively). This way the red edge analysis may become unnecessary. However, the variation of λ_{re} was found small for yellow-green to dark green leaves of maple and horse chestnut trees. Experiments performed by Horler *et al.* (1983) showed strong correlation between R_{540} and λ_{re} (r=0,842) and between λ_{re} and total leaf chlorophyll content for a number of species (e.g., corn: r=0,84, barley: r=0,74, winter wheat: r=0,92). For wheat, a variation of 10nm in λ_{re} was accompanied by a variation of 23 $\mu g/cm^2$ in chlorophyll content (day 181). On day 225 after sowing, the proportion was 5 and 38, respectively, showing a decrease of response to relative chlorophyll degradation.

Filella *et al.* (1995) carried out experiments on wheat with five levels of N fertilization. They found a linear relationship between three red edge parameters (λ_{re}, amplitude in the first derivative spectra, and sum of the amplitudes between 680 and 780nm in the same spectra) and canopy chlorophyll *a* concentration (the product of leaf concentration by LAI). Correlation values ranged from 0,849 to 0,878 and were higher than obtained through NDVI.

The maximum of the first derivative of reflectance in the green, near 525nm, correlated with chlorophyll and nitrogen concentration of sunflower leaves (r=0,704 and r=0,592, respectively) (Peñuelas *et al.*, 1994).

The first derivative spectra yielded better correlations to leaf percentage infection, in the visible region, than normal spectra (Malthus and Madeira, 1993). The experiment was carried out on field beans infected with *Botrytis fabae*. Maximum sensitivity was found at 525, 580, 700-760 and 780-940nm ($r \approx 0,9$). The red edge location was less correlated to infection ($r=0,57$) which is consistent with the fact that infection was not significantly correlated to chlorophyll *a* content. Second order derivative produced poorer results.

Yoder and Pettigrew-Crosby (1995) computed first difference transformations of leaf and canopy spectra of bigleaf maple as follows: $R'_\lambda = (R_{\lambda+4} - R_{\lambda-4}) / 8$. First difference transformations of log $(1 / R_\lambda)$ generally yielded better correlations between this variable and chlorophyll or N concentration than simple reflectance.

Stress detection moment

Carter (1996) detected herbicide stress on pine trees 16 days before they became visible measuring reflectance at 694nm or its ratio with R_{760}. Trenholm *et al.* (2000) could detect herbicide or N-stress in two turfgrass species by spectral data in the visible range prior to or simultaneously with perceptible symptoms.

Despite this promising results, Lorenzen and Jensen (1989), studying barley leaves infected by mildew, could only detect spectral changes three days after symptoms were already visible, pustules covering 30-50% of leaf area. Similarly, Malthus and Madeira (1993) were not able to detect spectral changes in field beans inoculated with *Botrytis fabae* before symptoms were evident.

3. Research objectives and methods

Remote sensing is, nowadays, a field of major importance. Countless applications were already developed and many more will be. The growing attention of industry and research is a proof of that. Remote sensing applications in agriculture are also rising.

3.1. Objectives

The present research will test the feasibility of visual and NIR spectrography to distinguish stressed from non-stressed leaves under greenhouse conditions.

More specifically, the objectives are:

- to evaluate the effect of nutrient shortage and diseases on leaf spectra, thereby identifying the spectral regions more suitable for discrimination purposes;
- to investigate to which extent can different treatments be discriminated on the basis of reflectance measurements:
    - nutrient deficient and well nourished leaves;
    - diseased and non-diseased leaves;
    - leaves infected with different diseases;
    - non-diseased and leaves infected with different diseases;
- to compare windowed data with averaged data;
- to assess the robustness of the discriminating model.

3.2. Materials and methods

A few plant treatments involving nutrient shortage and diseases were performed. Leaf spectra were then obtained through reflectance measurements under controlled conditions. Before data could be analysed it had to be processed (i.e., normalized) in order to minimize external influences.

Equipment

Reflectance spectra of leaves were obtained using an Imspector V9 spectrograph mounted on a visual monochromatic CCD camera (Digiteyes) (Figure 3). The spectrograph measures, along a line, the intensity of the light reflected by the leaves from about 430 to 900nm in bands 7nm wide. A Computar 3.6mm ½″ C-mount objective, with manual iris and focus control, was attached. A 50% reflection reference

(Spectralon SRS 50-010, 1.25″ diameter, constant over the range 300-2500nm) was used to calibrate all measurements.

The equipment was linked to a laptop through a NI-IMAQ 1408 measurement card. An easy to use Labview (National Instruments, 1999) program permitted a rapid image saving.

Figure 3 – Imspector V9 spectrograph mounted on a visual monochromatic CCD camera.

Measurements

Intact leaves (non-excised, adaxial surface up), usually 10 to 14 each time, were carefully placed over a black board 1m by 10cm. The reflectance reference was positioned roughly at the middle of the board. The spectrograph measured a line along the length of the board from a constant distance of about 1m. Because of this, each leaf resulted in several observations (typically from 40 to 60). As the enlightenment from the sun was generally not enough to obtain good measurements (with high signal-to-noise ratio), it was additionally provided by two halogen lamps.

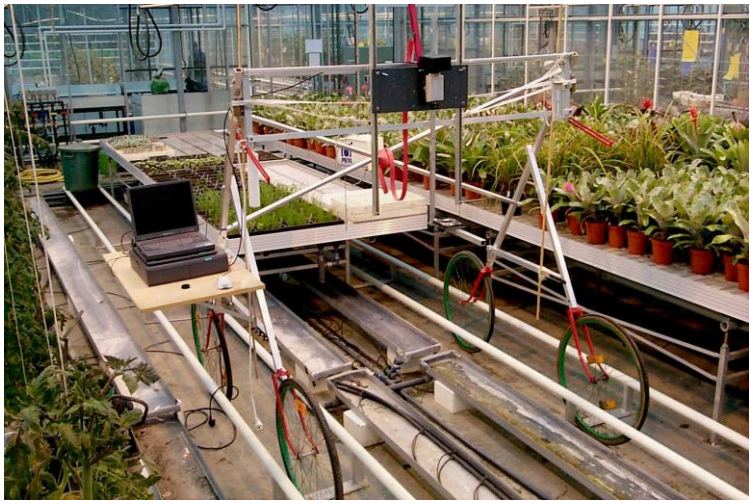

Figure 4 – Apparatus used in Leuven to carry out measurements. The "buggy" slid over the plants allowing an efficient and fast work.

Data normalization[5]

Each unprocessed image consisted of a 480 by 640 pixels grayscale (255 levels) bitmap, loaded into Matlab software (The MathWorks, Inc., 1999) as a matrix. Thus, it contained 480 observations for 640 variables (spectral range provided by the spectrograph but at the resolution of 0.75nm).

To obtain reflectance based images, the value of each pixel was divided by the corresponding value of the reference, and multiplied by 50%. Reference observations were, as a result, assigned the value of 0.5. A further normalization for enlightenment differences was performed. If *B* is the output matrix and *A* is the original one:

$$B(s,\lambda) = 50\% \times \frac{\sum_{i=400nm}^{900nm} A(ref,\lambda_i)}{\sum_{i=400nm}^{900nm} A(s,\lambda_i)} \times \frac{A(s,\lambda)}{A(ref,\lambda)} \qquad \text{Equation 5}$$

where s = row index on the observation axis;
λ = wavelength;
ref = row index of the reference on the observation axis.

All non-leaf observations (including the reference) were removed using the NDVI (740-760, 640-620). Matrix rows were deleted when the NDVI was lower than 0.35. This value was found to be precise enough to eliminate all undesirable values while maintaining observations of stressed leaves.

Windowing

Because the resolution of the image is higher than that of the spectrograph, the "raw" data are too noisy. Therefore, a median filtering was performed with a moving window 8nm wide. If C is the resulting matrix (Figure 5):

$$C(s,\lambda_i) = \frac{\sum_{i}^{i+7} B(s,\lambda_i)}{8} \qquad \text{Equation 6}$$

where s = row index on the observation axis;
λ_i = *i*th wavelength (from 1 to 633).

[5] - A Matlab file was built for this purpose and its code is shown in the Annex.

Averaging

For comparison purposes, in chapter 4.3 data was also averaged by bands 8nm wide, therefore reducing the amount of data. If C is the resulting matrix:

$$C(s, \lambda_i) = \frac{\sum_{i}^{i+7} B(s, \lambda_i)}{8} \qquad \text{Equation 7}$$

where s = row index on the observation axis;
λ_i = *i*th wavelength (from 1 to 633 in steps of 8).

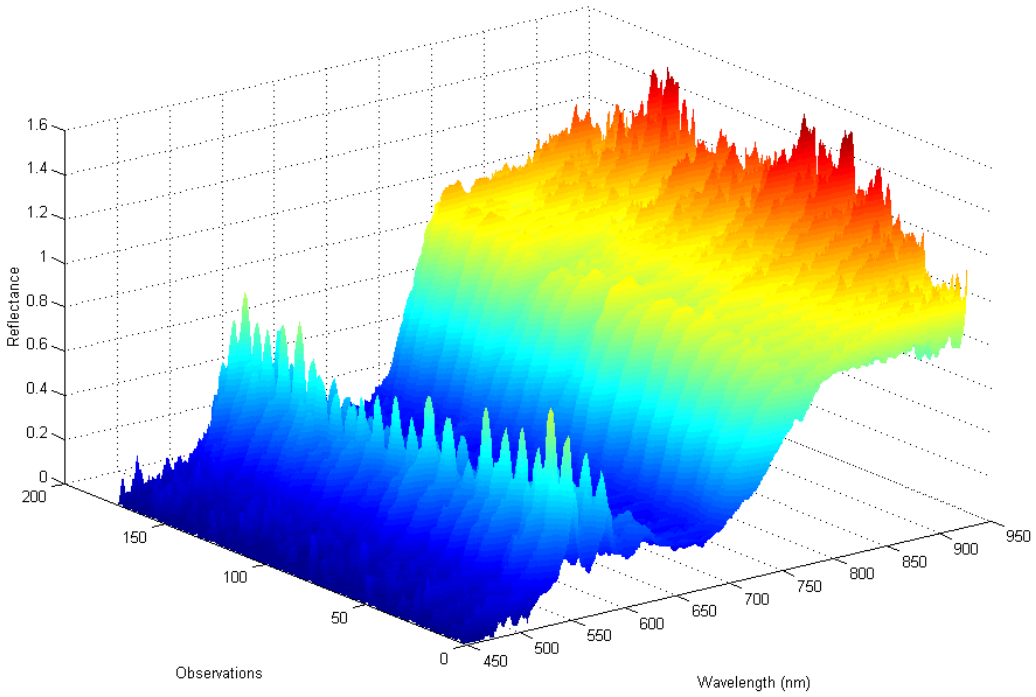

Figure 5 – Data resulting from windowed normalization. Only leaf spectra are, therefore, shown.

Data analysis

Resulting matrices from the same treatment were then concatenated and given a treatment code in a new column, forming this way a new, class assignment, variable.

Spectra from the long matrices to be compared were randomly assigned to one of two datasets: a *train* dataset (with 75% of the observations) and a *test* dataset (with the remainder 25%). As a result, both the train and the test datasets contain spectra from all the considered treatments. Since the correlation within the spectra is high, using the complete spectra for discrimination between treatments is not useful. The SAS

procedure *STEPDISC* (SAS Institute Inc., 1996) is able to find the most suitable wavebands for that purpose and sorts them according to their relevance.

STEPDISC was run on the train dataset. This stepwise selection first selects the variable that is expected to be the best discriminator among the available (not yet selected) variables. Before choosing another variable, a test is run to check if all of the previously selected variables remain significant. Non-significant variables are removed. The procedure stops when no new variables can be entered or removed from the discriminant model (Vrindts, 2000).

The selected wavebands were then used to draw up a classification criterion using the SAS procedure *DISCRIM*, which was run on both train and test datasets. The procedure automatically develops a discriminant criterion to classify each observation into one of the treatments.

It must be emphasised that the spectra in both datasets are not independent, because each leaf account for several observations, which in turn may have been allocated to different datasets.

Plant treatments

The research was made on plants grown under greenhouse conditions and comprised two different sets of treatments:

- nutrient stress, on barley, carried out in Leuven;
- disease stress, on wheat, carried out in Louvain-la-Neuve.

Nutrient stress, Leuven

Six nutrient treatments were tested (Table 8). Each of them was performed on six circular pots with a diameter of about 15cm, filled with a bed of rock wool. Each pot, in turn, contained about 7 to 9 barley plants, which were sown on 8 November, 2000.

Table 8 – The nutritional composition of the various treatments performed. Ion concentrations are expressed in mmol/L.

Name	**Interpretation**	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**NO_3^-**	**$H_2PO_4^{2-}$**	**SO_4^{2-}**	**Trace elements**
C1	Control	5.5	2.75	0.75	8.625	1.125	1.375	Yes
LowN	Low nitrogen	5.5	0	0.75	3.125	1.125	1.375	Yes
C2	Control	5.5	2.75	0.75	8.625	1.125	1.375	Yes
OnlyN	Only nitrogen	0	2.75	0	5.5	0	0	No
C3	Control	5.5	2.75	0.75	8.625	1.125	1.375	Yes
W	Only water	0	0	0	0	0	0	No

Disease stress, Louvain-la-Neuve

Two common wheat diseases were accessed: *Septoria tritici* (septoria leaf blotch, Figure 6) and *Puccinia striiformis* (yellow rust, Figure 7). A detailed description of the treatments can be found on Table 9. Each treatment was performed on 3 trays. The plants were sown on 27 October, 2000. *S. tritici* was inoculated on 1 December, 2000, and *P. striiformis* on 29 November, 2000. Quinoxyfen was applied in half of treatments to prevent the appearance of mildew, which could undermine or falsify the results.

Table 9 – Performed treatments and inoculated diseases.

Name	**Interpretation**
ST	Control
SS	*Septoria tritici*
SQT	Control treated with quinoxyfen
SQS	*Septoria tritici* treated with quinoxyfen
RT	Control
RR	*Puccinia striiformis*
RQT	Control treated with quinoxyfen
RQR	*Puccinia striiformis* treated with quinoxyfen

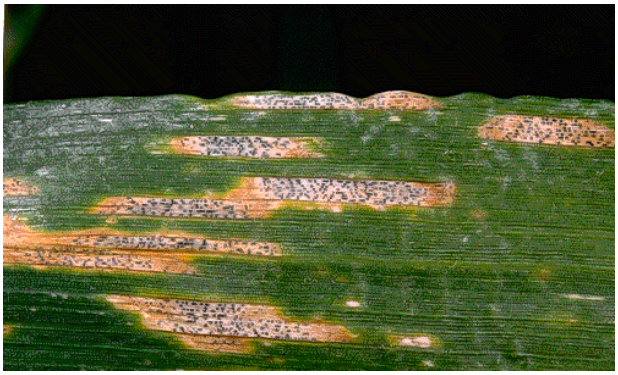

Figure 6 – A wheat leaf infected with septoria leaf blotch.

Figure 7 – A leaf infected with yellow rust.

4. Results and discussion

Results and discussion will be separated according to the primarily stress agent, or between measurements performed in Leuven and Louvain-la-Neuve. All data in sections 4.1 and 4.2 were obtained by the windowing method, whereas in chapter 4.3 a comparison between this method and the averaged one was made.

4.1. Nutrient stress, Leuven

A concise timetable showing the evolution of the experiment is shown on Table 10.

Table 10 – Development of the experiment.

Date	Experiment evolution
8/11/2000	Sowing
11/11/2000	Germination
20/11/2000	First visible symptoms of stress
12/12/2000	Measurements
14/12/2000	Measurements
18/12/2000	Measurements
17/01/2001	Measurements

Results are summarized in the following paragraphs. For each combination of treatments, the most discriminating wavebands as obtained from SAS and the lowest misclassification error are shown. The mean spectra for each treatment and respective standard deviation are also presented.

12 December, 2000

Measurements on 12 November were not analysed since the mean spectra of the controls were significant different from each other (Figure 8). Thus, the results were not reliable.

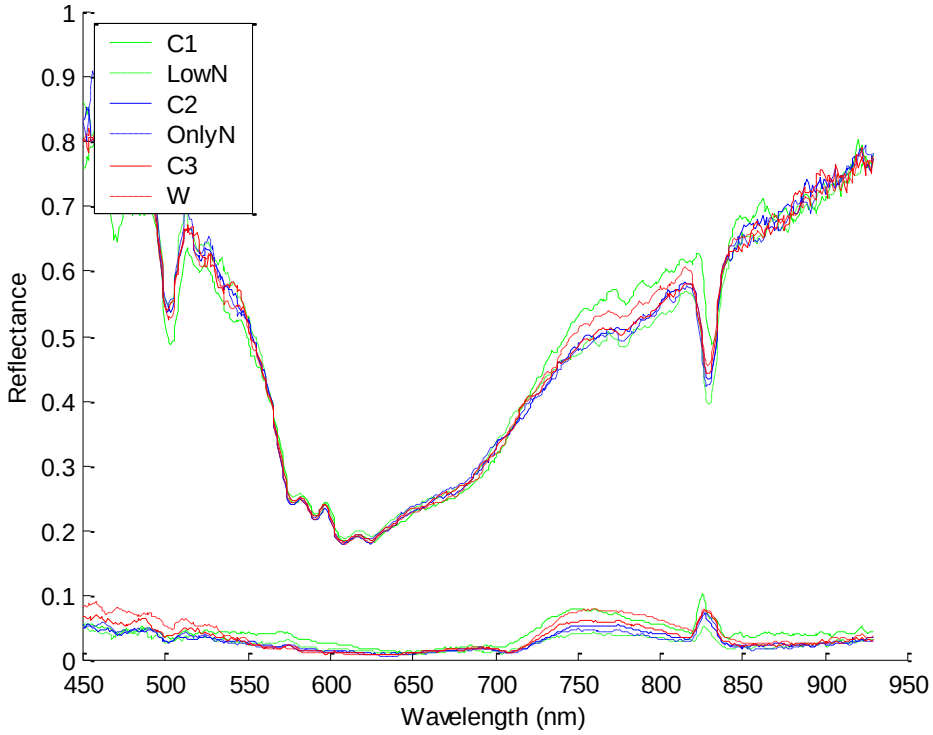

Figure 8 – Mean spectra for each treatment. Differences between controls are significant. Lower curves represent the standard deviation of the population.

14 December, 2000

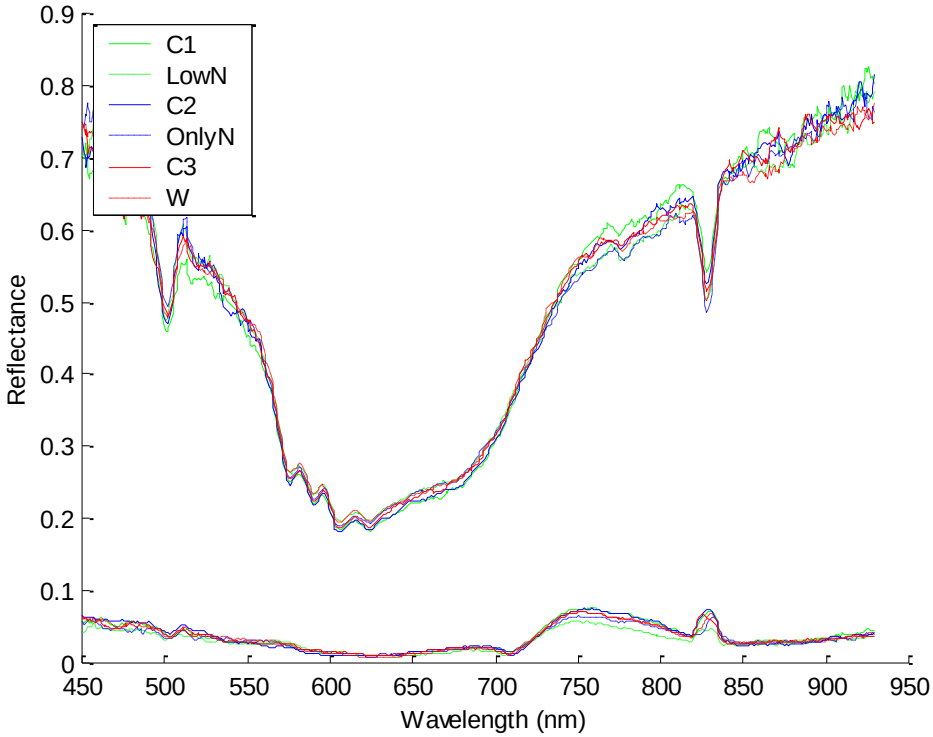

Figure 9 – Mean spectra for each treatment. Lower curves represent the standard deviation of the population.

Table 11 – Most discriminating wavelengths (nm) of indicated treatments, sorted by order of relevance. The lowest misclassification error shown, achieved with the number of wavelengths in parenthesis, is the same for both the test and train datasets.

	C1, LowN	C1, OnlyN	C1, W	C1, LowN, OnlyN	C1, LowN, OnlyN, W
Lowest error	0 (6)	0 (13)	0 (6)	0 (14)	0 (12)
Wavelength order					
1	860	455	863	860	860
2	856	926	873	856	856
3	911	906	924	852	863
4	919	482	664	849	864
5	879	667	840	455	865
6	881	893	455	453	455
7		881		461	924
8		863		846	456
9		855		924	906
10		883		906	908
11		895		908	460
12		839		666	486
13		926		870	
14				452	

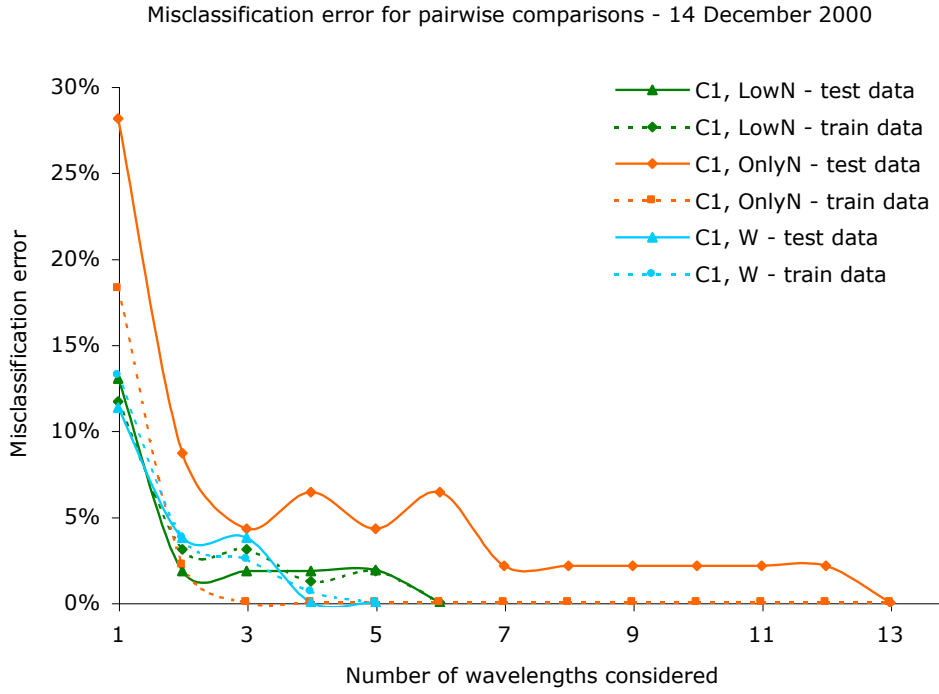

Figure 10 – General misclassification error for pairwise comparison obtained according to the number of wavelengths considered.

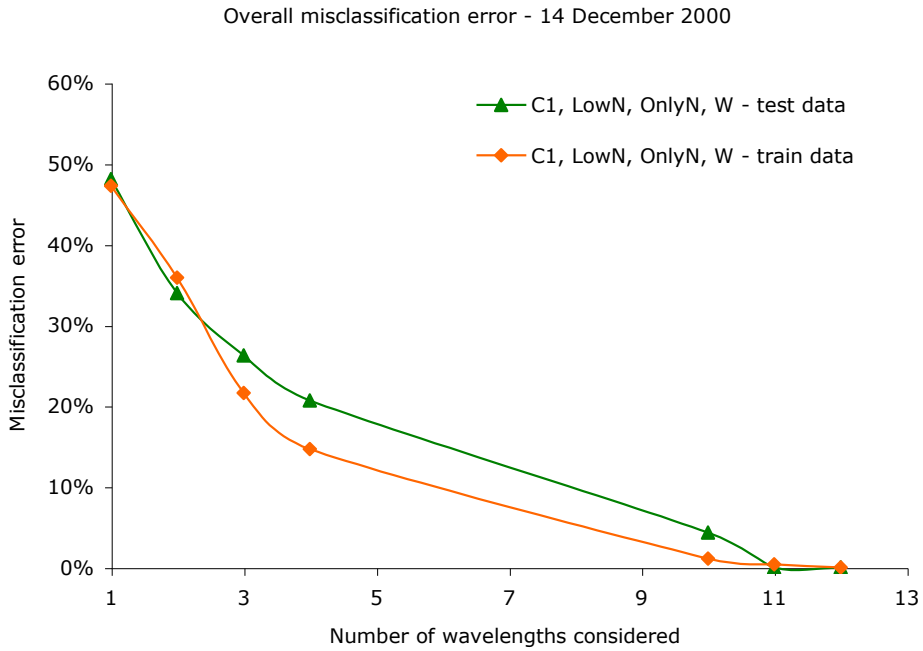

Figure 11 – Overall misclassification error obtained according to the number of wavelengths considered. The discrimination is between one control and all nutrient deficient treatments.

18 December, 2000

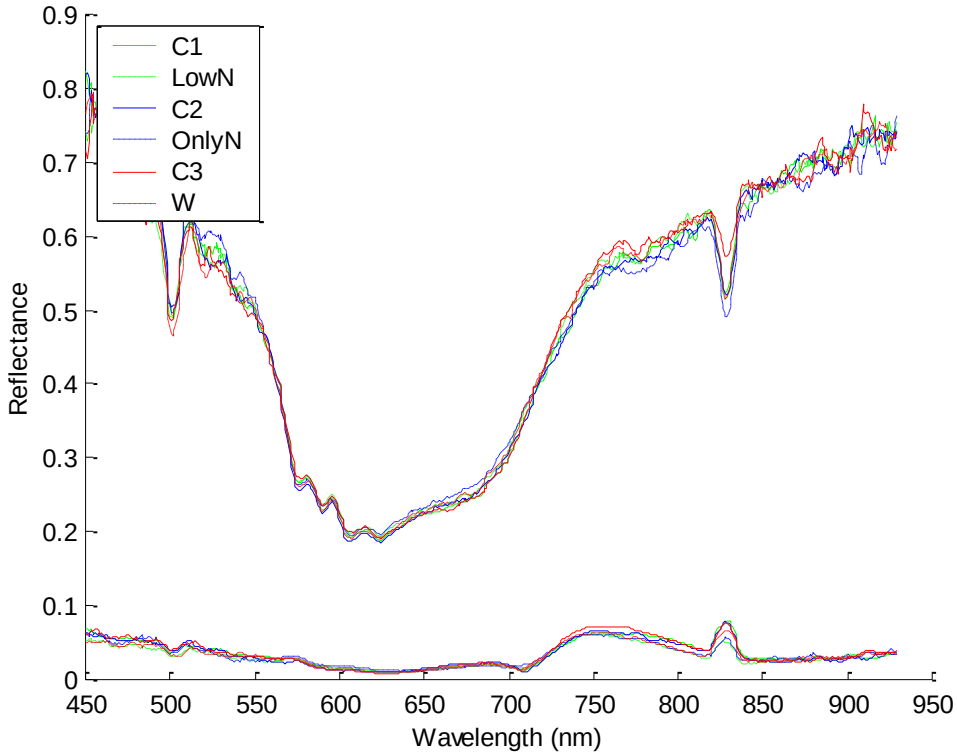

Figure 12 – Mean spectra for each treatment. Lower curves represent the standard deviation of the population.

Table 12 – Most discriminating wavelengths (nm) of indicated treatments, sorted by order of relevance. The lowest misclassification error shown, achieved with the number of wavelengths in parenthesis, is the same for both the test and train datasets.

	C1, LowN	**C1, OnlyN**	**C1, W**	**C1, LowN, OnlyN**	**C1, LowN, OnlyN, W**
Lowest error	0 (9)	0 (5)	0 (4)	0 (10)	0 (14)
Wavelength order					
1	470	905	505	906	470
2	473	899	695	913	473
3	694	680	815	899	505
4	451	468	809	914	695
5	461	488		915	493
6	794			490	482
7	497			857	494
8	792			451	491
9	497			768	491
10				751	452
11					452
12					451
13					456
14					455

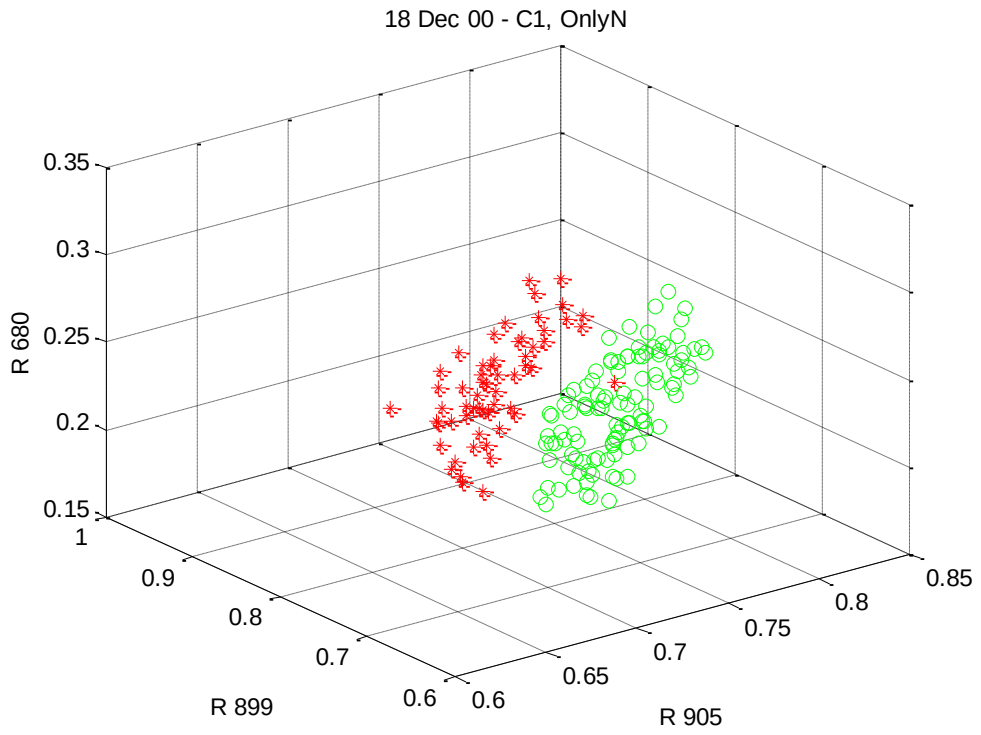

Figure 13 – Example of the discrimination between treatments C1 (green "○") and OnlyN (red "❄") using 3 wavelengths.

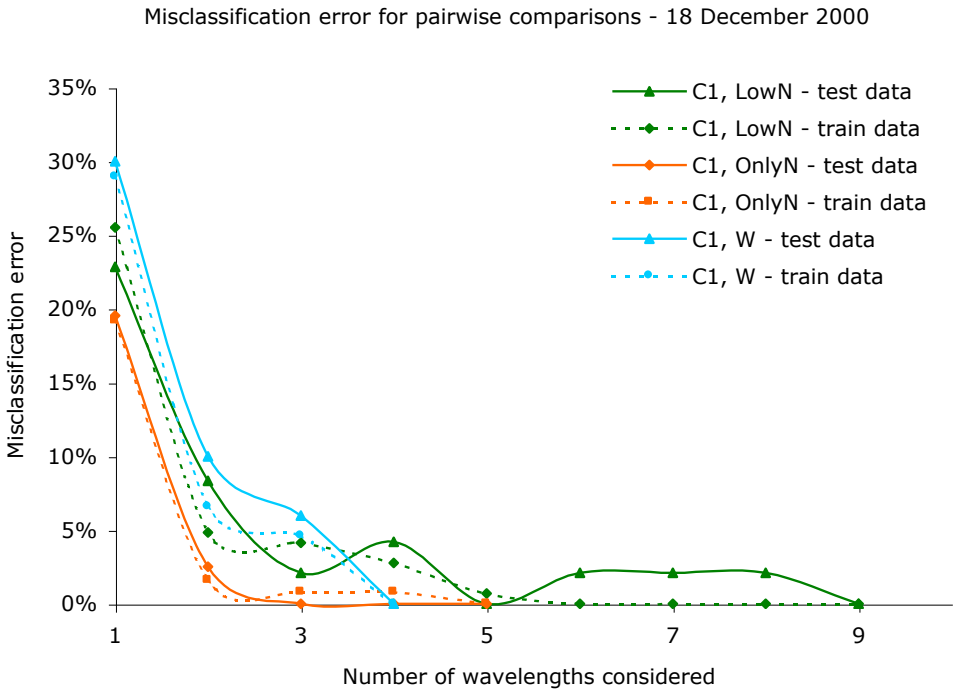

Figure 14 – General misclassification error for pairwise comparisons obtained according to the number of wavelengths considered.

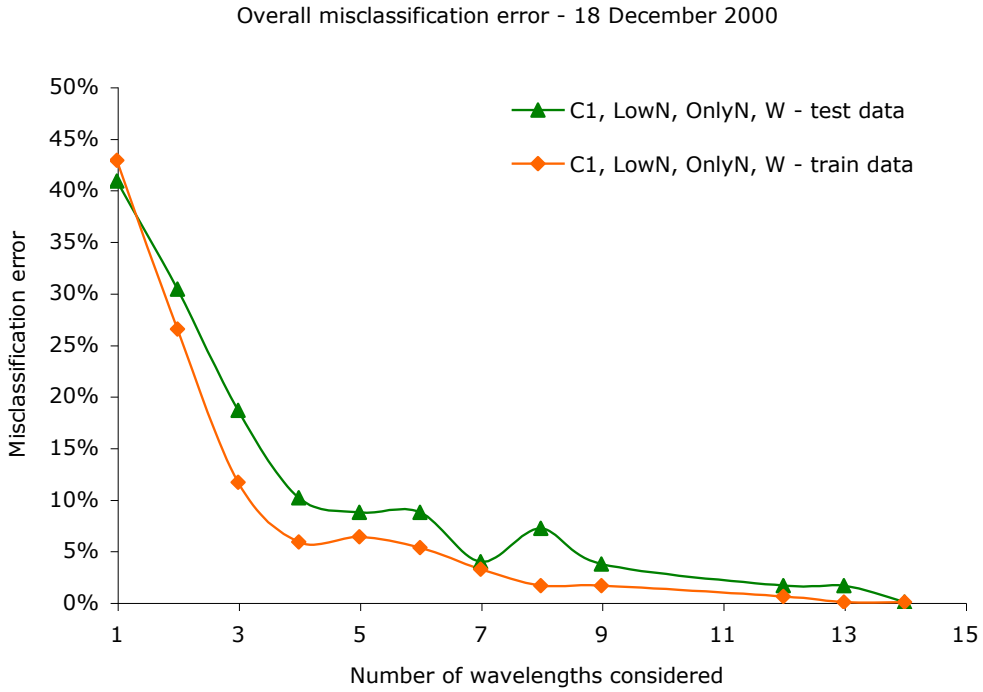

Figure 15 – Overall misclassification error obtained according to the number of wavelengths considered. The discrimination is between one control and all nutrient deficient treatments.

17 January, 2001

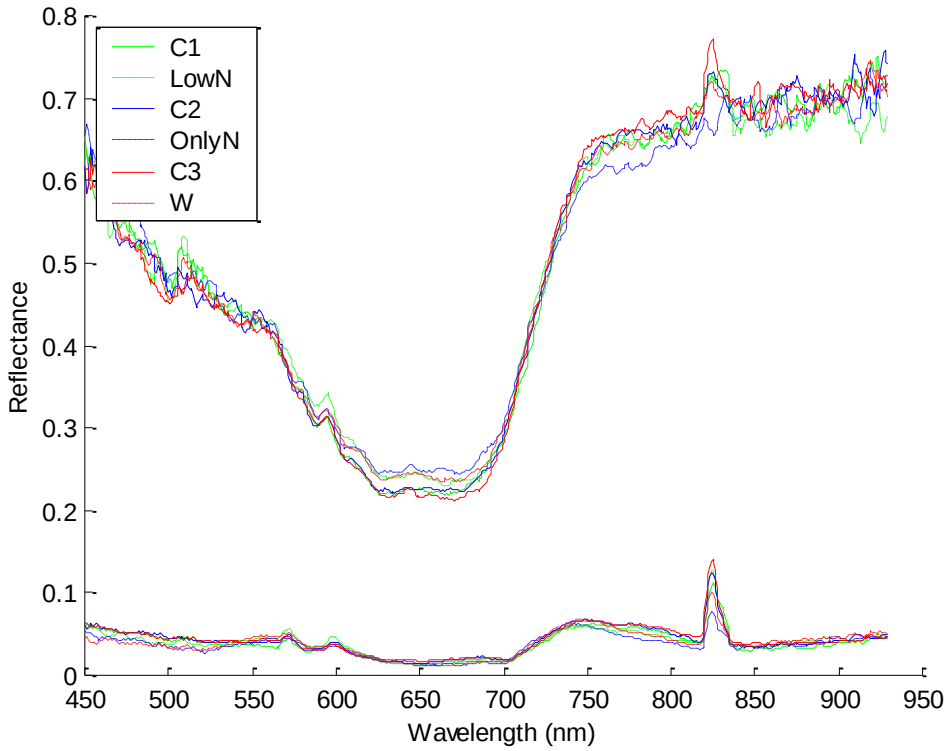

Figure 16 – Mean spectra for each treatment. Lower curves represent the standard deviation of the population.

Table 13 – Most discriminating wavelengths (nm) of indicated treatments, sorted by order of relevance. The lowest misclassification error shown, achieved with the number of wavelengths in parenthesis, is the same for both the test and train datasets.

	C1, LowN	**C1, OnlyN**	**C1, W**	**C1, LowN, OnlyN**	**C1, LowN, OnlyN, W**
Lowest error	0 (5)	0 (4)	0 (6)	0 (7)	0 (12)
Wavelength order					
1	914	644	651	662	662
2	891	846	791	509	509
3	673	859	794	492	492
4	887	452	886	457	452
5	884		865	906	451
6			855	891	466
7				887	453
8					477
9					476
10					494
11					912
12					911

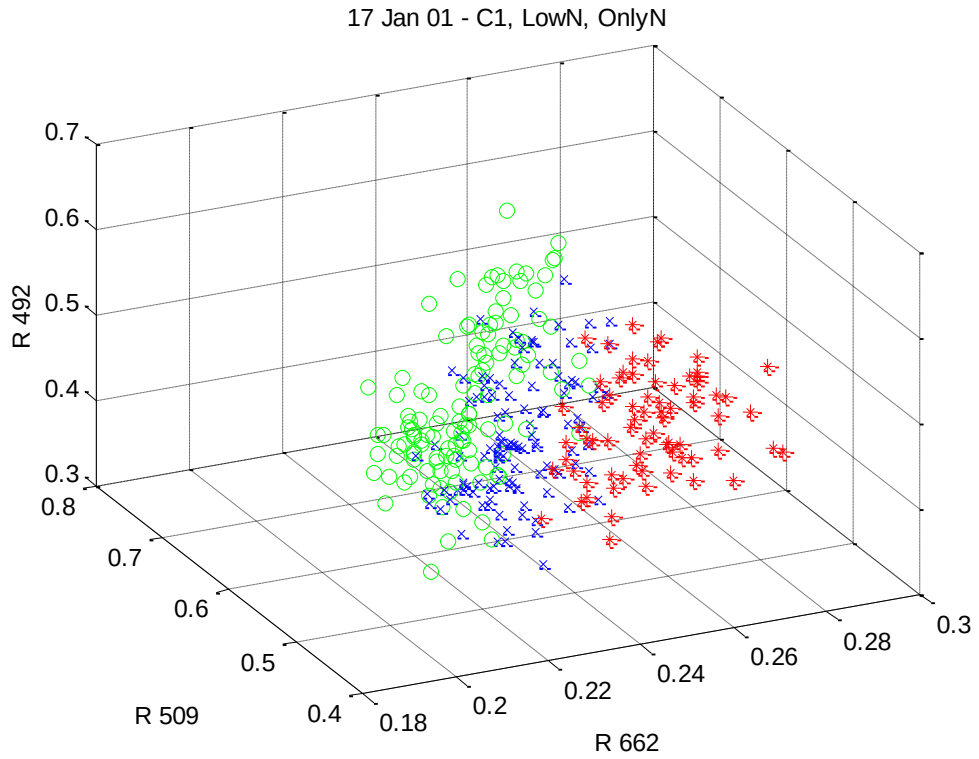

Figure 17 – Example of the discrimination between treatments C1 (green "○"), LowN (red "❄") and OnlyN (blue "×") using 3 wavelengths.

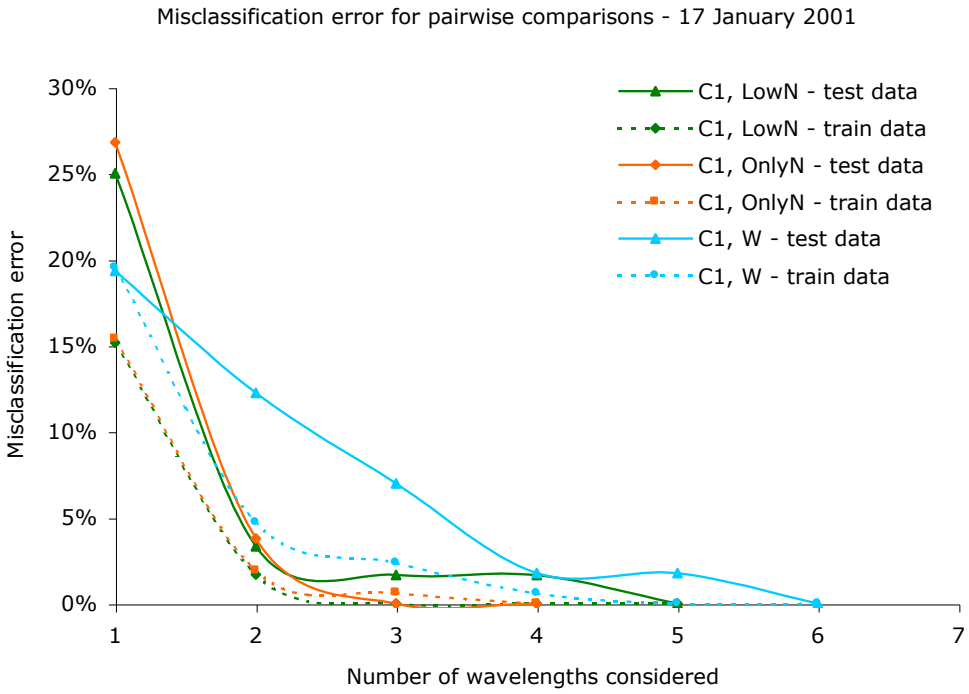

Figure 18 – General misclassification error for pairwise comparisons obtained according to the number of wavelengths considered.

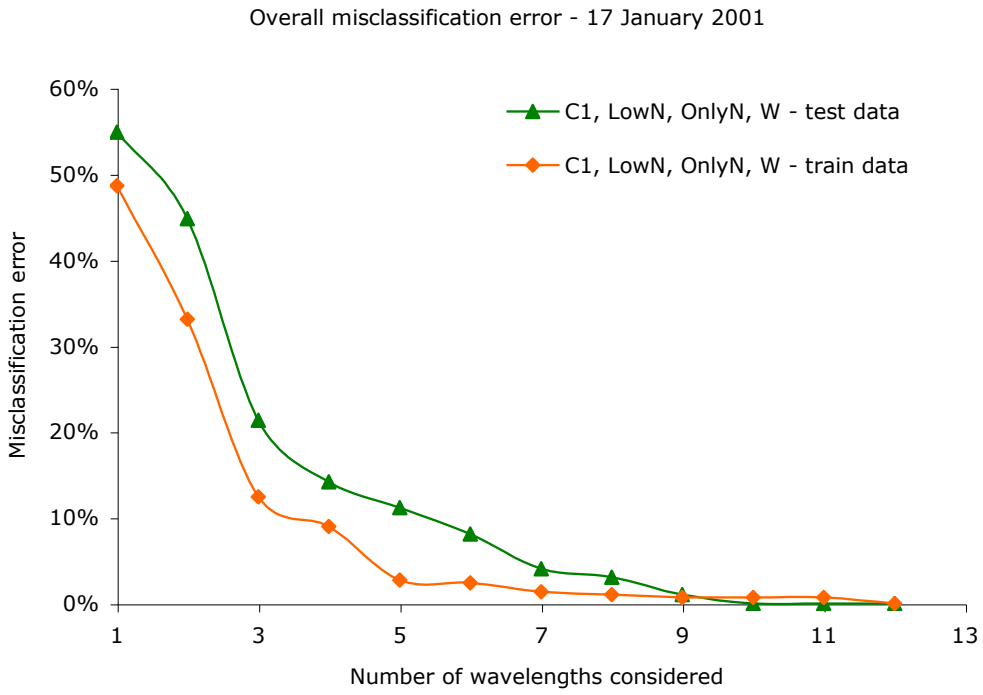

Figure 19 – Overall misclassification error obtained according to the number of wavelengths considered. The discrimination is between one control and all nutrient deficient treatments.

Discussion

The influence of nutrient stress on leaf spectra is readily seen in Figure 16, where stress is more developed. In the region 600-700nm the reflectance of all the controls is lower than that of nutrient deprived plants. In the NIR the inverse occurs, which is in agreement with literature.

Perfect discrimination between treatments was always obtained, that is, *all* observations were correctly classified by SAS using a certain number of wavebands – even between each kind of nutrient stress (low N, only N and water only) and the control. So, for each group of measurements, it is theoretically possible to develop a discriminating model based on the obtained wavebands.

Analysis of Figure 20 shows, however, that the most discriminant wavelengths were not always the same. In fact, they varied according to the measurement date. This is probably due to changes in leaf structure and composition which affected spectra in different ways. While in 14 December most of best variables concentrated around the band 856-865nm and 900nm, just 4 days later, on 18 December, they changed to the region 470-490nm. It is difficult to access what may have caused this alteration in such a little time, specially when symptoms were visible since 20 November. Strangely, the 1 month interval until the following measurement, in 17 January, caused a smaller change

in the most discriminating variables. As a result, it is not possible to determine exactly which are the best wavelengths for classification purposes, although results suggest that the band 450-500nm is suitable when stress is well developed. This roughly corresponds to the blue region, where chlorophyll *a* strongly absorbs.

Generally, results contradict literature, where the bands around 550nm and 710nm were often the most sensible to chlorophyll concentration decreases and, therefore, nutrient stress (see Table 5). The 450-500nm band is still strongly pigment dependent, but because chlorophyll absorption is very high, its concentration must decline severely before spectral changes are significant.

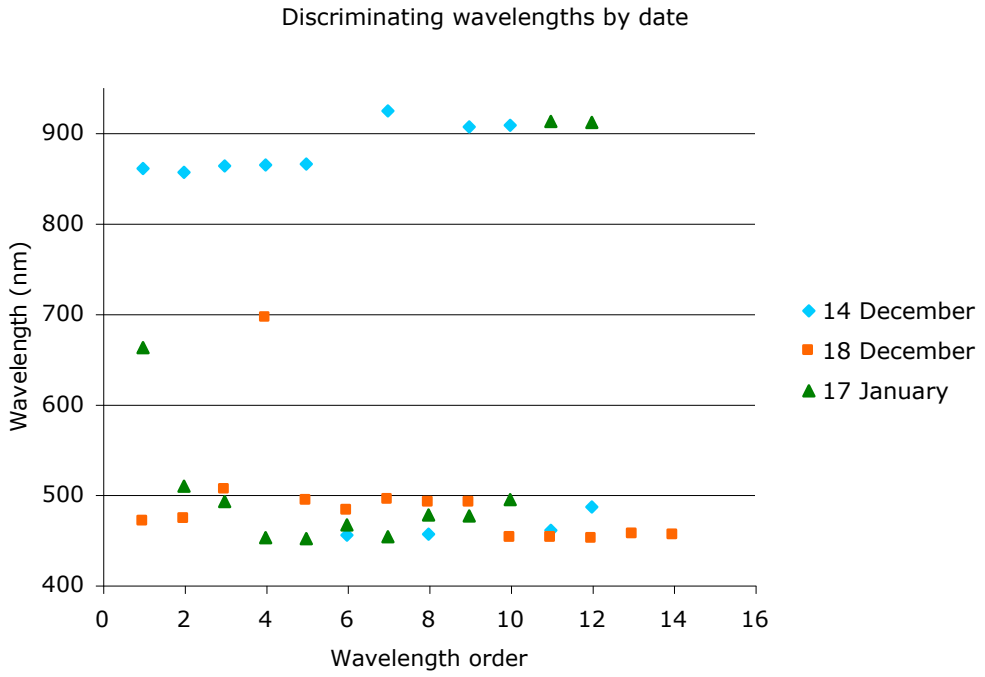

Figure 20 – Distribution of the most discriminating wavelengths of the combination C1, LowN, OnlyN and W, for each measurement date.

A sliding window 8nm wide was used to reduce the noise (see 3.2, "Data normalization"). Figure 20 also evidences that averaging reflectance across the spectrum, perhaps in 8nm wide bands, should also yield good results. Averaging would decrease the number of variables and increase the signal-to-noise ratio. It is important, though, that the averaged band is not too large, for it could ignore important narrow-band information.

4.2. Disease stress, Louvain-la-Neuve

A concise timetable showing the evolution of the experiment is shown on Table 14.

Table 14 – Development of the experiment.

Date	Experiment evolution
27/10/2000	Sowing
30/10/2000	Germination
29/11/2000	Inoculation of *Septoria tritici*
1/12/2000	Inoculation of *Puccinia striiformis*
4/12/2000	Measurements
8/12/2000	First visible symptoms of *P. striiformis*
11/12/2000	Measurements
16/12/2000	First visible symptoms of *S. tritici*

Results are summarized in the following paragraphs. For each combination of treatments, the most discriminating wavebands as obtained from SAS and the lowest misclassification error are shown. The mean spectra for each treatment and respective standard deviation are also presented.

4 December, 2000

Measurements of 4 December were not analysed since no significant differences between the spectra of treatments were observed (Figure 21).

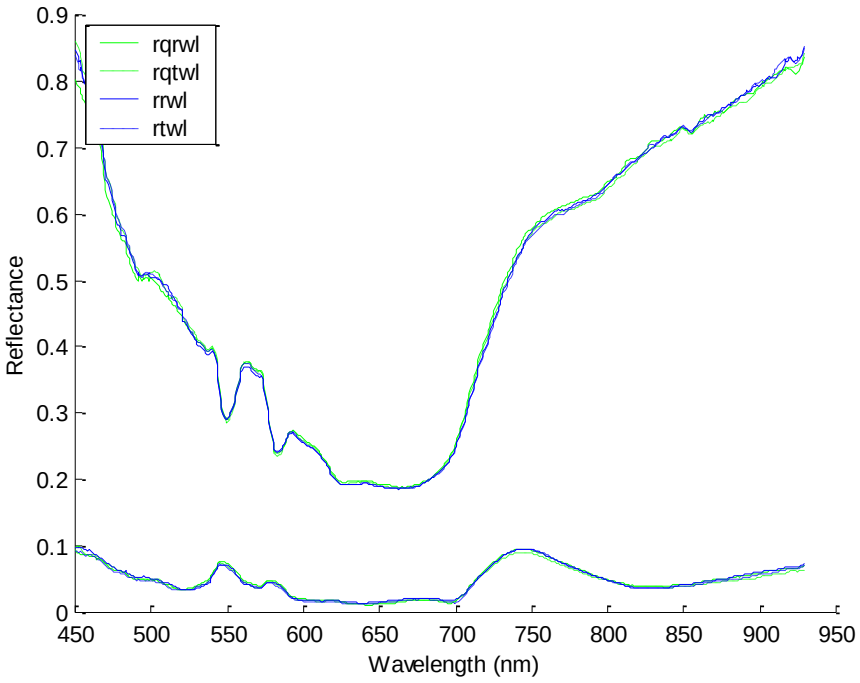

Figure 21 – Mean spectra for each treatment. Differences between treatments are not significant. Lower curves represent the standard deviation of the population.

11 December, 2000

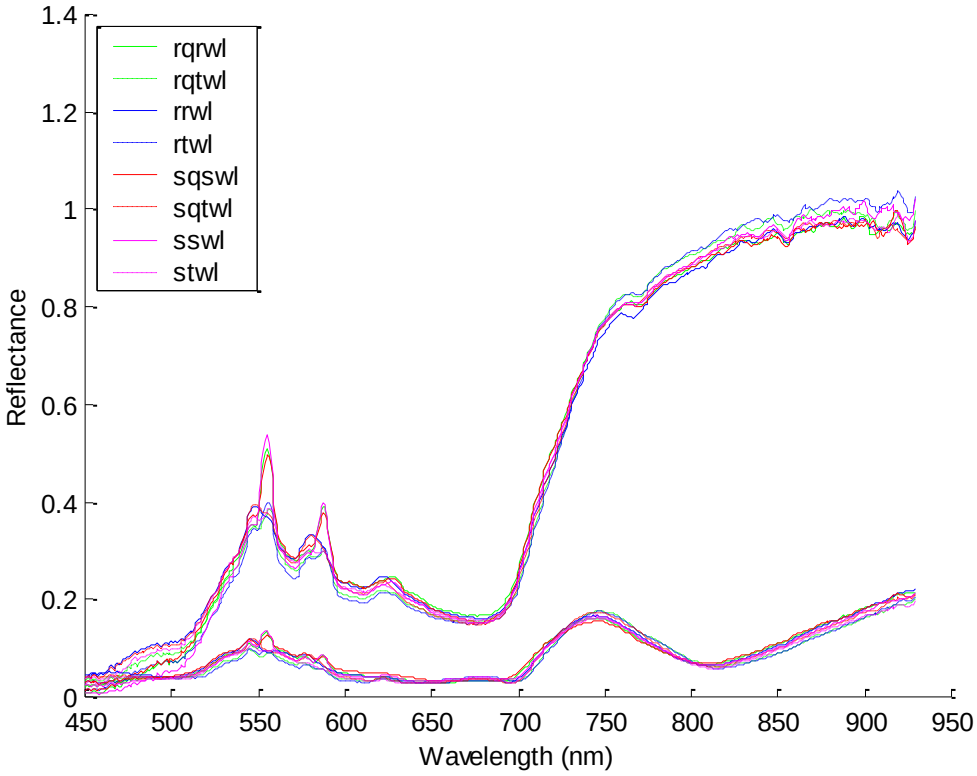

Figure 22 – Mean spectra for each treatment. Lower curves represent the standard deviation of the population.

Table 15 – Most discriminating wavelengths (nm) of indicated treatments, sorted by order of relevance. The lowest misclassification error, achieved with the number of wavelengths in parenthesis, is shown for both the test and train datasets.

	SS, ST	**SQS, SQT**	**RR, RT**	**RQR, RQT**
Lowest error (%)	Test: 0 (14) Train: 0.44 (14)	Test: 0.67 (13) Train: 0.22 (13)	Test: 5.66 (15) Train: 2.46 (15)	Test: 2.71 (14) Train: 2.62 (14)
Wavelength order				
1	494	556	473	588
2	587	551	614	583
3	592	559	590	703
4	584	549	584	592
5	546	577	592	907
6	702	562	842	693
7	586	587	580	819
8	544	488	572	903
9	549	550	588	810

10	553	569	556	809
11	548	583	560	898
12	542	546	563	892
13	559	548	553	847
14	622		855	840
15			858	

Table 16 – Most discriminating wavelengths (nm) of indicated treatments, sorted by order of relevance. The lowest misclassification error, achieved with the number of wavelengths in parenthesis, is shown for both the test and train datasets.

	SQS, RQR	**SS, RR**	**SQS, RQR, SQT**
Lowest error (%)	Test: 10.73 (15), 6.72 (25) Train: 8.55 (15), 3.15 (25)	Test: 0.34 (11) Train: 0 (11)	Test: 14.17 (15), 7.87 (25) Train: 14.18 (15), 7.32 (25)
Wavelength order			
1	677	492	481
2	669	556	589
3	765	551	583
4	761	559	583
5	810	548	592
6	816	578	702
7	805	711	581
8	803	863	577
9	794	562	547
10	785	586	550
11	554	620	546
12	885		553
13	903		549
14	883		548
15	907		549

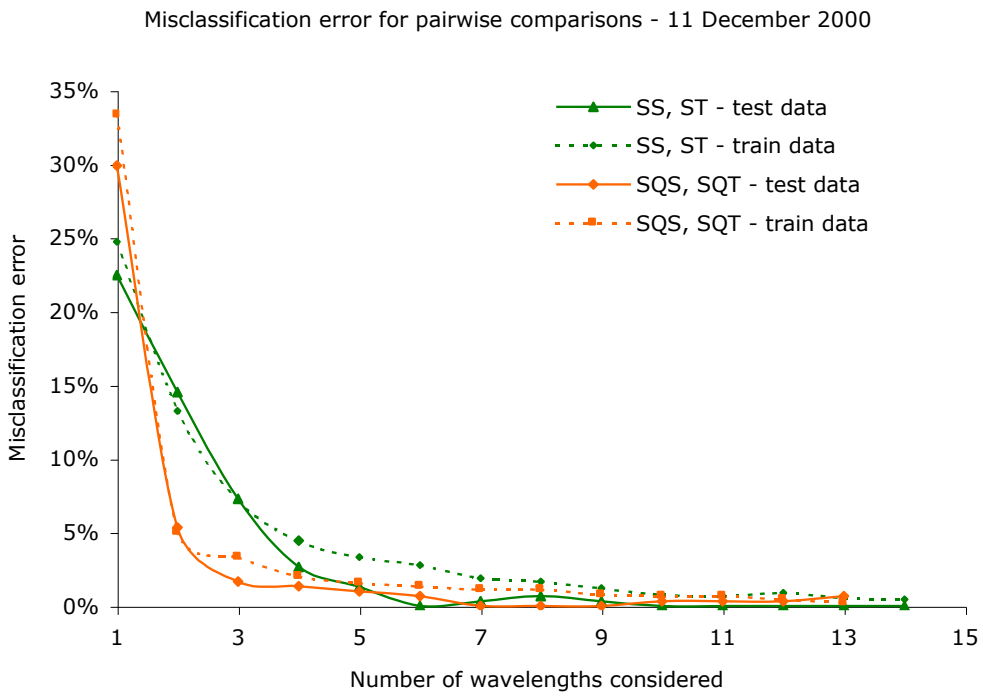

Figure 23 – General misclassification error for pairwise comparisons obtained according to the number of wavelengths considered.

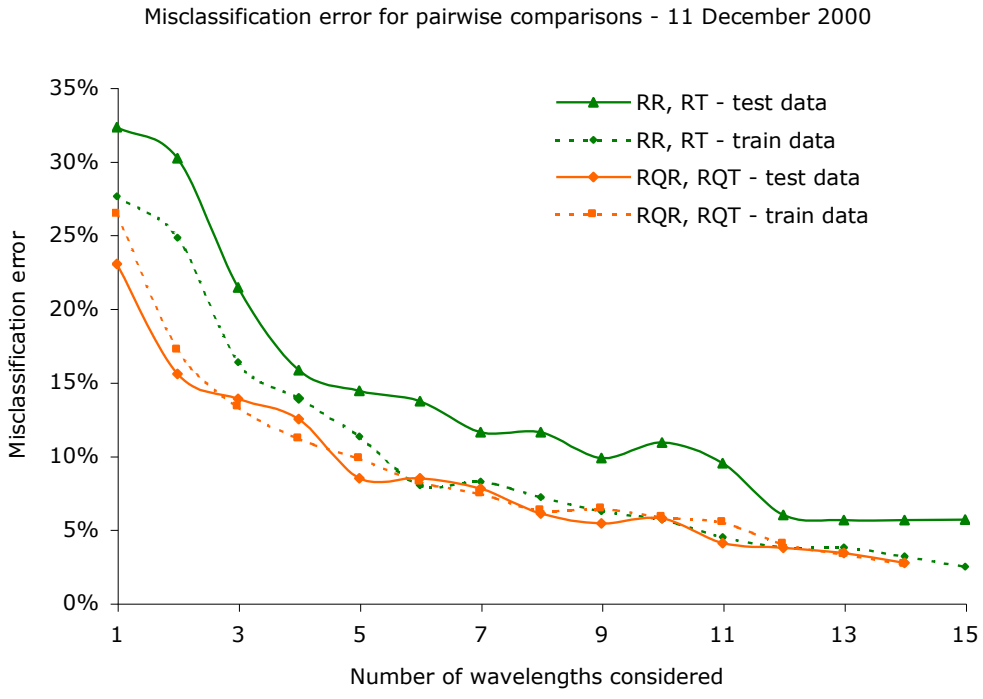

Figure 24 – General misclassification error for pairwise comparisons obtained according to the number of wavelengths considered.

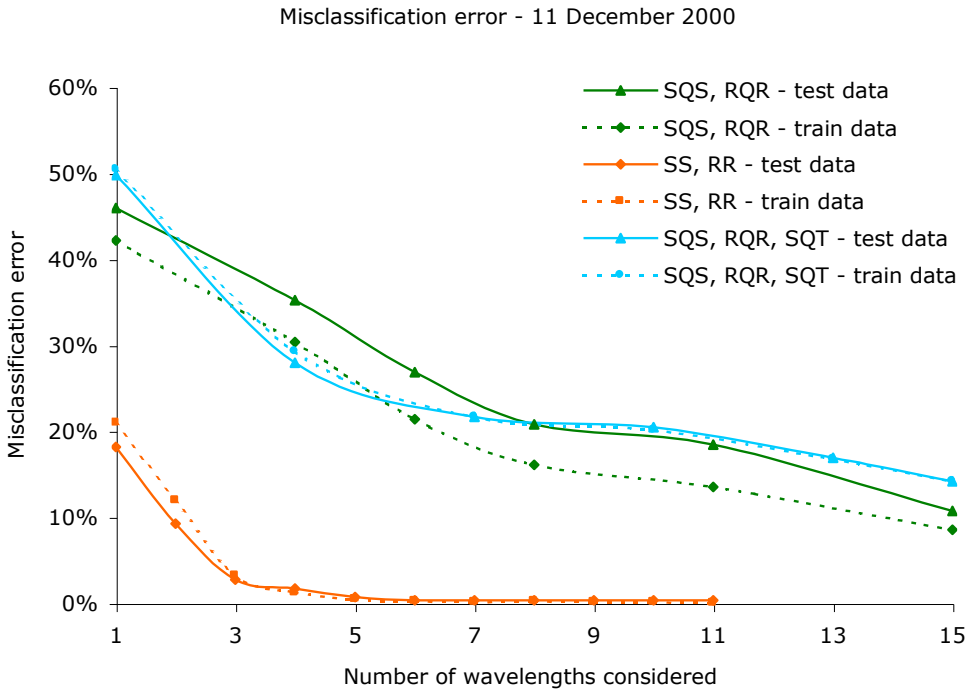

Figure 25 – General misclassification error obtained according to the number of wavelengths considered. The discrimination is between diseases or between diseases and a control.

Discussion

The influence of disease stress on leaf spectra can be assessed in Figure 22, where stress is more developed. In the region 550-700nm the reflectance of all the controls is lower than that of diseased plants. In the NIR the inverse occurs, which is in agreement with literature. No particular spectral features seem to identify each disease, though their discrimination was possible, even with a low misclassification error.

Very good discrimination was possible between each disease treatment and the respective control. The misclassification error was lower for treatments where quinoxyfen was applied (against mildew). On the others mildew may have masked some of the spectral characteristics of normal leaves, hence rendering more difficult the distinction from diseased ones. The classification was better for septoria than for yellow rust. Misclassification errors were less than 0.7% and 5.7%, respectively.

Discriminating wavebands varied with the combination of treatments considered (Figure 26) but results suggest the region between 540 and 590nm to be the most suitable. However, should only this region be used for RQR and RQT discrimination, an error around 10% would be obtained (Figure 24).

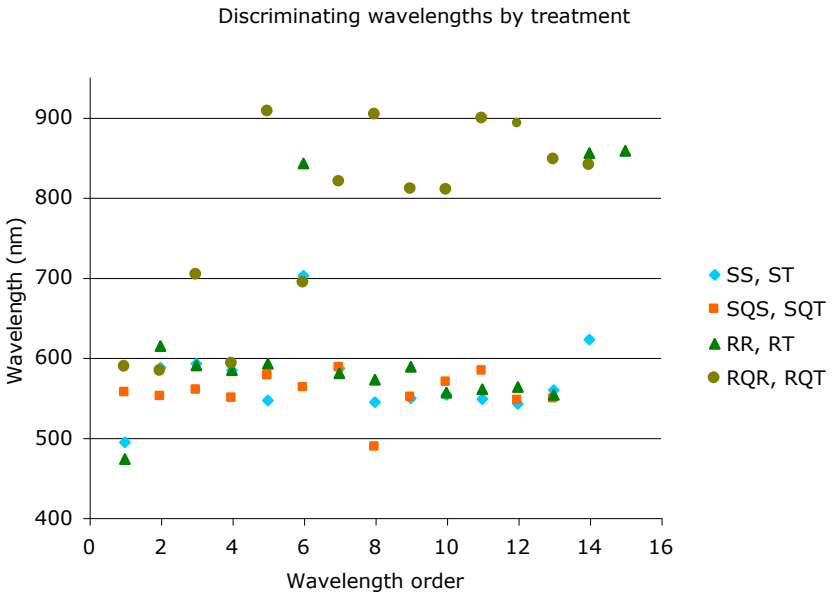

Figure 26 – Distribution of the most discriminating wavelengths of the combination C1, LowN, OnlyN and W, for each measurement date.

Discrimination between diseases very good between SS and RR and moderately good between SQS and RQR (treated with quinoxyfen). Misclassification errors were below 0.5% and 11%, respectively. If 25 variables are taken into consideration, the later value drops to 7%. The distinction between both diseases and control (SQS, RQR and SQT) was moderately good with 15 wavelengths (error≈14%) and good with 25 (error≈7.5%). Again, the best region for classification purposes seems to be 540-590nm. SQS and RQR, however, could not probably be discriminated based on the same spectral region (Figure 27).

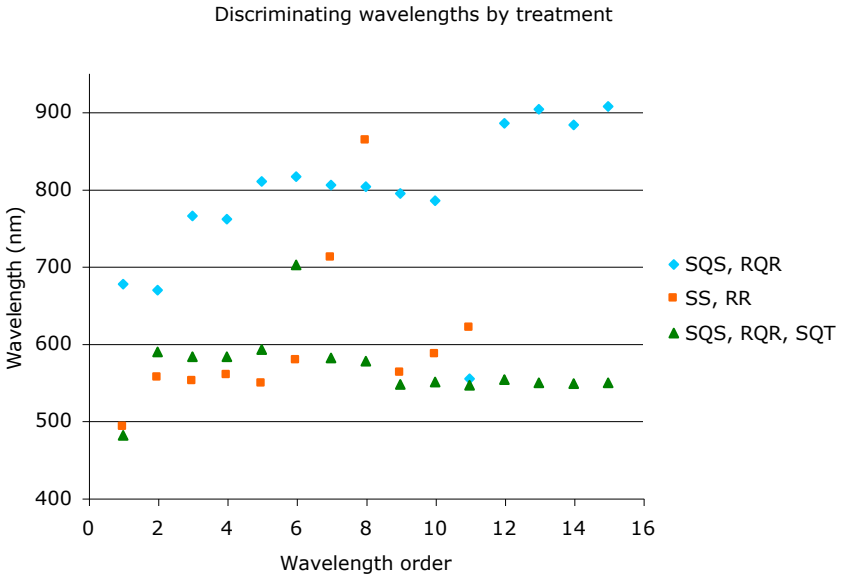

Figure 27 – Distribution of the most discriminating wavelengths of the combination C1, LowN, OnlyN and W, for each measurement date.

The region 540-590nm corresponds to the green peak of reflectance, where chlorophyll absorptance is moderate. Literature also refer to this band as being sensible for chlorophyll assessment and as a stress indicator (see Table 5). Therefore, results support the conclusion that disease stress is accompanied by a decrease in leaf chlorophyll concentration.

It is important to note that other regions of the spectra may have to be used to obtain a good discrimination between treatments.

Figure 23 to Figure 25 show the misclassification error according to the number of wavebands considered. On one hand, the discriminating model is more robust if it is based on a larger number of variables, provided they still enhance its efficiency. On the other hand, their number should be kept at a minimum for simplicity reasons. Thus, the optimal number of variables to take into consideration should be assessed depending on the application. It should be emphasized that beyond a certain number of wavebands the misclassification error starts to increase.

4.3. Windowed and averaged data

The same combination of treatments (SQS, RQR and SQT) was analysed with both the windowing and averaging methods to compare their efficiency. Results are shown on Table 17 and Figure 28.

Table 17 – Comparison of the most discriminating wavelengths (nm) obtained through the windowing and averaging methods. The lowest misclassification error, achieved with the number of wavelengths in parenthesis, is shown for both the test and train datasets.

	SQS, RQR, SQT (windowed data)	**SQS, RQR, SQT (averaged data)**
Lowest error (%)	Test: 14.17 (15), 7.87 (25) Train: 14.18 (15), 7.32 (25)	Test: 13.76 (15), 7.45 (25) Train: 14.17 (15), 9.80 (25)
Wavelength order		
1	481	486
2	589	595
3	583	589
4	583	480
5	592	697
6	702	559
7	581	565
8	577	553
9	547	546
10	550	715
11	546	920

12	553	902
13	549	896
14	548	890
15	549	866

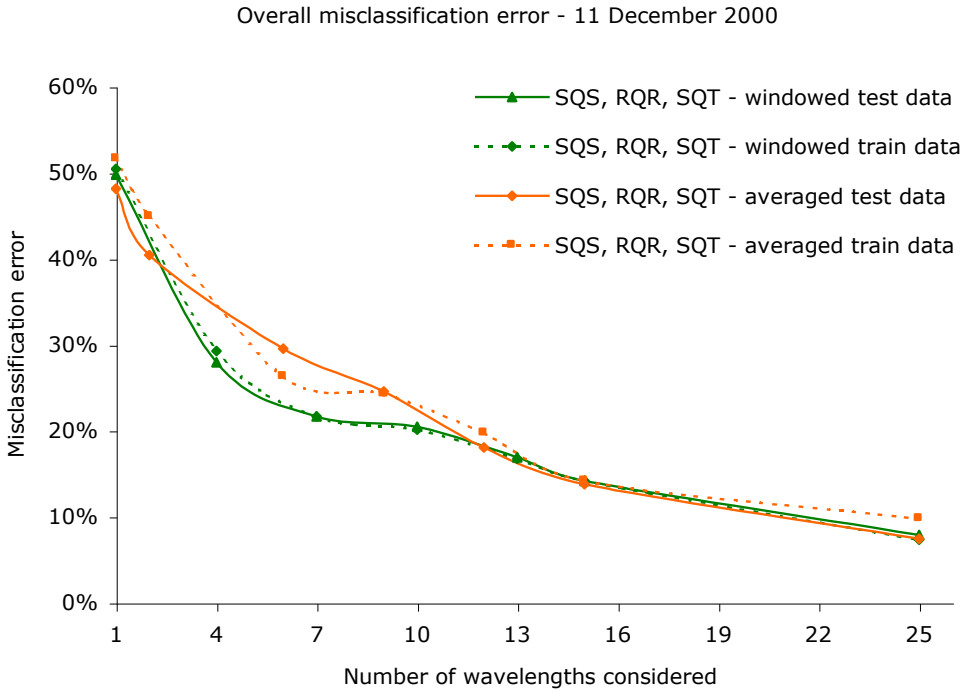

Figure 28 – Comparison of the overall misclassification error obtained through the windowing and averaging methods, according to the number of wavelengths considered.

Discussion

Misclassification errors obtained with both windowing and averaging methods were quite similar although, generally, windowed data yielded better results. This is probably because of the coarse definition of its spectra, allowing narrow-band based discrimination. Averaging data saves disk space and decreases computation time. Therefore, it could be used preferentially. Other bandwidths can be tested in both methods until an optimal size is found.

4.4. Model robustness

Model robustness, that is, the ability of the selected variables to discriminate different treatments and observations, was also assessed. Misclassification errors were obtained for different combinations of treatments using the same 5 or 15 wavebands resulting from the discriminating model SQS-RQR-SQT (cf. Table 16).

Table 18 – Comparison of the misclassification error obtained using the same 5 or 15 variables.

Treatments		5 wavebands		15 wavebands	
		Selected[a]	Best[c]	Selected[b]	Best[c]
SS, ST	Test	3.31	1.32	0.33	0
	Train	2.87	3.31	0.99	0.44
SQS, SQT	Test	3.00	1.00	0	0.33
	Train	3.88	1.56	0.55	0.33
RR, RT	Test	10.94	14.38	7.41	5.66
	Train	9.27	11.28	4.11	2.46
RQR, RQT	Test	15.28	8.44	8.15	3.05
	Train	14.68	9.78	8.87	2.73
SQS, RQR	Test	35.96	32.63	20.83	10.73
	Train	37.87	27.53	19.21	8.55
SS, RR	Test	2.06	0.69	0.34	0.34
	Train	1.37	0.46	0.34	0

[a] – wavelenghts (nm): 481, 589, 583, 583 and 592;

[b] – wavelenghts (nm): 481, 589, 583, 583, 592, 702, 581, 577, 547, 550, 546, 553, 549, 548 and 549;

[c] – the most discriminating wavebands obtained from the respective pairwise comparisons (please refer to Table 15 and Table 16).

Discussion

As would be expected, misclassification errors were almost always higher when the selected wavebands were used than when the most discriminating were. The only reason that may have accounted for the exceptions is any software problem. Yet, the difference was relatively small and good discrimination was generally possible with the selected, constant, wavebands. The classification was always better using 15 variables than with 5. Thus, a more robust model is attained if it is built using a larger number of wavebands. Their selection should be based on a broad discrimination model (one that is able to distinguish several treatments) so that the variables are, at least, expected to enhance the efficiency of all "sub-models".

4.5. Concluding remarks

Despite the encouraging results obtained, it is important to emphasize two aspects about data gathering and analysis:

a) in spite of using a black board over which the leaves were put, its reflectance was not close to zero as would be expected. The board strongly reflected in the blue (Figure 29). Therefore, it accounted for the unusually high reflectance observed in that region. Since the board was always the same, probably it did not influence significantly the results;

b) spectra were randomly assigned to train or test datasets. Because each leaf represented several observations, the train and test data were not independent. This allowed the development of a robust discriminating model which fitted well on both datasets, but which was not independently tested.

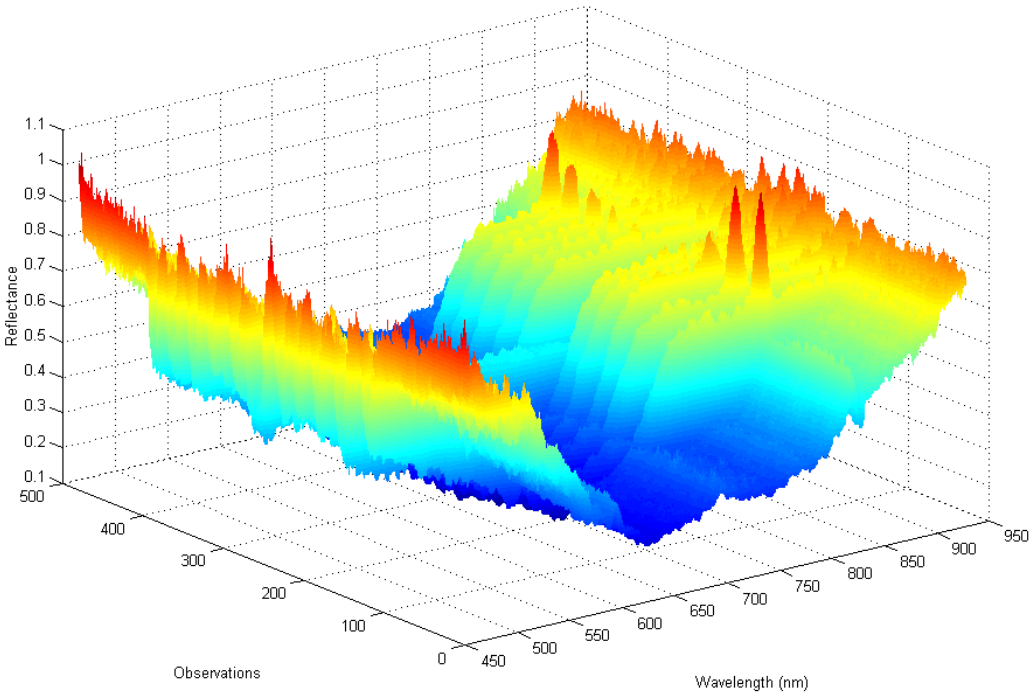

Figure 29 – Example of several spectra as obtained by the spectrograph. Leaves are clearly distinct, the rest being board's reflectance. Leaf reflectance from 400 to 600nm is abnormally high because the board strongly reflected in this region.

5. Conclusion

An increase of reflectance in visible wavelengths and a decrease in NIR was observed as a result of both nutrient and disease stress. This confirms several authors' conclusion that physiological responses to stress are similar regardless of the stress agent.

The most discriminating wavebands varied with the combination of treatments considered, but for nutrient stress the region 450-500nm was generally the most sensible whereas for disease stress it was 540-590nm. Likewise literature findings, NIR was usually a weaker indicator of stress.

A general idea of the results obtained is given in Table 19. Classification between nutrient stress treatments was always perfect taking between 4 and 14 wavelengths into consideration. As stress developed, the discrimination became possible using a lower number of variables. However, they varied between measurement dates.

Misclassification errors were also very low for disease stress treatments, though requiring more variables to reach a good discriminations. The discrimination between SQS, RQR and SQT, for instance, required 25 variables to reach 7.87%. Distinction of diseases was therefore possible, but no special spectral features were found to identify each of them.

Errors shown are just indicative. Actually, depending on the application envisioned, higher errors may be accepted using discriminating models based on less variables, thus simpler and faster. Good classifications (errors below ≈10%) were often possible using only 5 variables.

Table 19 - Highest misclassification errors (%) (in train or test datasets) achieved with the number of wavelengths in parenthesis, for each group of measurements.

	Treatments	**14 Dec**	**18 Dec**	**17 Jan**	**11 Dec**
Nutrient stress	C1, LowN	0 (6)	0 (9)	0 (5)	
	C1, OnlyN	0 (13)	0 (5)	0 (4)	
	C1, W	0 (6)	0 (4)	0 (6)	
	C1, LowN, OnlyN	0 (14)	0 (10)	0 (7)	
	C1, LowN, OnlyN, W	0 (12)	0 (14)	0 (12)	
Disease stress	SS, ST				0.44 (14)
	SQS, SQT				0.67 (13)
	RR, RT				5.66 (13)
	RQR, RQT				2.71 (14)
	SQS, RQR				6.72 (25)
	SS, RR				0.34 (11)
	SQS, RQR, SQT				7.87 (25)

Averaging data was found to minimize the amount of data stored and computation time while yielding similar results to windowed data. As a result, averaging could be the preferred method for noise reduction.

A more robust model was achieved using 15 variables than using only 5. The variables were chosen from a broad discriminating model (one that is able to distinguish between several treatments, such as SQS-RQR-SQT) and were used to separate "sub-models" (SS-ST, RQR-RQT, etc.). Misclassification errors obtained were usually higher than those resulting from the most suitable wavebands, but the difference was often slight. For the majority of cases, the classification was still good or very good, improving substantially when the number of variables considered increased from 5 to 15.

The results obtained confirm the literature conclusion that remote sensing of plant stress is feasible and can be used in agricultural management. In principle, significant pesticide and artificial fertilizer savings can be achieved, reducing the tremendous environmental impact of these chemicals. The role of precision farming in the deeper goal of a sustainable agriculture, however, is more difficult to assess, and should be the topic of a profound study.

6. Annex

The Matlab file used for data processing in shown.

```
% Copyright Nuno Quental
% Version 7.0
% 19/02/2001


% START

clear

% Selection of number of files and treatments

tmp = questdlg('Do you want to process or load data?','Process or load
data',...
   'Process','Load','');

if strcmp(tmp,'Process')
   t = 0;

   prompt = {'Number of treatments','Number of files per treatment'};
   tit = 'Number of treatments and files';
   tmp = inputdlg(prompt,tit,1);

   ntreat = str2num(tmp{1});
   nfiles = str2num(tmp{2});

elseif strcmp(tmp,'Load')
   [fname,pname] = uigetfile ('*.mat','Please select the file with the
data to load');
   load ([pname fname]);
   cd (pname);

   if t == ntreat
      tmp = msgbox('Data is loaded. All the files are processed.','Data
loaded');
      waitfor(tmp)
   else
      tmp = msgbox('Please finish processing your files.','Process data');
      waitfor(tmp)
   end

end


% PROCESSING

if t ~= ntreat

for t = t+1:ntreat

for h = 1:nfiles
```

```
% Load a bitmap file as a matrix

   ext = ['*.bmp'];
   tit = ['Please select the file to process n.' int2str(h) ' of treatment
n.' int2str(t)];
   S = ['[fname,pname] = uigetfile (ext,tit);'];
   eval (S);

   cd (pname);
   [del,filename,del,del] = fileparts(fname);

   A = double(imread(fname));

% Finding the maximum row

   col = sum(A,2);
   [del,irow] = max(col);

% Normalization for reflection

   A = A + eps;
   B = zeros(480,640);

   for i = 1:480
      B(i,:) = A(i,:)./A(irow,:);
   end

   B = B*0.5;

% Normalization for enlightenment

   col = sum(B,2);

   C = zeros(480,640);

   C = B*col(irow);
   for i = 1:480
      C(i,:) = C(i,:)/col(i);
   end

% Leaf recognition

   NIR = mean(C(:,221:248),2);
   R = mean(C(:,380:407),2);
   NDVI = (NIR-R) ./ (NIR+R);

   ndvi = 0.35;
   delrow = find(NDVI < ndvi);

   D = C;
   D(delrow,:) = [];

% Minimizing noise

   np = 8;
   m = size(D,1);
   n = 640-np+1;
   k = 0;

   do = 1;
   switch do
```

```
    case 0

    % Averaging

       E = zeros(m,fix(n/np));

       for j = 1:np:n
          k = k + 1;
          mval = mean(D(:,j:j+np-1),2);
          E(:,k) = mval;
       end

       n = size(E,2);

    case 1

    % Windowing

       E = zeros(m,n);

       for j = 1:n
          k = k + 1;
          mval = mean(D(:,j:j+np-1),2);
          E(:,k) = mval;
       end

       n = size(E,2);

    end

% Appending code columns

    F = fliplr(E);

    istr1 = lower(filename(1,1:4));
    istr2 = lower(filename(1,1:2));
    istr3 = lower(filename(1,1:3));

    CODE(1,:) = {'stan' 5 1};
    CODE(2,:) = {'sept' 5 2};
    CODE(3,:) = {'yr' 3 3};
    CODE(4,:) = {'rr' 3 4};
    CODE(5,:) = {'rt' 3 5};
    CODE(6,:) = {'ss' 3 6};
    CODE(7,:) = {'st' 3 7};
    CODE(8,:) = {'rqr' 4 8};
    CODE(9,:) = {'rqt' 4 9};
    CODE(10,:) = {'sqs' 4 10};
    CODE(11,:) = {'sqt' 4 11};

    icode = find(strcmp(istr1,CODE));
    if icode
       snameb = istr1;
    else
       icode = find(strcmp(istr2,CODE));
       if icode
          snameb = istr2;
       else
          icode = find(strcmp(istr3,CODE));
```

```
            if icode
               snameb = istr3;
            else
               if ~exist('repc')
                  tmp = warndlg('Codes not found!','Warning');
                  waitfor(tmp)
                  prompt = {'Repetition number position','Treatment code
position'};
                  tit = (filename);
                  tmp = inputdlg(prompt,tit,1);
                  repp = str2num(tmp{1});
                  treatp = str2num(tmp{2});
               end

               repc = lower(filename(1,repp));
               treatc = lower(filename(1,treatp));

               code2 = repc;
               code1 = treatc;

               snametxt = num2str(code1);

               if ~exist('snamemat')
                  snamemat = 'Please insert!';
               end
            end
         end
      end

      if icode
         code2 = filename(1,CODE{icode,2});
         code1 = num2str(CODE{icode,3});
      end

      prompt = {'Treatment code','Repetition number'};
      tit = (filename);
      def = {code1,code2};
      tmp = inputdlg(prompt,tit,1,def);

      F(:,n+1) = str2num(tmp{1});
      F(:,n+2) = str2num(tmp{2});

% Calculating the average spectra for each processed file

      SPEC = mean(F(:,1:n),1);

% Building cell array 'V' with the data

      if ~exist('V')
         V = cell(nfiles,4,ntreat);
         FINAL = cell(ntreat,4);
         VI = zeros(ntreat,11);
         STAT = cell(ntreat,2);
      end

      V{h,2,t} = F;
      V{h,3,t} = NDVI;
      V{h,4,t} = SPEC;
```

```
% End of the repetition number cicle

end


% FINAL DATA ASSEMBLY; ARRAYS 'FINAL', 'VI' AND 'STAT'

clear A B C D E F NDVI SPEC NIR R delrow mval

% Calculating the average spectra for the treatment

finalSPEC = mean(cat(1,V{1:nfiles,4,t}),1);

% Building a cell array with the final data

finaldata = cat(1,V{1:nfiles,2,t});
finaldata = finaldata(randperm(size(finaldata,1)),:);

finalNDVI = cat(1,V{1:nfiles,3,t});

FINAL{t,2} = finaldata;
FINAL{t,3} = finalNDVI;
FINAL{t,4} = finalSPEC;

% VI computation

if do == 1

W1 = FINAL{t,4}(w2p(900));
W2 = FINAL{t,4}(w2p(850));
W3 = FINAL{t,4}(w2p(800));
W4 = FINAL{t,4}(w2p(710));
W5 = FINAL{t,4}(w2p(700));
W6 = FINAL{t,4}(w2p(695));
W7 = FINAL{t,4}(w2p(680));
W8 = FINAL{t,4}(w2p(550));
W9 = FINAL{t,4}(w2p(451));
W10 = FINAL{t,4}(w2p(760));
W11 = FINAL{t,4}(w2p(750));

VI(t,1)  = (W1-W7) / (W1+W7);     %NDVI1
VI(t,2)  = (W2-W4) / (W2+W4);     %NDVI2
VI(t,3)  = (W10-W4) / (W10+W4);   %NDVI3
VI(t,4)  = (W7-W9) / (W7+W9);     %NPCI
VI(t,5)  = (W3-W9) / (W3-W7);     %SIPI
VI(t,6)  = (W2-W4) / (W2-W7);     %DATT
VI(t,7)  = W11 / W5;              %SR1
VI(t,8)  = W11 / W8;              %SR2
VI(t,9)  = W6 / W9;               %SR3
VI(t,10) = W1 / W7;               %SR4
VI(t,11) = W9 / W7;               %PSR

end

% Statistical computation

STAT{t,1} = std(FINAL{t,2}(:,1:n));
STAT{t,2} = var(FINAL{t,2}(:,1:n));
```

```
% DATA SAVING PER TREATMENT

fsize = size(filename,2);
snamee = filename(1,fsize-1:fsize);

if ~exist('snamemat')
   snamemat = snamee;
end

if ~exist('snametxt')
   snametxt = [snameb snamee];
end

prompt = {'Save name for MATLAB file','Save name for TXT file'};
tit = 'Save file';
def = {snamemat,snametxt};
sname = inputdlg(prompt,tit,1,def);
snamemat = sname{1};
snametxt = sname{2};

FINAL{t,1} = snametxt;

S = ['save ' snamemat '.mat V FINAL VI STAT nfiles ntreat ndvi t np
snamemat'];
T = ['save ' sname{2} '.txt finaldata -ascii -tabs'];
eval (S);
eval (T);
disp('Final data saved')

clear snametxt

% END OF TREATMENT CYCLE

end


% PLOTTING

STYLE{1} = 'g';
STYLE{2} = 'g--';
STYLE{3} = 'b';
STYLE{4} = 'b--';
STYLE{5} = 'r';
STYLE{6} = 'r--';
STYLE{7} = 'm';
STYLE{8} = 'm--';
STYLE{9} = 'k';
STYLE{10} = 'k--';

figure
hold on
xlabel('Wavelength (nm)')
ylabel('Reflectance')
title(snamemat)

for t = 1:ntreat

factor = (930-450) / n;
wave = 450:factor:930;
wave = round(wave(1:n));
plot(wave,FINAL{t,4},STYLE{t});
```

```
end

legend(FINAL{1:ntreat,1},2);

for t = 1:ntreat
   hSTAT = plot(wave,STAT{t,1},STYLE{t});
end

end

% END
```

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