# LASER INDUCED FLUORESCENCE FOR STRESS STUDIES ON MAIZE PLANTS UNDER CONTROL CONDITIONS

A DISSERTATION SUBMITTED IN FULFILLMENT

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# ABSTRACT

The growth of plants is affected by several environmental parameters of which light (particularly UV) is the most important. It is a well established fact that similar plants grow differently in different regions, due to the fact that the local environmental conditions vary from place to place. Stratospheric ozone depletion has caused an increase in the amount of ultraviolet-A (UV-A) and ultraviolet-B (UV-B) radiation reaching the earth's surface. In Namibia, excessive ultraviolet (UV) radiation is one of the main factors that adversely effects the growth of vegetation and therefore the study of its influence on the growth of plants is very vital. For this study, an advanced technique of Laser-Induced Fluorescence (LIF) is used.

When plant leaves are irradiated by ultraviolet (UV) radiation, fluorescence spectrum shows four peaks centered at wavelengths of 450nm, 530nm, 685nm and. The ratio of blue (450nm) to red (730nm) fluorescence differs from plant to plant and is considered a stress indicator. For laboratory measurement of fluorescence spectra from the leaves of maize plants grown under controlled conditions such as humidity, temperature, water and mineral stresses, and exposed to UV-A and UV-B radiation over different timelines are used. The emission spectrum of the third leaf from the bottom side of each maize seedling were obtained using a AvaLight- D(H)S Deuterium-Halogen light sources (337 nm), which was connected to Ava Spec-2048 Fiber Optic Spectrometer and a computer for displaying the results in a graph format. In this study, the intensity ratios of these peaks as $\frac{\mathbf{F410}}{\mathbf{F430}}$ , $\frac{\mathbf{F410}}{\mathbf{F550}}$, and $\frac{\mathbf{F550}}{\mathbf{F590}}$ corresponding to blue to green, blue to red, and chlorophyll fluorescence were calculated and analyzed. This study focused on maize plants due to socio-economic importance of maize in Namibia.

The ratio $\frac{F410}{F430}$ , that is blue to green, has decreased from 0.4 to 0.2, that is a 50% decrease, while the ratio $\frac{F410}{F550}$, that is blue to red, increase from 0.2 to 0.4, which is an increase of 100%. On the other hand, it was found that the ratio $\frac{F550}{F590}$ , the chlorophyll flurescence, has

decreased from 2.9 to 1.6, which is a decrease of 44.8%. The decease in the ratio $\frac{F550}{F590}$, the chlorophyll flurescence is due to the reduction in photosynthesis activity and hence the health status of plants, whereas the decrease in the ratio $\frac{\mathrm{F410}}{\mathrm{F430}}$ (blue to green fluorescence) reflects the chlorophyll and carotenoid pigmentation in green leaves. The increase in the ratio of $\frac{\mathrm{F410}}{\mathrm{F550}}$ (blue to red) with the increase of UV-A and UV-B radiation, could be due to the reabsorbtion on the red light in the the inner leaf with high concentrations of flavonoids and anthocynanins. Furthermore, it was found that UV-B was more destructive than UV-A.

From measurements taken of the growth parameters, such as number of leaves, width of the leave, diameter of the stem and height of seedling, the following were observed. When exposed to UV–A, the width of the leave reduces from 1.7 to 0.7 (58%), the diameter of the stem reduced from 0.4 to 0.1 (75%), and the height of the seedling from 41 to 19 (53%). In this study it is thus observed that UV and in particular UV-B has significant impacts on the total biomass and the growth of plants. This is in agreement with other reports that plants are more susceptible to long-wave UV-B irradiation and that this difference is more apparent from the changes in total area of leaves and dry mass of shoots, rather than from the parameters of chlorophyll fluorescence and net photosynthetic rate. It is recommended that the continued advancement of laser research and the further development of laser remote sensing techniques for atmospheric, environmental and further vegetative studies be actively promoted and motivated.

**Keywords:** Environmental stress, Growth parameters, Laser Induced Fluorescense; Maize plants, UV radiation

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# ACRONYMS

| | |
|---|---|
| **FIR** | Fluorescence Intensity Ratios |
| **FWHM** | Full-width at Half Maximum |
| **LAI** | Leave Area Index |
| **LASER** | Light Amplification Stimulating Emission of Radiation |
| **LIDAR** | Light detection and ranging |
| **LIF** | LASER-induced Fluorescence |
| **PAR** | Photosynthetically Active Radiation |
| **PRI** | Physiological Reflectance Index |
| **ROS** | Reactive Oxygen Species |
| **TEM** | Transverse Electromagnetic Mode |
| **UV** | Ultra-Violet radiation |
| **UV-A** | Ultra-Violet radiation A (400-320 nm), |
| **UV-B** | Ultra-Violet radiation B (320-280 nm) |
| **UV-C** | Ultra-Violet radiation C (280-100 nm) |

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# PUBLICATIONS BY THE CANDIDATE

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Thanks to my wife, Gisela for her encouragement and patience.

# DECLARATIONS

I, Alfred Adriaan van Kent, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.



…….. ……..

**Alfred Adriaan van Kent** **Date**

# CHAPTER 1: INTRODUCTION

## 1.1. FUNDAMENTALS OF LASERS AND ITS APPLICATIONS

### 1.1.1. GENERAL BACKGROUND ON LASERS

Laser is an acronym for Light Amplification by Stimulated Emission of Radiation. According to Silfvast (2004), a laser is a device that amplifies light and produces a highly directional, high intensity beam of light that most often a very fine frequency or wavelength. A laser is one of the most versatile tools ever invented in 1960 and since then, lasers are revolutionizing computers, communications and industries. Due to their unique properties, lasers are replacing electronic components with optoelectronics. One of the known applications of lasers is to perform delicate microsurgery on single cells. Furthermore, it has a very important and profound role in the field of remote sensing. The capability of lasers to enable conducting analysis at a long distance effectively adds a new dimension to remote sensing (Saito, Y., Saito, R., Kawahara, T. D., Nomura, A. and Takeda, S., 1999). A ground-based laser remote sensor is able to distinguish sodium atoms from the much larger concentration of other species at an altitude of 90 km, and thus makes lasers quite extraordinary.

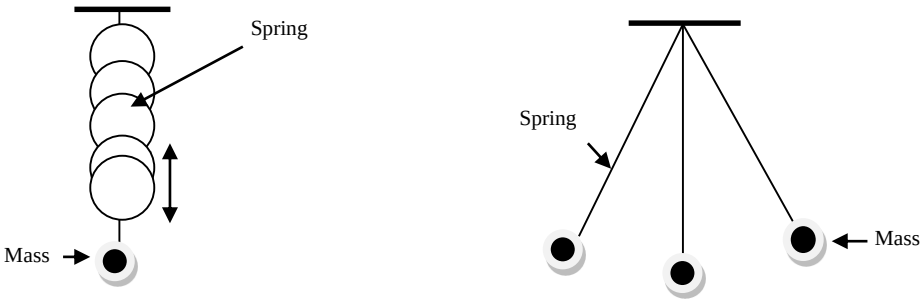
Lasers are very special source of coherent light as the light emitted by them is both spatially and temporally coherent. The main function of lasers is like an electronic component that generates and amplifies electronic signals. Lasers can produce and amplify radiation at any desired wavelength. In fact, a laser can be used as an amplifier as

well as an oscillator and hence it has unlimited applications in almost all branches of science, engineering, and technology. Today, it is used in medicine, agriculture, defense, environmental studies, computers, space research, industries, cutting and welding, mines and survey, telecommunications, measurement and analysis, laser printers, CD disk players, non-destructive techniques, chemistry, biology, etc.

### 1.1.2. DYNAMIC ASPECT

In order to understand the basic principles of lasers, the dynamic motion of a body, where the transfer of energy or the exchange of energy between two states is taking place, is considered. In a mechanical system of particles, the forces can change the momentum and energy of particles in the system. For example, in mechanical harmonic oscillator a mass connected to an elastic spring can oscillate up and down as shown in figure 1a. There are two kinds of energies, kinetic energy of the moving mass and the potential energy of the spring as stored up in the spring. However, the mass does not continue to oscillate forever because the oscillating mass interacts with the surrounding air, transferring its kinetic energy into acoustic, heat and frictional energy. Another example of a mechanical harmonic oscillator is an oscillating pendulum as shown in figure 1b. The mechanical motion of the system (mechanical harmonic oscillator) can be maintained by supplying additional energy to the system as in case of a clock pendulum. If the rate of externally supplied energy is equal to the energy loses of the system, a stationary oscillation system can be achieved. The energy of the system can be converted into useful energy. A laser is a special kind of optical oscillator, which converts energy into highly coherent light. The

energy to the optical oscillator is supplied by an excitation source (pump). However, this conversion is not so simple. Various conditions are considered in order to maximize the conversion of pump energy into coherent light and to control the coherent properties of the light.




(a) (b)

**Figure 1:** Mechanical harmonic oscillators (a) Spring and (b) Pendulum.

### 1.1.3. PROPERTIES OF LASER BEAM

All lasers have some unique characteristics but they operate under similar principles consisting of three main parts as shown in figure 2.

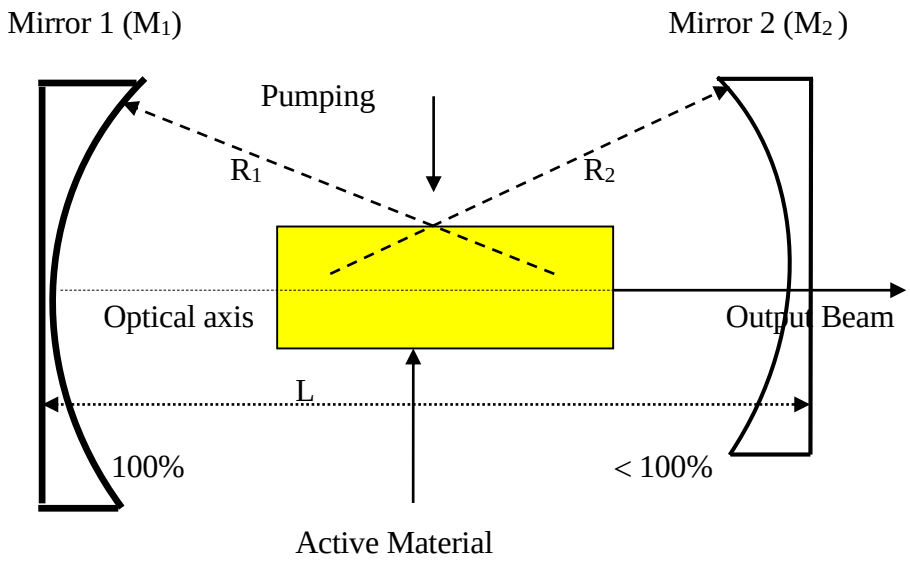



**Figure 2:** Basic components of a laser.

1. All lasers contain a gain or laser medium or active material to collect atoms, molecules or ions. This is the first requirement of a laser. The active material can be anything like gas, solid, liquid or a semiconductor.
2. Lasers have an excitation source called pump or power supply, which is required to excite the atoms from lower energy state to a higher energy state.
3. Optical cavity or resonator of length L, is another basic requirement of a laser. This consists of a set of mirrors $M_1$ (radius of curvature $R_1$) and $M_2$ (radius of curvature $R_2$) with the active material in between them to form a resonator. One of

the mirrors is 100 % reflective and the other one is less than 100 %. There are various configurations available.

The complete laser includes many other components such as prisms, gratings, and suitable absorber, etc. to direct the laser beam. They do not play any role to obtain the laser action but are very important for controlling the spectral and time-dependent properties of the laser beam.

The different lasers have some unique characteristics but they operate under similar principles consisting of the three main requirement mentioned above. The first requirement of a laser is the gain or laser medium or active material thay has atoms, molecules or ions that could be excited. We know that all material mediums contain atoms and these atoms are excited through a power source or pump. The active material can be anything like gas, solid, liquid or a semiconductor. As stated before the excitation source called pump or power supply is used to excite the atoms from lower energy state to a higher energy state. These excited atoms sooner or later decay from higher energy states to the lower energy states emitting highly coherent radiation of light.

This coherent radiation of light coming from decaying atoms, is amplified by a resonator (amplifier) that consist of a pair of mirrors with the active material in between them as shown in Figure 2. One of the mirrors is 100 % reflective and the other one is less than 100 % so that a powerful beam comes out through the mirror that is less reflective.

**Monochromaticity:**

Laser light is highly monochromatic (that is a laser emits a beam of light of single frequency). It is because a laser acts as an amplifier and in an amplifier only an electromagnetic beam of frequency given by $\nu = \frac{(En - Em)}{h}$ can be amplified. The oscillations of single frequency can occur only at the resonant frequencies of the cavity given by $\nu = \frac{(En - Em)}{h}$. The integers (n > m) represent the energy states and h is Planck's constant. The temporal coherence length $L_c$ is a measure of the temporal (longitudinal) coherence. The temporal coherence length determines the monochromaticity of the laser beam. Ordinary sources of light lack the property of monochromaticity.

**Directionality:**

Laser light is highly directional increasing its intensity and because of this property a laser beam can be focused on to a very small spot. The directionality of the laser beam is due to the fact that the active material is placed in a cavity called optical resonator.

**Coherence:**

The term coherence implies phase correlation between two points in a light beam. Light beams from two different sources cannot produce a permanent interference pattern because the light wave trains emitted are independent and have random phase relationship. Therefore for interference to occur the two beams are derived from the same source. They act as mutually coherent light beams since any discontinuities of fluctuations occurring in one beam will also appear in the other beam. There are two types of coherence *viz*., spatial and temporal.

**Spatial Coherence:**

Spatial coherence is a measure of the phase correlation between two points on the same wave front at a single time. It can be measured by passing a wave front through two points. If the light coming through the points forms an interference patterns, the light source is said to display spatial coherence. The contrast of the fringe pattern is the measure of the spatial coherence. A perfect spatially coherent light beam will produce fringe patterns that vary in intensity from zero to unity. This spatial coherence relates the directional properties of light. Light, which is spatially coherent, has well defined wave front. Most sources of light are finite in size and each microscopic point is propagating its own light, hence light is spatially incoherent with light waves going in many different directions. The laser, however, generates a spatially coherent light beam in which, all wave trains are in phase and as a consequence, it is highly directional and monochromatic.

**Temporal Coherence:**

If a wave at some point in space is split into two parts, delay one portion, and then recombine the two portions to form an interference pattern, one can measure the temporal coherence of a wave. The degree of temporal coherence is a measure of the correlation of a wave at one time with a wave at a later time. It may also be referred to as longitudinal coherence as opposed to transverse of spatial coherence. The time during which the wave is coherent, if multiplied by the velocity of propagation of the wave, gives the coherence length of the wave. Spatial coherence measured in two transverse directions determines the coherence area of the wave. Consequently the two measures, spatial and temporal coherence, describe the degree of coherence of a wave within a volume of space.

The characteristics of a source affect the temporal coherence of the wave. If a purely monochromatic wave is phase modulated, the bandwidth or spectral width of the wave must increase. If the modulating function is known, then in certain applications the temporal coherence can be preserved. If the phase modulation is random, the phase at one instant can be predicted for only a short time after the instant at which the phase is known. The time interval $\Delta t$ over which the phase can be predicted is related to the bandwidth of the signal because the bandwidth, or spectral width, imposes a limit on how rapidly the phase can vary. The minimum time interval over which the wave is coherent is given approximately by:

$$\Delta t = 1/\Delta \nu \qquad (1)$$

where, $\Delta \nu$ is the frequency corresponding to the bandwidth of the wave. The time difference $\Delta t$ is the coherence time of the wave and the corresponding length $c\,\Delta t$ is the coherence length.

The spatial (transverse or lateral) coherence represents the directionality. Spatial coherence is a measure of the transverse distance across the beam over which a constant phase relationship exists. The length in the transverse direction (perpendicular to the direction of propagation) over which the phase is constant is called the spatial coherence length $L_c = \frac{f\lambda}{D}$, where D is the diameter of the beam spot focused by a lens of focal length f. Coherence length can be a few mm and can be increased by using high quality lens.

The laser light has very high brightness as compared to any other natural or artificial light sources. Coherence is a measure of monochromaticity, directionality, and the phase consistency of laser light. Coherence is what makes laser light special and different from the light produced by any other source. Coherence is a direct measurement of the fringe visibility defined as $(I_{max} - I_{min})/(I_{max} + I_{min})$ where I is the intensity of fringe pattern.

One should remember that according to the uncertainty principle, a laser light cannot be perfectly monochromatic. It has always a finite line width (bandwidth) and has fluctuations in amplitude and phase, and hence it has always a spectral width greater than zero. It is because the energy levels are not like razor-sharp but are slightly fuzzy as shown in Figure 3(a) and 3(b). If $\lambda$ is the spectral wavelength then in terms of the temporal coherence length $L_c$ the bandwidth $\Delta\lambda$ is given by:

$$\Delta\lambda = \frac{\lambda^2}{2\,Lc} \tag{2}$$

with

$$L_c = c\,t_c \tag{3}$$

where, c is the speed of light, $t_c$ is the coherence time and $\Delta\nu$ is the frequency corresponding to the bandwidth. The temporal coherence (longitudinal coherence) is the relative phase relationship between the two waves at two different points (at two different times) along the direction of propagation of the two beams. The bandwidth can be measured in terms of wave numbers and frequencies also. It is numerically equal to the full-width at half maximum (FWHM) i.e. halfway down from the peak of the laser line (Figure 3c).

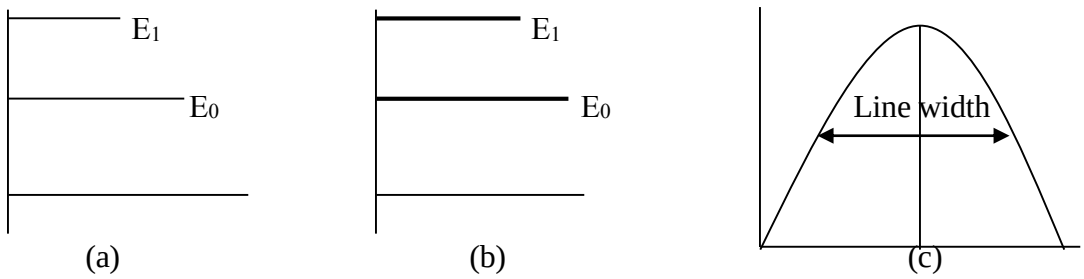



**Figure 3:** (a) Sharp energy levels, (b) energy levels with uncertainty, (c) full-width at half maximum.

The monochromaticity, directionality, and the phase consistency of laser light, are known as coherence. Because of the unique coherent properties of lasers, the applications of lasers are legion and span almost every field of human endeavor from medicine, science and technology to business and entertainment. Some of the applications of lasers and their use in devices such as supermarket bar-code readers, compact disk (CD) players, lecture theatre pointers, laser printers and holograms are well known. Their use in medicine, agriculture, telecommunications and industries is increasing every day. In medicine, they are used for wound healing, dentistry, pain relief, acupuncture, ophthalmology, gynecology, urology, angioplasty, photodynamic therapy, optical imaging and diagnostics. In agriculture, they are used in image processing, stress studies on vegetation, remote sensing and environmental monitoring. In telecommunications, optical fibres can carry more and reliable information than the conventional systems. In industries, lasers are used in welding, cutting, heat treatment and material processing. Some of the key applications of lasers are stated below in detail.

In order to narrow line width, the quality factor (Q factor) must be increased using high reflectivity external mirrors. The coherence time $t_c$ is the inverse of the bandwidth and can also be obtained by dividing the coherence length by the velocity of light.

### 1.1.4. TYPES OF LASERS

Various kinds of lasers have been fabricated for different applications. They have been categorized according to the radiation they emit. Lasers emitting UV radiation are called UV lasers. Those lasers whose radiation falls in the visible and infrared of the electromagnetic spectrum are known as visible and infrared lasers respectively.

Figure 4, gives the lasers spectrum from infrared to X-ray region.

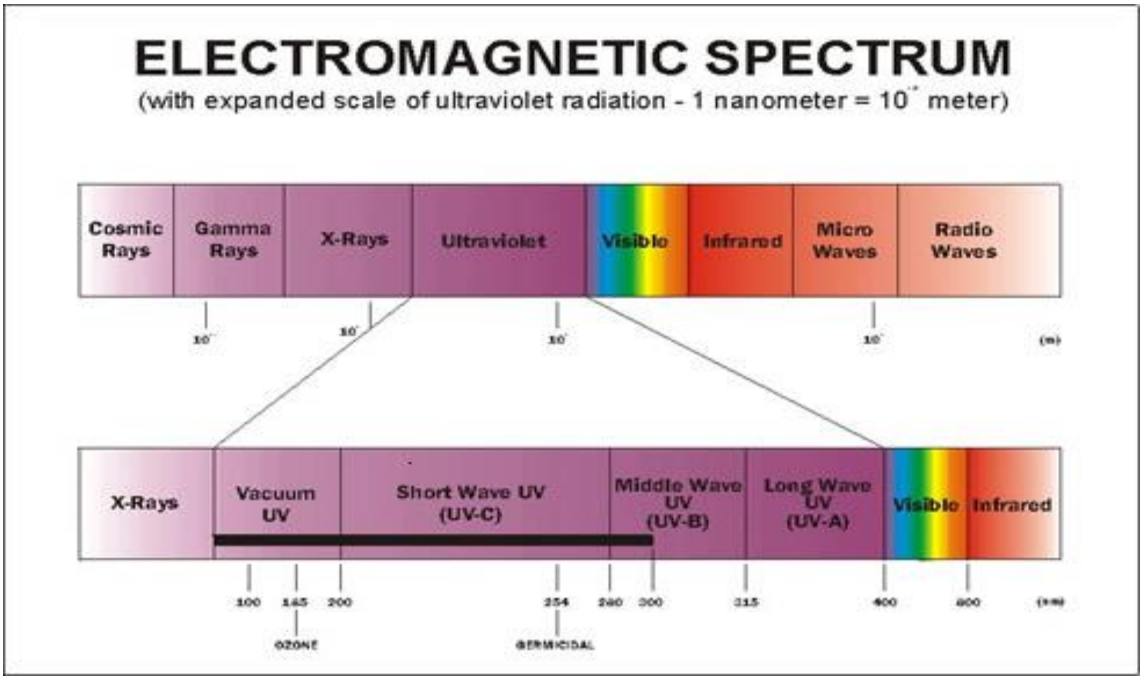



**Figure 4:** Electromagnetic spectrum from Infrared to X-ray region.

Based on their state, we find lasers such as the solid state lasers, (Solid state lasers are those in which the active medium is either insulating crystal or a glass), semiconductor lasers and gas lasers. None of these lasers can fulfill all the requirements at one time. Each laser has its own particular properties and specific applications like there are specific medicines for specific diseases.

Molecular gas lasers are available almost in whole range of electromagnetic spectrum with output power ranging from few milliwatt (mW) to several kilowatt (kW). Two molecular lasers that emit in the ultraviolet region of the electromagnetic spectrum are nitrogen ($N_2$) and excimer lasers. The properties of nitrogen laser are quite different from those of other molecular gas lasers such as carbon dioxide ($CO_2$) gas lasers. In $CO_2$, the transitions correspond to vibrations of the $CO_2$ molecule and produce radiation in the infrared. In the $N_2$ lsaer, the transitions correspond to different electronic energy states and the emitted radiation is in the ultraviolet region at 337.1 nm. The high frequency output of this laser, produced in short and high power pulses is very useful for many applications such as LIF. Another application of nitrogen laser is in pumping dye lasers. Nitrogen dye laser can be used advantageously as an excitation source in the UV region. There are mainly three categories of lasers namely, gas lasers, solid state lasers, and liquid lasers. Each category has numerous types of lasers. It is not possible to describe all types of lasers here in depth. Hence in this chapter we describe in brief only some selected lasers.

**He-Ne LASER**

The first gas laser to be invented was He-Ne laser as shown in Figure 5. The rate of production of these lasers is very high and much larger than that of all other gas lasers. The method of pumping is by electrical glow discharge from a dc voltage source of 1 to 2 kV at about 50 mA through a mixture of He and Ne as the active components, as shown in Figure 6. Most He-Ne lasers operate at a wavelength of 632.8 nm in the red part of the visible spectrum but operation in the short and long infra-red spectrum (1.3 μm and 3.39 μm) is also possible. The laser operates in continuous mode and the power ranges from 0.1 mW to over 100 mW output.

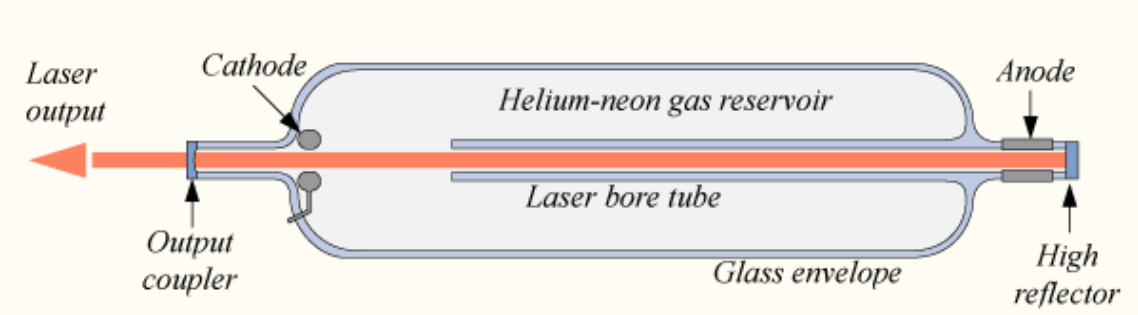



**Figure 5:** Diagram of a Helium-neon gas laser.

**ION LASER**

Another important class of gas laser is the ion lasers. Argon (Ar), Krypton (Kr) and Xenon (Xe) are the ionized gases most commonly used for lasing. These are four level systems. The current density for ion lasers required is about 1000 A/cm$^2$. These lasers operate in continuous mode and provide power output of the order of 10 W for cavities with a length of the order of 1 metre. No additive gases are required for operations and pumping occurs as a consequence of multiple collisions of Ar+ and Kr+ ions with energetic electrons.

## COPPER AND GOLD VAPOR LASER

These lasers provide the most efficient green source of light at 510 nm, however UV at 312 nm, yellow at 578 nm, and red at 628 nm transitions are also possible. These lasers are very useful for photodynamic therapy of tumors. They are also used in scientific and industrial research. Copper vapor lasers have been operated with average power of 40 W with pulse duration of 50 ns and repetition rate up to 20 kHz.

## ARGON LASER

The argon has nine different lasing transitions which will co-exist if the laser is pumped hard enough. These lasers are extensively used in surgery, particularly for the eye. The lasing action occurs at two lines at 488 nm and 515 nm.

## MERCURY LASER

Another useful ion laser is mercury ion laser oscillating at 615 nm in the red region. This system operates in very short pulses (a few microseconds) but the pulses occur at a very high rate so that the output appears continuous to the human eye.

## He-Cd and He-Se LASER

The two other most widely used ion lasers are He-Cd and He-Se lasers. These are generally included in the category of metal vapor lasers. The output power of both He-Cd and the He-Se lasers is of the order of 50 to 100 mW. He-Cd laser oscillates at 441.6 nm and 325.0 nm. The He-Cd laser may oscillate continuously at these wavelengths. He-Se laser emits about

15 possible lines ranging from 460 nm to 650 nm. More than one set of mirrors are required to obtain these lines. The output power of these lasers is about 10 to 20 mW.

**KRYPTON LASER**

This laser has several possible laser transitions, with two low threshold lines at wavelength of 568 nm and 647 nm. Because of high power density, such lasers are used in cutting and welding. Ion lasers are normally operated within multi-line output by using a simple wide band mirror resonator. When single narrow band operation is required, the laser is tuned to provide operation above threshold for one laser line by using a prism or a grating.

**TEA LASERS**

The other class of $CO_2$ lasers is Transversely Excited Atmospheric Pressure (TEA) and gas-dynamic $CO_2$ lasers. TEA laser is inexpensive and easy to construct. It can be made to work at atmospheric pressures as the name implies.

**CO and $H_2F$ LASERS**

The other categories of the molecular gas lasers are carbon monoxide and hydrogen fluoride lasers. These lasers are finding useful applications in medicine and material analysis.

**$H_2$ LASER**

This laser is a vibronic laser. It operates at 116 nm and output power may be up to 100 kW with pulse duration 1 ns. The applications of this laser are in scientific research and medicine.

## $N_2$ LASER

In this laser, radiative transitions occur between the electronic energy states. This laser oscillates at 337.1 nm in the ultraviolet region and peak power is about 100 kW with average power 5 mW in pulses 10 ns with repletion rate up to 100 Hz. The power supply should be 10 to 20 kV. These lasers are simple, inexpensive and easy to construct. This laser provides very powerful pulses and is used as a pump for dye lasers.

## EXCIMER LASER

These are very important class of molecular gas lasers involving transitions between different electronic states. These lasers are providing very strong and efficient UV sources which are used in scientific research and medical applications (lasik eye surgery).

## RUBY LASER

This was the first laser to be invented and has applications in high power pulse holography. It is a three level pulse laser. The lasing medium is a ruby rod, which is $Al_2O_3$ crystal containing 0.05% of $Cr^{3+}$ ions. The $Al_2O_3$ material has two broad absorption bands in the blue and green spectrum, which can be pumped by using a mercury vapor flash tube. The laser transition is made straight back to the ground state at 692.7 nm and 694.3 nm. These two laser lines depend upon temperature.

**Nd-YAG LASER**

**(Nd =** Neodymium and **YAG** = Yattrium Aluminium Garnet crystal) $Nd^{3+}$ ion is used as a laser center in these two solid state lasers. In the Nd-YAG laser (Figure 6), Neodymium ions, $Nd^{3+}$ are substituted for Y ions in the lattice of a Yattrium Aluminium Garnet (YAG) crystal. The Y host has several broad absorption bands that can be pumped by discharge lamp in a way that is similar to the ruby laser pumping method. The Nd-YAG laser is a four level laser. The laser lines are at 1.06 μm in the infrared region, however the laser can lase at 1.34 μm. Average power can be up to 1000 W and is used for cutting, drilling, welding, and medical applications such as photocoagulation.

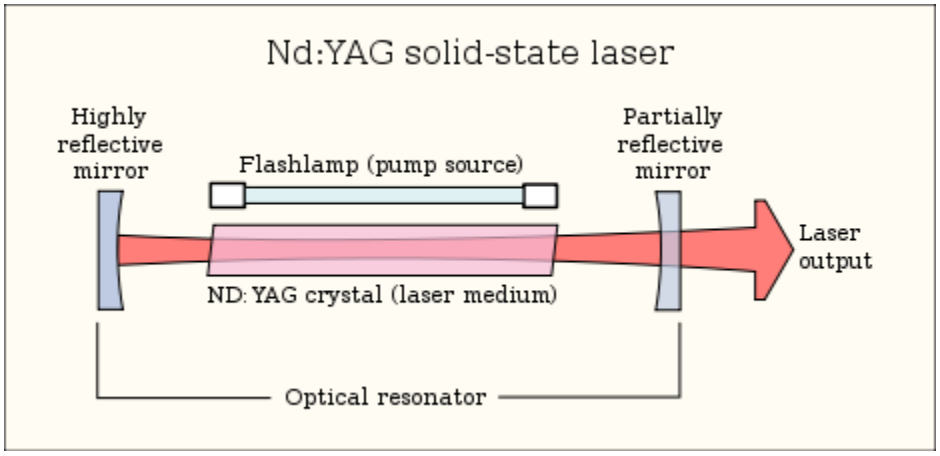



**Figure 6:** Nd:YAG solid-state laser

**Nd-GLASS LASER**

In this laser, the $Nd^{3+}$ ions (as the lasant) are embedded in a glass material (the host). Nd-glass lasers have higher efficiency than Nd-YAG lasers, so that more axial modes are excited. These lasers cannot operate in CW mode (continuous wave mode) because of poor thermal

properties of the glass host. The band width is about $10^{12}$ Hz with narrow pulse width of about $10^{-12}$ seconds.

**$HO_3$: YLF LASER**

The multiple doped $HO_3$: YLF laser is a low threshold solid state laser that operates in the 2 μm region at room temperature. It is eye-safe and has applications in atmospheric communication systems. Apart from the crystals used in lasers, a large number of other doped crystals have been successfully used in lasers e.g. trivalent chromium, trivalent neodymium or trivalent erbium in YAG or trivalent thulium in erbium doped YAG.

**Q-SWITCHED LASER**

Very narrow pulse with very high peak power can be produced from some lasers by switching the discharge voltage periodically called Q-switching. This can be done by spoiling the laser cavity or by interrupting lasing action (reducing its Q factor) so that very large carrier inversion (population inversion) can be achieved by strong sustained pumping system without lasing action and then returning the cavity to its unspoiled state (high Q factor) so that the population inversion is well above the threshold value. In this case substantial energy is stored in the cavity by the sustained pumping, and when the cavity Q factor is restored, most of the stored energy is released in a short high intensity pulse to bring the population inversion back to its threshold condition.

There are two techniques commonly used to produce Q-switching. The first method places a saturable absorber within the cavity. The second technique uses a bulk electro-optic switch consisting of electro-optic crystal, cross polarizers. . In this case, polarizer is aligned to accept linearly polarized wave with electric field aligned in plane of paper. Crystal axis is aligned so that there is equal coupling of the output from the polarizer into the ordinary and extraordinary waves.

**MODE-LOCKED LASER**

In a Continuous Wave (CW) laser, multiple axial modes are excited, photons excited in each mode are random sequences and each random sequence is independent of each other. Thus, the phases of the waves in each mode are uncorrelated and random, which means that the intensity of total output has random fluctuations. It is possible to lock these modes together so that they have a common phase at a particular time and position in the cavity. The effect of mode-locking is to convert the CW output into a train of output pulses, with a narrowness amplitude and spacing of the pulses dependent on the cavity length so that the numbers of multiple modes are excited.

**SINGLE-MODE LASER**

The multiple modes in the CW gas laser has the effect of providing a noisy broad band output and consequently the coherence length of such lasers is small, making them of little use for such applications as holography and coherent communication. Thus, there are many advantages to operate lasers with only one axial mode. This is done either by restrict the laser

cavity length so that only one axial mode is present or by placing frequency restricted transmission components in the cavity to reject all but one axial mode.

## COLOR CENTER LASER

The color center laser operates as a tunable laser operating in a limited wavelength range within 0.8 μm to 4 μm. Such lasers are most suitable for long distance fiber communications.

## SEMICONDUCTOR LASERS

The first semiconductor laser used a junction diode constructed of a single semiconductor (GaAs) and was known as homojunction diode laser. Homojunction means only one junction between p and n type, GaAs. Homo-junction device has a limited range of operating wavelength, has a high threshold pumping requirement, and can only be operated at cryogenic temperatures. Today's diode lasers are hetero-junction device and have a sandwich structure with the central region of a material that is different from that of the outside layers. A range of material can be used depending upon the wavelength of laser emission required. For example, lasers with operating wavelengths close to 0.8 μm, use GaAs as the central region and $Ga_1Al_xAs$ as the outer layers. These lasers use different pumping schemes than that of solid state lasers.

Diode lasers are easy to operate, easy to carry, high modulation bandwidth, high capacity, high power, tunable wavelength, low cost, large range of wavelength and compact pocket size, but disadvantages are high divergence, gaps in tuning core, asymmetric beam profile

and spectral width is not narrow. In the absence of the laser cavity, the device will produce spontaneous emission and operate as an incoherent light source. Such a diode, commonly referred to as a light emitting diode or LED and is in common use as an inexpensive incoherent source.

**LIQUID LASERS**

In general, good crystals are difficult to grow and are very expensive. The homogeneous liquids have a very high optical cavity and would not crack or shatter if the output power becomes very high. But thermal expansion and change in the refractive index due to the temperature rises cannot be ignored. This is controlled by cooling and circulating the liquid solution through the active region. One can, therefore, use tubes filled with liquid instead of laser rods.

**DYE LASERS**

Commercial dye lasers are pumped by nitrogen, argon, Nd-YAG, and excimer lasers, although Xe lamps can be used. Dye laser uses an organic dye dissolved in a suitable liquid such as ethyl alcohol, methyl alcohol or water is used as the active material. The output power is of the order of $10^3$ W/cm$^2$ and can be tuned to oscillate in a given dye over a range of 30 nm and by using different dyes it is possible to obtain oscillations at any wavelength from the near infrared to the near ultraviolet regions.

**CHEMICAL LASER**

The chemical reactions occurring in flames or in explosives, produce a large number of excited atoms and a large amount of light. Chemical energy is generally, the least expensive form of energy and is available in amounts per unit volume or per unit weight and is larger than that of the purely electrical energy. The release of chemical energy is associated with making and breaking of chemical bonds. In principle, the chemical reaction, once initiated, could proceed without any external source of power. This means, a chemical laser could be self-pumping. It could also be highly efficient. There are various chemical lasers available these days such as hydrogen-fluoride chemical laser, $H + Cl_2$ laser, $O + CS_2$ laser, etc. These lasers can lase between 1 to 10 μm.

**FREE ELECTRON LASER**

Such lasers operate between 100 μm to 400 μm and have energies of the order of 5 MeV. They have very useful applications in defense.

**$CO_2$ GAS LASER.**

The first $CO_2$ gas laser using pure $CO_2$ was reported by C.K.N. Patel at the Bell Laboratories in the USA in 1964, with output power in the range of 1mW. Within a year this output powers had been raised to 10W as a result of adding nitrogen to $CO_2$ and by addition of helium to the mixture power the existence of a $CO_2$ laser with an output of 8.8 kW were reported. The $CO_2$ gas laser has become one of the very important lasers and a large number of workers are working in the task of making machines of improved

efficiency, power and quality. Much of the design work has concentrated on two fundamental points, excitation of the gas using direct current (DC), alternating current (AC), radio frequency (RF) or gas dynamic methods and withdrawal of surplus energy from the gas, cooling it by conduction on the walls of the laser cavity or by the passage of the gas mixture through heat exchangers.

Various types of $CO_2$ lasers such as fast axial flow, sealed off, transverse flow, transversely excited atmospheric, gas dynamic, etc. are available but we will consider the axial flow gas type laser only as this laser is very simple in construction and provides many useful applications.

## 1.2. THEORETICAL BACKGROUND

### 1.2.1. ATOMIC ENERGY LEVELS

An atom consists of a positively charged nucleus and electrons that are moving in their own orbits around the nucleus. The electrons move faster when an atom absorbs some energy from an external source. When an electron absorbs some energy it goes to higher energy state and we call it its excited state. The important point is that only certain orbits are possible for a given electron when the atom absorbs a certain amounts of energy. The atom can lose the same energy it absorbed because the electron can return only to allowed lower energy orbits.

Consider a system of two non-degenerate energy levels (energy levels having different amounts of energy) of an atom as shown in Figure 7.

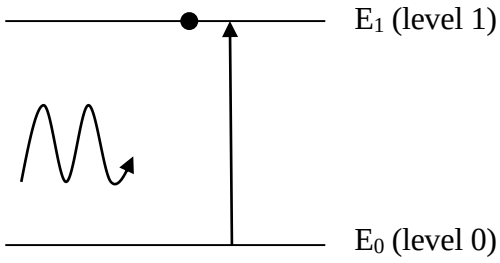



Figure 7: Absorption of energy.

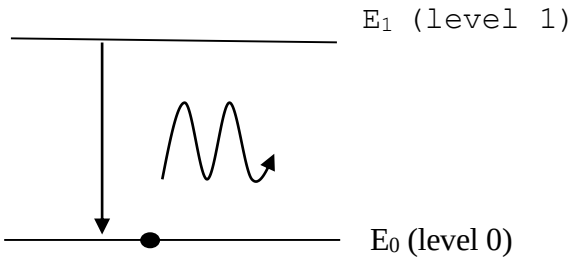



**Figure 8:** Emission of energy.

An atom in the ground state has energy $E_0$ (level 0), while an atom in the first excited state has energy $E_1$ (level 1). An atom in the ground state (energy level 0) can absorb only certain amounts of energy. For example, the atom in the ground state can absorb energy that is equal to $(E_1 – E_0)$ and moves to the first excited state as shown in Figure 7. If it absorbs $(E_2 – E_0)$, then it must move to the second excited state, and so on. The atom can lose energy $E = (E_1 – E_0)$ when it jumps from energy level 1 to energy level 0 as shown in Figure 8. It can never lose any other amounts of energy other than equal to $(E_1 – E_0)$. Hence, it must either keep all its energy or lose an amount equal to $(E_1 – E_0)$ all at once. It means that an atom cannot absorb or lose energy less than $(E_n – E_m)$, n and m are integers such that $n > m$ for a particular pair of energy levels. This is called the quantization of energy.

One of the ways that an atom can gain energy is to absorb a PHOTON from an external source of energy. Since an atom absorbs only certain amounts of energy, a partial absorption is not possible and hence an atom must absorb a whole photon. This means that the energy difference between the two levels of an atom must correspond exactly to the energy of the photon given by:

$$(E_1 - E_0) = h\nu = \frac{hc}{\lambda} \quad (4)$$

In this equation, ν is the frequency of the laser beam, c is the speed of light, $h = 6.625 \times 10^{-34}$ J.s, is Planck's constant and λ is the wavelength of the radiation. This equation shows that the energy of a photon has restriction on wavelength of light that can be absorbed by a given atom or molecule. This means that an atom will absorb light of wavelength λ or will give off light of wavelength λ given by $\lambda = hc/(E_1 - E_0)$. The energy, which is not corresponding to the wavelength λ given by $\lambda = hc/(E_1 - E_0)$, will not be absorbed by the atom.

A molecule is composed of two or more atoms and hence it has more complex energy levels than an atom. In a molecule, three types of energy levels are possible, electronic energy levels due to the motion of the electrons around the nuclei, vibration energy levels due to the motion of nuclei, and rotational energy levels due to the molecule. All kinds of energy of a molecule are quantized and hence one can draw a diagram for its electronic, vibration, and rotational levels. The molecule may have translational energy but it is not quantized and hence it is not important to be considered hereafter.

### 1.2.2. BOLTZMANN'S LAW

Boltzmann's law is one of the fundamental laws of thermodynamics, and discusses the population of energy levels when the atoms are in equilibrium. It is a very important law and helps to understand the principle and working of a laser. According to this law, as shown in Figure 9, the number of atoms $N_i$ in an energy state i in thermal equilibrium at absolute temperature T is given by:

$$N_i = N_0\, e^{\{-(Ei/kT)\}} \qquad 5(a)$$

where, $k = 1.38 \times 10^{-23}$ Joule/$^oK$ is Boltzmann's constant and $N_0$ is the total number of atoms present in the material at absolute temperature. The total number of molecules are distributed such that $\sum N_i = N_0$. In case of degenerate, sublevels of the level $|i\rangle$ with total angular momentum $J_i$, equation 5(a) is given by

$$N_i = (g_i/Z)\, N_0\, e^{\{-(Ei/kT)\}} \qquad 5(b)$$

where the degeneracy $g_i = 2J_i + 1$ gives the number of degenerate sublevels and the partition function Z is given by:

$$Z = \sum g_i\, e^{\{-(Ei/kT\}} \qquad 5(c)$$

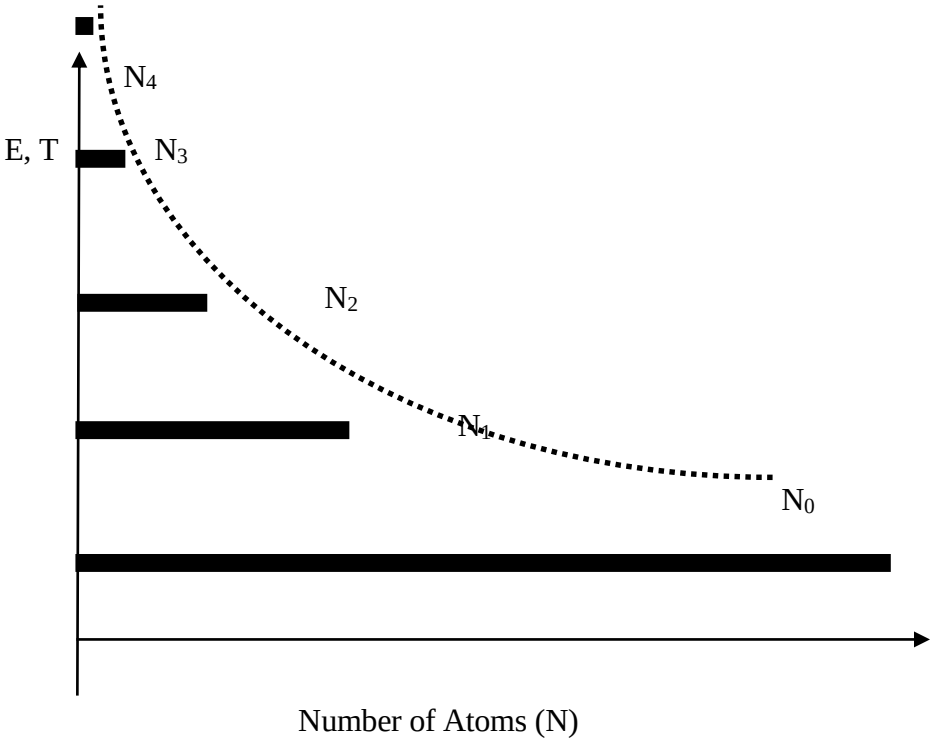



**Figure 9:** Boltzmann' distribution law at thermal equilibrium

Equation 5(a) shows that the number of atoms will always be more in ground state than the higher energy state under thermal conditions.

As the temperature increases, more atoms are raised to higher energy states but still the number of atoms in a higher energy state is found to be less than the next lower energy state. Under thermal conditions, it is impossible to have more atoms in higher energy state than lower energy state. One of the most important conditions for lasing action, in general, is a population inversion, which means there must be more atoms or molecules in higher energy state (excited state) than in the lower state. Without a population inversion, a beam of light directed through the medium with photon energy $h\nu = (E_2 - E_1)$ will simply be absorbed.

The number of atoms per unit volume in a given energy state is called the population of that energy state.

If we consider two-level-energy system only for which $E_1$ and $E_2$ are the energies of the respective levels and $N_1$ and $N_2$ are the number of atoms per unit volume in each level. The ratio $N_2/N_1$ at thermal equilibrium is given by:

$$N_2/N_1 = e^{\{-(E2 - E1)/kT\}} = e^{\{-(hf/kT)\}} \quad (6)$$

It can be seen from equation 6 that $N_2$ can never exceed $N_1$ for an equilibrium situation since T cannot be negative. Thus, a population inversion refers to a non-equilibrium situation in which $N_2$ is greater than $N_1$. The population inversion is possible only if some excitation process called optical pumping is used. The optical pumping can be referred to as a "*Negative Temperature*" condition because equation 6 tells us that $N_2$ will be greater than $N_1$ only if T is negative.

### 1.2.3. FUNDAMENTAL PROCESSES

When an electromagnetic wave of frequency f interacts with some material, three types of fundamental transitions are possible. These are called stimulated absorption, spontaneous emission, and stimulated emission as shown in figure 10.

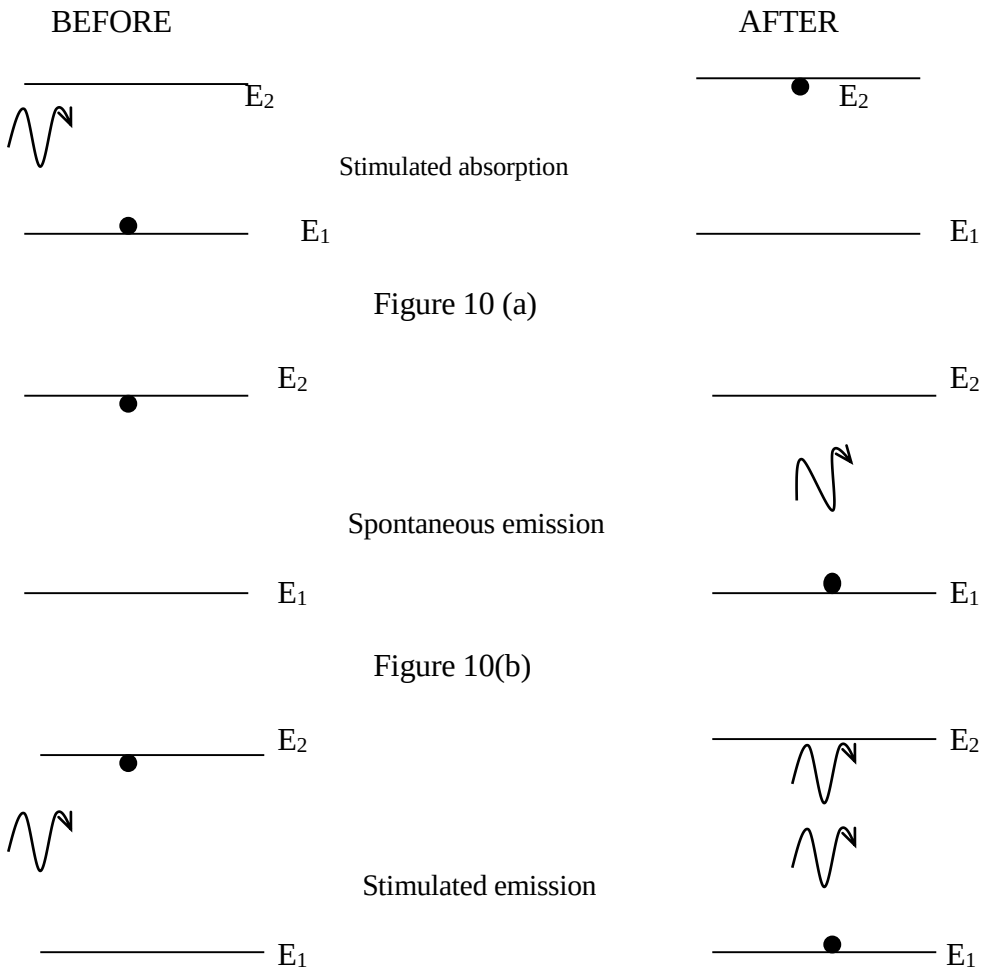



**Figure 10:** Three fundamental processes: (a) Stimulated absorption, (b) spontaneous emission, and (c) stimulated emission

**Stimulated Absorption:**

When a photon with energy equal to the energy difference between the two energy states is absorbed, the atom is raised to a higher energy state, causing an excitation. This is called stimulated (forced) absorption as shown in Figure 10(a). In an electronic transition, the

electron is raised to a higher energy state and in a molecular transition, the vibration energy of the molecule is increased.

Let us consider that the atom is initially lying in a lower energy level depicted by $E_1$ under BEFORE condition as shown in Figure 10(a). If $E_1$ is the ground state, the atom will remain in the ground state until some external energy is supplied to it. Let us consider that an electromagnetic wave of frequency given by $\nu = (E_2 - E_1)/h$ is incident on the material. In this case, an atom in the lower energy state $E_1$ absorbs a photon having energy $h\nu$, and the atom is raised to energy state $E_2$. The energy difference $(E_2 - E_1)$ required by the atom to undergo this change is obtained from the external incident electromagnetic wave. This is the stimulated absorption process through which the atom is forced to absorb energy and go the higher energy state. The probability $(\frac{dN1}{dt})_{ab}$ that an atom will absorb a photon (the rate equation) for absorption process depends on the energy density $\rho_f$ and the number of atoms per unit volume $N_1$ and can be written as:

$$(\frac{dN1}{dt})_{ab} = - B_{12}\, \rho_f\, N_1 \qquad (7)$$

where, $N_1$ is the number of atoms per unit volume, in energy state $E_1$ at a given time t. The quantity $\rho_f$ is the energy density and $B_{12}$ is the probability per unit time per unit energy density that a given atom will be excited from state $E_1$ to state $E_2$. The unit of $B_{12}$ is $m^3Hz/Joule\text{-}s$ and is simply called the Einstein B coefficient or absorption probability.

**Spontaneous Emission:**

When the atoms are in excited states (as a result of stimulated absorption), sooner or later they will drop spontaneously to a lower energy state and a photon is given off with the same energy as absorbed during the process of stimulated absorption. This is called spontaneous emission as shown in Figure 10(b).

Let us consider that at any time t, there are $N_2$ atoms per unit volume in the upper energy state $E_2$. The rate of decay of these atoms due to spontaneous emission will be proportional to $N_2$. Thus

$$\left(\frac{dN2}{dt}\right)_{sp} = - A_{21} N_2 \qquad (8)$$

where, $A_{21}$ is the probability per unit time that a given atom in an excited state will spontaneously undergo a transition to state $E_1$ from $E_2$ state. It is called the Einstein A coefficient or spontaneous emission probability. The product of $N_2$ and $A_{21}$ gives the number of spontaneous transitions per unit volume per unit time (rate per unit volume). The quantity $t_{sp} = 1/A_{21}$ is called the spontaneous emission lifetime. The numerical value of $A_{21}$ and $t_{sp}$ depends only on the particular transition involved.

**Stimulated Emission:**

While the atom is still in an excited state, the incident photon from the external source may cause it to undergo a transition before it decays spontaneously. In this case, the incident photon forces the photon emitted by the excited atom to move in the same direction and hence the gain is achieved as we have two photons now as shown in Figure

10(c). The stimulated photons have the same phase, same polarization, same frequency and travel in the same direction as the stimulating photon. Therefore the stimulated emission process is coherent. In fact the whole stimulated transition process is considered as a wave process of coherent waves and not as a photon process.

Let us suppose that in the case of stimulated emission, the atom is in a higher energy state $E_2$ (excited state) and that an electromagnetic wave of frequency $\nu$ given by $(E_2 - E_1)/h = \nu$ is incident on the material. Since this wave has the same frequency as the atomic frequency, this wave will force the atom to undergo a transition from energy state $E_2$ to energy state $E_1$. In this case the energy difference $(E_2 - E_1)$ is delivered in the form of an electromagnetic wave, which adds to the incident photon as shown in Figure 10(c). In this case the rate of the stimulated emission can be written as:

$$\left(\frac{dN2}{dt}\right)_{st} = - W_{21} N_2 \qquad (9)$$

The rate at which transitions from state $E_2$ to state $E_1$ occur as a result of stimulated emission. The term $W_{21}$ is called the stimulated transition probability and has the dimension of $(\text{time})^{-1}$ as the Einstein Coefficient A has. Here $W_{21}$ does not only depend on the particular transition but also on the intensity of incident electromagnetic wave. Thus $W_{21}$ can be written as

$$W_{21} = \rho_f B_{21} \qquad (10)$$

where, $\rho_f$ is the energy density (spectral quantity) and therefore has the units of $J.m^{-3}.s^{-1}$. The term $B_{21}$ is the probability per unit time per unit spectral energy density that a given

atom will undergo stimulated transition from state $E_2$ to state $E_1$. $N_2$ is the number of atoms per unit volume in the upper state. Combining equations 9 and 10, the rate is given by:

$$\left(\frac{dN2}{dt}\right)_{st} = - P_f B_{21} N_2 \qquad (11)$$

### 1.2.4. CONDITION AT THERMAL EQUILIBRIUM

In case of thermal equilibrium condition, the number of upward transitions must be equal to the downward transitions. so that

$$(dN_1/dt)_{ab} = (dN_2/dt)_{sp} + (dN_2/dt)_{st}$$

$$N_1 \rho_f B_{12} = N_2 A_{21} + N_2 \rho_f B_{21} \qquad 12(a)$$

$$N_2 A_{21} = N_1 \rho_f B_{12} - N_2 \rho_f B_{21} \qquad 12(b)$$

$$= \rho_f (N_1 B_{12} - N_2 B_{21}) \qquad 12(c)$$

$$\rho_f = N_2 A_{21}/(N_1 B_{12} - N_2 B_{21}) \qquad 12(d)$$

$$\rho_f = A_{21}/[(N_1/N_2) B_{12} - B_{21}] \qquad 12(e)$$

Substituting $N_1/N_2$ from the Boltzmann's distribution law (Equation 6), we get,

$$\rho_f = A_{21}/[B_{12}\, e^{(h\nu/kT)} - B_{21}] \qquad (13)$$

According to Planck's black body radiation theory, the spectral radiation density $\rho_f$ at a frequency $\nu$ at a given temperature T, is given by:

$$\rho_f = (8 \pi h \nu^3/c^3)/\{e^{(h\nu/kT)} - 1\} \quad (14)$$

Since equations 13 and 14 must be the same, therefore comparing these equations, we get following two important conditions

$$B_{12} = B_{21} \quad (15a)$$

$$A_{21} = (8 \pi h \nu^3/c^3) B_{21} \quad (15b)$$

Equation 15a tells that the probability of stimulated emission is the same as that of stimulated absorption. This means that for a given radiation density for an atom, a stimulated radiation from energy state $E_2$ to energy state $E_1$ is as probable as stimulated absorption from energy state $E_1$ to energy state $E_2$.

This condition, Equation 15b, shows that the term $(A_{21}/B_{21})$ is proportional to $\nu^3$. That is, the ratio of the spontaneous emission to the stimulated emission is proportional to $\nu^3$. It means that the probability of spontaneous emission dominates over stimulated emission more and more as the energy difference between the two energy states increases.

Equation 15b clearly tells us that the higher frequency lasers are more difficult to produce than lower frequency lasers. This was probably the reason why high frequency lasers like x-ray laser could not be possible in the beginning. These days such lasers are possible to

make but very special technique is used. Such lasers are very expensive and are not for common use.

### 1.2.5. PUMPING SCHEMES

A process of raising atoms from a lower energy state to a state of higher energy is called pumping process. A number of different pumping techniques are used to achieve the population inversion. Different schemes are used for different types of lasers. For example, optical pumping is not suitable for two energy level lasers such as a diode laser. The electrical pumping is most suitable in this case.

**Optical Pumping**:

The optical pumping method was used for the first time in the ruby laser. A ruby rod was irradiated with an intense optical wide band. The laser rod absorbs the red component of the wide band source and the number of atoms $N_2$ increases at the expense of $N_1$ and hence the population inversion is obtained. Nd-YAG laser also uses optical pumping.

**Electrical Pumping:**

The second laser to be invented was He-Ne gas laser, which uses discharge pumping called electrical pumping. In this method, a high electric field is applied to a gas mixture to break the gas down. The high velocity particles transfer their kinetic energy to the atoms through collision.

**Injected Pumping:**

Semiconductor diode lasers are injection pumped. A forward current is injected across a junction diode. The injected carriers can invert the population to provide optical gain.

**Chemical Pumping:**

A chemical reaction, either controlled or uncontrolled can produce population inversion. This is chemical pumping. This is used in a laser where the products of an explosive reaction are directed into a cavity to produce photons.

**Dynamic Pumping:**

Another method is dynamic pumping where the population inversion of a gas is produced by pumping it through a choke to change the velocity of the molecules and consequently to produce population inversion.

## 1.3. APPLICATIONS OF LASERS

### 1.3.1. SURFACE HARDENING

Laser surface hardening (heat treatment) is a process whereby a defocused beam is scanned across a hardenable material to raise the temperature near the surface above the transformation temperature. There are various advantages of laser surface hardening such as in this case very low amount of total input to the part produces is required and the selective areas can be hardened without affecting the surrounding area.

### 1.3.2. LASER WELDING

There are basically two types of laser welding, continuous laser welding and pulse laser welding. $CO_2$ laser can be used for both. The main advantage of this technique is that minimum heat input is required resulting with very little distortion. This technique provides very high strength welds and can weld some metals difficult to weld by other techniques especially dissimilar metals. This provides an automated process that can produce very precisely located welds in areas difficult to reach with some other techniques.

### 1.3.3. LASER CUTTING

Industrial laser cutting is done with CW or pulsed $CO_2$ and high-repetition rate pulsed Nd-YAG lasers. The process is generally gas-assisted in both the techniques of continuous laser cutting and pulse laser cutting.

### 1.3.4. LASER MARKING

Laser marking is a process whereby serial numbers or identification, including logos are placed on parts by evaporating a small amount of material with a pulsed $CO_2$ or Nd-YAG laser. The main advantages of the technique are high-speed, easily automated, no mechanical contact with part is required and can be done through transparent enclosures. Hole piercing is also done with pulsed $CO_2$, Nd-YAG and Nd-Glass lasers.

### 1.3.5. LASERS IN OPHTHALMOLOGY

Various lasers such as $CO_2$, dye laser, diode and excimer laser are used in ophthalmology. The main use of lasers in ophthalmology is to ablate, create adhesion, make holes, coagulate, destroy, disrupt, diagnose and to vaporize tissue for therapeutic or diagnostic purposes and the treatment of retinal detachment and photocoagulation. In photocoagulation therapy the temperature of the tissue is increased from 37$^{o}$C to at least 50 $^{o}$C producing denaturation of protein and coagulation in the region of the absorbent tissue element. In photodynamic therapy the temperature rise is minimum usually 1$^{o}$C or less.

### 1.3.6. LASERS IN OTOLARYNGOLOGY

Lasers are also used in otolaryngology. Otolaryngology is a surgical specialty that deals with the treatment of the disorders of the ear, nose, throat, and oral cavity. There is an extremely rich system of blood vessel in the area of the head and neck which create a problem of excessive amount bleeding in standard surgical procedures. This problem is solved in new Laser equipment which is capable of being accurately manipulated in the areas of restricted access.

### 1.3.7. LASERS IN NEUROLOGY

The field of neurosurgery deals with the surgical treatment of various neural tissues in the skull, the spine, and elsewhere in the body. The laser can be used for incising tissue, boring holes in the skull, vaporizing lesions, and catuerlizing blood vessels. The laser can convert

neoplasms to vapor in a very short period of time. The extent of damage around a lesion that has been treated with a laser is less than a millimeter. This is an advantage in treating areas in the brain where it is essential that the least possible amount of tissue be destroyed. $CO_2$ surgical laser can be used to coagulate blood vessels up to 1 mm in external diameter in neurosurgery.

### 1.3.8. LASERS IN BURN SURGERY

Lasers are also used in burn surgery. There are three kinds of burns; a first-degree burn is simple erythema or redness of the skin, a second-degree burn is erythema plus the formation of blisters, and a third-degree burn is the through and through necrosis of the epidermis and the dermis of the skin. Third-degree burns are often charred black. The UV lasers and the $CO_2$ surgical laser can be used for the excision of the burns.

### 1.3.9. LASERS IN GASTROENTEROLOGY

Gastroenterology is a field of medicine like ophthalmology and deals with the study of diseases of the digestive system. It is a specialty in which recent technical developments such as fiber optic endoscope have been adopted to improve diagnostic technique. Endoscopes are used to go down into the stomach or up into the colon for the purpose of obtaining a biopsy or observing a given anatomical abnormality. $CO_2$ (penetration depth in tissue 0.05 mm), Nd:YAG (penetration depth in tissue 0.8 mm) and argon lasers (penetration depth in tissue 0.2 mm) are used in gastroenterology. These lasers can also be used in dermatology which is the medical specialty concerned with diseases of the skin.

### 1.3.10. APPLICATIONS OF LASERS IN AGRICULTURE

Lasers have immense applications in agriculture. Study of UV effects on plants is an important area from a Namibian point of view, as lot of UV is present in solar radiation in Namibia (Singh, 2002). In Namibia, due to extreme dryness the soil soaks up a lot of water. In addition, the farmers use large amounts of fertilizer and other minerals to enhance soil fertility. The implications of these practices are hardly studied scientifically in Namibia. The laser induced fluorescence signal emitted from vegetation is highly correlated to the photosynthesis and can serve as an indicator for plant health (Smorenburg, Visser & Court, 2000). Therefore, the use of laser techniques to study the effect of lack of vital mineral in the soil as well as water stresses due to lack of rain on plant growth and the health study under local environmental conditions will be very worthwhile in Namibia.

Laser induced fluorescence is a new powerful technique in the field of remote sensing and has been used successfully in detecting vegetation stress and in monitoring water quality. The growth of plants is affected by several environmental parameters of which light (particularly UV) is the most important. UV induced blue-green and far-red fluorescence from wheat leaves have been studied by Meyer, Cartelat, Moya & Cerovic (2003). Various authors, Arfsten and Schaeffer (1996); Balakumar (1993); Ballare (1991); Barnes (1990, 1996), D'Ambrosio, Szabo and Lichtenthaler (1992); Teramura (1980, 1984, 1986, 1987, 1990); Singh (2000); Drilias, George, Efi, Yiola and Yiannis (2004), Day (1993), have

studied the effect of enhanced UV-B on vegetation. Ambasht and Agrawal (1995) studied the physiological growth of maize plants.

The fluorescence spectra of different plants depends on the photosynthetic activity of these species and environmental stress factors such as 'acid rain', the presence of heavy metals, nutrient stress, drought, etc. The spectrum of a plant excited by UV pulsed nitrogen gas laser (337 nm) shows at least four spectral bands which can characterize the plant and serve to identify it. The fluorescence intensity ratios (FIR) at these wavelengths are used for these purposes. The intensity ratios of the blue and green emission bands at 425 and 530 nm with respect to the chlorophyll fluorescence bands at 690 and 740 nm have great potential in determining the health of plants (Sailaja, Chandrasekhar, Rao and Das 2004; Daughtry, Walthall, Kim, Brown de Colstoun and McMurtrey, 2000; and Anderson and Buah-Bassuah, 2004). Remote sensing of the health status of vegetation using UV laser induced fluorescence (LIF) has already been pursued by many workers, (Anderson *et al.* 2004; Saito *et al.* 1999; Wood, McMurtrey and Newcomb, 1984 and Wood, Newcomb & McMurtey, 1985).

## 1.4. STATEMENT OF THE CURRENT PROBLEM

There are various factors such as light, water, minerals, gravity etc., which affect both mankind and plant organisms. Light has profound effect on the growth of the vegetation. Light has a spectrum well beyond the visible on either side, i.e. UV and infrared. Recent measurements of ozone levels have led to a concern that the stratospheric ozone layer is

being depleted as a result of contamination with man-made chlorofluorocarbons. Concomitantly, the amount of solar UV-B radiation reaching the Earth's surface is increasing. UV-B radiation has been shown to be harmful to living organisms, damaging DNA, proteins, lipids and membranes. Plants use sunlight for photosynthesis and are unable to avoid exposure to enhanced levels of solar UV-B radiation. Thus, mechanisms by which plants may protect themselves from UV radiation are of particular interest. Hollosy (2002) summarized the main aspects of ultraviolet radiation on plants at physiological and biochemical level, with particular emphasis on protective structures and mechanisms. It should further be noted that the content of UV in solar radiation varies from one location to other.

Plants may also suffer from other stresses that may be induced by water, minerals, dissolved metals. Fluorescence techniques are also useful in studying the physiological state of the plants (Chappelle, Wood and McMurtrey, 1984a; Broglia, 1993; Stober and Lichtenthaler, 1993 and Tezuka, Yamaguchi and Ando, 1994). When plant leaves are irradiated by UV radiation, fluorescence spectrum shows four peaks centered at 450 nm, 530 nm, 685 nm and 730 nm. The ratio of blue (450 nm) to red (730 nm) fluorescence differs from plant to plant and is considered a stress indicator. It responds to any environmental changes including water stress. The ratio of blue (450 nm) to green (530 nm) fluorescence reflects the chlorophyll and carotenoid pigmentation of green leaves. The chlorophyll fluorescence is highly correlated to the mechanism of photosynthesis, and

hence the health status of plants can be studied (Chappel et al. 1984; Chappelle, Wood Jr, Newcomb, and McMurtrey, 1985) easily using LIF.

## 1.5. DEFINITION OF THE PROBLEM

There is considerable amount of UV radiation present in Namibia and its influence on the growth of plant is very vital. It is known that the growth of similar species of plants in different regions is different. Stratospheric ozone depletion has caused an increase in the amount of ultraviolet-A (UV-A) and ultraviolet-B (UV-B) radiation reaching the earth's surface (Gao, Zeng, Slusser, Heisle, Grant, Xu, & He, 2004). It is suggested that the maximal ozone depletion and peak UV-B levels will occur during the next decade, with a return to pre-1980 levels of stratospheric ozone and UV-B by the middle of this century (Day & Neale, 2002; Gao et al. 2004). It is observed that plants do not grow tall in Namibia due to certain stress factors; one of the most important is exposure to UV radiation. Having established this fact, which is also very vital for the advancement of knowledge, it may be desirable to search species that are least influenced by the environmental stresses. Therefore, this project will have the direct impact on our local environmental development.

## 1.6. MAIN OBJECTIVES OF THE STUDY

The main objectives of this research were to carry out fluorescence studies on stresses in maize in order to understand the mechanism of environmental stress on the maize plants and the effect on their growth. For this study maize seeds and its plants were considered,

and this is due to its socio-economic importance, as well as the fact that maize is one of the staple food in Namibia. Through this project a laboratory facility was created at the University of Namibia to carry out experimental work in this important field of laser techniques.

The effect of different kinds of stresses on plants grown under different controlled conditions in a greenhouse and plants grown under normal field conditions could be investigated. Key activities to be covered through this study are:

1. Literature collection and purchase of laser equipment;
2. Purchase of other relevant component and items;
3. Setting up the laboratory for field and controlled conditions;
4. Development of laser induced fluorescence techniques to detect environmental and controlled stresses on plants;
5. Measurements of growth parameters under different stress;
6. Laboratory measurements of fluorescence spectra from plants grown under different controlled and field conditions;
7. Studying fluorescence intensity ratios and relate them to the growth of the plants; and
8. Studying fluorescence spectra from the seeds and comparing with the plants grown under different conditions.

## 1.7. SCOPE AND LIMITATION OF THE STUDY

Although laser applications are broad and provide accurate information, they are very expensive to be adopted or to replace existing techniques easily. This is why very little work has been done in this area using laser equipment. Laser-induced fluorescence (LIF) application on plants has been considered for the present study.

It will be the first attempt in Namibia to use LIF in plant study. Since the study of plants is much localized and LIF technique is very sensitive, we expect new important information regarding the status of Namibian plants under local environmental stresses. Some of the local environmental stresses for the current study would be:

1. excessive UV-A and UV-B in solar radiation (Due to ozone depletion),
2. water stress (Namibia being the driest country in Sub-Sahara Africa), and
3. temperature stress, etc.

The objectives of this research are to carry out fluorescence studies on maize plants under controlled stresses in order to understand their growth parameters and to relate this mechanism with the environmental stresses on similar plants. The potential applications of lasers are broad, as stated above, but for the purpose of this study the focus will be on one or two areas of application. The most important applications of UV lasers suitable to the current socio-economic development of Namibia can be found in medicine and agriculture. The most relevant economic sector of research using laser in Namibia is agriculture. Furthermore, it is clear that not all possible aspects of agriculture can be

covered through this study. In addition, it is not possible to consider all types of plants (vegetation) in Namibia. Due to the above limitations, we will consider the application of laser in the study of LIF on plants of maize only. Experimentation will involve irradiating the plant's leaves with UV radiation (such as 337 nm from a Nitrogen laser) and recording the fluorescence spectra and then carrying out statistical analysis. The study will investigate the health status and LIF spectra of these plants under different controlled conditions such as UV, temperature, and water stresses, etc.

# CHAPTER 2: LITERATURE REVIEW

## 2.1. GENERAL STUDIES ON THE EFFECTS OF UV-A AND UV-B ON PLANTS

It is well known that life of mankind and plant kingdom on earth is affected by light through photosynthesis activity. There are many other parameters that affect the growth of plants but light alone can be considered the most important parameter that governs the health and growth of plants. It can be taken as an essential stress indicator of health status of plants. The solar radiation contains some natural UV that is filtered out through the atmosphere to the ground. Therefore, as soon as seedling breaks through the soil surface it is exposed to natural UV radiation. The plants grow continuously under the stress of UV. The continuous growth of plants indicates that they can tolerate or adapt to natural radiation and can survive. It is observed that when plants are grown in a greenhouse without UV radiation they cannot tolerate UV exposure to greater extent. These plants experience UV shock when they are planted outside and this shock can kill these plants. It is also known that plants adapted to UV radiation are impaired in their development, structure and function by increased radiation. The epidermis usually protects the inner leaf with high concentrations of flavonoids and anthocynanins, which absorb the UV radiation safely. UV-induced reduction in photosynthetic activity shows, however, some UV radiation penetrates into the lower cell layers.

In the following section, dealing with the effect of natural solar UV radiation, we will review the subject and will highlight the work done in this field up to 2006. The effects

of UV radiation on the growth of plants and their physiological conditions will be discussed in greater depth.

### 2.1.1. EFFECT OF NATURAL SOLAR UV RADIATION

The effect of natural solar UV radiation on the growth of tomato and radish plants was studied (Tezuka, Toshihiro & Ikuko, 1993) using polyvinyl chloride films with different UV transmission. It was found that the growth increases with an increase in chlorophyll content and photosynthetic activity. Many workers (Klein, 1978; Bornman, 1989; Takeuchi, Akizuki, Shimizu and Kondo, 1989; Teramura, 1983; Teramura, Ziska, & Sztein, 1991; Hashimoto and Tajima, 1980; Cen and Bornman, 1990) have studied the effects of UV radiation on various plants. Cultivation of crops in a greenhouse covered with plastic film such as polyvinyl chloride, which is opaque to UV, has been used extensively.

The effect of solar UV on plant growth was studied using UV-transparent and UV-cut polyvinyl chloride film, both of which transmitted uniformly about 85% of the visible waveband (Hashimoto, Kondo, & Tezuka, 1993). Various workers (Lees, Evans, & Brown, 1991; Holzwarth, 1986; Hodges and Moya, 1988; Owens, Webb, Alberte, & Mets, 1988) have worked on the characterization of the time-resolved fluorescence properties of photosynthetic material. Lees *et al.* (1991) found that photosynthetic development, measured as light induced oxygen evolution is impaired. They considered that this was as

a consequence of source in the medium inhibility photoautotrophic growth as pointed by Murshiga and Skoog (1962).

It is not yet clear whether the use of opaque plastic film is appropriate for the cultivation of crops in all cases. It is also not fully understood whether natural solar UV radiation (400-290 nm) consisting of the UV-A and almost all UV-B regions on the earth's surface is also deleterious for the growth of plants. Tezuka *et al*. (1993) investigated the effects of radiation with UV from the natural solar spectrum on the growth, photosynthesis and other physiological activities of plants. In order to determine whether solar UV radiation induces the inhibition of plant growth and photosynthesis compared to visible radiation (no solar UV), a series of polyvinyl chloride films were employed (Brandle, Campbell & Sisson, 1977; and Van, Garrard & West, 1976, 1977). The content of (total) chlorophyll a and b can be expressed on the basis of leaf area (Arnon, 1949) taking leaves homogenized and extracted thrice in chilled 80% acetone in a glass homogenizer (Tezuka, Sekiya & Ohno, 1980; Tezuka, Chitose & Yukio, 1989)

### 2.1.2. UV-A RADIATION

The quality and quantity of solar radiation that reaches the earth's surface are also subject to wide fluctuations owing to weather conditions and seasons, but radiation in the solar UV region plays a vital and key role as a limiting factor in the growth of plants (Sato and Kumagai, 1991). The influence of natural solar on the ground is changeable. The effects of a constant influence of near UV (especially UV-A) radiation from UV lamps, instead

of changeable influence of natural solar UV on the growth and physiological activities of radish plants were investigated by Tezuka et al. (1993). These plants were grown in plastic frames covered with UV non-transmitting polyvinyl chloride films. He found that the growth of plants was promoted by the UV-A radiation, and the promotion by UV-A radiation was associated with an increase in chlorophyll content and photosynthetic activities. It has positive role in the biosphere such as in UV-A dependent vision. UV radiation is an important physical stressor for many exposed organisms including plants. The effects of UV exclusion and its importance in interpreting the data were discussed by Cockell, Southern and Herrera (2000).

Studies in the literature of UV effects on plants measure the ambient exposure with radiometers and spectroradiometers and provide a measure of the UV exposure to plants. Factors such as the inclined surfaces of some of the individual plant canopy, self-shading and shading of the canopy by neighboring plants, the UV exposure to the plant canopy is much less than the ambient exposure. A more accurate measure of the UV exposure to the plants (Parisi, Wong & Galea, 1996) is the dosimetric technique.

The UV treatment is affected by photosynthetically active radiation (PAR) levels (Giannini, Pardossi & Lercari, 1996; Teramura, 1980; Warner & Caldwell, 1983; Mirecki & Teramura, 1984; Cen & Bornman, 1990; Adamse, Britz & Caldwell, 1994). Whether the presence or absence of activated photomorphogenic, photosynthetic and photoprotective systems during the UV treatment, affect plant sensitivity to UV radiation

or not, has not been investigated so far. For example, the possible role of photosynthetic process and of the xanthophyll cycle activated by wavelengths > 500 nm has not been determined because UV irradiation always been given in the presence of PAR.

### 2.1.3. EFFECTS OF UV-A ON MAIZE GROWTH

Singh, Dube and Gupta (1998) studied the effects of UV-A radiation on the growth of maize plants and their fluorescence spectra. The emission spectra of the second leaf from bottom side of each maize plant excited by 337 nm were obtained using spex 1680, 0.22m double monochromator known as Spex Fluorolog made in USA. The spectra consisted of two peak bands in the blue-green region at 435 nm (F435) and 525 nm (F525) respectively and two peak bands in the red region at 684 nm (F684) and 740 nm (F740) respectively. The ratios of these peaks as ($\frac{F435}{F525}$), ($\frac{F435}{F684}$) and ($\frac{F684}{F740}$) were calculated in each case. The ratio of the blue to green ($\frac{F435}{F525}$) was found to be increased and the ratio of blue to red ($\frac{F435}{F684)}$ and chlorophyll fluorescence ($\frac{F684}{F740}$) ratio were found to be decreased. It might be due to the fact that the intensity of red peaks was increased due to reabsorption of light when the plants were treated with UV-A, and hence the ratios ($\frac{F435}{F684}$) and ($\frac{F684}{F740}$) were reduced.

### 2.1.4. UV-B RADIATION

UV-B radiation (280–320 nm) is an environmental challenge affecting a number of metabolic functions through the generation of reactive oxygen species (ROS). Plants protect themselves from this harmful radiation by synthesizing flavonoids, which act as a screen inside the epidermal cell layer, and by making adjustments to the antioxidant systems at both cell and whole organism level. Carletti, Masi, Wonisch, Grill, Tausz and Ferretti (2003) described the flavonoid content, the photosynthetic pigment composition and the proline, tocopherol and ascorbate content in UV-B exposed maize plants.

The effects of UV radiation on various kinds of plants have been studied by many workers (Klein, 1978; Hashimoto & Tajima, 1980; Teramura, 1983, 1991; Bornman, 1989; Bornman & Vogelmann, 1991; Bornmann & Teramura, 1993; Takeuchi *et al.* 1989; Tevini, Iwanzik & Thoma, 1981; Tevini, Thoma & Iwanzik, 1983; Tevini & Pfister, 1985; Tevini & Teramura, 1989; Tevini, Braun & Fieser, 1991; Tevini, 1994; Ziska, Teramura & Sullivan, 1992; Ziska, Teramura, Sullivan & McCOY, 1993; Appenroth,1993; Braun & Tevini, 1993) found that UV-B radiation (280-320 nm) inhibits photosynthetic activity and the growth of plants. These inhibitory effects are caused by the supplementary radiation (high intensity) of UV-B in wavelength regions of natural solar UV or by the radiation of UV-B including a part of wavelength region of UV-C that is below 280 nm. It has already been pointed out but not confirmed that the promotion of the growth of plants is caused by the increase in the chlorophyll content and the activities of photosynthetic and respiration in response to solar UV radiation.

The solar spectrum reaching the earth's surface does not extend below approximately 290 nm because ozone in the stratosphere effectively absorbs all radiation of shorter wavelengths (Campbell, 1980; Tezuka, 1990, 1991). Therefore, the natural solar radiation of the UV-B region at the earth's surface is between 290 and 320 nm. Many reports (Hashimoto & Tajima, 1980; Teramura, 1980, 1983; Ambler, 1975; Haldall, 1964; Sisson & Caldwell, 1975, 1976; Van et al. 1976, 1977) have shown that UV-B radiation inhibits photosynthesis and growth of plants.

A reduced stratospheric ozone layer may affect plants in several different ways and alter species composition and productivity since shorter wavelengths UV-B (280-320 nm) radiation will increase even with a relative small decrease in ozone. Caldwell (1981, 1983, 1984) studied several species and found that the plants native to higher latitudes are often found to be more sensitive to UV radiation than those native to lower latitudes. Changes in the internal spectral regime of leaves have been measured in plants exposed to enhanced level of UV-B radiation (Bornman and Vogelmann, 1991). Detailed studies of PSII have shown that the functional integrity between the water splitting complex and P680 on the oxidizing side of PSII is impaired with exposure to UV-B radiation (Renger, Völker, Eckert, Fromme, Hohm-Veit & Graberet, 1989). The reducing side of PSII is also a sensitive UV target. They found that the chloroplast protein $D_1$ is rapidly degraded on exposure to UV radiation. This degradation is thought to be mediated by the semiquinone anion radical. Bornman (1991) studied UV radiation as an environmental stress in plants

and found that the exposure to UV-B radiation changes the light microenvironment of the leaves.

Indeed the effects of increased UV-B radiation on plant growth and crop productivity are still of much speculation and, obviously, a scientific challenge (Stapleton, 1992). Several authors (Adamse et al. 1994; Lercari & Lipucci, 1991; Lercari & Sodi, 1990; Middleton and Teramura, 1994) have discussed the interaction between higher plants and UV-B radiation in the lake of advancement of the knowledge. The action spectra and the kinetics of the responses can identify the causal factors involved in UV mediated responses. Ultraviolet induced inhibition of elongation growth appears to be a general phenomenon (Tevini & Teramura, 1989; Barnes, 1990, 1996; Lercari, Sodi & Sbrana, 1989, Lercari, Sodi & Di Paula, 1990, Lercari, Bretzel & Piazza, 1992). Bertram and Karlsen (1994) and Bertram and Lercari (1996) measured the short and long term stem elongation responses to UV radiation.

UV-B radiation sources emit small but biologically very effective UV-C radiation, which is not present in the solar radiation at the earth surface. Even the best sun tracking system supplementing solar UV-B by a definite amount of artificial UV-B will suffer from these inconveniences and uncertainties. Continuous UV-B irradiation of barley seedlings disturbs the vertical growth, which may be due to destruction of IAA in the leaf tips. Plants possibly protect themselves against increased UV radiation by an increase synthesis

of pigments in the epidermis. It has been shown that UV-B radiation at about 300 nm effectively stimulates the synthesis of flavone glycosides and anthocynanins.

Barka, Kalantari, Makhlouf, and Arul (2000) and Barka (2001) exposed tomato (*Lycopersicon esculentum* L.) to a low level (3.7 kJ $m^{-2}$) of UV-C (254 nm) radiation, which is defined as a beneficial level. They observed a delay in fruit ripening by at least one (1) week for treated fruit. They investigated the changes in the activities of different enzymes involved in defense mechanisms in tomato fruit in response to a beneficial level of UV-C. The irradiation leads to an increase in the guaiacol peroxidase and ascorbate peroxidase activities, whereas catalase activity remains similar to the control. The activities of superoxide dismutase and ascorbate oxidase were significantly reduced after UV-C exposure. In UV-C-treated fruit, an increase of lipoxygenase and phenylalanine ammonia lyase activities occurred within the first 5 days, followed by a second period in which these activities were below those of the control. This study suggests that the level of UV-C used induced a rapid but moderate accumulation of photo-oxidation products, to which plants react by stimulating their defense mechanisms against oxidation. This activation may explain the delay observed in ripening and senescence of irradiated tomato fruit.

Barka (2000) further studied the effects of a hormic dose (3.7 kJ $m^{-2}$) of UV-C (254 nm) on changes in fruit membrane lipids perox-idation markers during storage using tomato (*L. esculentum*) fruit. There were two distinct response phases following the treatment. A

significant induction of lipid peroxidation markers (lipofuscin-like compounds, malondialdehyde, aldehydes, pentane, ethane, hydrogen peroxide, and efflux of electrolytes including potassium and calcium) occurred within the first 5 days. This induction suggests that the cell membrane was the primary target of UV-C irradiation. After this period, the level of all of these peroxidation markers become lower in UV-C-treated fruit than in control fruit, suggesting the induction of a defense or repair mechanism, probably involving production of antioxidants and activation of antioxidative enzyme. Within the second phase, any changes in lipid peroxidation activity reflected the fruit ripening senescence process rather than the UV-C effect. Kovacs and Keresztes (2002) also studied the effects of gamma and UV-B radiation on plant cells. Tendel and Hader (1995) studied the effects of UV radiation on orientation movements of higher plants. Low UV-B level do not induce direct damage but may effect movement reactions in higher plants. UV-B was found to impair (damage) phototropic and gravitropic reactions of shoots, photonastic reactions of leaf joints and opening movements of fluorescence. Giannini et al. (1996) studied the relationship between the levels of ambient photosynthetically active radiation (PAR) and applied UV dose with regard to expansion growth, biomass production, leaf water relation and gas exchange of plant under greenhouse conditions.

Sutherland, B. H., Takayanagi, Sullivan & Sutherland, J. C. (1996) studied UV responses of plants grown under controlled conditions of day length, temperature and illumination. Enhanced UV-B radiation affects crop species and varieties in a differential way

depending on their sensitivity, which is determined by their genetic and enzymatic ability to accumulate UV protecting pigments and to repair UV-B damage. This has been studied by many workers Robberecht, Caldwell & Billings (1980); Tevini, *et al*., 1991; Pang and Hays, 1991; Teramura, Perry, Lydon, McIntosh & Summers, 1984; Teramura, Sullivan & Lydon, 1990; Teramura *et al.*, 1991; Barnes, 1993, 1996; Dai, Peng, Chavez & Vergara, 1994; Caldwell and Flint, 1994; and Caldwell, Bjoorn, Borman & Flint, 1995.

The most important parameter for global irradiance at the surface of the earth is the solar elevation above the horizon. The spectral alteration in the UV-B range is mainly caused by ozone layer through which solar radiation must pass (Seckmeyer, 1989; Green, 1983). Since cloudiness is a very decisive factor for global irradiance, it is difficult to define general criteria for its influence, however the small contribution of transmitted UV-B radiation can not be neglected since the photochemical and damaging effects of plants increase exponentially with decreasing wavelength.

### 2.1.5. EFFECTS OF UV-B ON MAIZE GROWTH

As in the case of UV-A, Singh (2000) also studied the effect of UV-B on the growth parameters of maize plants. The emission spectra of second leaf from bottom side of each plant excited by 337 nm were obtained using spectrofluorimeter, model Florolog-2, make Spex – USA known as Spex Fluorolog having excitation bandwidth 3.77 nm and emission bandwidth 1.7 nm. Similar effects were observed which could be due to a photooxidative destruction of the phytohormone indole acetic acid followed by lower cell wall

extensibility (Ros & Tevini, 1995). The width of the second leaf of each plant was found to be increased slightly. The ratios of blue to green ($\frac{F435}{F525}$ ), blue to red ($\frac{F435}{F684}$) and chlorophyll fluorescence ratios ($\frac{F684}{F740}$) were found to be decreased. This showed that the intensity of green and red peaks was increased under the treatment of UV-B radiation. This might be due to the decrease in the chlorophyll content and the phtosynthetic activity. The ratios of blue to green, blue to red and chlorophyll fluorescence ratio were decreased due to the decrease in the photosynthetic activity and chlorophyll content. It was clear from this study that these ratios could be taken as stress indicators and could be used to study the health status of a plant at an early stage.

It is well known that depletion of the stratospheric ozone layers is causing an increase in UV-B radiation reaching the earth. This has raised a major concern about the effects of increasing UV-B levels on plants. Mackerness (2000) made predictions based on the expected rise in UV-B radiation in the next few decades. He indicated that the growth, development, and yield are going to be affected because of rise in UV-B radiation. However, because of little information about UV-B effect on plants is not fully known, measures should be taken to protect crops from increasing UV-B radiation in the environment.

When studying the effects of UV-B radiation on plants, Smith, Anderson, Fischer & Webb (2002) found that there was a decrease in dry weight and this reflects the cumulative effect of many small disruptions in plant function. Measurements of chlorophyll concentration

and the level of UV-absorbing compounds are also used to gauge plant health during and after UV-B exposure. When a variety of vegetable crop plants were screened for UV-tolerance, it was found that the levels of chlorophyll and UV-absorbing compounds did not correlate with sensitivity. Biomass accumulation was, however, correlated with UV-sensitivity; plants that accumulated more biomass over a two-week period were more likely to be UV-B sensitive. This suggests that a rapid growth rate renders plants more sensitive to the injurious effects of UV-B radiation.

Skórska (2000) stated that pea plants are more susceptible to long-wave UV-B irradiation (305 nm – 320 nm, 7.7 kJ $m^{-2}$ $d^{-1}$, 4 weeks) in comparison with the triticale. This difference is more apparent from the changes in total area of leaves and dry mass of shoots, rather than from the parameters of chlorophyll fluorescence and net photosynthetic rate. In fact, UV-B can change the anatomical features of plants, inhibit photosynthesis, slow down their growth, reduce biomass, and lower the crops yield (Caldwell, 1977).

Teramura and Murali (1986) as well as Teramura and Sullivan (1987) discussed that the photosynthetic apparatus of some plant species appeared to be well-protected from direct damage from UV-B radiation. Leaf optical properties of these species apparently minimize exposure of sensitive targets to UV-B radiation. However, damage by UV-B radiation to Photosystem II and Rubisco has also been reported. Secondary effects of this damage may include reductions in photosynthetic capacity, RuBP regeneration and quantum yield. Furthermore, UV-B radiation may decrease the penetration of PAR, reduce

photosynthetic and accessory pigments, impair stomatal function and alter canopy morphology, and thus indirectly retard photosynthetic carbon assimilation. Subsequently, UV-B radiation may limit productivity in many plant species. In addition to variability in sensitivity to UV-B radiation, the effects of UV-B radiation are further confounded by other environmental factors such as $CO_2$, temperature, light and water or nutrient availability. Therefore, we need a better understanding of the mechanisms of tolerance to UV-B radiation and of the interaction between UV-B and other environmental factors in order to adequately assess the probable consequences of a change in solar radiation.

The effects of enhanced UV-B radiation on the needle anatomy of loblolly pine (*Pinus taeda* L.) and Scots pine (*Pinus sylvestris* L.) were studied in the field under supplemental UV-B radiation supplied by a modulated irradiation system. Enhanced UV-B radiation caused different responses in these two species. The needles of loblolly pine had larger amounts of tannin in the lumen of epidermal cells and more wall-bound phenolics in the outer epidermal walls of UV-B-treated needles, whereas the most pronounced effect on Scots pine needles was increased cutinization. In both species, the outer epidermal cell walls thickened and the needle cross-sectional and mesophyll areas decreased. This suggests that more carbon may have been allocated to the protection mechanisms at the expense of photosynthetic area. The difference in response between these species suggests that the response to UV-B radiation is not mediated by a single mechanism and that no generalization with regard to the effects of UV-B on conifers can be made.

The effects of long-term elevated UV-B radiation on silver birch (*Betula pendula* Roth) seedlings were studied by Tegelberg & Julkunen-Tiitto (2001) over some growing seasons in an outdoor experiment. Changes in growth appeared during one growing season; the stems of the UV-B treated seedlings were thinner and their height tended to be shorter compared with that of the control seedlings. In contrast, there was no UV-B effects on biomass, bud burst, bud dry weights, leaf area, and rust frequency index or chlorophyll concentrations in any of the summers. During the three-year study, the flavonols were significantly increased by the elevated UV-B only in other growing season.

Rousseaux, Scopel, Searies, Caldwell, Sala & Ballaré (2001) studied the effects of solar ultraviolet-B radiation (UV-B) on the growth of the dominant plant species of a shrub-dominated ecosystem in Tierra del Fuego. This part of southern Argentina can be under the direct influence of the Antarctic 'ozone hole' during the austral spring and lingering ozone-depleted air during the summer. The plant community is dominated by an evergreen shrub (*Chiliotrichum diffusum*) with an herbaceous layer of *Gunnera magellanica* and *Blechnum penna-marina* in the interspaces between the shrubs. Inspections of ozone trends indicate that the springtime and summertime ozone column over Tierra del Fuego has decreased by 10–13% from 1978/9 to 1998/9. In a set of well-replicated field plots, solar UV-B was reduced to approximately 15–20% of the ambient UV-B using plastic films. Polyester films were used to attenuate UV-B radiation and UV-transparent films (~90% UV-B transmission) were used as control. Treatments were imposed during the growing season beginning in 1996 and continued for three complete growing seasons. Stem elongation of the shrub *C. diffusum* was not affected by UV-B attenuation in any of the three seasons studied. The results suggest that the increase in UV-B radiation associated with the erosion of the ozone layer might be affecting the functioning of this

ecosystem to some degree, particularly by inhibiting the growth of some plant species and by altering plant–insect interactions.

Nine populations of white clover (*Trifolium repens* L.) were grown (Hofmann, Campbell, & Fountain, 2003) for 12 weeks with supplemental application of UV-B radiation under controlled environmental conditions. Drought conditions were applied during the last four weeks of the experiment. Under well-watered conditions, UV-B decreased white clover growth on average by 20%. Cultivars bred for agricultural performance were sensitive to UV-B, while slow-growing ecotypes were UV-B-tolerant. After four weeks of water stress, there were no significant population differences in UV-B responsiveness. UV-B sensitivity decreased with increasing exposure to drought and with longer duration of UV-B irradiation, suggesting that the detection and extent of the UV-B with plants and drought interaction depends on the duration of stress. The population comparisons indicate that low constitutive growth rate and adaptation to other forms of stress may be related to UV-B tolerance under well-watered conditions, but not during extended periods of drought.

It emerged recently that there is an inter-relationship between drought and UV-B radiation in plant responses, in that both stresses provoke an oxidative burst. Alexieva, Sergiev, Mapelli & Karanov (2001) compared the effects and interaction of drought and UV-B in wheat and pea. Increases in anthocyanin and phenols were detected after exposure to UV-B. The increases do not appear to be of sufficient magnitude to act as a UV-B screen. UV-B application caused greater membrane damage than drought stress, as assessed by lipid peroxidation as well as osmolyte leakage. An increase in the specific activities of

antioxidant enzymes was measured after UV-B alone as well as after application to plants under drought conditions. Proline increased primarily in drought-stressed pea or wheat. Proline may be the drought-induced factor which has a protective role in response to UV-B. The physiological and biochemical parameters measured indicate that the UV-B light has stronger stress effects than drought on the growth of seedlings of both species. The two environmental stresses acted synergistically to induce protective mechanisms such that the either stress reduces the damages caused by the other stress.

The content and distribution of UV-absorbing phenolic compounds was investigated (Semerdjieva, Sheffield, Phoenix, Gwynn-Jones, Callaghan & Johnson, 2003) in leaves of three species of *Vaccinium* co-existing at a site in north Sweden. *Vaccinium myrtillus* L., *Vaccinium vitis-idaea* L., and *Vaccinium uliginosum* L. exhibit markedly different strategies, in terms of localization and content of leaf phenolics and in their responses to UV-B enhancement. *Vaccinium myrtillus* contained the highest concentration of methanol-extractable UV-B-absorbing compounds, which was elevated in plants exposed to enhanced UV-B. In *V. vitis-idaea*, most phenolic compounds were cell wall-bound and concentrated in the walls of the epidermis; this pool increased in response to UV-B enhancement. It is suggested that these two plants represent extreme forms of two divergent strategies for UV-B screening, the different responses possibly being related to leaf longevity in the two species. The response of *V. uliginosum* was intermediate between the other two, with high concentrations of cell wall-bound phenolics in the epidermis but with this pool decreasing, and the methanol-soluble pool tending to increase, after exposure to enhanced UV-B. One explanation for this response is that this plant is deciduous, like *V. myrtillus*, but has leaves that are structurally similar to those of *V. vitis-idaea*.

The primary leaf of wheat (*Triticum aestivum* L. cv Maris Huntsman) was used as a model system to examine how elevated UV-B (280–320 nm) radiation affected growth (Hopkins, Bond, & Tobin, 2002). A reduction in the rate and duration of growth of the primary leaf, in response to UV-B, was the result of changes in both the rate and extent of cell division and elongation. The supply of cells into the elongation zone was reduced, and this, coupled to a reduction in the rate of elongation, resulted in reduced leaf growth. This analysis of the spatial distribution of growth provided a means of calculating the age of cells within the leaves. Cells of UV-B-treated leaves were found to age more quickly than those of the controls. This analysis will enable future studies to take account of age-related changes when interpreting the response of plants to any number of environmental stresses that affect leaf development.

Plants raised under field conditions are acclimated to ambient levels of solar UV-B radiation. Jansen (2002) discussed the possibility that some UV-B driven morphogenic responses do not involve a dedicated photosensory system, but rather are a consequence of UV-B induced changes in secondary metabolism. UV-B induced flavonoid aglycones and phenol-oxidizing peroxidases can affect, respectively, polar auxin transport and auxin catabolism, and hence plant architecture. Integration of genetic, photobiological, biochemical and physiological approaches is necessary to fully appraise the ecophysiological role of UV-B radiation in controlling plant architecture.

The effect of UV-B radiation on leaf and stem length, photosynthetic $O_2$ evolution, levels of carbohydrates and nitrates, and extractable activities of some of the enzymes involved in C and N metabolism was evaluated by Ghisi, Trentin, Masi, and Ferretti (2002) in barley (*Hordeum vulgare* L. cv. Express) seedlings during the 9 days following transfer to an UV-B enriched environment. The results showed that under our experimental conditions UV-B radiation scarcely affects the photosynthetic competence of barley leaves. Nevertheless, this treatment induced significant alterations of the enzyme activity of nitrate after a few days of treatment. The effects were not confined to the exposed tissue, but were detectable also at the root level. In fact, nitrate decreased in response to UV-B in both leaf and root tissue, whereas glutamine was affected only in the root. In contrast, nitrate content was not influenced by the treatment, neither in root nor in leaf tissue, whilst leaf sucrose diminished in exposed plants only on the last day of treatment.

Tegelberg and Julkunen-Tiitto (2001) studied the impact of increased ultraviolet-B (UV-B) radiation on the secondary chemistry of *Salix myrsinifolia* (dark-leaved willow). For nearly two decades, the loss of stratospheric ozone above the high latitudes of the Northern Hemisphere has increased UV-B radiation (280–320 nm) over the long-term mean. To determine the effects of increased UV-B radiation on willows, the plantlets, three clones of *S myrsinifolia* were grown under ambient or enhanced UV-B irradiance. After the 2-week indoor experiment, the concentrations of UV-B-screening phenolics and low-UV-B-screening phenolics in fresh leaves were investigated and the biomass of leaves, stems and roots were determined. As expected, the total amount of flavonoids in willow leaves

clearly increased when plantlets were exposed to higher UV-B irradiation. However, the degree of increase of individual compounds varied.

Galatro, Simontacchi, and Puntarulo (2001) studied the effect of ultraviolet-B (UV-B) exposure on oxidative status in chloroplasts isolated from soybean (*Glycine max* Hood). Chloroplasts were isolated from soybean leaves excised from either control seedlings or those exposed to UV-B radiation for 4 days. The results suggested that the increased content of lipid radicals and oxidized proteins in the chloroplasts isolated from leaves exposed to UV-B could be ascribed to both the lack of antioxidant response in the lipid soluble fraction and the modest increase in the soluble antioxidant content.

Zalala, Scopel and Ballare (2001) also studied the effects of ambient UV-B radiation on soybean crops and the impact on leaf herbivory by anticarsia gemmatalis. The pollen germination characteristics, flower and pollen morphology in response to enhanced UV-B radiation in soybean (Glycine max) was also discussed. Stratospheric ozone depletion by anthropogenic chlorofluorocarbons has lead to increases in UV-B radiation (280–320 nm) along the Antarctic Peninsula during the austral spring. Ruhland and Day (2000) manipulated UV-B levels around plants of Antarctic hair grass and Antarctic pearlwort for one field season near Palmer Station along the west coast of the Antarctic Peninsula. Treatments involved placing frames over naturally growing plants that either (1) held filters that absorbed most biologically effective radiation (2) held filters that transmitted most UV-B, or (3) lacked filters that is ambient UV-B. There were no significant

differences in concentrations between UV-B treatments but concentrations of insoluble ferulic acid in *D. antarctica* tended to be higher under ambient and near-ambient UV-B than under reduced UV-B. They also examined bulk-leaf concentrations of soluble (methanol extractable) UV-B-absorbing compounds and found that concentrations were higher in plants exposed to near-ambient and ambient UV-B than in plants exposed to reduce UV-B. They further assessed the UV-B-screening effectiveness of leaves that had developed on plants at the field site with a fiber-optic microprobe. While the leaves of Antarctic vascular plants are relatively effective at screening UV-B, the levels of UV-B in Antarctica are sufficient to reduce leaf epidermal cell size and leaf elongation in these species, although the mechanisms for these reductions remain unclear.

Kakani, Reddy, Zhao, and Mohammed (2003) and also Kakani, Reddy, Zhao, and Gao (2004) determined the effects of UV-B radiation and atmospheric carbon dioxide concentrations on leaf senescence of cotton by measuring leaf photosynthesis and chlorophyll content and to identify changes in leaf hyperspectral reflectance occurring due to senescence and UV-B radiation. Plants were grown in controlled-environment growth chambers at different levels of UV-B radiation. No interaction was detected between $CO_2$ and UV-B for any of the measured parameters. Significant interactions were observed between UV-B and leaf age for photosynthesis and stomatal conductance. The hyperspectral reflectance between 726 nm and 1142 nm showed interaction for UV-B radiation and leaf age. In cotton, leaf photosynthesis can be used as an indicator of leaf senescence, as it is more sensitive than photosynthetic pigments on exposure to UV-B

radiation. This study revealed that, cotton leaves senesced early on exposure to UV-B radiation as indicated by leaf photosynthesis, and leaf hyperspectral reflectance can be used to detect changes caused by UV-B and leaf ageing.

An experiment was conducted by Zhao, Reddy, Kakani, Koti and Gao (2005) in sunlit controlled environment growth chambers to determine the physiological mechanisms of fruit abscission of cotton grown in high temperature and enhanced UV-B radiation. High temperature did not negatively affect either leaf net photosynthetic rates or abscission of cotton squares (floral buds with bracts) but significantly decreased boll retention. Plants exposed to 7 kJ UV-B radiation retained 56% less bolls than the 0 kJ UV-B control plants. The high UV-B radiation significantly increased square abscission. It was indicated that non-structural carbohydrate limitation in reproductive parts was a major factor associated with fruit abscission of cotton grown under high temperature and enhanced UV-B radiation conditions.

Liu, Iii, and McClure (1995) and Liu and McClure (1995) studied the effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves. They also studied the effects of UV-B on activities of enzymes of secondary phenolic metabolism in barley primary leaves. They discussed barley leaf adaptation to UV-B as a development response dependent on conditions of plant growth.

It has been suggested that the combination of $CO_2$ and UV-B radiation may differentially affect plant growth and morphogenic parameters, and elevated $CO_2$ may ameliorate the effects of UV-B radiation. Lavola, Julkunen-Tiitto, De la Rosa, Lehto, and Aphalo (2000) and Lavola, Aphalo, Lahti, and Julkunen-Tiitto (2003) studied the effect of increasing UV-B radiation on secondary plant compounds in Scots pine and the effects of increased atmospheric $CO_2$ concentration and UV-B radiation on growth and the accumulation of different types of secondary metabolites in silver birch (*Betula pendula* Roth). UV-B radiation significantly increased biomass and the accumulation of phenolic acids and flavonoids in seedlings. Elevated $CO_2$ concentration increased the activities of all the enzymes studied and the accumulation of condensed tannins in leaves, especially with UV-B radiation. Because the observed UV-B induction of flavonoids was smaller under a high $CO_2$ concentration, it was suggested that the excess of carbon in the atmosphere may moderate the effect of UV-B by increasing the metabolic activity of leaves (high enzyme activities) and by changing the allocation of internal carbon between different primary and secondary metabolites in the plant.

Plant responses to above-ambient or supplemented levels of solar ultraviolet-B radiation (UV-B) are typically subtle because targets or receptors in plants become saturated. Coleman and Day (2004) examined the response of *Gossypium hirsutum* (cotton) and *Sorghum bicolor* (sorghum) to filter exclusions that provided five levels of biologically effective UV-B. As UV-B dose increased or approached ambient, individual leaves of *S. bicolor* were smaller, but plants produced more tillers and leaves. In *G. hirsutum*,

individual leaves as well as total plant leaf area were smaller, but plants produced more branches. Bulk concentrations of soluble UV-B absorbing compounds increased with UV-B dose in both species. The intensity of UV-induced blue fluorescence from leaf surfaces was strongly correlated with bulk concentrations of wall-bound UV-B absorbing compounds, and this signal has the potential to provide a rapid, non-invasive method to estimate concentrations of these compounds, which are time-consuming to extract. While both species were responsive to solar UV-B, responses did not appear to become saturated as doses approached ambient levels.

Effects of UV-B on growth, flavonoids, ferulic, and photosynthesis in barley primary leaves were discussed by Liu et al. (1995). They found that UV-A had no significant effects on growth, ferulic acid, and photosynthesis in barley primary leaves were but it slightly increased flavonoids accumulation as UV-B did. UV-B also did not significant affect leaf area, fresh weight, dry weight, total chlorophyll, total carotenoids or photosynthetic quantum efficiency. Effects of spring and summer levels of UV-B radiation on the growth and reproduction of a temperate perennial forbs were discussed by McCloud and Berenbaum (2000).

Correia, Coutinho, Bjorn, and Torres-Pereira (2000) studied the effect of an increase UV-B radiation on the growth and yield of maize (*Zea mays L.*) at different level of nitrogen under Mediterranean field condition. They found that the response of grain yield was smaller with enhanced UV-B radiation. Mineuchi, Takahashi, and Tatsumoto (2001) used

laser induced fluorescence technique to study the effects of UV-B radiation on the peanut leaves. They evaluated the vitality decrease of leaves by measuring the change in a specific fluorescence peak ratio. Feng, An, Tan, Hou, and Wang (2000) investigated several higher plants to determine the response of pollen to UV-B radiation and cumulative effects of UV-B exposure time of pollen germination and tube growth.

Nithia, Shanthi, and Kulandaivela (2005) studied the effects of different responses to UV-B enhanced solar radiation in radish and carrot. Radish and carrot plants with underground storage organs grown in the field were exposed to ambient (UV-A or 20% UV-B) enhanced solar radiation till their root yield stage. They found that effect of enhanced UV-B on radish was positive and on carrot was negative. Radish plants showed a significant increase in shoots and root fresh mass, increase in the contents of chlorophyll, carotenoids, flavonoids, root yield, etc. On the other hand carrot showed a decrease in all these parameters.

Flavonoid induction by UV-B was recently investigated by Casati, and Walbot (2005) in five maize landraces from high. In their natural habitats these landraces receive much higher UV-B fluence than plants at lower altitudes at similar latitudes and would be predicted to have UV-B tolerance by recurrent selection against UV-B stress. They identified two flavones that are induced by UV-B in leaves of high-altitude lines: maysin and its biosynthetic precursor rhamnosylisoorientin. They concluded that maize plants from high altitudes respond to UV-B radiation by accumulating UV-absorbing flavones

in leaves; in contrast, these compounds are present at only very low levels in inbred lines and are not regulated by UV-B.

Field-grown plants show only subtle responses to supplemental UV-B radiation in many aspects of growth, yet plants grown under low visible light (as in most growth chambers and greenhouses) show much more discernible changes. Stephan, Martyn, Caldwell, and Ron (2005) assessed a specific aspect of UV-B sensitivity in plants grown under lower photosynthetically active radiation (PAR). They conducted field experiments at near-ambient PAR and enhanced UV-B, and also with reduced irradiance in both wavebands, on three species. Each of these species occurs in both open and shaded habitats. They found the grass *Setaria viridis* sensitive to UV-B radiation only when grown at lower irradiances, while the forbs *Geranium viscosissimum* was only sensitive to UV-B at the higher irradiances. In the grass *Elymus glaucus*, UV-B sensitivity did not appear to be influenced by the irradiance levels. Species appear to respond differently to these changes in irradiance levels, and an array of physiological and anatomical mechanisms are likely involved.

The effects of ultraviolet-B (UV-B) radiation on seven cotton and six soybean genotypes were evaluated by Reddy, Koti, and Kakani (2005) in sunlit controlled-environment chambers under optimum water, nutrient and temperature conditions. Plants were exposed to different level of UV-B radiation. Growth and physiological responses were measured and quantified. Higher UV-B significantly reduced dry matter production, plant height,

leaf area in all genotypes compared to control plants in both the crops; however, significant genotypic differences in the magnitude of the UV-B induced changes were observed. The observed genotypic differences suggested that it is possible to breed and select UV-B tolerant soybean and cotton genotypes for a niche environment.

Lettuce appears to be a valuable crop to use to study phytochemical-environment interactions. Eight cultivars each of red and green leaf lettuce were raised in a greenhouse with supplement of UV radiation, either UV-A (wavelengths greater than 315 nm) or UV-B (wavelengths greater than 290 nm). Several phytonutrients were analyzed in leaf flours to identify lines with large differences in composition and response to UV-B. There were large differences between cultivars in levels of phenolic compounds under control conditions and also large differences in UV-B effects.

Although our understanding of the effects of UV-B radiation on annual and crop plants has improved considerably over the past three decades (Beneski, Bassman, Slusser & Gao, 2005) knowledge of effects on perennial plants, particularly trees, remains much more limited. Understanding the effects of enhanced UV-B radiation on forest trees has been hampered by an inability to develop realistic dose-response relationships, which in turn, has resulted from lack of instrumentation sufficient to accurately determine biologically effective UV irradiances within the canopy. Measurement of UV radiation above a plant canopy provides little information about the radiation environment of individual leaves within the canopy. This is important because whole plant response to UV-radiation is an

integrated response of all of the leaves, though obviously not all leaves contribute equally. Within leaves, stress stimuli may induce compensatory responses in unaffected portions of the same leaf, in other adjacent leaves, and even in remote leaves. It is not known if this is true for UV radiation; the only way of finding out is to have a probe small enough to measure diminutive and precise locations within the canopy. Results are considered in the context of precision, accuracy and logistical attributes. Sufficient precision and accuracy were shown to suggest that, with proper calibration, this inexpensive and highly portable instrument can be used to make precise measurements of solar UV-B radiation within tree canopies.

Zhang, Yu, and Tang (2005) investigated whether increased solar UV-B radiation (280-315 nm) could suppress the growth of marine microalgae through effects on their antioxidant systems. Two marine microalgae species, Platymonas subcordiformis (Wille) Hazen and Nitzschia closterium (Ehrenb.) W. Sm, were exposed to a range of UV-B radiation and both showed reductions in their growth rates, and the chlorophyll a (Chl a) and carotenoid (Car) contents when UV-B radiation dose increased. When the exogenous glutathione (GSH) was added, the effects of UV-B radiation on the growth of the two species were alleviated. These results suggest that enhanced UV-B radiation suppressed the antioxidant systems and caused some active oxygen species to accumulate, which in turns retarded the development of the marine microalgae.

Keski-Saari, Pusenius, and Julkunen-Tiitto (2005) studied the ability of tree seedlings to respond to two environmental factors, elevated UV-B radiation and availability of nitrogen (N), at the beginning of their development. Seeds of two birch species, *Betula pubescens* Ehrh (common white birch) and *B. pendula* Roth (silver birch), were germinated. The experimental design consisted of a constant 50% increase in UV-B radiation (including a slight increase in UV-A), a UV-A control (a slight increase in UV-A) and a control. The seedlings were fertilized with three levels of nitrogen. Growth of the seedlings was not significantly affected by enhanced UV-B, but was increased by increasing nitrogen. No significant interaction between UV and nitrogen was detected, and the responses of the two species were highly similar to UV-B, while the responses to nitrogen regimens varied slightly more between species.

Influence of UV-A exerted on UV-B damaged peanut leaves was examined by Fukuchi, Takahashi, and Tatsumoto (2004) using the Laser Induced Fluorescence (LIF) method. The vitality index of the leaves was estimated from the measurement of the induction kinetics of chlorophyll fluorescence that was done in parallel with LIF spectra measurement. Visible ray irradiation of several hours to the UV-B damaged leaves recovered the vitality index of the leaves, while UV-A irradiation for the same period to the UV-B damaged leaves decreased the vitality index remarkably. No significant decrease of the vitality index was seen by the UV-A irradiation of several hours to the control (damage-less) leaves. It was understood that the UV-A irradiation to the UV-B damaged leaves causes the synergistic decrease of the vitality index. It is known that UV-

absorbing pigments induced by UV-B irradiation are able to reduce not only UV penetration into the leaf but also photo-system II damage of the leaves. In this experiment, it is thought that the UV-absorbing pigments were decomposed by the UV-A irradiation and so further decreased in the vitality index of the leaves.

Sensitivity to UV-B radiation (280–320 nm) varies widely among rice cultivars. Hidema and Kumagi (2006) studied that UV-resistant rice cultivars are better able to repair Cyclobutane Pyrimidine Dimers (CPDs) through photorepair than are UV-sensitive cultivars. Their results suggested that spontaneously occurring mutations in the CPD photolyase gene cause different degrees of sensitivity to UVB in rice and that the resistance of rice to UVB radiation could be increased by increasing the photolyase function through conventional breeding or bioengineering. MacKenzie, Saadé, Le Bureau, and Schoen (2005) also studied genomic mutation in arabidopsis thaliana exposed to UV-B radiation.

Qaderi and Reid (2005) studied the growth and physiological responses of canola plants (Brassica napus) to UV-B and $CO_2$ under controlled environment conditions and found many significant relationships between morphological, physiological, and chemical parameters. Pancotto, Sala, Robson, Caldwell, and Scopel (2005) studied the direct and indirect effects of solar ultraviolet-B radiation on long-term decomposition of barley. They found that UV-B treatment applied during the growth of plants decreased the decay rate. The effect of UV-B during growth on decomposition was considered likely the result of concentrations of nitrogen.

Epidermally located ultraviolet (UV)-absorbing phenolic compounds, flavonoids and hydroxycinnamic acid esters (HCAs), can shield the underlying tissues in plants against harmful UV-radiation. Burchard, Bilger and Weissenböck (2000) investigated developing rye primary leaves grown under supplementary UV-B radiation. From the fourth to the tenth day after sowing, epidermal located flavonoids increased in age- and irradiation-dependent manner, whereas mesophyll flavonoids and epidermal HCAs, mainly ferulic acid and *p*-coumaric acid esters, were constitutively present and did not vary in their contents over the observed time period. There was an excellent correlation between epidermal UV-A and UV-B absorbance as assessed by chlorophyll fluorescence measurements and contents of epidermal flavonoids. When absorbance of the abaxial and adaxial epidermal layers were compared, it became apparent that in fully expanded primary leaves epidermal tissues from both sides were equally effective in absorption of UV-radiation. It is shown that in early stages of development the epidermal HCAs are the dominant UV-B protective compounds of the primary leaf.

A newly developed laboratory fluorescence imaging system was used to obtain fluorescence images of freshly excised cucumber (*Cucumis sativus* L.) leaves in spectral bands centered in the blue (F450), green (F550), red (F680), and far-red (F730) spectral regions that resulted from a broad-band (300-400 nm) excitation source centered at 360 nm. Means of relative fluorescence intensities (RFI) from these spectral fluorescence images were compared with spectral fluorescence emission data obtained from excitation wavelengths at 280 nm of dimethyl sulfoxide extracts from these leaves. All three

fluorescence data types were used to assess UV-B induced physiological changes. Plants exhibited well known foliar growth and pigment responses to UV-B exposure (e.g., increased UV-B absorbing compounds and decreased leaf area, chlorophyll *a* content; and lower chlorophyll *a/b* and chlorophyll/carotenoid pigment ratios). The results were found in agreement with the validity of the imaging technique as a non-destructive diagnostic tool for assessing UV-B stress damage in plants.

Helsper, Ric de Vos, Maas, Jonker, Van der Broeck, Jordi, Pot, Keizer, and Schapendonk (2003) studied the effect of supplemental UV-A exposure on selected antioxidants and pigments in tissues of rosa and fuchsia hybrida species. They found that light absorption at 355 nm of leaf extract was significantly increased upon UV-A exposure. The major protection towards UV-A exposure in leaves originates from absorption of radiation and not from scavenging reacting oxygen species. Gartia, Pradhan, Joshi, Biswal, and Biswal (2003) mentioned that UV-A irradiation guards the photosynthetic apparatus against UV-B-induced damaged. They said that in clusterbean leaves UV-B radiation caused a reduction in contents of chlorophyll and carotenoids and in the efficiency of photoystem 2. The counteracting effect of UV-A radiation against UV-B induced is impairment.

Shinkle, Atkins, Humphrey, Rodgers, Wheeler, and Barnes (2004) examined the influence of short-term exposure of different UV wavebands on the fine-scale kinetics of hypocotyl growth of dim red light-grown cucumbers (*Cucumis sativus* L.) and other selected dicotyledonous seedlings to evaluate: (1) whether responses induced by UV-B radiation (280–320 nm) are qualitatively different from those induced by UV-A (320–400 nm) radiation, and (2) whether different wavebands within the UV-B elicit different responses. Responses to brief (30 min) irradiations with 3 different UV wavebands all included transient inhibition of elongation during irradiation followed by wavelength specific responses. Irradiations with proportionally greater short wavelength UV-B (37% of UV-

B between 280 and 300 nm) induced inhibition of hypocotyl elongation within 20 min of onset of irradiation, while UV-B including only wavelengths longer than 290 nm (and only 8% of UV-B between 290 and 300 nm) induced inhibition of hypocotyl elongation with a lag of 1 to 2 h. The response to short wavelength UV-B was persistent for at least 24 h, while the response to long wavelength UV-B lasted only 2 to 3 h.

The UV-A treatment induced reductions in elongation rates of approximately 6 to 9 h following exposure followed by a continued decline in rates for the following 15 to 18 h. Short wavelength UV-B also induced positive phototropic curvature in both cucumber and *Arabidopsis* seedlings, and this response was present in nph-1 mutant *Arabidopsis* seedlings defective in normal blue light phototropism. Reciprocity was not found (Shinkle *et al*., 2004) for the response to short wavelength UV-B. The short wavelength and long wavelength UV-B responses differed in dose–response relationships and both short wavelength responses (phototropic curvature and elongation inhibition) increased sharply at wavelengths below 300 nm. These results indicate that different photosensory processes are involved in mediating growth and morphological responses to short wavelength UV-B (280–300 nm), long wavelength UV-B (essentially 300–320 nm) and UV-A. The existence of two separate types of hypocotyl inhibition responses to UV-B, with one that depends on the intensity of the light source, provides alternate interpretations to findings in other studies of UV-B induced photomorphogenesis and may explain inconsistencies between action spectra for inhibition of stem growth.

An outdoor experiment in which potted plants were exposed to below ambient, ambient, and above ambient UV-B radiation, was conducted in order to evaluate the radiation effects (Gaberscik, Novak, Trošt, Mazej, Germ & Björn, 2001). The amount of photosynthetic pigments and photochemical efficiency of PSII were not affected, but the amount of UV-B absorbing compounds was lower in plants grown under reduced UV-B.

This change was measurable after only fourteen days in reproductive shoots, while in the vegetative shoots, it was not detectable until after three months. The results reveal that under simulated 17% ozone depletion the harmful effects of UV-B on the measured parameters were negligible.

Patches of vegetation of six common species growing on Léonie Island (67°35′S, 68°20′0W), Antarctic Peninsula region were covered (Lud, Huiskes, Moerdijk & Rozema, 2001) with either UV-B transparent perspex screens or UV-B absorbing screens. Uncovered plots served as a control. Temperature and relative humidity were monitored during the austral summer under and outside the screens. The mean effective PSII quantum efficiency showed significant differences among the species, but not between the UV-B treatments. It was concluded that the temperature and the moisture status of the vegetation obscured any possible influence of UV-B treatment on the effective PSII quantum efficiency.

Kyparissis, Drilias, Grammatikopoulos, and Manetas (2001) grew seedlings of ceratonia siliqua L. for 1 year in the field under ambient or ambient plus supplemental UV-B radiation and received two levels of additional irrigation during the summer dry period. Plants receiving additional irrigation showed significantly higher leaf number, plant height and chlorophyll content at the end of the summer, but these differences were abolished at the final harvest. Plants growing under enhanced UV-B radiation had significantly fewer leaves and less nitrogen content at the end of the dry period, but these

effects were also abolished at the final harvest, during which significant UV-B induced increases in stem dry mass were observed. None of the other measured parameters (mean leaf area, leaf dry mass, leaf thickness, UV-B absorbing compounds, phenolics, tannins and photochemical efficiency of PSII) were affected by either treatment. Combined UV-B and water effects were not significant. They concluded that although some minor responses to enhanced UV-B radiation were evident, C. siliqua is resistant against UV-B radiation damage at the level applied.

Charophycean algae are mainly freshwater organisms and are thought to be the algae most closely related to higher land plants. De Bakker, Van Beem, Van de Staaij, Rozema, & Aerts (2001), reported that responses of charophycean algae to UV-B radiation might be more related to those observed in the higher land plants than those of other `lower' algal groups. Under elevated UV-B radiation algal length was reduced. There was no induction of UV absorbing compounds under enhanced UV-B. This might relate to a sensitive response to UV-B radiation. The charophycean algae show similar adaptations to UV-B radiation as terrestrial plants, while not having UV-screens as occur in many angiosperms. Vegetative reproduction (bulbils) increased in the presence of UV-B radiation, while generative reproduction (antheridia and oogonia) decreased.

To test the hypothesis that plant source-sink relations are important in determining response to UV-B radiation, a short-term (45 d) field experiment was conducted by Gwynn-Jones (2001) at Abisko Scientific Research Station, Abisko, Sweden (68° N). Tillers of the grass Calamagrostis purpurea were grown outdoors at levels of UV-B

radiation representing 25% ozone depletion. Growth, respiration, photo-assimilate allocation and UV-B protective compounds were subsequently measured. Robson, Pancotto, Flint, Ballaré, Sala, Scopel, and Caldwell (2003) found that near-ambient UV-B caused reduced height growth but had no effect on biomass production of Sphagnum but it reduced the leaf and rhizome growth of Tetroncium magellanicum.

UV-B induced stress response in three barley cultivars was discussed by Hideg, Rosenqvist, Váradi, Bornman, and Vincze (2005). Effects of UV irradiation on barley and tomato leaves were discussed by Gilbert, Skotnica, Weingart, and Wilhelm (2004). Yanqun, Yuan, Haiyan, and Jianjun (2003) discussed the intra-specific differences in physiological response of 20 wheat cultivars to enhanced UV-B radiation under field conditions. Influences of light and UV-B on growth and sporulation on the green alga Ulva pertusa kjellman were discussed by Han, T., Han, Y. S., Kim, K. Y., Kim, J. H., Shin, Kain, Callow, J. A. and Callow M. E. (2003). They observed a significantly lower incidence of sporulation in UV-B irradiated plants with the degree of reduction being greater in those exposed to higher UV doses.

Gwynn-Jones (2001) studied short term effects of enhanced radiation on photo-assimilate allocation and metabolism. There were no significant effects of enhanced UV-B on total plant dry weight, leaf area, shoot: root ratio, leaf weight ratio, leaf area ratio, specific leaf area, tiller number per plant or blade thickness of this species. However, the amount of UV-B absorbing compounds and respiration rates were significantly increased in young and mature leaves. Increases in leaf respiration were accompanied by alterations in plant

carbohydrate allocation at enhanced UV-B. The amount of soluble root carbohydrates was reduced following UV-B exposure. Enhanced UV-B also caused increases in the soluble sugar: starch ratio of young leaves, the stem and total aboveground biomass. The importance of source-sink relations and constitutive versus induced defense are discussed in relation to UV-B response.

To determine whether effects of $CO_2$ enrichment on faba bean (cv. Minica) growth are modified by UV-B radiation, the effects of enhanced $CO_2$ on growth and photosynthetic characteristics were studied at four UV-B levels (Tosserams, Visser, Groen, Kalis, Magendans & Rozema, 2001). Faba bean was sensitive to enhanced UV-B radiation as indicated by decreases in total biomass production. Growth stimulation by $CO_2$ enrichment was greatly reduced at the highest UV-B level. $CO_2$ and UV-B interactions on biomass accumulation were related to loss of apical dominance. Both $CO_2$ and UV-B radiation affected biomass partitioning, UV-B effects being most pronounced. Effects of $CO_2$ and UV-B on faba bean growth were time-dependent, indicating differential sensitivity of developmental stages. $CO_2$ and UV-B effects on photosynthetic characteristics were rather small and restricted to the third week of treatment. $CO_2$ enrichment induced photosynthetic acclimation, while UV-B radiation decreased light-saturated photosynthetic rate. It is concluded that the reduction in biomass production cannot be explained by UV-B-induced effects on photosynthesis.

Searles, Flint, Díaz, Rousseaux, Ballaré, and Caldwell (2002) studied plant response to solar ultraviolet-B radiation in a Southern South American *Sphagnum* peatland. They said that if the ozone layer is substantially covered, there would be only modest effects of increased solar UV-B on plant growth. The influence of enhanced UV-B radiation on Batrachium trichophyllum and Potamogeton alpinus-aquatic macrophytes with amphibious character was studied by Germ, Mazej, Gaberscik, and Hader (2002). Shukla,

Joshi, and Kakkar (2002) studied the **s**ynergistic action of UV-B radiation and cadmium on the growth of wheat seedlings. Hakala, Jauhiainen, Koskela, Käyhkö, and Vorne (2002) found the consequences of increased UV-B on crops and the sensitivity of different varieties of barley, wheat, oats, clover, timothy, potato, etc. Masi, Ghisi, and Ferretti (2002) reported that UV-B radiation caused a significant increase in leaf of Zea mays L during night. Levizou and Manetas (2001) presented the combined effects of enhanced UV-B radiation and additional nutrients on growth of two Mediterranean plant species.

Leaves of Zea mays were subjected to different scenarios of UV-B radiation in a sun simulator to determine the cellular vitality at the microscopic level and the contents of carbohydrates and photosynthetic pigments. Barsig and Malz (2000) showed that the leaf morphology and fine structure of sugar maize leaves are only slightly affected by UV cut-off wavelengths down to 288 nm. At a microscopic level, a number of epidermal cells are affected by supplemental UV-B. The leaf dry weight was not influenced by enhanced UV-B. The amount and structure of starch grains in leaf chloroplasts did not differ between UV treatments. There was no clear impact of enhanced UV-B on sucrose content. Carbohydrate partitioning was more significantly influenced by leaf exposure than by UV treatment. Only glucose was decreased under high UV-B. Changes in photosynthetic pigments were limited to a slight destructive effect of UV-B on chlorophyll b. They concluded that sugar maize leaves have adapted efficiently to cope with supplemental UV-B radiation. Changes in epidermal cell layer due to their shield function may indicate that this remarkable resistance against enhanced UV-B radiation is not unlimited.

UV-B radiation (290 -315 nm) is expected to increase as the result of stratospheric ozone depletion. Within the environmental range, UV-B effects on host plants appear to be largely a function of photomorphogenic responses, while effects on fungal pathogens may include both photomorphogenesis and damage. The effects of increased UV-B on plant-pathogen interactions have been studied by Paul (2000) in only a few pathosystems. The most crop diseases are greatly affected by stratospheric ozone depletion within the limits currently expected. However, the lack of a detailed understanding of the mechanisms by which UV-B influences plant-pathogen interactions in most pathosystems is a significant limit to such predictions.

Nogués and Baker (2000) studied the effects of drought on the photosynthetic characteristics of three Mediterranean plants (olive, Olea europea L.; rosemary, Rosmarinus officinalis L.; lavender, Lavandula stoechas L.) exposed to elevated UV-B irradiation in a glasshouse, over a period of weeks. Exposure of plants to elevated UV-B radiation (0.47 W $m^{-2}$) prior to and during the drought treatment had no significant effects on the growth or photosynthetic activities of the plants. Consequently, it is predicted that increasing UV-B due to future stratospheric ozone depletion is unlikely to have any significant impact on the photosynthetic productivity of olive, lavender and rosemary in the field.

Field studies were conducted by Yuan, Yanqun, Jianjun, Haiyan, Jilong, and Zhide (2000) to determine the potential for alterations in physiology and the intra-specific variation in

sensitivity of 20 wheat (Triticum aestivum) cultivars to enhanced UV-B (UV-B, 280-315 nm) radiation. The supplemental UV-B radiation was 5 kJ $m^{-2}$, simulating a depletion of 20% stratospheric ozone. Out of 20 wheat cultivars (from South China, North China and Mexico) tested, 13 showed significant changes in total chlorophyll content. However, some species had an increased chlorophyll a/b ratio under enhanced UV-B. The effect of UV-B on flavonoid content also showed intra-specific differences, a significant increase for one cultivar, decreases in 12 cultivars and no effect on the other seven cultivars. They found large intra-specific differences for the different parameters measured but there was no clear correlation between them under UV-B radiation.

Pea (*Pisum sativum* L.) and bean (*Phaseolus vulgaris* L.) plants were exposed to enhanced levels of UV-B radiation in a growth chamber. Bolink, Van Schalkwijk, Posthumus, and Van Hasselt (2001) found that the pigments were increased in UV-B treated pea plants compared to controls, but in bean no significant differences were found between treatments. However, in bean plants thiol concentrations were significantly enhanced by UV-B treatment, and UV-absorbing compounds increased in both species, indicating a higher antioxidant capacity. An increased leaf thickness, together with increases in antioxidant capacity could have contributed to the higher protection against photo-inhibition in UV-B treated plants.

The effects of heat shock (HS), UV-B irradiation, and the consecutive action of these factors on the growth, development, and water supply of seven-day-old melon seedlings were investigated by Borisova, Bugaje, Rakitin, Vlasov, and Kuznetsov (2001).

Depending on the HS severity, they observed growth stimulation (after treatment at 45°C for 1 h), growth retardation (after treatment at 45°C for 2 h or at 48°C for 1 h), or complete growth inhibition and cell death (after treatment at 45°C for 3 h or at 55°C for 1 h). UV-B irradiation, depending on its duration, stimulated (5–10 min), retarded (60 min), or resulted in complete growth inhibition and plant death (90 min). HS treatment (at 45°C for 1 h) prior to UV-B irradiation (for 1 h) favorably affected both the growth and water balance of seedlings. Apparently, the HS pretreatment increases the tolerance of seedlings to high doses of UV-B radiation.

Day, Ruhland, and Xiong (2001) examined the influence of solar UV-B radiation (280-315 nm) on the performance of Antarctic vascular plants by placing filters that either absorbed or transmitted most solar UV-B over tundra along the Antarctic Peninsula for four consecutive growing seasons. They found no effect of UV-B exposure on the numbers of spikelet or seeds produced per unit of ground surface area. While seeds from plants exposed to UV-B tended to be lighter, germination rates were similar between UV-B treatments. The relative reductions in leaf elongation rates in D. antarctica attributable to UV-B exposure increased from the first (23%) through the fourth (43%) growing season, and relative reductions in leaf longevity in C. quitensis tended to increase from the first (9%) through the fourth (19%) growing season, suggesting that UV-B growth responses tended to be cumulative over successive years.

Kumagai, Hidema, Kang, and Sato (2001) reported a thorough investigation made of the variations in growth and grain yield in response to increased exposure to UV-B radiation of Japanese lowland rice (*Oryza sativa* L.) in a cool rice-growing region. The two cultivars

were grown in a lowland field with or without supplemental UV-B radiation, which was provided by UV-B-emitting fluorescent lamps, with a 0.1-mm-thick cellulose diacetate film as a filter. In both cultivars, significant decreases in tiller number as the result of supplemental UV-B radiation were observed. Furthermore, decreases in grain size from supplemental UV-B radiation were recorded in all seasons. When the temperature and the amount of sunshine were lower, the tiller number, the dry mass of aboveground parts and the panicle number were significantly reduced by supplemental unfiltered UV-B radiation. These results indicate that supplemental UV-B radiation has a positive effect on the growth and grain development of rice, which may be enhanced by unusual climatic conditions such as lower temperature and less sunshine, in cool rice-growing regions.

Khalilov, Ahmadov, and Kadirov (2002) studied the effects of UV-B radiation on plasma membrane of water plant cells were investigated by using microelectrode methods. Bjorn, Widell, and Wang (2002) showed that plants have evolved under the influence of UV-B radiation and have acquired systems for monitoring it and investing appropriate resources for protection against it, i.e., filters quenchers of radicals and reactive oxygen species, and repair systems. A hypothesis for how plants monitor radiation was presented by Khalilov *et al*. (2002). Costa, Gallego, and Tomaro (2002) studied the effect of UV-B radiation sunflower cotyledons and confirmed that UV-B radiation undoubtedly induces antioxidant defense system in sunflower cotyledons, allowing plant survival in spite of the oxidative stress generation. However, more research is necessary to elucidate the precise role that the antioxidant system plays under UV-B stress. Jayakumar, Eyini, Lingakumar, and

Kulandaivelu (2002) studied the effects of enhanced UV-B radiation on growth and photosynthetic activities were investigated in fronds of the aquatic fern *Azolla microphylla* Kaulf plants.

Recent measurements of ozone levels have led to concern that the stratospheric ozone layer is being depleted as a result of contamination with man-made chlorofluorocarbons. Concomitantly, the amount of solar UV-B radiation reaching the Earth's surface is increasing. UV-B radiation has been shown to be harmful to living organisms, damaging DNA, proteins, lipids and membranes. Plants use sunlight for photosynthesis and are unable to avoid exposure to enhanced levels of solar UV-B radiation. Thus, mechanisms by which plants may protect themselves from UV radiation are of particular interest. Hollosy (2002) summarized the main aspects of ultraviolet radiation on plants at physiological and biochemical level, with particular emphasis on protective structures and mechanisms.

Gaberscik et al. (2001) studied the effect of enhanced UV-B radiation on buckwheat, an important high elevation crop, in order to estimate its vulnerability in changing UV-B environment. Plants were grown in outdoor experiments from July to October under reduced and ambient UV-B levels, and an UV-B level simulating 17% ozone depletion in Ljubljana. At the end of the experiment, growth rate and production of seeds were estimated. The seeds collected from plants exposed to different UV-B treatments were tested for germination capacity. Total UV-B absorbing compounds during plant

development were increased by UV-B radiation, photosynthetic pigments (chlorophyll a and b and carotenoids) decreased. Photosynthetic rate was lowered in an early stage of development. UV-B treatment resulted in the increase in the transpiration rate and consequently the decrease in water use efficiency (WUE). The germination of seeds collected from treated plants revealed on average about 95% success, independently of the treatment, but the time needed for germination was the shortest for seeds developed under enhanced UV-B level treatment. Enhanced UV-B radiation affected water relations and production of buckwheat, but not the potential of seeds for germination.

Zavala and Botto (2002) discussed the impact of present-day solar UV-B radiation on seedling emergence, its association with the accumulation of UV-absorbing compounds, and the growth and yield of radish (*Raphanus sativus* L.). Two field experiments were conducted at intermediate latitudes in South America (Buenos Aires, Argentina) using two cultivars of radish (`Scarlet Globe' and `Sparkler National'). Solar UV-B reduced the emergence of seedlings by nearly 20% for the Scarlet radish cultivar, and delayed emergence for both cultivars by least one day. Ambient UV-B affected the biomass partitioned to tubers, resulting in an increase of at least 17% in tuber diameter and 26% in tuber fresh weight at the end of the life cycle. They showed that the early effects of UV-B on seedling development involve a cost for young plants, but it appeared to be advantageous to increase the carbon partitioning to the tubers at harvest. These findings suggested ways to improve the yield of radish crops.

Pinto, Edwards, Riquelme, and Ku (2002) showed that the exposure of bean plants grown in the greenhouse, where UV-B is low, to ambient levels of UV-B light stimulated nodulation more than 2.5-fold. Reduction of UV-B radiation to 3% of ambient levels for outdoor-grown plants through use of Mylar filters consistently reduced nodulation by 45%. The increase in nodulation caused by UV-B was mainly due to an increase in number and size of nodules. The amounts of UV-B-absorbing compounds in roots of UV-B-exposed plants increased almost 5-fold. However, the composition of UV-B-absorbing compounds remained very similar. Exposing leaves to UV-B also significantly increased release of these compounds from roots to the medium. These results suggest that UV-B radiation enhances nodulation, and that a signal may be transported from shoot to roots to play a role in nodulation.

The stratospheric ozone depletion and enhanced solar ultraviolet-B (UV-B) irradiance may have adverse impacts on the productivity of agricultural crops. The effect of UV-B enhancements on agricultural crops includes reduction in yield, alteration in species competition, decrease in photosynthetic activity, susceptibility to disease, and changes in structure and pigmentation. Many studies have examined the influence of supplemental UV-B irradiance on different crops, but the effect of UV-B irradiance on cotton (*Gossypium hirsutum L*.) crops has received little attention. Cotton is one of the most versatile of all the crops. It is a major fiber crop of the world and a major source of trade and economy in many countries. Gao et al. (2002 & 2003) provided quantitative examination of the effects of elevated UV-B irradiance on cotton plant. The tested cotton

crop was grown under natural and four regimes of supplemental UV-B irradiance in the field. With UV-B irradiance increased 9.5% throughout the growing season, the negative impacts on cotton growth included reductions in height of 14%, in leaf area of 29%, and in total biomass of 34%. Coleman and Day (2004) also made contribution to the study of response of cotton to UV-B radiation.

Rousseaux, Flint, Searies, and Caldwell (2004) conducted some field experiments assessing UV-B effects on plants using two contrasting techniques: supplementation of solar UV-B with radiation from fluorescent UV lamps and the exclusion of solar UV-B with filters. They compared these two approaches by growing lettuce and oat simultaneously, under three conditions: UV-B exclusion, near-ambient UV-B (control) and UV-B supplementation (simulating a 30% ozone depletion). For oat, solar UV-B had a greater effect than supplemental UV-B on main shoot leaf area and main shoot mass, but supplemental UV-B had a greater effect on leaf and tiller number and UV-B–absorbing compounds. For lettuce, growth and stomatal density generally responded similarly to both solar UV-B and supplemented UV-B radiation, but UV-absorbing compounds responded more to supplemental UV-B, as in oat. Because of the marked spectral differences between the techniques, experiments using UV-B exclusion are most suited to assessing effects of present-day UV-B radiation, whereas UV-B supplementation experiments are most appropriate for addressing the ozone depletion issue.

*Vigna unguiculata* (L.) Walp. (cowpea), *Glycine max* (L.) Merr (soybean) and *Phaseolus vulgaris* (L.) (common bean) plants were exposed to UV-B radiation at above- and below-ambient levels, and their effects on growth, symbiotic performance and root concentration of metabolites were assessed by Chimphango (2003). Moderately and highly elevated UV-B exposures averaging 32% and 62% above ambient had no effect on plant total dry matter, nodule number, nodule mass, nodule size, N fixed or root concentration of flavonoids, anthocyanins, soluble sugars and starch in the three species studied. However, N concentrations were markedly reduced in roots of *G. max* and *P. vulgaris*, and in leaves of *P. vulgaris*, which contrasted with the significant increase in stems and leaves of *V. unguiculata*. Below-ambient UV-B exposures averaging 22% of ambient also altered growth and metabolism of these legumes. Total plant dry matter, nodule number, nodule dry mass, N fixed and root starch concentrations in *V. unguiculata* decreased relative to both visible and UV-A radiation controls, whereas in *G. max* and *P. vulgaris*, these parameters were not altered. Root concentrations of flavonoids and anthocyanins in all species tested were also unchanged with below-ambient UV-B exposures. Taken together, growth and symbiotic function of these species remained unaltered with exposure to above-ambient UV-B, but differed in their response to below-ambient UV-B radiation.

Egert and Tevini (2003) found that biologically effective ultraviolet-B (UV-B) radiation did not significantly influence leaf length or leaf peroxidase activity of chives (*Allium schoenoprasum* L.). However, correlation and regression analyses with different climatic parameters revealed that increased UV-B radiation enhanced ascorbate peroxidase activity

in chive leaves whereas guaiacol peroxidase was inhibited. Lydon et al. (1986) also studied the effects of UV-B radiation on the growth and productivity of field soybean.

Seventeen herb, shrub and tree species of commercial and ecological importance in southern Africa were exposed at one location to UV-B (280–315 nm) radiation approx. 35% above clear-sky background (control). Musil, Chimphango, and Dakora (2002) examined how UV-B affects canopy area, dry mass, and some biochemical and morphological properties of leaves. They also investigated whether differences between species are related to growth form of the plants and found that there was no pattern of response to UV-B related to growth form. Leaves of trees had altered chlorophyll *a* and *b*, carotenoid and flavonoid concentrations, but those of shrubs or herbs did not. Non-structural carbohydrates were unaffected. Smaller canopy areas and dry masses were observed under enhanced UV-B, but these were not statistically different among growth forms. There was a general insensitivity of species to elevated UV-B. Only five species had significantly altered leaf biochemical and morphological properties, canopy area and dry mass, the changes differing in magnitude. There was no consistent pattern of change in leaf thickness or biochemical composition with increased UV-B. Correlation analyses did not support the view that growth is less negatively affected in species with thick leaves or in those where leaf thickness increases, or in species with naturally high leaf flavonoid contents or that are able to synthesize additional flavonoids in response to UV-B enhancement. The analyses did not support the hypothesis that growth was inhibited by starch accumulation in leaves under elevated UV-B.

However, changes in leaf shape did correlate with canopy area and dry mass, showing the importance of photomorphogenetic changes caused by UV-B which affect species' performance. Musil *et al.* (2002) concluded that generalizations on plant sensitivity to UV-B based on growth form and functional type could be misleading, and that the great majority of economically important species of the region are likely to be insensitive to future UV-B increases. Notable exceptions include the Colophospermum mopane tree ecotypes chota and leslie and the arable annual Vigna unguiculata, both of which are traditional sources of livelihood to rural African populations and of importance to African industry and agriculture.

Sullivan, Gitz, Peek, and McElrone (2002) and Sullivan, Gitz, Peek, and McElrone (2003) evaluated the chemical composition and deposition patterns of UV-absorbing compounds in three tree species and assayed these species for possible effects on gas exchange and photosynthetic carbon assimilation. Branches of mature trees of sweetgum (*Liquidambar styraciflua*), tulip poplar (*Liriodendron tulipifera*) and red maple (*Acer rubrum*) were exposed to supplemental levels of UV-B radiation over three growing seasons. Controls for UV-A were also measured by exposing branches to supplemental UV-A only, and additional branches not irradiated were also used for controls. These species demonstrated contrasting chemical composition and deposition patterns with poplar being the most responsive in terms of epidermal accumulation of phenolics including flavonols and chlorogenic acid and hydroxycinnamates. Sweetgum and red maple showed increases primarily in hydroxycinnamates, particularly in the mesophyll in red maple. Leaf area was

marginally influenced by UV exposure level. Assimilation was generally not reduced by UV-B radiation in these species and was enhanced in red maple by both UV-B and UV-A and by UV-A in sweetgum. These finding are consistent with a hypothesis that epidermal attenuation of UV-B would only be reduced in poplar, which accumulated the additional epidermal screening compounds. It is possible that photosynthetic efficiency was enhanced in red maple by the increased absorption of blue light within the mesophyll. Stomatal conductance was generally reduced, and this led to an increase in water use efficiency in red maple and poplar.

As soon as seedling breaks through the soil surface it is exposed to natural UV radiation (Tevini, 1981, 1983, 1985). The continuous growth indicates that plants can tolerate or adapt natural radiation. When plants are grown in a greenhouse without UV radiation they experience a UV shock when planted outside, which can kill the plant. Even plants adapted to UV radiation are impaired in their development, structure and function by increased radiation. The epidermis usually protects the inner leaf with high concentrations of flavonoids and anthocynanins, which absorb in the UV range. UV-induced reduction in photosynthetic activity shows that some UV radiation penetrates into the lower cell layers. The effect may be very heterogeneous. Continuous UV-B irradiation of barley seedlings disturbs the vertical growth, which may be due to destruction of IAA in the leaf tips. Plants possibly protect themselves against increased UV radiation through an increase in synthesis of pigments in the epidermis. Excellent work has been reported on

effects of UV radiation on plant growth (Lichtenthaler and Rindele, 1988; Pangopoulos, 1990, 1992; and Saito, 1999).

The biotically produced oxygen accumulated slowly in the atmosphere, and was converted into ozone in the upper layers by solar UV radiation. The gradually forming ozone layer effectively protected life from short wavelength UV radiation. A reduced stratospheric ozone layer may affect plants in several different ways and alter species composition and productivity since shorter wavelengths UV-B (280-320 nm) radiation will increase even with a relative small decrease in ozone. Caldwell (1989) studied several species and found that the plants native to higher latitudes are often found to be more sensitive to UV radiation than those native to lower latitudes. Changes in the internal spectral regime of leaves have been measured in plants exposed to enhance level of UV-B radiation.

The sunlight reaching the earth surface has a waveband down to approximately 280nm. The plants receive many beneficial effects as well as harmful effects of UV of the sunlight. The effect of solar UV on plant grown under UV-transparent and UV-cut polyvinyl chloride film, both of which transmitted uniformly about 85% of the visible waveband was studied (Hashimoto, 1993). Various workers (Lees, 1991; Holzwarth, 1986; Hodges and Moya, 1988; Owens, 1988) have worked on the characterization of the time-resolved fluorescence properties of photosynthetic material (Sparrow *et al.*, 1986, 1989, 1990). Lees *et al*. (1991) found that photosynthetic development measured as light induced oxygen evolution is impaired.

The effect of natural solar UV radiation on the growth of tomato and radish plants was studied (Tezuka, 1991 & Tezuka, 1993) using polyvinyl chloride films with different UV transmission. The growth of the tomato plants exposed to UV-A (400-320 nm) radiation (UV-C-320 film, transmission above 320 nm) was greater than that of plants exposed to no UV radiation (UV-C-400 film, transmission above 400 nm). It was found that the growth increases with an increase in chlorophyll content and photosynthetic activity. Many workers (Bornman, 1989, 1991, 1993; Takeuchi et al., 1989; Teramura et al., 1991; Hashimoto and Tajima, 1980; Teramura, 1983 & Sullivan, 1992) have studied the effects of UV radiation on various plants. The solar spectrum reaching the earth's surface does not extend below approximately 290 nm because ozone in the stratosphere effectively absorbs all radiation of shorter wavelengths (Tezuka, 1990). Therefore the natural solar radiation of the UV-B region at the earth's surface is between 290 and 320 nm. Many reports (Hashimoto and Tajima, 1980; Teramura, 1983; Ambler, 1975; Brandle et al., 1977; Haldall, 1964; Sisson and Caldwell, 1976) have shown that UV-B radiation inhibits photosynthesis and growth of plants. Hence cultivation of crops in a greenhouse covered with plastic film such as polyvinyl chloride, which is opaque to UV, is used extensively.

It is not yet clear whether the use of opaque plastic film is appropriate for the cultivation of crops in all cases. It is also not fully known whether natural solar UV radiation (400 to 290 nm) consisting of the UV-A region or almost all UV-B regions on the earth's surface is deleterious for the growth of plants. Tezuka et al. (1993) and Ziska et al. (1993) investigated the effects of radiation with UV from the natural solar spectrum on the

growth, photosynthesis and other physiological activities of plants. In order to determine whether solar UV radiation induces the inhibition of plant growth and photosynthesis compared to visible radiation (no solar UV), a series of polyvinyl chloride films were employed (Brandle et al., 1977). The content of (total) chlorophyll a and b can be expressed on the basis of leaf area taking leaves in homogenized with 80% acetone in a glass homogenizer (Tezuka et al., 1980; Tezuka et al., 1989).

The fluorescence emitted by the chlorophyll of plants and bacteria is a process in competition with photosynthetic reactions. The changes in chlorophyll fluorescence reflect variations in the photosynthetic activity of the organisms (plants). Many workers (Schreiber, 1984; Lichtenthaler and Rinderle, 1988; Renger and Schreiber, 1986) have shown that fluorescence is an early indicator of damage to the photosynthetic apparatus induced from environmental stress factors. Therefore, the fluorescence-measuring techniques on intact leaves of higher plants are more useful because they are non-destructive and provide simple tool to monitor vegetation. There are various methods of measuring the fluorescence induced kinetics (it is called the Kautsky effect), which occurs when dark-adapted leaves are irradiated (Lichtenthaler and Rinderle, 1988; Renger and Schreiber, 1988; Krause and Weis, 1984).

A faster, more recent and reliable technique is based on the measurement of the chlorophyll fluorescence spectrum, which consists of two broad bands at about 685 and 735 nm. The relative intensity of these two peaks is used as an indicator (Lichtenthaler

and Rinderle, 1988; Mazzinghi, 1991). This method is of particular interest since chlorophyll fluorescence spectra can be easily measured in remote sensing from ground (Castagnoli et al., 1986) or aircraft (Hoge et al., 1983) platform. Most of the information provided by fluorescence kinetics data, which are possible only as contact measurements on single leaves, can now be obtained in remote sensing detection of the fluorescence spectra. Today multi-spectral chlorophyll fluorescence images (Edner et al., 1992) are available to study the plant fluorescence spectral properties.

The origin of the two bands in the chlorophyll fluorescence spectrum of intact leaves has been discussed (Butler, 1978; Briantais et al., 1986; Krause and Weis, 1991). At room temperature, the chlorophyll fluorescence in both spectral regions is mostly emitted from the photosystem II (PSII) reaction centre. Emission by photosystem I (PSI) contribute with only a minor component at 740 nm. However, when excitation energy distribution on PSI increases with respect to PSII, a corresponding increase in the longer wavelength fluorescence band relative to the shorter wavelength band is observed (Bradburg and Baker, 1983; Kyle et al., 1983; Murata and Satoh, 1986). The peak fluorescence ratio $\frac{\mathrm{F685}}{\mathrm{F735}}$ is found to be decreased during the Kautsky kinetics (Hak et al., 1990).

One of the most important factors, which greatly affect the leaf fluorescence spectrum is the absorption of the light emitted by chlorophyll itself. The effect is most important for the spectral region around 685 nm, resulting in chlorophyll fluorescence spectra in which the two peaks have similar intensities. The $\frac{\mathrm{F685}}{\mathrm{F735}}$ fluorescence ratio is inversely proportional

to the leaf chlorophyll content (Lichtenthaler & Rinderle, 1988). At very low chlorophyll concentrations, leaf fluorescence spectra match those of isolated chloroplasts with high $\frac{F685}{F735}$ ratios. Estimation of the re-absorption effect on the leaf fluorescence properties of different plant species are compared (Lichtenthaler & Rinderle, 1988).

Many authors have studied the effects on UV radiation on various kinds of plants. Significant contribution were made by authors such as (Hashimoto & Tajima, 1980; Teramura, 1983; Bornman, 1989; Takeuchi et al., 1989; Tevini et al., 1989; Teramura et al., 1991; Tevini et al., 1991; Ziska et al., 1992; Appenroth, 1993; Siffel et al., 1992, Murali & Teramura, 1985; Mirecki &Teramura, 1984; Kulandaivelu, 1989; Lydeon et al. 1986; Braun and Tevini, 1993; Dohler, 1985 & Parisi et al., 1996). Many of them showed that UV-B radiation (280 to 320 nm) inhibits photosynthetic activity and the growth of plants. These inhibitory effects are caused by the supplementary radiation (high intensity) of UV-B in wavelength regions of natural solar UV or by the radiation of UV-B including a part of wavelength region of UVC that below 280 nm. It has already been pointed out but not confirmed that the promotion of the growth of plants is caused by the increase in the chlorophyll content and the activities of photosynthetic and respiration in response to solar UV radiation (Rau & Hofmann, 1996; Warner & Caldwell, 1983; Caldwell 1977, 1984, 1995).

The quantitative and qualitative changes in solar radiation at the earth's surface throughout the year are more marked in the UV than in the visible region. The quality and quantity

of solar radiation that reaches the earth's surface are also subject to wide fluctuations owing to weather conditions and seasons, and radiation in the solar UV region play a vital and key role as a limiting factor in the growth of plants (Sato & Kumagai, 1991). Instead of using changeable fluence of natural solar UV one can use a constant fluence of UV-A. The effects of a constant fluence of near UV (especially UV-A) radiation from UV lamps on the growth and physiological activities of radish plants grown in plastic frames covered with UV non transmitting polyvinyl chloride film were investigated by Tezuka et al. (1994). He found that the growth of the plants was promoted by the UV-A radiation, and the promotion by UV-A radiation was associated with an increase in chlorophyll content and photosynthetic activities.

Tendel and Hader (1995) studied the effects of UV radiation on orientation movements of higher plants. Low UV-B level does not induce direct damage but may effect movement reactions in higher plants. UV-B was found to impair (damage) phototropic and gravitropic reactions of shoots, photonastic reactions of leaf joints and opening movements of influourescences. UV radiation impaired growth responses as well as turgid reactions. Giannini (1996) studied the relationship between the levels of ambient Photosynthetically Active Radiation (PAR) and applied UV dose with regard to expansion growth, biomass production, leaf water relation and gas exchange of plant under greenhouse conditions.

Indeed the effects of increased UV-B radiation on plant growth and crop productivity are still of much speculation and, obviously, a scientific challenge (Stapleton, 1992). Several authors (Adamse et al., 1994; Stapleton, 1992; Lercari, 1991; Lercari & Sodi, 1990; Middleton and Teramura, 1994) have presented and discussed the interaction between higher plants and UV-B radiation in the lake of advancement of the knowledge. The action spectra and the kinetics of the responses can identify the causal factors involved in UV mediated responses. Ultraviolet induced inhibition of elongation growth appears to be a general phenomenon (Tevini and Teramura, 1989; Barnes, 1990, Lercari et al., 1989, 1990, 1992). Bertram and Lercari (1996) and Bertram and Karlsen (1994) measured the short and long term stem elongation responses to UV radiation on S. Splendent plants by using linear voltage transducers interfaced with data loggers. The high sensitivity of these transducers make it possible to characterize accurately the time courses of UV induced responses by measuring in the same plant (a) very fast growth responses of the order of a few minutes, (b) very slow responses of the order of hours and days and (c) short and long term responses.

UV-B radiation sources emit small but biologically very effective UVC radiation, which is not present in the solar radiation at the earth surface. Even the best sun tracking system supplementing solar UV-B by a defined amount of artificial UV-B will suffer from these inconveniences and uncertainties. Recently, Sutherland et al. (1996) studied UV responses of plants grown under controlled conditions of day length, temperature and illumination. Enhanced UV-B radiation affects crop species and varieties in a differential way

depending on their sensitivity, which is determined by their genetic and enzymatic ability to accumulate UV protecting pigments and to repair UV-B damage (Robberecht et al., 1980; Tevini et al., 1991; Pang and Hays, 1991; Teramura et al., 1991; Barnes et al., 1993; Dai et al., 1994; Caldwell et al., 1994; Teramura et al., 1990; Caldwell and Flint, 1994).

Environmental issues such as the influence of fluorocarbons and nitric oxide on the earth's protective shield of ozone, the effect of carbon dioxide and volcanic dust on the climate, the formation of photochemical smog, oil pollution, and acid rain have drawn to our attention, the ease with which the biosphere can be perturbed. Vegetative problems, untimely rains, and droughts are to be considered seriously at the same time. Laser-based techniques can find solutions to some of these problems more effectively.

UV-B radiation causes a multitude of physiological and biochemical changes in plants, including inhibition of photosynthesis (Bornman & Teramura, 1993; Hidema & Kumagi, 2006). This inhibition is due to reduced levels of chlorophyll (Chl), chloroplast proteins such as Rubisco (ribulose-1, 5-bisphosphate carboxylase / oxygenase) and LHCII (light-harvesting chlorophyll a/b-binding protein of photosystem II) (Hidema & Kumagi, 2006), and photosynthesis-related gene expression (Hidema & Kumagi, 2006; Strid, Chow & Anderson, 1996). Allen, Mckee, Farage & Baker (1997), reported that loss of Rubisco is a primary factor in UV-B inhibition of photosynthesis in oilseed rape. Further, it was apparent that the UV-B induced reduction in Rubisco was greater in UV-sensitive, than in UV-resistant strains (Hidema and Kumagi, 2006).

It has been reported that, in chloroplasts under illumination, the large subunit (LSU) of Rubisco is directly fragmented into two polypeptides by reactive oxygen species (ROS) (Ishida, Makino and Mae, 1999; Ishida, Shimizu, Makino and Mae, 1998). Other authors have demonstrated that UV-B generated ROS induce photo damage to Rubisco (Caldwell, 1993) and that ROS cause proteolytic degradation of the LSU (Desimone, Henke & Wagner, 1996). Thus, the generation of ROS is thought to be involved in UV-B induced degradation of Rubisco in rice. John, Morris, Jordan, Thomas and Mackerness (2001) reported that, in *Arabidopsis*, exposure to UV-B radiation induces expression of senescence-associated genes (SAGs), including SAG12, which encodes a cysteine protease (Hidema and Kumagi, 2006; Noh and Amasino, 1999). This result suggests that the formation of some kind of protease may also be involved in the enhancement of Rubisco degradation in plants (Hidema and Kumagi, 2006).

Singh, Dube & Gupta (1998) studied the effects of UV-A radiation on the growth of maize plants and their fluorescence spectra. The emission spectra of the second leaf from the bottom side of each maize plant excited by 337 nm were obtained using spex 1680, 0.22m double monochromator known as Spex Fluorolog made in USA. The spectra consisted of two peak bands in the blue-green region at 435 nm (F435) and 525 nm (F525) respectively and two peak bands in the red region at 684 nm (F684) and 740 nm (F740) respectively. The ratios of these peaks as $(\frac{\mathrm{F435}}{\mathrm{F525}})$, $(\frac{\mathrm{F435}}{\mathrm{F684}})$ and $(\frac{\mathrm{F684}}{\mathrm{F740}})$ were calculated in each case. The ratio of blue to red $(\frac{435}{\mathrm{F684}}\mathrm{F})$ and chlorophyll fluorescence $(\frac{\mathrm{F684}}{\mathrm{F740}})$ ratio were found to have decreased. This might be due to the fact that the intensity of red peaks was increased due

to reabsorption of light when the plants were treated with UV-A, and hence the ratios ($\frac{F435}{F684}$) and ($\frac{F684}{F740}$) were reduced (Kent & Singh, 2006, 2008; Singh, Dube & Gupta, 1998).

LIF obtained from intact leaves under different conditions and their quantitative relation to stress detection has been discussed in detail by Broglia (1993). Fluorescence of plant tissues is considered a serious problem in immuno fluorescence microscopy investigation of signified tissues and tissues with vacuolar deposits of phenolic compounds and in some cases it has been eliminated by the use of strong reducing substances (Kent & Singh, 2006, 2008). Oxidized chlorophylls that emit at 540 nm are released to vacuoles from chloroplasts. Broglia (1993) found a decrease of laser induced green fluorescence and increase of chlorophyll fluorescence in *Vicia faba* when epidermal tissue had been removed. This shows that the epidermis is both an important protect ion against UV radiation and a possible topological source of green fluorescence (Kent & Singh, 2006, 2008)

There are two potential primary mechanisms involved in UV-B-induced physiological and biochemical damage. DNA lesions, such as cyclobutane pyrimidine dimer (CPD) and pyrimidine (6–4) pyrimidone photoproducts, interfere with DNA replication and transcription (Hidema and Kumagi, 2006; Britt, 1996). The second mechanism is through modification of proteins by photo-oxidation, or by reactive oxygen species (ROS) and free radicals produced during photosensitization (Hidema and Kumagi, 2006; Caldwell, 1993;

Britt, 1996). These modifications include cross-linking, aggregation, denaturation and degradation (Hidema & Kumagi, 2006).

Gao et al. (2004) investigated the effects of supplementary UV-B radiation on the growth, yield and seed qualities of maize under field conditions. Increased UV-B radiation caused a significant reduction in dry matter and yield, and affected seed quality as follows: protein, sugar and starch levels decreased, whereas lysine levels increased. Taken together, the results of this study suggest that the enhanced solar UV-B radiation as predicted by atmospheric models will result in reduction of growth and yield of maize crops in the future. This has been confirmed from this study that the enhanced UV-A and UV-B radiation causes a significant reduction in the growth of maize plants and hence in the yield in turn. However, to have proper protection, the excessive exposure of both UV-A and UV-B should be avoided to have better growth of yield. Also, it has been observed that maize can sustain certain level of UV-A, but excessive exposure will be dangerous to plants whereas exposure of UV-B of any level is harmful to plants.

The continuous growth of plants indicates that they can tolerate or adapt to UV radiation to survive. The epidermis usually protects the inner leaf with high concentrations of flavonoids and anthocyanins, which absorb the UV radiation safely. The UV treatment is affected by Photosynthetically Active Radiation (PAR) levels (Adamse, 1994 & Giannini, 1996). Whether the presence or absence of activated photomorphogenic, photosynthetic and photoprotective systems during the UV treatment affect plant sensitivity to UV

radiation or not has not been investigated so far. For example, the possible role of photosynthetic process and of xanthophyll cycle activated by wavelengths >500 nm has not been determined because UV irradiation is always been given in the presence of PAR (Hedimbi, Naikaku & Singh, 2012).

## 2.2. UV LASERS FOR LASER-INDUCED FLUORESCENCE

Remote sensing techniques are widely used these days to study plants. One of the most important features of these techniques is the ability of differentiating and identifying species of plants. In remote sensing applications, laser-induced fluorescence (LIF) is a powerful technique, which can be used for early remote sensing of stress conditions in plants (Chappelle et al. 1984). There are several advantages in the use of pulsed UV lasers as excitation sources. For example, the use of this excitation spectral range (200 nm – 400 nm) is recommended by the international eye-safe regulation in remote sensing.

Another advantage is that the powerful and reliable laser sources are available in the whole spectral range of UV. The continuous fluorescence can be obtained from a high repetition rate pulsed excitation. Furthermore, the fluorescence emission spectra may have a range as wide as possible and hence the increasing information contents in the LIF technique (Chappelle et al. 1989) that can be collected. In this section, we will review UV lasers for Laser Induced Fluorescence and will highlight the work done in this field up to 2006. This study will mainly concentrate on the analysis of fluorescence spectra induced by laser wavelength and chlorophyll fluorescence.

### 2.2.1. CHLOROPHYLL FLUORESCENCE

Laser induced chlorophyll fluorescence (Wood, 1984, 1985) is a power tool to study the health and growth of plants through chlorophyll fluorescence. Furthermore, remote sensing of the health status of vegetation is possible by using UV laser induced fluorescence. Light induced fluorescence is an important deexitation mechanism of sharing light energy transformation in leaves (Butler & Kitajima, 1975) through photosynthesis. Chappelle (1994) and Krajicek and Vrbova (1994) discussed the laser induced fluorescence spectra of vegetation and of plants.

The fluorescence spectra of different vegetation have different shapes. Moreover, the shape of the fluorescence spectra depends on the photosynthetic activity of these species and environmental stress factors such as acid rain, the presence of heavy metals, nutrient stress, drought, etc. The process of photosynthesis reflects the biological condition of plants, grasses, crops, etc., under the influence of environment stress. The spectra of a plant excited by UV pulsed nitrogen gas laser (337 nm) show at least four wavelengths which can be used to characterize plants (Andrews & Demidov, 1995). The ratios of LIF at these wavelengths are used for studying the health of plants. This is the principle of relative measurement in LIDAR detection. However, it still requires proper understanding of how laser light interacts with such a complicated system as the photosynthetic apparatus of intact plants. Buschmann et al. (2000) gave an overview on the florescence imaging of plants.

The in vivo chlorophyll fluorescence excited by different visible lights has been extensively studied (Schreiber and Schliwa, 1987; Lichtenthaler, 1988; Barber, 1989), and a good amount of knowledge of its correlation to the mechanism of photosynthesis has been obtained. Chappelle et al. (1984; 1985; 1989, 1990) have obtained a large number of correlations between LIF spectra and different plant types such as monocots, dicots, hardwoods, and algae, their nutritional deficiencies and their stress conditions. Broglia (1993) used an eximer laser and collected spectra from leaves of different species and in different stress conditions. The investigation of the origin of the blue-green fluorescence was done by observing fluorescence changes induced by actinic light on the phytosynthetic apparatus (Senorer, 1986). In active chloroplast suspensions blue fluorescence from photosynthetically reduced nicotinamide adenine dinucleotide phosphate (NADPH) were detected.

The fluorescence emitted by the chlorophyll of plants and bacteria is a process in competition with photosynthetic reactions. The changes in chlorophyll fluorescence reflect variations in the photosynthetic activity of the organisms (plants). Many researchers (Schreiber, 1984; Lichtenthaler & Rinderle, 1988; Renger & Schreiber, 1986) have shown that fluorescence is an early indicator of damage to the photosynthetic apparatus induced from environmental stress factors. Therefore, the fluorescence-measuring techniques on leaves of higher plants are more useful because they are non-destructive and provide simple tool to monitor vegetation. Various methods of measuring

the fluorescence induced kinetics (the Kautsky effect) which occurs when dark-adapted leaves are irradiated (Lichtenthaler & Rinderle, 1988; Krause & Weis, 1984).

A faster, more recent and reliable technique is based on the measurement of the chlorophyll fluorescence spectrum, which consists of two broad bands at about 685 nm and 735 nm. The relative intensity of these two peaks is used as an indicator of the health of plants (Lichtenthaler & Rinderle, 1988; Mazzinghi, 1991; Rinderle and Lichtenthaler, 1988). This method is of particular interest since chlorophyll fluorescence spectra can be easily measured in remote sensing from ground (Castagnoli et al. 1986) or aircraft (Hoge et al. 1981, 1983) platform. Most of the information provided by fluorescence kinetics data, which are possible only as contact measurements on single leaves, can now be obtained in remote sensing detection of the fluorescence spectra. Today multispectral chlorophyll fluorescence images (Edner et al. 1992) are available to study the plant fluorescence spectral properties.

The origin of the two bands in the chlorophyll fluorescence spectrum of leaves has been discussed (Briantais et al. 1986; Krause & Weis, 1991). At room temperature, the chlorophyll fluorescence in both spectral regions is mostly emitted from the photosystem II (PSII) reaction centre. Emission by photosystem I (PSI) contributes with only a minor component at 740 nm. However, when excitation energy distribution on PSI is increased with respect to PSII, a corresponding increase in the longer wavelength fluorescence band relative to the shorter wavelength band is observed. This was studied extensively by

Kyle et al. 1983; and Murata and Satoh, 1986. The fluorescence ratio $\frac{F685}{F735}$ was also observed to be decreased during the Kautsky kinetics (Hak et al. 1990).

One of the most important factors, which greatly affect the leaf fluorescence spectrum, is the absorption of the light emitted by chlorophyll itself. The effect is most important for the spectral region around 685 nm, resulting in chlorophyll fluorescence spectra in which the two peaks have similar intensities. The $\frac{F685}{F735}$ fluorescence ratio is inversely proportional to the leaf chlorophyll content (Lichtenthaler and Rinderle, 1988). At very low chlorophyll concentrations, leaf fluorescence spectra match those of isolated chloroplasts with high $\frac{F685}{F735}$ ratios. Estimation of the reabsorption effect on the leaf fluorescence properties of different plant species are compared (Lichtenthaler & Rinderle, 1988).

A simple method for the estimation of the reabsorption effect on the chlorophyll fluorescence spectrum on intact leaves (Agati, 1993) involves the calculation of the laser-induces-chlorophyll fluorescence spectrum effectively emitted inside the leaf from the measured chlorophyll fluorescence spectrum by taking into account the transmittance and reflectance properties of the leaf. The model was validated on an aurea mutant of tomato and its isogenic wild type. These two genotypes possess very different absorption properties owing to their chlorophyll contents and chloroplast ultra structures and hence provide useful material to compare fluorescence spectra of the same plant species under extremely different reabsorption conditions.

In recent years, the measurements of chlorophyll a fluorescence have been considered as an indicator for characterizing light reactions photosynthesis (Schreiber & Bilger, 1993, Schreiber, Bilger & Neubauer 1995). Bilger, Schreiber, and Bock (1995) determined the quantum efficiency of photosynthesis II and non-photochemical quenching of chlorophyll fluorescence in the field. Recently, Rascher, Liebig, and Luttge (2000) studied the effective quantum yield of photosystem II under momentary ambient light conditions in the field. They increased the light intensity in short intervals and the light-response curves were recorded instantly within a few minutes time.

Blue-green fluorescence and the red/far-red chlorophyll (Chl) fluorescence emission spectra of green tobacco leaves and of *Aurea* leaves in different stages of senescence were measured by Subhash et al. (1999) using excitation at 355 nm in order to test the suitability of the fluorescence ratios green/red and green/far-red together with other ratios as stress and pigment indicators. Young yellowish-green leaves of the *Aurea* mutant tobacco showed a much higher blue-green fluorescence emission and lower chlorophyll fluorescence as compared to the green wild type tobacco. As a consequence, the fluorescence intensity ratios blue/green ($\frac{F440}{F520}$), blue/red ($\frac{F440}{F690}$), blue/far-red ($\frac{F440}{F740}$), green/red ($\frac{F520}{F690}$) and green/far-red ($\frac{F520}{F740}$), as determined from the measured fluorescence emission spectra of the adaxial and abaxial leaf sides, were quite different for both leaf types and were functions of the content of chlorophylls and carotenoids. As chlorophyll levels decreased in senescing leaves of the *Aurea* tobacco plant, the chlorophyll *a*/*b* ratio

remained high at 3.3 to 4.0, while the ratio of chlorophylls to carotenoids declined slightly. Values of the chlorophyll fluorescence ratio $\frac{F690}{F740}$ for adaxial and abaxial leaf-sides increased with yellowing, whereas $\frac{F440}{F520}$ tended to decrease with the senescence-induced decline in chlorophyll content. Ratios $\frac{F440}{F690}$ and $\frac{F520}{F690}$ were found to increase after an initial decrease, with leaf age and senescence. The same trend was found for $\frac{F440}{F740}$ and $\frac{F520}{F740}$ which exhibited maximum sensitivity with respect to chlorophyll breakdown. Whereas the $\frac{F690}{F740}$ ratio for abaxial versus adaxial leaf surfaces correlated linearly, with the abaxial leaf-side values always being higher than the adaxial leaf-side, values for both sides corresponded in a curvilinear fashion with leaf chlorophyll content. Changes during early stages of leaf development were seen not only in the most commonly used red/far-red ratio, $\frac{F690}{F740}$, but to some extent also in $\frac{F440}{F520}$. The results indicate that changes in photosynthetic activity due to senescence and chlorophyll breakdown can easily be detected and qualified via fluorescence ratios that are based on the UV-A excited fluorescence bands F440, F520, F690 and F740. These ratios provide a solid basis for the laser-induced remote sensing of the state of health and chlorophyll content of vegetation. The fluorescence ratios green/red and green/far-red were found to be very suitable complementary indicators of early stress events in plants.

### 2.2.2. LASER INDUCED FLUORESCENCE

UV laser induced fluorescence emission of green plants, according to Chappelle et al. (1984); Gunther et al. (1991); Lichtenthaler et al. (1989, 1990, 1991, 1992) has become of increased interest in recent years, in particular the application of remote fluorescence LIDAR (light detection and ranging) techniques for screening the state of health of terrestrial vegetation.. Green leaves excited by UV light emit red fluorescence between 650 and 800 nm (known as chlorophyll fluorescence) and a fluorescence emission in the blue-green spectral region (Chappelle et al. 1984; Lang & Lichtenthatler, 1991; Lichtenthatler and Stober, 1990).

The blue-green and red fluorescence emission of green wheat and soybean leaves as induced by UV light was determined in plants grown in a phytochamber and in the field (Stober & Lichtenthaler, 1993). The red chlorophyll fluorescence possesses two maxima near 690 nm (F690) and 735 nm (F735) (Lichtenthaler & Buschmann, 1987; Lichtenthaler, Buschmann & Rinderle, 1986; Virgin, 1954). The blue-green fluorescence of green plants possesses a blue maximum near 450 nm (F450) and a green shoulder near 530 nm (F530) respectively. The ratio of blue to red fluorescence, $\frac{F450}{F690}$ exhibits a clear correlation to the irradiance applied during the growth of the plants. On the other side the chlorophyll fluorescence ratio $\frac{F690}{F735}$ and the ratio of blue to green fluorescence $\frac{F450}{F530}$ are not influenced by the irradiance applied during plant growth. The blue fluorescence F450 is slightly decreased whereas the red chlorophyll fluorescence is decreased much with

increasing irradiance applied during the growth of the plants and hence the ratio $\frac{F450}{F690}$ is greatly increased. The decrease in the chlorophyll fluorescence with increasing irradiance is due to the accumulation of UV light absorbing substance in the epidermal layer, which considerably reduces the UV laser light, which passes through the epidermis and excites the chlorophyll fluorescence of the chloroplasts in the subepidermal mesophyll cells (Stober & Lichtenthaler, 1993).

The origin of the blue-green fluorescence is not yet fully known and understood. It is speculated that major signal comes from phenolic components located in the cell walls and the vacuoles of both the epidermal layer and the mesophyll cells (Goulas, Moya and Schmuck, 1990; Lang & Lichtenthaler, 1991; Lichtenthaler et al. 1991). Chappelle et al. (1991) considered NADPH as a source of blue-green fluorescence but Lang and Lichtenthaler (1991) did not accept this.

The ratio of the blue to red fluorescence $\frac{F450}{F690}$ differs from plant to plant and therefore can be used as possible indicator (Chappelle et al. 1985; Lichtenthaler et al. 1991, 1992). This ratio increases with increasing age of conifer needles and it responds substantially to environmental changes including water stress (Gunther et al. 1991; Lichtenthaler et al. 1991). The ratio of the blue to green fluorescence $\frac{F450}{F530}$ represents the chlorophyll and carotenoid pigmentation of green leaves (Stober & Lichtenthaler, 1992). It also exhibits a

linear response both to the total chlorophyll content and to the weight ratio of the chlorophylls to carotenoids (Stober & Lichtenthaler, 1993).

LIF obtained from intact leaves under different conditions and their quantitative relations to stress detection have been discussed in detail (Theisen, 1988). Fluorescence of plant tissues is considered a serious problem in immuno fluorescence microscopy investigation of signified tissues and tissues with vacuolar deposits of phenolic compounds, and in some cases it has been eliminated by the use of strong reducing substances (Knox & Singh, 1985). Oxidized chlorophylls that emit at 540 nm are released to vacuoles from chloroplasts (Matile et al. 1988). Broglia (1993) found a decrease of laser induced green fluorescence and increase of chlorophyll fluorescence in Vicia Faba when epidermal tissue had been removed. This shows that the epidermis is both an important protection against UV radiation and a possible topological source of green fluorescence (Lichtenthaler & Stober, 1990).

The laser induced chlorophyll fluorescence is affected by the light scattered inside the leaf (Markin et al. 1981). The direct fibre optic measurement shows that the scattering increases the intensity distribution on the superficial layers of the leaf and therefore the effective absorption of radiation in the leaf is caused by the diffuse propagation due to scattering structure. It is known that wild type scatters more light than the aurea mutant and that the scattering is higher in chloroplasts with stacked thylakoids (as in wild type), than in chloroplasts with isolated lamellar thylakoids (as found in the aurea). The blue

shift mutant with respect to wild type can be explained by a lower scattering contribution in addition to a lower chlorophyll content.

Consequently, the lower values of $\frac{F685}{F735}$ in the wild type than in the aurea mutant is due to:

1. A higher fluorescence reabsorption at 685 nm due to the higher scattering properties and chlorophyll content; and
2. A greater contribution at 685 nm due to the increases excitation of top layers by scattered light.

Leaf area index (LAI) is an important determinant of canopy photosynthesis, evapotranspiration and competition among crop plants and weeds. Direct methods of measuring LAI are labor-intensive. Previous indirect methods can be inaccurate under some sky conditions and fail to distinguish between green and senescent leaves. Most indirect methods are also somewhat invasive, in that the instrument must be placed below the crop canopy. Denison and Russotti (1997) modified the inclined-point quadrate method to estimate LAI, by substituting a laser beam for the traditional metal probe. Laser-induced chlorophyll fluorescence is used to detect living leaves (or other organs containing chlorophyll), allowing the crop canopy to be scanned from above. Field tests with sudangrass, wheat, and maize found reasonably good correlations ($r^2$ = 0.76 to 0.98) between LAI estimates from our prototype instrument and from direct harvest of leaves. None of the crops tested had significant dead leaf area. This new method can also provide greater detail on the spatial distribution of LAI (e.g., across crop rows) than previous

methods. Because of the limited range at which laser-induced chlorophyll fluorescence can be detected, this approach probably would not be useful for research on forests or tree crops.

A laser-induced fluorescence imaging system was developed (Kim et al. 2003) to capture multispectral fluorescence emission images simultaneously from a relatively large target object. With an expanded, 355-nm Nd:YAG laser as the excitation source, the system captures fluorescence emission images in the blue, green, red, and far-red regions of the spectrum centered at 450, 550, 678, and 730 nm, respectively, from a 30-cm-diameter target area in ambient light. Images of apples and of pork meat artificially contaminated with diluted animal feces have demonstrated the versatility of fluorescence imaging techniques for potential applications in food safety inspection. Regions of contamination, including sites that were not readily visible to the human eye, could easily be identified from the images.

### 2.2.3. LIF STUDY ON MAIZE

Nutrient deficiencies in plants cause impaired and abnormal growth along with some other visible symptoms due to the deficiency of that particular element. However, visible symptoms are sometimes difficult to relate to the nutrient deficiencies in field conditions due to effects of environmental conditions such as weather, disease, and insect infection. They pointed out that the environmental conditions can produce symptoms similar to the symptoms produced by nutrient deficiencies. Chappelle et al. (1989) studied the effects of

nutrient deficiencies on laser induced fluorescence spectra of intact maize plants. They found that potassium deficiency in corn was most observable. Holub et al. (2000) demonstrated fluorescence lifetime imaging in real time of *Zea mays* leaves and showed the differences in the fluorescence lifetime due to photosystem I and photosystem II. Their technique could measure the fluorescence lifetimes at the maximum (P level) fluorescence during the chlorophyll a fluorescence transient (induction) in photosynthetic organism.

Laser induced fluorescence techniques were used to study the deficiencies of nitrogen and sulfur in corn by Samson et al. (2000). They tried to establish a relationship between changes in LIF emission spectra and plant growth inhibition. The corn plants were grown in the greenhouse and fertilized for 35 days with nutrient solutions of different concentrations of nitrogen and sulfur. They took LIF spectra remotely using a compact multiwavelength fluorescence LIDAR and found that UV-induced fluorescence could be used to detect and discriminate of nitrogen and sulfur deficiencies in corn plants. They found that deficiency in nitrogen showed a significant increase in the $\frac{\mathrm{F460}}{\mathrm{F740}}$ ratio. It was concluded that the reason of this could be in the lowered UV transmittance by the leaf epidermis, which could be estimated using the $FRF_{ex360}/FRF_{ex440}$ ratio.

Intact vegetation produces two overlapping broadband fluorescence emissions at about 335 nm and 440 nm when exited with 280 nm wavelength of light (Corp et al. 1997). However, Chappelle et al. (1984) got four maxima near 440 nm, 525 nm, 685 nm and 740 nm. Separation between the two fluorescence bands can be used as a nondestructive tool

to remotely sense variations in protein concentration due to nitrogen supply. Recently, Corp et al. (2005) studied leaf and canopy fluorescence properties of corn (*Zea mays L*) grown under varying levels of nitrogen concentration. They found that the fluorescence response from corn leaves when excited between 350 nm and 380 nm exhibited a number of significant correlations among single bands (peaks) and bands ratios to measure the growth conditions of plants. They concluded that a relationship exists between nitrogen supply and in vivo fluorescence emissions from corn leaves.

Plant damage caused by environmental changes can be studied using laser induced fluorescence technique. Mineuchi et al. (2001) used laser induced fluorescence technique to study the effects of UV-B radiation on the peanut leaves. They evaluated the vitality decrease of leaves by measuring the change in a specific fluorescence peak ratio. They found that an early detection and an evaluation of UV-B stress could be possible by applying the method of remote sensing. Takeuchi et al. (2002) investigated leaf samples irradiated by UV-B and found that at two wavelengths, namely 685 nm and 735 nm, the LIF lifetimes became shorter during UV-B irradiation. Takeuchi et al. (2002) established a relationship between the lifetimes and the plant's activities under UV-B irradiation.

Metal stress was induced in maize (*Zea mays L.*) by the addition to the soil of a range of concentration of either ethylene-diamine-tetra-acetate (EDTA) of citric acid (CA) as chelating agent and measurements were taken using a sensor (Colls & Hall, 2005). The sensor was capable of detecting plant fluorescence at 762 nm and 688 nm. Significant

differences were estimated in the fluorescence responses of those plants for which high concentrations of EDTA were added compared to those for which CA or no chelating agent was added. These plants also exhibited higher tissue metal concentrations and showed visible stress. These results are in support of the use of plant fluorescence as potential tool for an early indication of phototoxic metal stress.

Laser-induced fluorescence (LIF) is an active sensing technique capable of capturing immediate and specific indications of changes in plant physiology and metabolism as they relate to the concentration and photosynthetic activity of the plant pigments. Reflectance is a passive sensing technique that can be used to capture differences in the concentration of the primary plant pigments. Fluorescence and reflectance were compared (McMurtrey et al. 1991) for their ability to measure levels of plant stress that are of agronomic importance in corn (*Zea mays* L.) crops. Laboratory LIF and reflectance spectra were made on leaves from field grown corn. Changes in the visible region of the spectrum were compared between groups of plants fertilized with seven different levels of nitrogen (N) fertilization. A pulsed nitrogen laser emitting photons at a wavelength of 337 nm was used as a fluorescence excitation source. Differences in maximum intensity of fluorescence occurred at 440 nm, 525 nm, 685 nm, and 740 nm. Significant separations were found between levels of N fertilization at several LIF wavelength ratios. Several reflectance algorithms also produced significant separations between certain levels of N fertilization.

Singh et al. (1998) studied the growth of Indian maize plants under UV-A treatment. The fluorescence spectra of the second leaf from bottom side of each plant excited by 337 nm gave four maxima, two in the blue-green region at (F435) and (F525) respectively and two in the red region at (F684) and (F740) respectively. Under UV-A treatment, a clear reduction was observed in the total weight, full width and the length of the second fully expanded leaf, and the length of the stem of each plant. The ratio of blue to red ($\frac{F435}{F684}$) and the chlorophyll fluorescence ratio ($\frac{F684}{F740}$) were decreased due to the decrease in the photosynthetic activity. In case of UV-B stress, the ratios of blue to green, blue to red and chlorophyll fluorescence ratio were decreased due to the decrease in the photosynthetic activity and chlorophyll content. It was clear from this study that these ratios could be taken as an effective indicator of UV stress (Subhash et al. 1995) and could be used to study the health status of a plant at an early stage.

Laser induced chlorophyll fluorescence techniques have been used for forest decline research (Lichtenthaler et al. 1990) and for the detection of different nutrient stresses in plants (Chappelle et al. 1984; Heisel et al. 1996; Schuerger et al. 2003; Subhash & Mohanan, 1994). The principle of the technique is that the laser light is absorbed by chlorophyll molecules which dissipate the energy by emitting fluorescence light. Laser induced chlorophyll fluorescence measurements to detect the nitrogen concentration of wheat (*Triticum aestivum* L) at canopy level under ambient conditions were studied by Schachti et al. (2005). They increased the amount of nitrogen fertilizer to induce variations in the nitrogen uptake of canopies. A clear differentiation between the nitrogen treatments

was observed. They found that nitrogen supply of wheat canopy is detectable at the field level with the laser induced chlorophyll fluoresce sensor.

Laser-induced red chlorophyll fluorescence spectra of rice leaves were studied using a Jobin Yvon double monochromator by Subhash and Mohahan (1994). A He-Ne laser was used as the excitation source, and *in vivo* fluorescence emission spectra were recorded with a resolution of 2 nm in the 650–800 nm range. They found that the fluorescence spectra showed a broad band at 690 nm. This band is usually observed around 735 nm resolved into three prominent bands at 725 nm, 745 nm, and 750 nm. In addition, a new band was also observed at 705 nm. The fluorescence induction kinetics (Kautsky effect) were measured at 690 nm, 705 nm, and 725 nm, and the plant vitality index Rfd and the ratios of fluorescence intensities $\frac{F690}{F705}$and $\frac{F690}{F725}$ were determined for rice grown under normal and nutrient deficient conditions. The results showed that the *Rfd* values and the fluorescence intensity ratios related to the 705 nm band had great potential for remote sensing stress effects in plants. Velentini *et al.* (1994) has also shown the similar results saying that the fluorescence ratio $\frac{F690}{F730}$ is a good index for vegetation remote sensing.

Cecchi et al. (1994) used remote sensing technique in several vegetation monitoring experiments. Remote sensing, spectroscopy, and ecophysiology data were analyzed. Ludeker et al. (1999) used laser fluorescence technique to detect the vitality of Scots pines. Since the fluorescence LIDAR measurements present an average over different twinges and needles of different age, they concluded that LIDAR measurements from the ground

can yield useful information about the vitality states of trees. Therefore, a differentiation between stressed and non-stressed trees is quite obvious. Induction kinetics of chlorophyll fluorescence (the Kaustky effect) and delayed fluorescence of some cold climate species were studied both in laboratory and in situ experiments by Kharuk *et al.* (1994). They wanted to find out if this could be as an indicator of plant health. They found that parameters of induction curves (normalized variables fluorescence, time of its half decreases) could be used as indicators of plant health.

### 2.2.4. TERRESTRIAL VEGETATION

Direct measurement of chlorophyll fluorescence at a distance was considered by many workers (Field et al. 1995). Gunther et al. (1994) and Velentini et al. (1994) used the ratio of fluorescence $\frac{F690}{F730}$ while laser based technique was used by Cerovic et al. (1996). Recently, Ananyev et al. (2005) and Kolber et al. (2005) developed a technique based on laser induced fluorescence transient (LIFT) to remotely measure photosynthetic properties in terrestrial vegetation from a distance up to 50 m. Laser induced fluorescence transient technique is useful for nondestructive, remote and real time characterization of photosynthetic properties and estimation of photosynthetic rates in terrestrial vegetation. The stationed LIFT instrument can perform continuously within the radius of 50 m and hence continuous measurements are possible. They used 665 nm laser beam targeted on to leaves of the plants such as canopy of cottonwood and oak trees. The fluorescence emission spectra was collected at 690 nm by a 250 mm reflective telescope and processed in real time.

Stewart Jr. et al. (2005) used the application of remote sensing of transgenic plants using green fluorescent protein (GFP) from the jellyfish *Aequorea victoria*. They described laser induced fluorescence imaging and laser induced fluorescence spectroscopy of GFP transgenic plants in ambient light. They used Nd:YAG laser emitting 355 nm, however they got better signal-to-noise ratio with 390 nm. They used a dye laser giving about 8 mJ of energy at 290 nm. The laser beam was passed through a liquid light guide and projected as a uniform source on to the plants. Green fluorescent protein and other fluorescent protein bioreporters can be used to monitor transgenes in plants. GFP is a valuable marker for transgene presence and expression, but remote sensing instrumentation for stand-off detection has lagged behind fluorescent protein marker biotechnology. However, both biology and photonics are needed for the monitoring technology to be fully realized. In this section, we describe laser-induced fluorescence imaging and laser-induced fluorescence spectroscopy of GFP-transgenic plants in ambient light towards the application of remote sensing of transgenic plants producing GFP.

Laser-induced fluorescence emission of green terrestrial vegetation is an important area of research in remote LIDAR techniques. The remote sensing of blue and red fluorescence signatures is very suitable tool to determine the state of health of plants. Stober et al. (1992) discussed some of the major factors which determine the intensities of the UV-laser (337 nm) excited blue fluorescence near 450 nm and the red chlorophyll fluorescence between 650 nm and 800 nm as well as the ratio of the blue to the red fluorescence $\frac{\mathrm{F450}}{\mathrm{F690}}$. In contrast to green plants that are grown in a phytochamber or at low light intensities,

plants grown in the field at a high photon flux density (PFD) showed high values for the ratio of the blue to the red fluorescence $\frac{F450}{F690,}$ which was due to a relatively low intensity of the red chlorophyll fluorescence. The strongly increased ratio $\frac{F450}{F690}$ was primarily caused by a much reduced penetration depth of the exciting UV light into the leaf, which seemed to be due to substances absorbing in the epidermal layers. *Etiolated* wheat leaves exhibited stronger blue fluorescence intensity than green leaves and showed also a small maximum around 530 nm (green fluorescence). White leaves of wheat treated with the bleaching herbicide norfluorazone ($10^{-1}$ M) and leaves from which the photosynthetic pigments had been extracted by acetone showed a fourfold and tenfold increase of the blue fluorescence, respectively. Isolated chloroplasts and thylakoids did not exhibit a blue-green fluorescence.

### 2.2.5. PLANT CLASSIFICATION

Stober et al. (1992) concluded that the blue fluorescence could be mainly caused by phenolic plant substances located in the cell wall and or vacuoles of leaves. The partial reabsorption of the emitted blue and red fluorescence by the photosynthetic pigments was thought to modify the shape of the fluorescence spectra. Time resolved chlorophyll fluorescence spectra of intact leaves were studied by Schmuk and Moya (1994). It is known that UV excitation radiation causes plants to produce relatively a stronger fluorescence emission. Spectral characteristics and of blue green fluorescence excited by UV laser on leaves of unrelated species were studied in detail by Bongi et al. (1994).

According to them, the blue-green fluorescence emission comes mainly from other epidermal layers. They found that the fluorescence emissivity is not linked to a short term metabolic arrangements but it follows long-term epidermis adaptations to draught, and excessive radiation.

The capacity of a plant for photochemistry depends on various factors including stresses caused by environmental conditions. The cholorophyll fluorescence has been used as a standard method for investigating plant classification, chlorophyll contents monitoring, and plant stressed detection (Stober & Lichtenthaler 1992; D'Ambrosio et al. 1992; Chappelle et al. 1985; Lichtenthaler & Miehe, 1997). Ndao et al. (2005) collected laser induced fluorescence emission spectra of leaves of some tropical plants using a compact fiber-optic fluorosensor with continuous wave blue diode laser and an integrated digital spectrometer. They confirmed that the fluorescence ratios can act as stress and strain indicators. The fluorescence intensity ratio $\frac{F690}{F735}$ is an early indicator of the plant physiological state. However the physiological information obtained about plants health from fluorescence measurements must be supplied by compliments information obtained from passive reflectance and absorbance measurement.

The fluorescence intensity at about 685 nm corresponds to the fluorescence of PHII cholorphyll in an intact leaf. This wavelength range for LIDAR studies of in vivo fluorescence has been used by many workers (Lukin et al. 1989; Saito et al. 1998). Saito et al. (1998) investigated the laser-induced fluorescence spectra of living leaves of seven

different trees by using a 355 nm pulsed Nd:YAG laser. The shapes of the spectra (360-800 nm) varied depending on the season and growing conditions. Generally, red fluorescence (>650 nm) was larger during summer to autumn, which offers information on the activity of photosynthesis, and blue-green fluorescence (<650 nm) was relatively large in early summer and late autumn to winter, which offers information on the progress of growth and senescence. The spectral shapes also varied depending on the organic constituents inside the leaves. Separation of the spectra into their components was tried to identify the leaves constituents.

Mineuchi et al. (2001) used laser induced fluorescence technique to study the effects of UV-B radiation on the peanut leaves. They evaluated the vitality decrease of leaves by measuring the change in a specific fluorescence peak ratio. Laser-induced fluorescence of birch, pine, and aspen trees was studied by Astafurova et al. (2001). They concluded that LIDAR (remote) probing could be used for identifying trees species and assessing the condition of leaf and needle canopies. Such a method could be used to monitor natural resources from an aircraft.

## 2.3. STATUS OF CURRENT STUDY

Laser Induced Fluorescence (LIF) is a non-destructive technique. It is very suitable for remote sensing applications. It can be used for detecting stresses in plants and also studying the health status of plants (Singh and Gupta, 1998 and Singh, 2000). In plants a major part of the solar radiation is used for photosynthesis and then absorbed by the

chlorophyll and carotenoid. A small portion of the absorbed energy is released as heat and fluorescence giving lot of information about the plants in terms of four maximum bands near 450 nm, 525 nm, 680 nm and 740 nm. These four bands can provide very useful information about the health status and the level of stresses in plants. Recent studies have shown that the intensity ratios of the blue and green emission bands at 425 nm and 530 nm with respect to the chlorophyll fluorescence bands at 690 nm and 730 nm have great potential in determining the health status of plants.

LIF has been proposed as a most suitable technique for early remote sensing of stress conditions in local plants. Several advantages are there in the use of pulsed UV lasers as excitation sources. For example, the use of this excitation source in the UV spectral range is recommended by the international eye-safe regulation in remote sensing. Powerful and reliable laser sources are available in this spectral range. Efficient time discrimination from ambient light and continuous fluorescence can be obtained from the high repetition rate of pulsed excitation. Further the fluorescence emission from plant for a wide spectral range can easily be collected. This fluorescence emission gives increasing important information being the LIF technique. Laser based techniques provide high accuracy and therefore it is proposed to carry out more precise measurements on plants under the laboratory conditions. Hence, using LIF technique it will be possible to know more precisely what happens to the plants when they are subjected to different stresses and when the field conditions change.

## 2.4. KEY ISSUES EMANATING FROM LITERATURE

Mackerness (2000) made predictions based on the expected rise in UV-B radiation in the next few decades. He indicated that the growth, development, and yield are going to be affected because of rise in UV-B radiation. Stapleton (1992) argued that the effects of increased UV-B radiation on plant growth and crop productivity are still of much speculation and, obviously, a scientific challenge. Campbell (1980) and Tezuka (1990, 1991) indicated that the solar spectrum reaching the earth's surface does not extend below approximately 290 nm because ozone in the stratosphere effectively absorbs all radiation of shorter wavelengths. Therefore, the natural solar radiation of the UV-B region at the earth's surface is between 290 and 320 nm. A reduced stratospheric ozone layer may affect plants in several different ways and alter species composition and productivity since shorter wavelengths UV-B (280-320 nm) radiation will increase even with a relative small decrease in ozone. Caldwell, (1981, 1982, 1983, 1984) studied several species and found that the plants native to higher latitudes are often found to be more sensitive to UV radiation than those native to lower latitudes.

Many reports (Hashimoto & Tajima, 1980; Teramura, 1980, 1983; Ambler, 1975; Haldall, 1964; Sisson & Caldwell, 1975, 1976; Van, 1976, 1977) have shown that UV-B radiation inhibits photosynthesis and growth of plants. The same conclusion was also reached by the following authors (Hashimoto & Tajima, 1980; Teramura, 1980, 1983; Ambler, 1975; Haldall, 1964; Sisson & Caldwell, 1975, 1976; Van, 1976, 1977).

Fluorescence techniques are also useful in studying the physiological state of the plants (Chappel et al. 1984; Broglia, 1993; Strober & Lichtenthaler 1993 and Tezukla et al. 1994). When plant leaves are irradiated by UV radiation, fluorescence spectrum shows four peaks centered at 450 nm, 530 nm, 685 nm and 730 nm. The ratio of blue to red fluorescence differs from plant to plant and is considered a stress indicator. It responds to any environmental changes including water stress. The ratio of blue to green fluorescence reflects the chlorophyll and carotenoid pigmentation of green leaves. The chlorophyll fluorescence is highly correlated to the mechanism of photosynthesis, and hence the health status of plants can be studied (Chappel et al. 1984, 1985) easily using LIF. Lichtenthaler and Rinderle (1988); Mazzinghi (1991); Rinderle and Lichtenthaler (1988) is of the opinion that a faster, more recent and reliable technique is based on the measurement of the chlorophyll fluorescence spectrum, which consists of two broad bands at about 685 nm and 735 nm. The relative intensity of these two peaks is used as an indicator of the health of plants.

The continuous growth indicates that plants can tolerate or adapt natural radiation. When plants are grown in a greenhouse without UV radiation they experience a UV shock when planted outside, which can kill the plant. Even plants adapted to UV radiation are impaired in their development, structure and function by increased radiation. The epidermis usually protects the inner leaf with high concentrations of flavonoids and anthocynanins, which absorb in the UV range. UV-induced reduction in photosynthetic activity shows that some UV radiation penetrates into the lower cell layers.

One of the most important factors, which greatly affect the leaf fluorescence spectrum is the absorption of the light emitted by chlorophyll itself. The effect is most important for the spectral region around 685 nm, resulting in chlorophyll fluorescence spectra in which the two peaks have similar intensities. The F685/F735 fluorescence ratio is inversely proportional to the leaf chlorophyll content (Lichtenthaler & Rinderle, 1988).

However, to have proper protection, the excessive exposure of both UV-A and UV-B should be avoided to have better growth of yield. Also, it has been observed that maize can sustain certain level of UV-A, but excessive exposure will be dangerous to plants whereas exposure of UV-B of any level is harmful to plants. When studying the effects of UV-B radiation on plants, Smith (2000) found that there was a decrease in dry weight and this reflects the cumulative effect of many small disruptions in plant function.

Liu (1995) and Liu and McClure (1995) studied the effects of UV-B on flavonoids, ferulic acid, growth and photosynthesis in barley primary leaves and have suggested that the combination of $CO_2$ and UV-B radiation may differentially affect plant growth and morphogenic parameters, and elevated $CO_2$ may ameliorate the effects of UV-B radiation. This was also the view of Lavola (2000, 2003).

Hopkins (2002) was of the view that. a reduction in the rate and duration of growth of the primary leaf, in response to UV-B, was the result of changes in both the rate and extent of

cell division and elongation. The supply of cells into the elongation zone was reduced, and this, coupled to a reduction in the rate of elongation, resulted in reduced leaf growth. Cells of UV-B-treated leaves were found to age more quickly than those of the controls. The effects of increased UV-B on plant-pathogen interactions have been studied by Paul (2000) in only a few pathosystems. The most crop diseases are greatly affected by stratospheric ozone depletion within the limits currently expected. However, the lack of a detailed understanding of the mechanisms by which UV-B influences plant-pathogen interactions in most pathosystems is a significant limit to such predictions.

# CHAPTER 3: MATERIALS AND METHODS

## 3.1. METHODS APPLIED

The conditions of humidity, water, soil fertility, light, and temperature were standardized and then the plants were grown under these controlled or laboratory conditions. The fluorescence spectra were gathered from the leaves of the maize plants and studied. The maize plants were given different level of photo and water stresses for its photosynthesis activity studies. The vegetation fluorescensor is housed in the laser laboratory at the University of Namibia.


**Figure 11:** Avaspec 2048 Spectrometer.

Laboratory conditions were set up in the laser laboratory that is located in the University of Namibia, Faculty of Science in Windhoek, by making a greenhouse of suitable size. Laboratory measurements of fluorescence spectra from maize plants grown under different controlled conditions were gathered.

Fifteen pots were filled with sandy soil and three maize seeds were planted at a depth of one centimeter in each pot. The pots were watered with 100 ml of water every third day for two weeks. The seedlings were divided into two groups, one for white light and the second group was further subdivided into two subgroups. One of the subgroups was exposed to UV-A radiation while the other subgroup was exposed to UV-B radiation. A Philips, F40T, 12/B1, Actinic light source was used as a source for UV-A exposure (Figure 12).


**Figure 12:** Philips Actinic light source used for UV-A exposure.

The other light source was a Philips, TL. 40W/12RS, J2) lamb lights used as a source for UV-B exposure (Figure 13).


**Figure 13:** Philips Light source used for UV-B exposure.

The exposure of UV-A and UV-B was given from up to 10 hours at two hour intervals. Each experiment was done using three replicates.

### 3.2. PLANT GROWTH AND CONDITIONS

First, the seeds of maize were screened based on approximately equal size and weight and then were soaked in 0.1% of $HgCl_2$ solution for about 10 minutes and then washed properly in distilled water. For planting the seeds, small size buckets, with equal amount of clean sand were used and in each bucket three seeds at about the same depth was planted. These buckets were then placed in complete darkness for some hours for germination. The LIF spectra from a particular leaf of the maize plants were studied. After some days when the seeds have germinated, the poorly germinated plants were discarded and the plants of nearly equal height, health and look were retained. These plants were divided into several groups consisting of a few plants in each group (Figure 14).

One of the groups were transferred into Chamber 1 and the other group into Chamber 2 and the third group into Chamber 3. The different chambers were used to grow maize plants under different controlled conditions, for example, in the first chamber (Chamber 1) white light source (400 -700 nm), was used and in the second chamber (Chamber 2) another light source emitting radiation at 360 nm (UV-A) in addition to the white light was used. In the third chamber (Chamber 3) a light source emitting radiation at 290 nm (UV-B) in addition to the white light was used.

After some days of continuous treatment of white light in Chamber 1 and the treatment with white light plus UV-A and UV-B in Chamber 2 and 3, the plants were taken out with root.


**Figure 14:** Picture of maize plants (1-White light, 2-White light +UV-A, 3- Wite light + UV-B).

The growth parameters such as the weight of each plant, length of stem, and the length and width of the second leaf were measured. To study the effects of UV-A and UV-B on plants, all other conditions such as water, temperature were kept identical. The measurements of the emission spectra from the leaf were obtained using vegetation fluorescensor (spectro-fluorometer). After the exposure of the maize seedlings, the

emission spectrum of the third leaf from the bottom side of each maize seedling excited by 337 nm was obtained, using a AvaLight- D(H)S Deuterium-Halogen light sources, which is connected to Ava Spec-2048 Fiber Optic Spectrometer and a computer, Avasoft 6.2 software for displaying the results in a graph format.

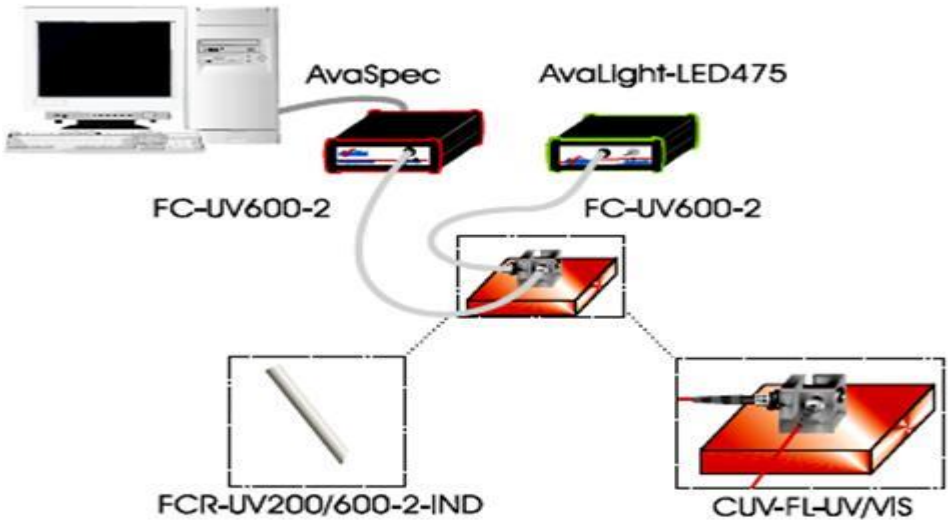



**Figure 15:** Exprimental setup to obtain LIF spectra form leaves.

The ratios of the peaks of the emission spectra were calculated and then studied. The growing conditions are given below:

WL = plants growing in white light (400-700 nm) only;

UV-A = plants growing in WL plus some additional constant and continuous treatment of UV-A (360 nm);

UV-B = plants growing in WL+ some additional treatment of UV-B (290 nm).

### 3.3. WORKING HYPOTHESIS

One of the main objectives of this research work was to carry out fluorescence studies on stresses in maize plants in order to understand the mechanism of local environmental (in Namibia) effects on plants. This was done on plants grown under controlled conditions. In this study, the most relevant and important seeds such as of maize plants were considered.

There is considerable amount of UV radiation present in Namibia as indicated by Singh (2000) in the "International Workshop on renewable energy and its applications for rural development, Windhoek, August 2002". Therefore, the study of UV influence on the growth of plant is very vital. It is known that the growth of similar plants in different regions is different. It is observed that plants do not grow tall in Namibia due to certain stress factors; one of the most important factors may be the excessive exposure of UV radiation to plants.

Having established this fact, which is also very vital for the advancement of knowledge, it may be desirable to study the health status of plants that are influenced by the environmental stress. Therefore, this project will have the direct impact on our local environmental development.

# CHAPTER 4: RESULTS, DISCUSSIONS, CONCLUSION AND RECOMMENDATIONS

## 4.1. RESULTS

In Tables 1 and 2, the fluorescence intensity ratios of maize leaf exposed to UV-A and UV-B respectively, are presented.

**Table 1:** The fluorescence intensity ratios of maize leaves (3rd leaf) exposed to UV-A with their respective standard errors (of three replicates).

| UV-A (hours) | **F410/F430** | **F410/F550** | **F550/F590** |
|---|---|---|---|
| 0 | 0.4±0.02 | 0.2±0.03 | 2.9±0.2 |
| 2 | 0.3±00.3 | 0.2±0.02 | 2.7±0.3 |
| 4 | 0.3±0.04 | 0.3±0.04 | 2.1±0.2 |
| 6 | 0.3±0.03 | 0.3±0.02 | 1.7±0.4 |
| 8 | 0.3±0.02 | 0.6±0.03 | 1.7±0.1 |
| 10 | 0.2±0.01 | 0.6±0.02 | 1.6±0.2 |

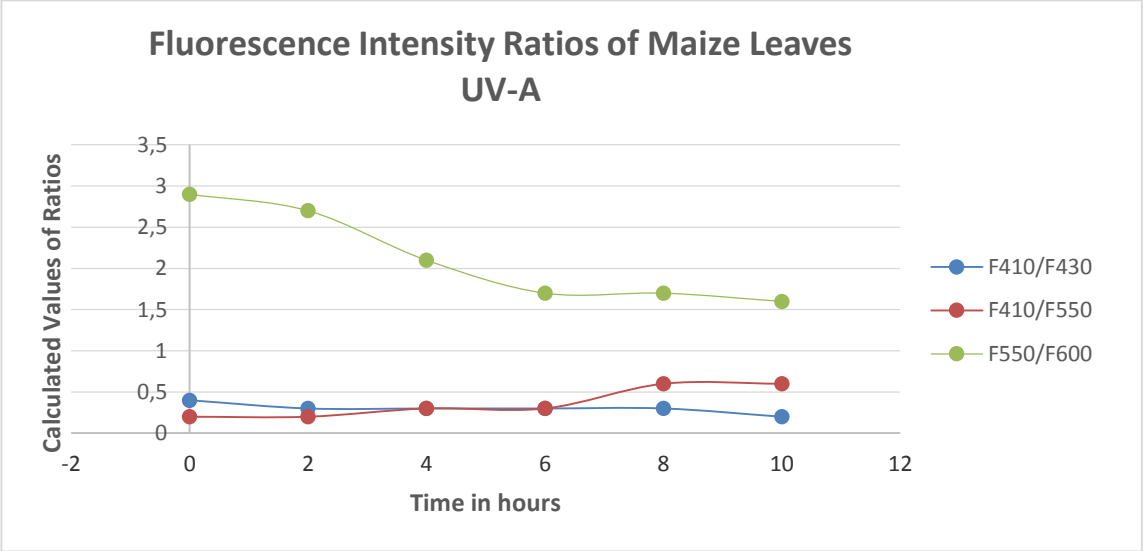



**Figure 16:** Fluoresence Intensity Ratios of Maize Leaves – exposed to UV-A

**Table 2:** The fluorescence intensity ratios of maize leaves (3rd leaf) exposed to UV-B with their respective standard errors.

| UV-B (hours) | F410/F430 | F410/F550 | F550/F590 |
|---|---|---|---|
| **0** | 0.4±0.02 | 0.3±0.02 | 2.5±0.2 |
| **2** | 0.3±0.05 | 0.3±0.04 | 2.2±0.3 |
| **4** | 0.3±0.03 | 0.4±0.08 | 2.1±0.4 |
| **6** | 0.3±0.02 | 0.4±0.03 | 2.0±0.4 |
| **8** | 0.2±0.01 | 0.4±0.04 | 1.4±0.2 |
| **10** | 0.2±0.03 | 0.4±0.02 | 1.1±0.5 |

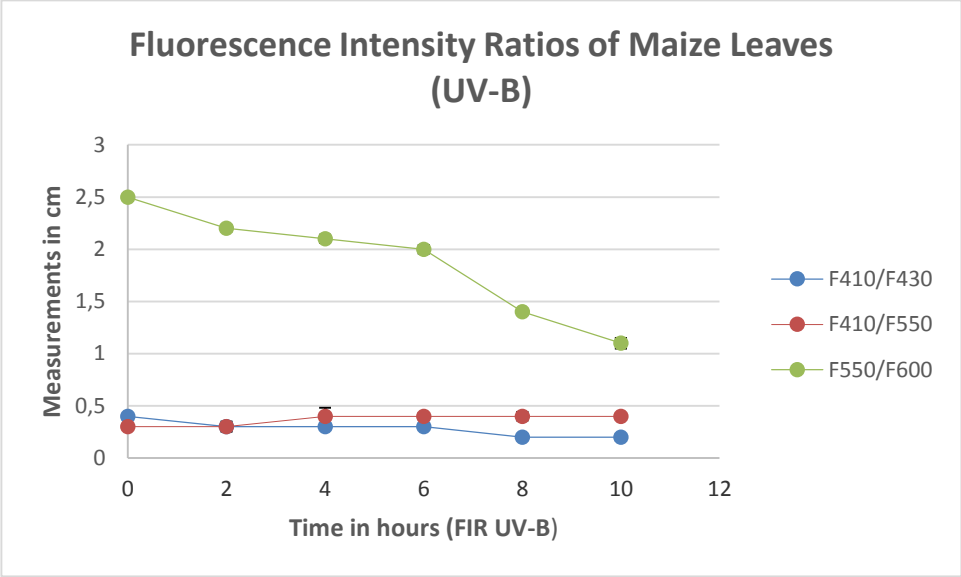



**Figure 17:** Fluoresence Intensity Ratios of Maize Leaves – exposed to UV-B

While in Tables 3 and 4, the mean of each health parameter at different times of exposure to UV-A and U-VB with their standard errors, are shown. Health parameters include the width of the leaf, diameter of the stem and the height of the seedling.

**Table 3:** Show the mean of each health parameter at different times of exposure to UVA with their standard erors.

| Hours | No. of leaves | Width of the 2nd leaf - cm | Diameter of the stem - cm | Height of the seedling - cm |
|---|---|---|---|---|
| **Control** | 3 | 1.7±0.04 | 0.4±0.03 | 41±4.1 |
| **0** | 3 | 1.7±0.02 | 0.4±0.02 | 41±3.8 |
| **2** | 3 | 1.3±0.03 | 0.3±0.03 | 35.4±4.1 |
| **4** | 3 | 1.3±0.02 | 0.3±0.04 | 30.8±4.7 |
| **6** | 4 | 1.2±0.03 | 0.2±0.01 | 25.7±4.9 |
| **8** | 4 | 1.1±0.02 | 0.2±0.01 | 23.2±3.4 |
| | | | | |
| **10** | 4 | 0.7±0.04 | 0.1±0.01 | 19.3±0.6 |

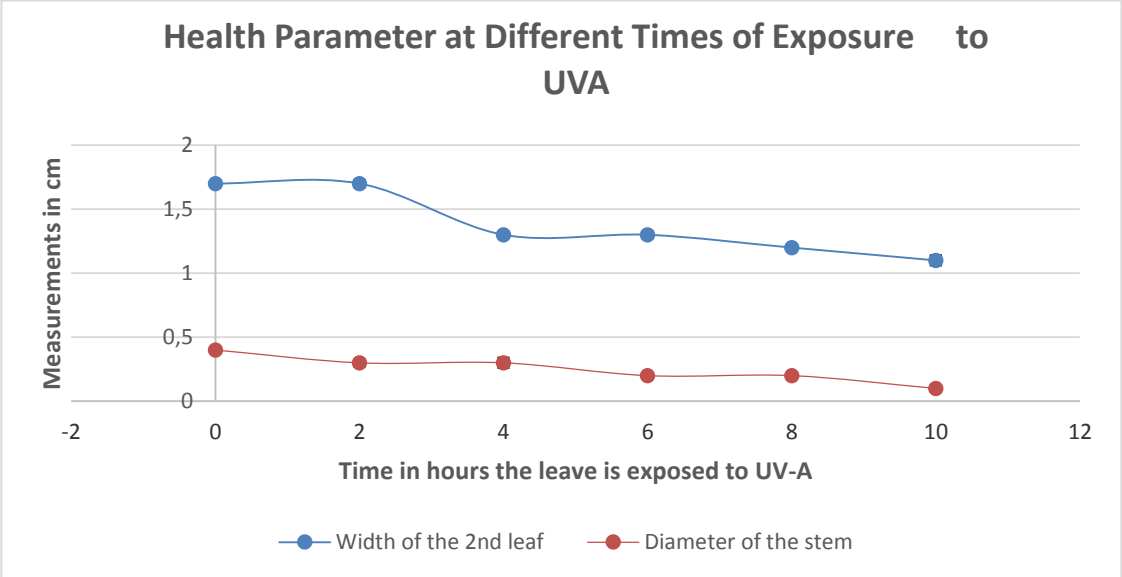



**Figure 18:** Health parameters at different times of exposure to UV-A.

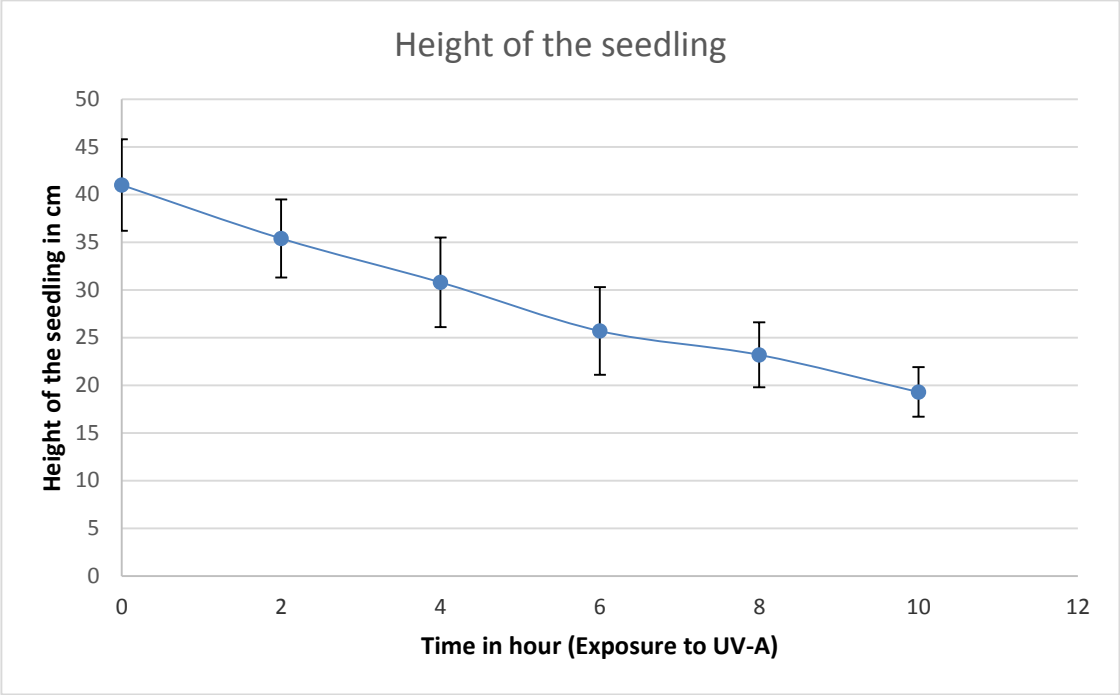



**Figure 19:** Health parameters (Height) at different times of exposure to UV-A.

**Table 4:** Show the mean of each health parameter at different time of exposures to UVB with their standard errors **.**

| Hours | No. of leaves | Width of the 2nd leaf cm | Diameter of the stem cm | Height of the seedling cm |
|---|---|---|---|---|
| **Control** | 3 | 1.7±0.04 | 0.4±0.03 | 41.0±4.2 |
| **0** | 3 | 1.6±0.02 | 0.3±0.06 | 40.0±4.3 |
| **2** | 3 | 1.2±0.03 | 0.2±0.04 | 27.7±5.8 |
| **4** | 3 | 1.1±0.02 | 0.2±0.03 | 26.3±3.2 |
| **6** | 3 | 0.4±0.04 | 0.2±0.05 | 20.7±2.5 |
| **8** | 3 | 0.4±0.05 | 0.2±0.02 | 18.7±1.9 |
| **10** | 3 | 0.3+/-0.01 | 0.1±0.005 | 12.3±0.6 |

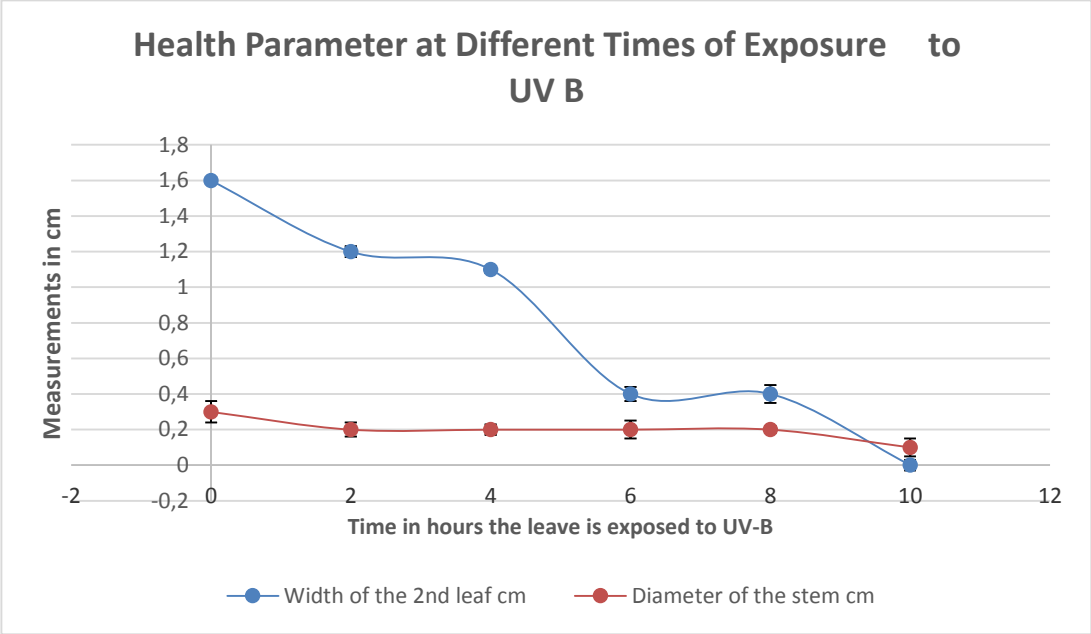



**Figure 20:** Health parameters at different times of exposure to UV-B

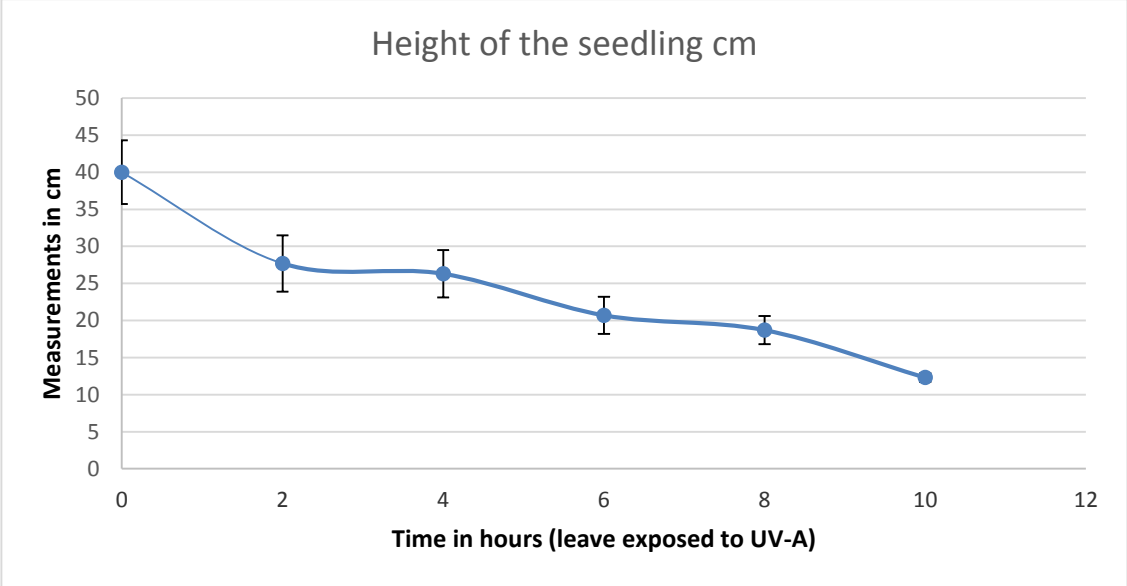



**Figure 21:** Health parameters (Height) at different times of exposure to UV-A.

The Figures below, from Figure 22 (a –e) and Figure 23 (a-e), show the Laser Induced Fluorescence spectra of maize leaves exposed to 2 to 10 hours of UV-A and UV-B radiation, respectively. Four peaks are observed at F410, F430, F550 and F590. From these figures it is clear that the size of the four peaks are reducing over time, thus after ten hours of exposure the Chorophil peak is one third of the size of the peak representing two hours of exposure, in both UV-A and UV-B.

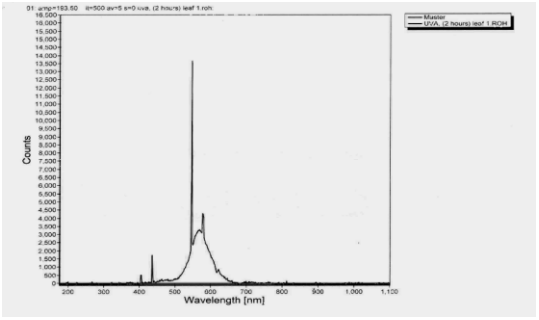



Figure 22 (a): LIF spectrum exposed to 2 hours of UV-A radiation.

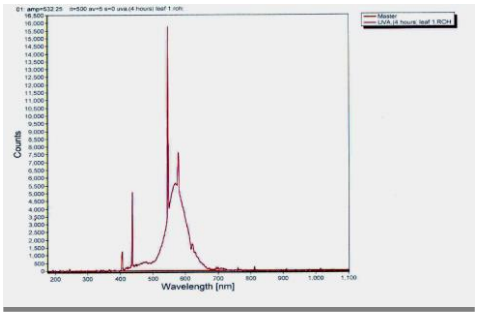



Figure 22(b): LIF spectrum exposed to 4 hours of UV-A radiation

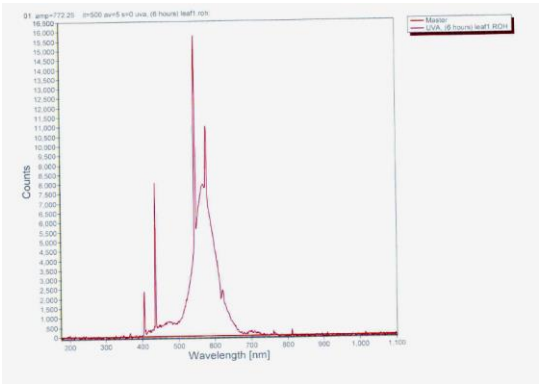



Figure 22(c) LIF spectrum exposed to 6 hours of UV-A radiation.

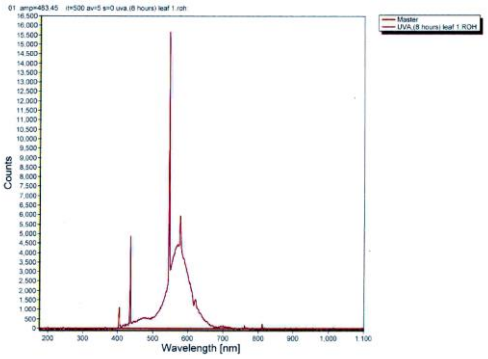



Figure 22 (d): LIF spectrum exposed to 8 hours of UV-A radiation

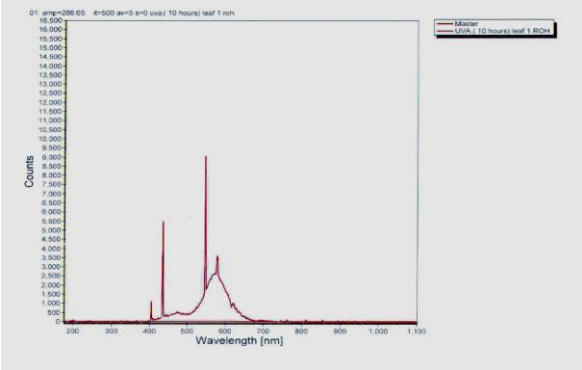



Figure 22 (e) : LIF spectrum exposed to 10 hours of UV-A radiation.

**Figure 22:** LIF spectra for Maize exposed for 2h, 4h, 6h, 8h and 10h of UV-A radiation.

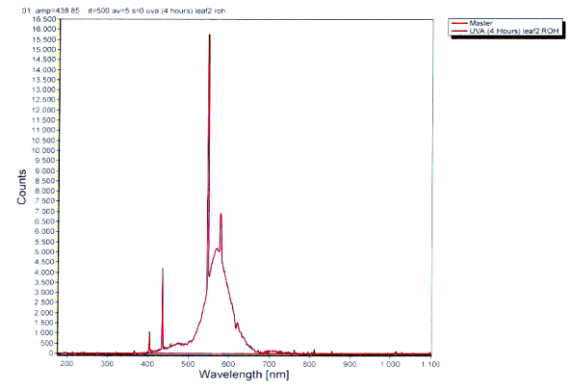



Figure 23 (a): LIF spectrum exposed to 2 hours of UV-B radiation.

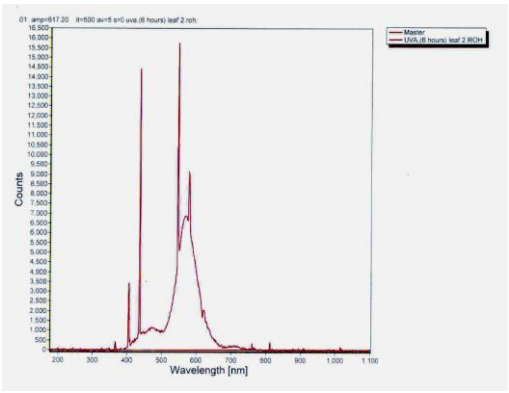



Figure 23 (b) LIF spectrum exposed to 4 hours of UV-B radiation

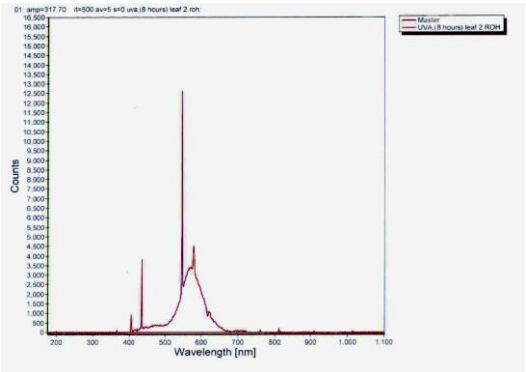



Figure 23 (c): LIF spectrum exposed to 6 hours of UV-B radiation

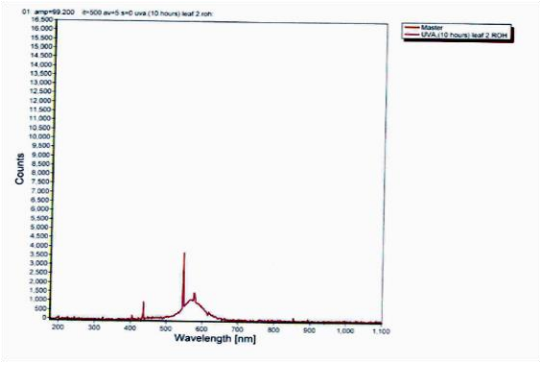



Figure 23 (d): LIF spectrum exposed to 8 hours of UV-B radiation

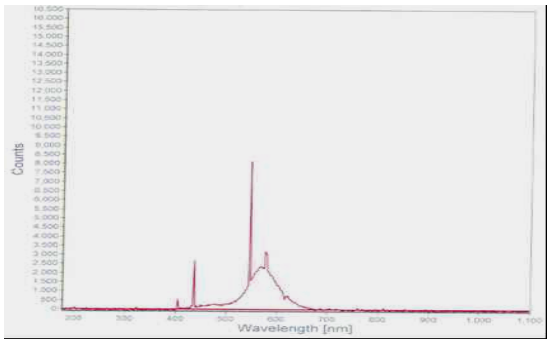



Figure 23 (e): LIF spectrum of Maize leaves exposed to 10 hours of UV-B radiation•

**Figure 23:** LIF spectra for Maize exposed for 2h, 4h, 6h, 8h and 10h of UV-B radiation.

## 4.2. DISCUSSIONS

The emission spectrum of the third leaf from the bottom side of each maize seedling were obtained using a AvaLight- D(H)S Deuterium-Halogen light sources, which is connected to Ava Spec-2048 Fiber Optic Spectrometer and a computer for displaying the results in a graph format as can be seen in Figure 22 (a –e) and Figure 23 (a-e).

The laser induced fluorescence spectrum was taken from the bottom of 1$^{st}$, 2$^{nd}$, or 3$^{rd}$ leaf of each plant grown under normal conditions and under stress of UVA or UVB exposure but for this study the spectra taken for 3$^{rd}$ leaf of each plant are shown in the Figure 22 (a –e) and Figure 23 (a-e). For each plant, the kind of stress given to the plant and time of stress are shown. For example, the Figure 22 (a) is for UVA stress of two hours given to the leaf 3 of each plant, Figure 22 (b) is for UVA stress of 4 hours given to the leaf 3 of each plant, and so on. Each spectrum has four peaks at 410 nm, 430 nm, 550 nm, and 590 nm. The intensity ratios of these peaks as $\frac{F410}{F430}$, $\frac{F410}{F550}$, and $\frac{F550}{F590}$ corresponding to blue to

green, blue to red, and chlorophyll fluorescence were calculated and analyzed. From Table 1, exposure of the maize leaf to UV-A, it was found that the ratio $\frac{F410}{F430}$ , that is blue to green, has decreased from 0.4 to 0.2, that is a 50% decrease, while the ratio $\frac{F410}{F550}$, that is blue to red, increase from 0.2 to 0.4, which is an increase of 100%. On the other hand it was found that the ratio $\frac{F550}{F590}$ , the chlorophyll flurescence, has decreased from 2.9 to 1.6, which is a decrease of 44.8%.

In the case of Table 2, exposure of the maize leaf to UV-B, it was found that the ratio $\frac{F410}{F430}$ , that is blue to green, has decreased from 0.4 to 0.2, that is a 50% decrease, while the ratio $\frac{F410}{F550}$, that is blue to red 0.3 to 0.4, which is an increase of 33%. On the other hand it was found that the ratio $\frac{F550}{F590}$ , the chlorophyll flurescence, has decreased from 2.5 to 1.1 which is a decrease of 56%.

It was also observed from the analysis of the ratio, $\frac{F410}{F430}$ , that is blue to green and the ratio, $\frac{F550}{F590}$ , the chlorophyll flurescence, that there was a decrease with increase in exposure to UV-A and UV-B. According to Chappel et al. (1984); Broglia, (1993); Strober and Lichtenthaler, (1993) and Tezukla et al. (1994) the ratio blue to green fluorescence reflects the chlorophyll and carotenoid pigmentation of green leaves. The chlorophyll fluorescence is highly correlated to the mechanism of photosynthesis, and

hence the health status of plants can be studied (Chappel et al. 1984, 1985) easily using LIF.

The decease in the ratio $\frac{F550}{F590}$ , the chlorophyll flurescence is due to the photosynthesis activity and hence the health status of plants, whereas the decrease in the ratio $\frac{\mathrm{F410}}{\mathrm{F430}}$ (blue to green fluorescence) reflects the chlorophyll and carotenoid pigmentation in green leaves, but the ratio of $\frac{\mathrm{F410}}{\mathrm{F550}}$ (blue to red) was found to be increased with the increase of UV-A and UV-B radiation.

Lichtenthaler and Rinderle (1988); Mazzinghi (1991); Rinderle and Lichtenthaler (1988) indicated that the epidermis usually protects the inner leaf with high concentrations of flavonoids and anthocynanins, which absorb light in the UV range. UV-induced reduction in photosynthetic activity shows that some UV radiation penetrates into the lower cell layers.

The decrease in $\frac{\mathrm{F550}}{\mathrm{F590}}$ (chlorophyll fluorescence) and the $\frac{\mathrm{F410}}{\mathrm{F430}}$ (blue to green) ratio could be as a result of the reduction in photosynthetic activity and increase intensity of the red peak due to re-absorption of light when plants are treated with UV-A and UV-B. While the increase in the ratio of $\frac{\mathrm{F410}}{\mathrm{F550}}$ (blue to red) with the increase of UV-A and UV-B radiation, could be due to the reabsorbtion on the red light in the the inner leaf with high

concentrations of flavonoids and anthocynanins. Further, it was found that UV-B was more destructive than UV-A as shown in Table 1 and 2.

From Tables 3 which summarises the growth parameters, such as number of leaves, width of the leave, diameter of the stem and height of seedling, the following can be observed. When exposed to UV–A, the width of the leave reduces from 1.7 to 0.7 (58%), the diameter of the stem reduced from 0.4 to 0.1 (75%), and the height of the seedling from 41 to 19 (53%).

While in Table 4, which summarises the growth parameters, such as number of leaves, width of the leave, diameter of the stem and height of seedling, when exposed to UV-B, the following can be observed. The width of the leave reduces from 1.7 to 0.0 (100%), the diameter of the stem reduced from 0.4 to 0.1 (75%), and the height of the seedling from 41 to 12.3 (70%).

Skórska (2000) observed that plants are more susceptible to long-wave UV-B irradiation and that this difference is more apparent from the changes in total area of leaves and dry mass of shoots, rather than from the parameters of chlorophyll fluorescence and net photosynthetic rate. While Caldwell (1977), observed that UV-B can change the anatomical features of plants, inhibit photosynthesis, slow down their growth, reduce biomass, and lower the crops yield. The reduction in growth parameters reflects

accumulative effects of many small disruptions in plant function, as also observed by Smith (2000) and Caldwell (1977).

In this study, it is thus observed that UV-A and in particular UV-B has significant impacts on the total biomass and the growth of plants. From the two Tables it is clear that the effect of UV-B on all growth parameters is much more significant.

## 4.3. CONCLUSIONS

The development of laser technology in African countries is growing very fast. Recently, South Africa launched an African Laser Centre (ALC) at Pretoria. ALC is ready to help the University of Namibia to develop laser applications in the fields of agriculture and medicine. The University of Namibia organized an international conference on laser applications to bring awareness in optical sciences and to realize the importance of lasers and their applications in areas suitable to Namibia such as agriculture and medicine.

This project has contributed to the further development of the University of Namibia laser laboratory, in order to carry out experimental work in this important field. This technique can be used to establish how the growth of plants is affected by UV radiation in Namibia, in particular the fact that the study suggests that the enhanced solar UV- radiation as predicted by atmospheric models will result in reduction of growth and yield of crops in the future. Having established the effects that UV radiation has on Namibian vegetation, Namibia could use these results to develop strategies to reduce the impact of UV on plants.

In this study it was found that the ratio $\frac{F550}{F590}$ (fluorescence) has decreased by 44.5% when maize plants were exposed to UV-A and by 56% when exposed to UV-B and this is due to the reduction in photosynthesis activity, whereas the ratio of $\frac{F410}{F430}$ (blue to green) was found to have decreased by 50% with the increase of UV-A and UV-B radiation but the ratio of $\frac{F410}{F550}$ (blue to red) was found to increase by 100% with increase in UV-A and with 33% with increase in UV-B radiation.

It is further observed that UV-A and in particular UV-B has significant impacts on the total biomass and the growth of plants. It is also clear that the effect of UV-B on all growth parameters is much more significant than that of UV-A. UV-B has a wavelength range of 280-320 nm. Since UV-B has a shorter wavelength than UV-A, it is the most damaging ultraviolet radiation (Gao et al. 2004). This is a result of Ozone depletion, and thus these effects could intensify in the future.

## 4.4. RECOMMENDATIONS

Namibia has lot of potential in various fields where lasers can play a key role, particularly in agriculture, defense, medicine and minerals detection. Some of these applications will be developed in Namibia and the following could be recommended for the future:

- Enhancement of the research activities and facilities in contemporary optics and laser applications at the University of Namibia to be used in physical, biological and chemical studies;

- Actively promote and motivate the continued advancement of laser research and the further development of laser remote sensing techniques for atmospheric, environmental and further vegetative studies. Promotion could include the introduction of laser science and technology in the secondary education curriculum;
- The UNAM, Faculty of Science in collaboration with the UNAM Engineering Faculty, to develop a potable diagnostic device that could be used in the field studies, using this technique of Laser Induced Fluoroscence;
- Explore the use of Remote Sensing using light aircraft for LIF studies;
- The UNAM Nuclear Physics Team to measure radiation from the Sun, and to develop UV-maps;
- Develop mathematical models to predict effects of UV radiation on plant, as well as assessing plant deseases, soil composition, and studying UV-radiation near cities; and
- Enhance collaboration in laser Technology and its applications with African Laser Centre of South Africa and other institutes of interest.

There are numerous useful applications of lasers that could be used as a spring board to build local capacity to develop technologies in fields like defense and agriculture. Finally, UNAM could lead the development of a comprehensive National Doctorial Studies Programme to enhance Namibia's research capacity. In addition, UNAM could enhance the use science and technology techniques to address social and economic challenges of Namibia people.

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