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# Vegetation Stress: an Introduction to the Stress Concept in Plants

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**Summary**

This is a presentation of the essentials of the present stress concept in plants, which has been well developed in the past 60 years. Any unfavorable condition or substance that affects or blocks a plant's metabolism, growth or development, is to be regarded as stress. Plant and vegetation stress can be induced by various natural and anthropogenic stress factors. One has to differentiate between short-term and long-term stress effects as well as between low stress events, which can be partially compensated for by acclimation, adaptation and repair mechanisms, and strong stress or chronic stress events causing considerable damage that may eventually lead to cell and plant death. The different stress syndrome responses of plants are summarized in a scheme. The major abiotic, biotic and anthropogenic stressors are listed. Some stress tolerance mechanisms are mentioned.

Stress conditions and stress-induced damage in plants can be detected using the classical ecophysiological methods. In recent years various non-invasive methods sensing different parameters of the chlorophyll fluorescence have been developed to biomonitor stress constraints in plants and damage to their photosynthetic apparatus. These fluorescence methods can be applied repeatedly to the same leaf and plant, e.g. before and after stress events or during recovery. A new dimension in early stress detection in plants has been achieved by the novel high resolution fluorescence imaging analysis of plants, which not only senses the chlorophyll fluorescence, but also the bluegreen fluorescence emanating from epidermis cell walls which can change under stress induced strain. This powerful new technique opens new possibilities for stress detection in plants.

*Key words: Bluegreen fluorescence, chlorophyll fluorescence, damage, resistance, long-term stress, strain, stress-factors.*

## Introduction

In the past ten years the number of scientific publications, found in journals of botany, plant physiology, ecophysiology and plant biochemistry dealing with plant stress and plant stress detection increased enormously. This process is still continuing and will proceed in an even more enhanced way in the future. Several books, e.g. *Stress and Stress Coping in Cultivated Plants* (McKersie and Leshem, 1994), *Plant Adaptation to Environmental Stress* (Fowden et al., 1993) and proceedings of symposia (Alscher and Cumming, 1990) or plant stress reviews (Larcher, 1987; Lichtenthaler, 1988) have appeared, which describe either plant stress concepts or particular aspects of plant stress, and also name various stressors and stress constraints.

The term «plant stress» is used by most authors in a very broad sense which justifies the establishment of a unifying concept of plant stress. This is fully correct since a multitude of stressors with different modes of action can induce, besides very specific effects, the same or at least similar overall responses in the plant. Plants do not have many response possibilities to stress, but respond, besides specific acclimation, in general with either a high-light type or a low-light type growth or adaptation response (Lichtenthaler, 1984).

From the literature it appears, however, that many authors regard almost every little modification and change of metabolic pathways, growth responses and development pattern of plants as stress responses and stress effects. In this respect the terms «plant stress and stress responses» are «over stressed». The term «stress» should not be applied to mere and fast readjustments of metabolic fluxes, e.g. of photosynthetic rates or respiration and transpiration rates as induced by changes in the photon flux density (sunlight ⇔ clouds), a decrease in temperature or an increase in air humidity. The plants are acclimatized and respond flexibly to such steadily re-occurring switches of cell metabolism and physiological activities as a response to changing environmental conditions. Moreover, diurnal changes in metabolic activities, growth pattern and cell division activities, which are regularly found at the day/night changes in the evening or at the night/day changes in the morning, do not represent stress effects, but can only be regarded as a reorientation of metabolic and growth activities according to the preferential day or night occurrence of certain metabolic processes. In addition, the plants can respond to environmental changes not only by fast acclimations, but also by particular long-term adaptations, e.g. of leaf size and thickness, stomata density, structure and function of chloroplasts as well as enzyme levels to either high-light or low-light growth conditions. Depending on their type and nature, these adaptations may take place within 1 or 2 days or in one week latest. With such adaptation responses plants can avoid stress constraints and adapt in an optimal way to new and changing outdoor growth conditions (Lichtenthaler et al., 1981; Lichtenthaler and Meier, 1984; Meier and Lichtenthaler, 1981).

Despite their capacity for fast acclimation of metabolic fluxes and the somewhat slower adaptation responses as well as certain stress tolerance mechanisms, plants are often exposed to sudden short-term or long-term stress events which reduce cell activity and plant growth to a minimum. This can lead to a severe damage eventually causing cell death if the stress coping mechanisms or repair mechanisms of plants are overworked. There exist many either natural or anthropogenic stress factors, which, depending on their intensity and duration, can cause damage to plants. These stresses can also be characterized as abiotic or biotic stresses.

In order to better differentiate between regular acclimation and adaptation responses of plants on one hand, and stress effects, stressors and stress constraints on the other hand, one needs a unifying general stress concept of plants. In the past 60 years the stress concept (Seleye, 1936) had successively been developed for plants by various authors (Stocker, 1932, 1947; Larcher, 1987; Levitt, 1980; Lichtenthaler, 1988; McKersie and Leshem, 1994). This concept seems to be hardly known to the botanical community although the term stress is presently being used in many publications. During the first international «Vegetation Stress Conference» in Munich, June 1995, the present stress concept of plants was therefore presented as an introduction speech, and is also exposed here at the beginning of this vegetation stress volume. This review not only surveys the different stress approaches, but also gives some examples for stress detection in plants, e.g. by the non-invasive chlorophyll fluorescence techniques, and very recently also by including the bluegreen fluorescence of plants.

## Definition of plant stress

The original general stress concept for living organisms was developed by H. Selye (1936, 1956) and can be summarized in the following two sentences: «*All agents can act as stressors, producing both stress and specifc action*» and «*There exist stressor specific responses and non-specific general responses*». J. Levitt (1980) defined stress as: «*Any environmental factor potentially unfavorable to living organisms*».

On the basis of various observations in plants, and also under inclusion of the original concept on drought resistance of the botanist Stocker (1932 and 1947), the plant ecophysiologist Larcher (1987) summarized the stress concept of plants, and he stated that «*Every organism experiences stress, although the way in which it is expressed differs according to its level of organization*». From the botanist's point of view he described stress as a «state in which increasing demands made upon a plant lead to an initial destabilization of functions, followed by normalization and improved resistance» and also «*If the limits of tolerance are exceeded and the adaptive capacity is overworked, the result may be permanent damage or even death*». Larcher (1987) also stated that «stress contains both destructive and constructive elements and that stress is a selection factor as well as a driving force for improved resistance and adaptive evolution».

### *Eu-stress and dis-stress*

Lichtenthaler (1988), who took up a proposal of W. Larcher given in a personal discussion, extended the stress concept of plants by differentiating between eu-stress and dis-stress, in which case eu-stress is an activating, stimulating stress and a positive element for plant development, whereas dis-stress (as seen in the English word distress) is a severe and a real stress that causes damage, and thus has a negative effect on the plant and its development. As formulated by Lichtenthaler (1988): «*A mild stress may activate cell metabolism, increase the physiological activity of a plant, and does not cause any damaging effects even at a long duration. Such mild stimulating stress is favorable for the plant*». In any case one has to consider that stress is a dose-dependent matter. At fairly low concentrations a stressor, e.g. a herbicide, can stimulate plant metabolism and plant growth, as has been observed in the case of various herbicides and plant growth regulators. Thus, very low doses of a stressor and a xenobiotic can, in fact, have the opposite effect than higher doses. Whether this applies to all stressors has yet to be proved. However, at a concentration 10 or 100 times higher the same xenobiotics will cause damage to the plant and induce early senescence finally leading to death if the stressor is not removed. Such damaging stressor concentrations and all other stress constraints at higher doses are negative for the physiology and development of plants, and thus represent a definite stress in the sense of a dis-stress. Within this concept, real stress shows up when a certain threshold of a stressor, which can no longer be compensated for.by the plant, is exceeded. When this threshold of stress-tolerance or stress-resistance has been passed, a short-term high level stress can principally induce the same damage as a long-term low level stress. The applicability of the «stressor dose – stress effect relationship» seems to be obvious, but has

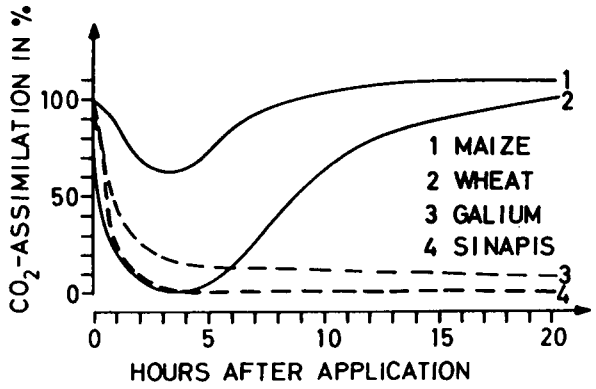


**Fig. 1:** Inhibition of photosynthetic $CO_2$ assimilation in the crop plants maize and wheat and the weeds *Galium* and *Sinapis* after spraying of leaves with the herbicide bentazon at doses equivalent to 1 kg ha$^{-1}$ (from Lichtenthaler et al., 1982).

not been proved so far in all cases and thus, more research is required in this field.

One should keep in mind that the transition between eu-stress and dis-stress is fluent. The relative position of the stress tolerance threshold depends not only on the plant species, but also on the type of stressors applied and on the predisposition of the plant, i.e. the growth condition and vitality before the stressor starts to act. Plants also differ in their stress coping capacity. This can be illustrated with the example of the application of herbicides in agricultural crops in order to kill weeds. Many crop plants possess the capacity to detoxify herbicides by introduction of a hydroxyl group to the aromatic ring of the herbicide which is then glycosylated to an inactive compound that can no longer bind to its target protein (Devine et at., 1993; Hock et at., 1995). However, this detoxifying capacity is often not present in the weeds to be controlled and the latter will eventually die off. An example is shown in Figure 1 with the application of the herbicide bentazon which blocks the photosynthetic electron transport by binding to the $Q_B$-binding protein of photosystem II instead of $Q_B$. After bentazon application the photosynthetic rates initially decline in the crop plants wheat and maize as well as in the weeds *Galium* and *Sinapis* (Lichtenthaler et al., 1982). After several hours, the photosynthetic capacity of maize and wheat is, however, restored since both crop plants possess the ability to hydroxylate, glycosylate and detoxify bentazon. Consequently, the weeds possess a much lower stress tolerance than the crop plants wheat and maize, which exhibit the herbicide detoxifying metabolism.

### *President Clinton 1995 and Plant Stress Research*

In a public speech President B. Clinton regarded a 1 million US$ government-financed «study of plant stress» as an example of wasted funds, since he mistook plant stress as meaning emotional stress of plants. The topic of concern was a governmental grant to Texas Tech University to run a laboratory for the «development of drought-resistant wheat and pasture grasses». Animal or human stress on one hand and plant stress on the other hand, however, are different matters. This should be better emphasized by plant physiologists and made clear to the public. In his letter and response to B. Clinton's remarks, James N. Siedow (1995), the present president of the American Society of Plant Physiology, also gave a clear definition of stress in plants: «*Plant stress refers to a wide range of biological and environmental stresses that crops and other plants are subjected to daily. These include drought, cold and heat, weeds, insects and a host of diseases including those caused by viral, fungal and bacterial pathogens.*»

### *Stress Concept in Physics and Botany*

The stress concept has also been developed in physics, and there the terms stress, strain and damage are well defined. This stress concept can also be applied to plants (see Lichtenthaler, 1988). According to this, these stress terms used in physics mean:

**Stress:** is a state of the plant under the condition of a force applied.

**Strain:** is the response to the stress and to the force applied to the plant (i.e. the expression of stress before damage occurs)

**Damage:** is the result of too high a stress, which can no longer be compensated for.

In botany and plant physiology the term «strain» is rarely used and often not known. Strain is usually replaced by stress responses. Based on the stress concepts in physics it is clear that there can be stress and strain in plants, and that a damage does not necessarily occur even when the plant is under long-term stress and continuous strain. With specific strain (and limited vitality) the plant can survive also under continuous stress constraints although at much reduced metabolic activities and growth rates. An example may be given here. In the Northern Black Forest at Herrenalb a 170 year old pine (*Pinus silvestris* L.) grows on the portal and walls of a former Romanic monastery church, ca. 4 m above ground, but its roots are only found above ground in the stones of this wall and they have no access to soil and water. Thus, under continuous stress (primarily water stress) and strain this pine managed to survive in this unfavorable location and to grow within 170 years to a ca. 9 m high tree which visually appears fully intact and healthy (Fig. 2). The growth limitations set by this location are, however, documented by much less needles per needle year, as well as much shorter and thinner twigs as compared to pines growing in locations with more optimal growth conditions. Reducing the leaf or needle area, i.e. the area for transpiration, is one of the major water stress-coping mechanisms found in broad-leaf and conifer trees.

### *The different phases induced by stress*

Based on the original stress concept of Selye (1936, 1956) and taking into account the results of Stocker (1932, 1956) one has to differentiate among the plant's stress responses three phases (Larcher, 1987) to which a fourth has been added by Lichtenthaler (1988). Before stress exposure the plants are in a certain standard situation of physiology which

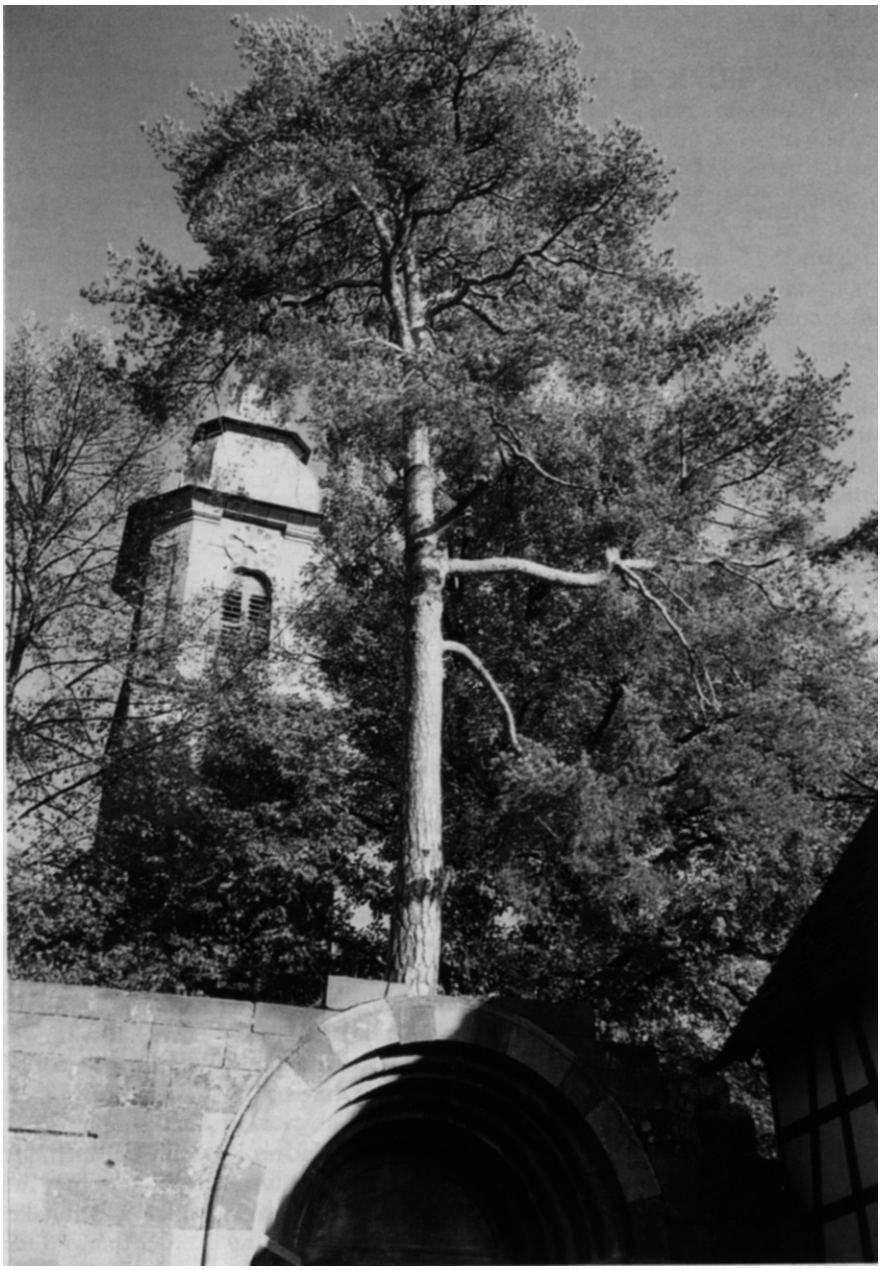

**Fig. 2:** Pine (*Pinus silvestris* L.) having grown for ca. 170 years under continuous water stress on the sandstone portal of a former Romanic monastery church at Herrenalb, Black Forest. The pine roots have no contact to soil and ground water, but end in the wall 2 m above ground (Height of the pine: ca. 9 m).

is an optimum within the limits set by the growth, light and mineral supply conditions of the location. Stressors or complex stress events will then lead to the three stress response phases, and later to the regeneration phase after removal of the stressors if the damage had not been too severe. These are the consecutive four phases:

**1. Response Phase:** (beginning of stress)
**alarm reaction**
- deviation of the functional norm
- decline of vitality
- catabolic processes exceed anabolism

**2. Restitution Phase:** (continuing stress)
**stage of resistance**
- adaptation processes
- repair processes
- hardening (reactivation)

**3. End Phase:** (long-term stress)
**stage of exhaustion**
- stress intensity too high
- overcharge of the adaptation capacity
- chronic disease or death

**4. Regeneration Phase:** **partial or full regeneration** of the physiological function when the stressor is removed and the damage was not too high.

At the beginning of stress the plants react with a decline of one or several physiological functions, such as the performance of photosynthesis, transport or accumulation of metabolites and/or uptake and translocation of ions. Due to this decrease in metabolic activities, the plants deviate from their normal physiological standard and their vitality declines. Acute damage will occur fast in those plants which possess no

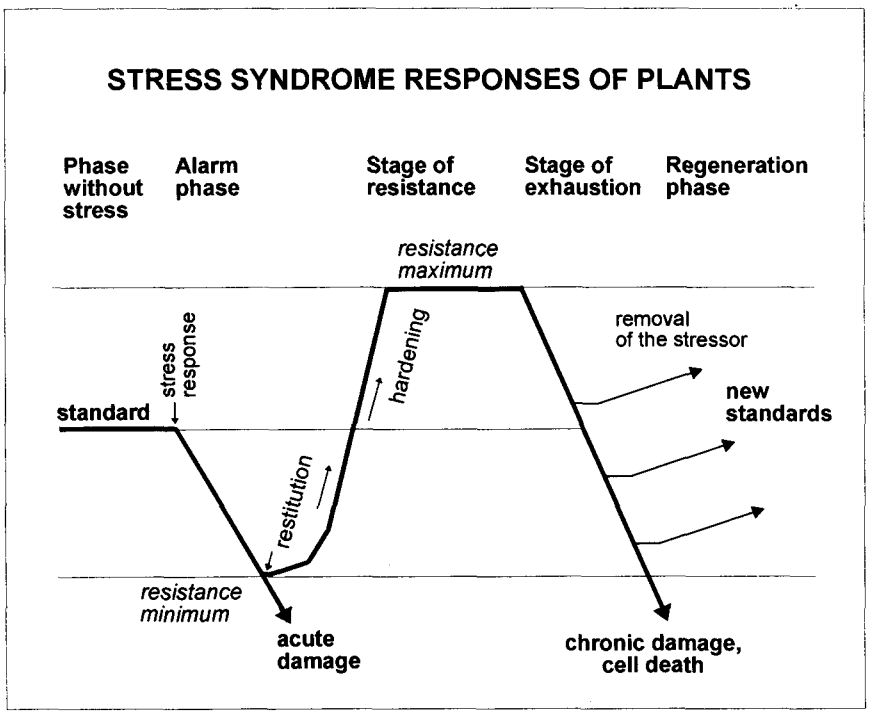


**Fig. 3:** General concept of the phase sequences and responses induced in plants by stress exposure. Plants growing at a physiological standard condition will respond to and cope with stress. After removal of the stressor(s), new standards of physiology can be reached depending on the time of stressor removal as well as the duration and intensity of the stress.

or only low stress tolerance mechanisms, and thus have a low resistance minimum (Fig. 3). During this alarm phase most plants will, however, activate their stress coping mechanisms by fast acclimations of their metabolic fluxes as well as activating repair processes and long-term metabolic and morphological adaptations. This is also called the general alarm syndrome GAS (McKersie and Leshem, 1994). GAS may also stand for general acclimation syndrome or general adaptation syndrome. Repair processes and adaptations will not only lead to a restitution of the previous physiological functions, but also to a hardening of plants by establishing a new physiological standard, which is an optimum stage of physiology under the changed environmental conditions and which corresponds to the plants' *resistance maximum* (Fig. 3). At long-term stress and a stress-dose overloading the plants' stress coping mechanisms, the stage of exhaustion (end phase) shows up in which physiology and vitality are progressively lost. This causes damage and finally cell death. However, when the stressors are removed in the right time before the senescence processes become dominant, the plants will regenerate and move to new physiological standards (regeneration phase). The time and stage of exhaustion at which the stressors are removed defines to which new physiological standard within the resistance minimum and maximum the plants will move (Fig. 3).

How long the plant will stay at the new physiological standard depends on external and internal factors. In field plants this is certainly not too long. Endogenous changes in the development program of plants have always been associated with changes in their physiology program and activity, and have resulted again in a new physiological standard. Furthermore, the next stress events will show up soon, and these again require a re-orientation of the plant's physiology standard to a new «optimum» within the limited possibilities set by the stress constraints. One should keep in mind that stress exposure of plants is not a rare event, but can occur daily, since there exist many different stressors which often act simultaneously. Therefore, stress and strain are routine events in a plant's life. Continuous stress and strain does, however, not mean that a damage must necessarily occur in a plant. If intensity and duration of stress are not too high and long, the plants will orient themselves within the range set by the resistance minimum and maximum (Fig. 3), and in such cases damage symptoms are not detectable. With respect to such findings one has to differentiate between the detection of stress and strain on one hand and the detection of clear damage symptoms on the other hand. Both processes may require different methods of detection, since the methods for damage detection may not allow to screen stress or strain of plants. If one wants to take countermeasures against stress and strain in order to avoid damage and to guarantee an optimum growth and harvest of plants, one should not wait until damage symptoms are visually detectable but respond much earlier. And this requires an early and efficient stress and strain detection in plants.

### *Stress constraints and stressors*

There exist many stress events and a multitude of stressors in the life cycle of plants. The different kinds of stress factors (stressors) acting on land plants are listed in Table 1 under the grouping of natural stress factors (I) and anthropogenic stress factors (II). One can also list the various kinds of stressors under biotic and abiotic stress factors which is as valid as the grouping given in Table 1. With respect to the new large scale tree and forest decline detected in 1983 in the Northern hemisphere (Europe, USA, Russia, China) (Lichtenthaler and Buschmann, 1984; Rennenberg et al., 1996; Wellburn, 1994) and which is still progressing, it was desired to contrast the

**Table 1:** List of natural and anthropogenic stress factors acting on terrestrial vegetation.

**I. Natural stress factors:**
- **high irradiance** (photoinhibition, photooxidation),
- **heat** (increased temperature),
- **low temperatures** (chilling),
- **sudden and late frost,**
- **water shortage** (desiccation problems),
- **natural mineral deficiency** (e.g. nitrogen shortage),
- **long rainy periods,**
- **insects,**
- **viral, fungal and bacterial pathogens.**

**II. Anthropogenic stress factors:**
- **herbicides, pesticides, fungicides,**
- **air pollutants,** e.g. $SO_2$, NO, $NO_2$, NOx,
- **ozone** ($O_3$) and **photochemical smog,**
- **formation of highly reactive oxygen species** ($^1O_2$, radicals $O_2^{\cdot-}$ and $OH^{\cdot}$, $H_2O_2$)
- **photooxidants** (e.g. peroxyacylnitrates),
- **acid rain, acid fog, acid morning dew,**
- **acid pH** of soil and water,
- **mineral deficiency** of the soil, often induced by acid rain (shortage of the basic cations K, Mg, Ca, often Mn and sometimes Zn),
- **over-supply of nitrogen** (dry and wet $NO_3$-deposits),
- **heavy metal load** (lead, cadmium, etc.),
- **overproduction of $NH_4^+$** in breeding stations (uncoupling of electron transport),
- **increased UV-radiation** (UV-B and UV-A),
- **increased $CO_2$ level and global climate change.**

potential anthropogenic stress factors (most of which showed up only in the past 40 years) against the many natural abiotic and biotic stress factors to which the trees had been exposed to for a very long time.

One has to consider that the stressors listed in Table 1 rarely act individually and separately on the plant. Usually, several stress factors act simultaneously on the plant, such as the frequently combined heat, water and high-light stress at dry, sunny and warm summer periods. In addition, on plants there often act primary stressors or stress events, which considerably reduce the plants' vitality, such as air pollution followed by secondary stressors, such as bark beetles or particular fungi, which further lower the plant's vitality and will eventually lead to the dying-off of the tree.

### *Light adaptation and stress tolerance*

Plants can adapt their leaf morphology as well as the structure and function of their photosynthetic apparatus to the incident light intensity. This adaptation response is best visualized in the formation of sun and shade leaves of trees which possess not only a different morphology and chemical composition, but also different rates of photosynthesis (Lichtenthaler, 1984). High-light plants and sun leaves exhibit a smaller leaf area (to reduce the transpiration rate) and are thicker (e.g. longer palisade parenchyma cells, or even two rows of palisade cells as in beech) than shade leaves or leaves of low-light plants. They possess sun-type (high-light) chloroplasts with higher rates of photosynthetic quantum conversion and net $CO_2$ assimilation, and also a higher light compensation point and a higher light saturation point of the overall photosynthetic process (Lichtenthaler et al., 1981; Lichtenthaler and Meier, 1984; Meier and Lichtenthaler, 1981). High-light or sun-type chloroplasts possess much lower amounts of the light-harvesting chlorophyll *a/b* proteins, the LHCPs, a lower degree of stacking of thylakoids, less thylakoids per chloroplast, but more photosynthetic electron transport chains and photosynthetic reaction centers per total chlorophyll compared to low-light or shade-type chloroplasts. The latter, in turn, exhibit much higher and wider grana stacks and have invested in a large light-harvesting antenna to overcome the light shortage at their shade or low-light location (Lichtenthaler and Meier, 1984; Meier and Lichtenthaler, 1981).

The sun-type (high-light) or shade-type (low-light) modification of leaves and chloroplasts is a true adaptation response of plants. The sun and shade leaf modification can only be expressed during leaf growth, but the adaptation of chloroplast ultrastructure and photosynthetic function to high-light (sun) or low-light (shade) growth conditions is possible throughout the vegetation period, and takes about one week in order to fully convert shade-chloroplasts into sun-chloroplasts *or vice versa.* These adaptations to either high or low irradiance make sense from a physiological point of view. High-light plants and sun leaves are better adapted to high-light exposure than low-light plants and shade leaves. Sun leaves with an extreme full sun light exposure can reduce their LHCPs to very low amounts in order to avoid absorption of excess light which cannot be used in photosynthetic quantum conversion.

In other words, high-light plants and sun leaves are much better protected against high-light stress than low-light plants or shade leaves. By a thicker cuticula, more flavonols in their epidermis, etc. they are also better protected against UV-A and UV-B stress or damage as is being shown via fluorescence excitation spectra (Schweiger et al., 1996). This indicates that a high-light adaptation of leaves and chloroplasts is also associated with a higher stress tolerance. The light adaptation capacity of plants is genetically fixed. Many plants are so-called «low-light plants» which grow in the shade of others or in locations with low irradiance. Their adaptation capacity is relatively low, and they cannot grow or do not survive at full sun light (plant group 1 in Fig. 4 A). Other plants, e.g. most of our crop plants, are light plants, which need a high irradiance to yield a reasonable growth and grain yield, but their adaptation capacity is also fairly narrow (plant group 3 in Fig. 4 A). In addition, there exist plants possessing a very wide adaptation range, such as beech (plant group 2) with its sun and shade leaves and their extreme modification capacity of chloroplast ultrastructure and function.

The wider the range of the adaptation capacity of the plant the better they are protected against various stress factors. The «light plants» (group 3 in Fig. 4 A) will be under stress when the irradiance falls below their genetically possible adaptation range. Low light plants (group 1 in Fig. 4 A), in turn, are under stress when the irradiance of their location exceeds their light adaptation capacity. However, plants with a wide range of adaptation capacity (group 2) can respond very flexibly to changes in irradiance, and are thus much better protected against high light stress and photoinhibition. This basic principle of adaptation capacity range and relative stress

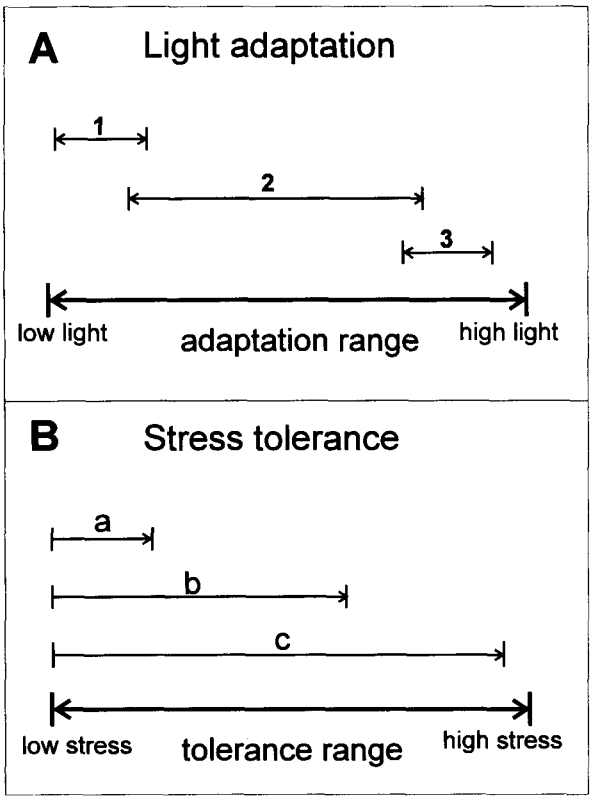


**Fig. 4:** Light adaptation and stress tolerance range of plants. **A)** The adaptation capacity of leaves and chloroplasts to high-light or low-light growth conditions is low for the plant groups 1 and 3, and high for the plant group 2. **B)** The plants a, b and c possess a low, medium or high stress tolerance, respectively.

tolerance exposed here for light adaptation also applies to all the other adaptation responses plants may possess.

Like the adaptation capacity of plants, also the tolerance capacity of plants is genetically fixed. Some plants possess a low, medium or high stress tolerance as shown in Fig. 4 B. Although light adaptation processes are one essential factor of a relative stress tolerance of a plant, there exist many more factors which determine the overall stress tolerance of plants, such as stress-coping mechanisms and the capacity of repair processes. Flexibility of cell metabolism and its fast acclimation to changes in environmental conditions as found e.g. in the books by Alscher and Wellburn (1994) and Smirnoff (1995), is a first essential step in stress avoidance. In plants with a low stress tolerance the capacity of the different stress-coping mechanisms is very low, and some of the mechanisms may not exist at all. Thus, such plants reach very fast an acute stage of damage, since their stress resistance minimum has fallen short already at a low stress threshold.

*Stress coping mechanisms*

There exist many stress-coping mechanisms which show up depending on the type and strength of stress, such as proline accumulation during drought and salinity, polyol accumulation (e.g. mannitol, sorbitol) at water stress conditions, formation of heat shock proteins, formation of radical scavenging compounds (ascorbate, glutathione, α-tocopherol), increase of the level of superoxide dismutase, formation of UV-A and UV-B absorbing pigments in the epidermis layer which protect the photosynthetic apparatus in the leaf mesophyll against damaging UV-radiation (Schweiger et al., 1995), or within the thylakoids the fast photoreduction (within minutes) of the carotenoid violaxanthin to zeaxanthin functioning at high-light conditions in the photoprotection of the photosynthetic apparatus (Lichtenthaler and Schindler, 1992; Schindler, Demming and Adams II, 1993; Schindler and Lichtenthaler, 1994). Those plants that are particularly tolerant to photoinhibition, such as the tobacco aurea mutant Su/su, even double their zeaxanthin amounts by *de novo* biosynthesis within a 5 h high light exposure (Schindler and Lichtenthaler, 1994). The exact mechanism of the photoprotective action of zeaxanthin is not yet known, it has, however, in some cases an indirect influence on the quenching of chlorophyll fluorescence. But many chlorophyll fluorescence quenching processes proceed independently of zeaxanthin. A non-enzymic oxidation of zeaxanthin to violaxanthin by either the highly reactive oxygen species ($^1O_2$, $O_2^{\cdot -}$, $OH^{\cdot}$) formed at high-light conditions and/or by detoxifying epoxy groups being formed at double bounds of thylakoid lipids has also been proposed a possible photoprotection mechanism of zeaxanthin (Lichtenthaler and Schindler, 1992; Schindler and Lichtenthaler, 1996).

One essential mechanism in the preservation of reasonable, though reduced photosynthetic rates at an excess of high-light, is the partial inactivation of photosystem II centers by photoinhibition (destruction of the D1-protein) (Godde et al., 1996; Krause and Weis, 1991; Schindler and Lichtenthaler, 1994), a process which protects the remaining photosystem II centers from photodestruction. Partial photoinhibition thus guarantees to still maintain sufficient photosynthetic net $CO_2$ assimilation rates at high-light conditions in order to allow plant growth and development.

*Stress detection by chlorophyll fluorescence*

Stress conditions and stress-induced damage in plants can be detected using the classical ecophysiological methods of measuring the rates of photosynthesis, respiration and transpiration, the stomata conductance and water potential, as well as content and ratios of the photosynthetic pigments (chlorophylls and carotenoids) or the concentration of stress metabolites. Most stress factors, even if they do not directly affect the composition of the photosynthetic apparatus or its functions, will affect the photosynthetic process in the long run. At physiological conditions about 80 to 90 % of the absorbed light energy will be dissipated from excited chlorophyll *a* (Chl*) via photosynthetic quantum conversion, whereas de-excitation by heat emission (ca. 5 to 15 %) and the red + far-red chlorophyll fluorescence (0.5 to 2 %) are much lower (Fig. 5 A). Under stress, the photosynthetic quantum conversion declines, however, and correspondingly heat emission and chlorophyll fluorescence increase considerably (Fig. 5 B).

Thus, stress-induced changes in the photosynthetic quantum conversion as well as damages to the photosynthetic apparatus can easily be detected via the non-invasive method of

measuring the chlorophyll fluorescence induction kinetics (Kautsky effect), or particular chlorophyll fluorescence ratios (Rfd-values as vitality index, ratio F690/F735, Fo/Fm, Fv/Fo) (Lichtenthaler, 1988, 1990; Lichtenthaler and Rinderle, 1988) as well as different quenching coefficients such as qP and qN (Schreiber et al., 1986; Krause and Weis, 1991). Kautsky and Hirsch (1931) already described the inverse relationship between photosynthetic quantum conversion and chlorophyll fluorescence (see review Lichtenthaler, 1992). The chlorophyll fluorescence shows maxima in the red region near 685 to 690 nm and the far-red region near 730 to 740 nm (Fig. 6). The Rfd-values are best measured at both regions (Rfd 690 and Rfd 730), in which case the values and amplitude changes induced by stress are higher in the 690 nm than in the 740 nm region. From both Rfd-values one can also determine the stress adaptation index (Lichtenthaler and Rinderle, 1988), which provides information on the stress exposure and the strain on the photosynthetic apparatus. The height of the Rfd-values is a measure of the potential photosynthetic capacity of a leaf and is correlated to the photosynthetic net $CO_2$-assimilation (Tuba et al., 1994; Babani et al., 1996).

From the chlorophyll fluorescence induction kinetics one is able to directly determine the ratio of the chlorophyll fluorescence emission in the red (F690) and far-red fluorescence maximum (F740) and this ratio F690/F740 is an indicator of the *in vivo* chlorophyll content (Hák et al., 1990; Lichtenthaler et al., 1990; D'Ambrosio et al., 1992). Although the various kinds of chlorophyll fluorescence measurements provide valuable information on the state of health or stress exposure of the photosynthetic apparatus, they have an essential disadvantage. It is a fact that the fluorescence data can be collected only from one leaf point per measurement, and one needs to determine the fluorescence signals of various leaf points in order to obtain an approximate realistic picture of the functional state or damage of the photosynthetic apparatus and its function.

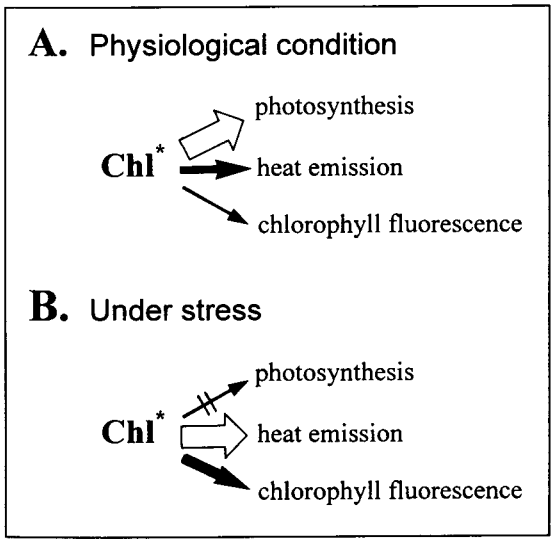


**Fig. 5:** De-excitation of the excited states of chlorophyll *a* by photosynthetic quantum conversion (photosynthesis), heat emission and red and far-red chlorophyll *a* fluorescence **A)** under physiological and **B)** under stress conditions. The thickness of the arrow indicates the relative proportions of the three de-excitaton processes.

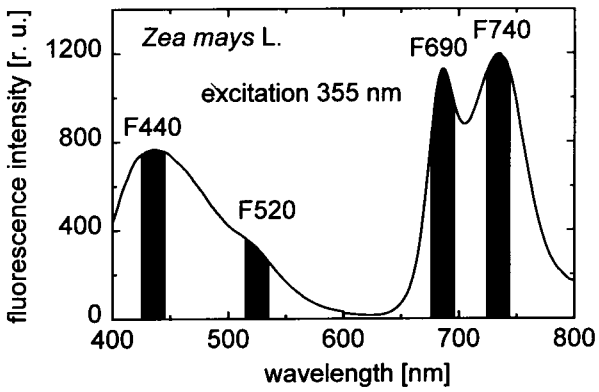


**Fig. 6:** UV-laser induced fluorescence emission spectrum of a plant leaf with maxima or shoulders in the blue (F440), green (F520), red (F690) and far-red (F740) spectral region. The four fluorescence bands applied in fluorescence imaging and stress detection of plants are indicated.

*Stress detection by fluorescence imaging*

In order to overcome this problem we developed, in a close cooperation with physicists, the first high resolution laser-induced fluorescence imaging system (LIF imaging method), which allows to simultaneously image the chlorophyll fluorescence signatures F690 and F740 at all points of a leaf (Lang et al., 1994, 1996; Lichtenthaler et al., 1995, 1996). Since plants also possess a blue (F440) and green fluorescence emission (F520), when excited with UV-A radiation (Fig. 6), we included the blue and green fluorescence in the fluorescence imaging of leaves. In contrast to chlorophyll fluorescence, the blue and green fluorescences do not show any variable but are constant during the light-induced fluorescence induction kinetics (Stober and Lichtenthaler, 1993 a). The blue and green fluorescence is primarily emitted from diverse plant phenolics (e.g. hydroxy cinnamic acids, flavonols) of the plant epidermis cell walls (Lang et al., 1991; Lichtenthaler et al., 1992; Stober and Lichtenthaler, 1993 b, Stober et al., 1994). The blue-green fluorescence emission is not evenly distributed over the whole leaf surface but is particularly high in the leaf vein regions (Lang et al., 1994; Lichtenthaler, 1996). The red + far-red chlorophyll flurescence emission, in turn, is low in the leaf vein region and high in the intercostal, vein-free leaf regions. This can also be seen in the fluorescence images of a variegated leaf of *Codiaeum* where the chlorophyll is unevenly distributed over the leaf surface (Fig. 7).

Small local differences in bluegreen and red + far-red fluorescence emission as well as fluorescence gradients over the leaf surface can easily be sensed via the high resolution fluorescence imaging system. Fluorescence imaging in the four fluorescence bands sets a new standard in the detection of stress effects in plants. We found that the fluorescence ratios blue/red (F440/F690), blue/far-red (F440/F740) and red/far-red (F690/F740) are very sensitive to any changes in envi-

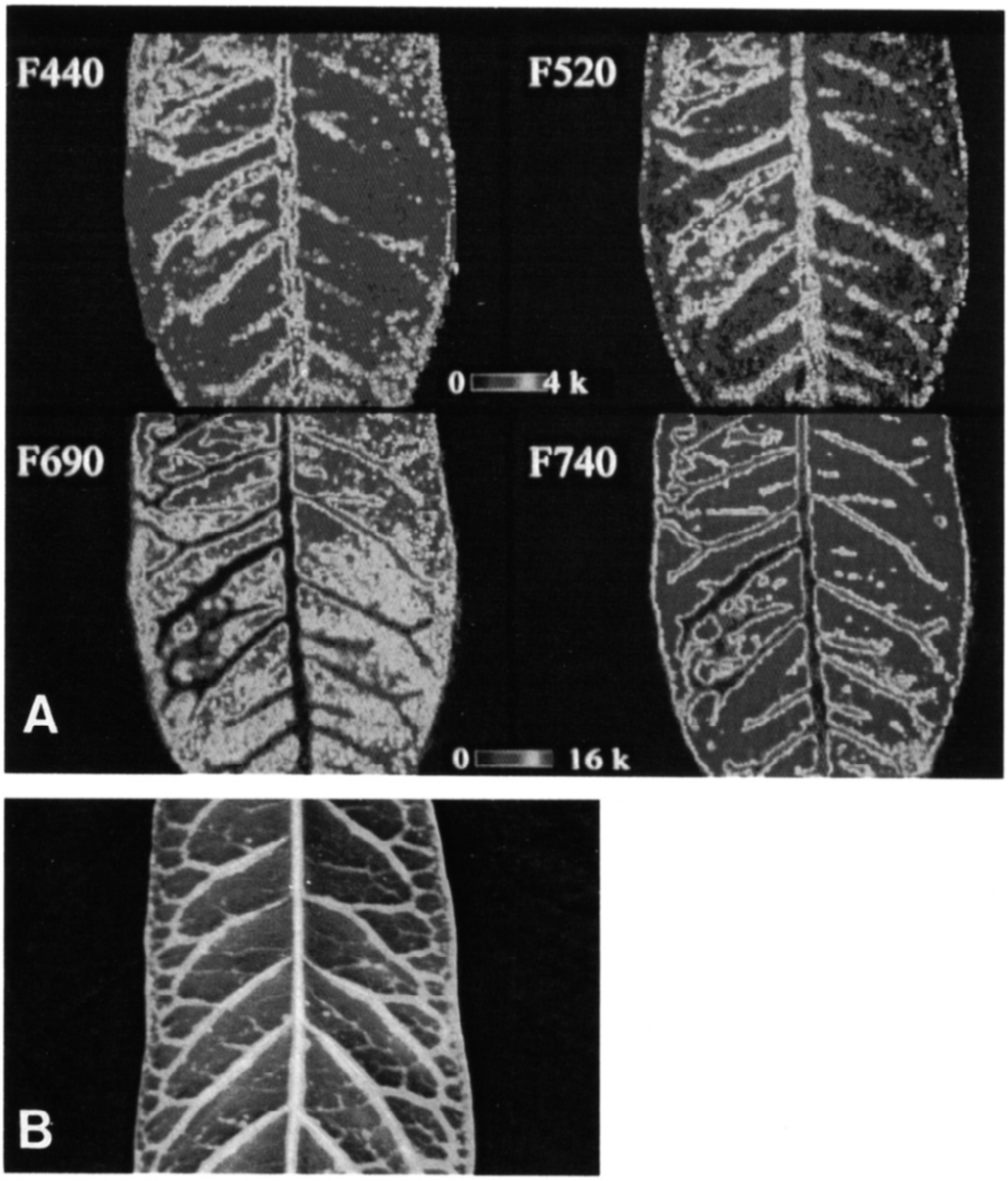


**Fig. 7: A.** False colour fluorescence images of the blue (F440), green (F520), red (F690) and far-red (F740) fluorescence emission in a young green leaf of *Codiaeum variegatum* L. Fluorescence intensities increase from dark blue via green, yellow to red (see color scale in the figure). **B.** Color photograph of a leaf of *Codiaeum.*

ronment and growth conditions of plants. These ratios are thus early indicators of stress and strain-induced changes in the photosynthetic function of leaves (Lang et al., 1996; Lichtenthaler et al., 1996). Laser-induced fluorescence imaging thus provides a very early and much better and precise stress and damage diagnosis than the point measurements of chlorophyll fluorescence applied so far. This new LIF method can thus be recommended to all those interested in stress detection in plants.

*Photon energy flow in plant leaves*

Plant stress modifies in multiple ways the energy flow of photons (sun light) through the leaf with the result that the absorption, reflectance and transmittance properties of leaves are changed. Stress also changes the relative proportions of absorbed ligth energy, which are used for photosynthetic quantum conversion, chlorophyll fluorescence, blue-green fluorescence or heat emission as is shown in Fig. 8. This is why red + far-red chlorophyll fluorescence and blue-green fluorescence kinetics and images can successfully be applied in stress detection of plants.

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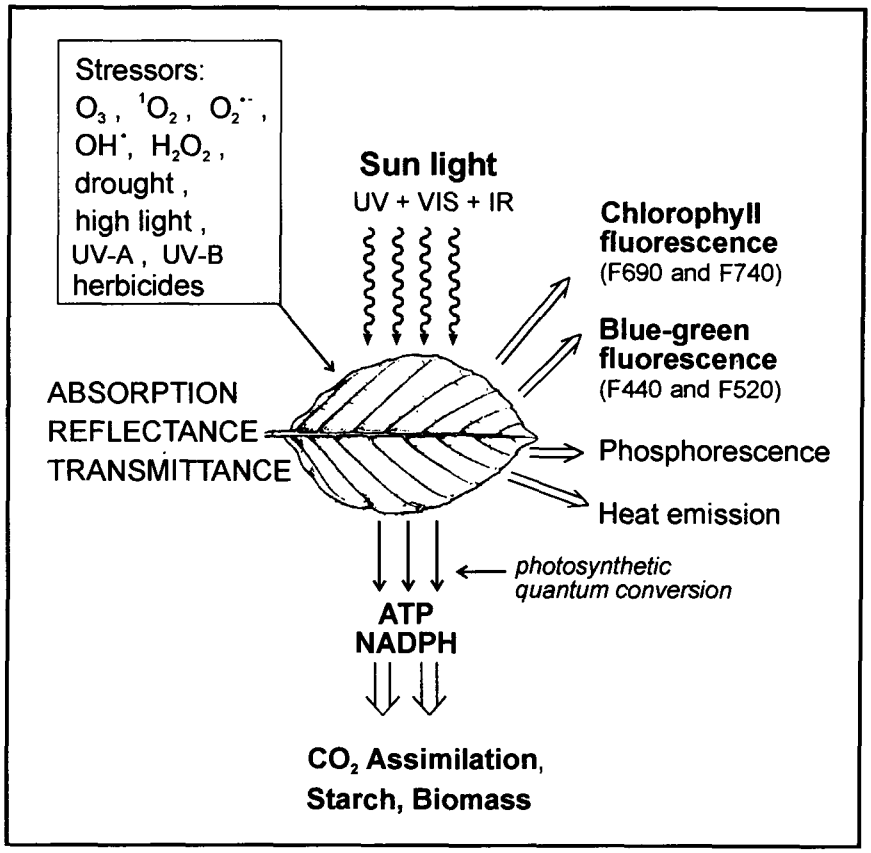


Scheme of photon energy flow in plant leaves

**Fig. 8:** Scheme of photon energy flow and dissipation in plant leaves which is modified (either blocked or enhanced) by a multitude of natural and anthropogenic stressors, such as highly reactive oxygen species, herbicides, UV-A and UV-B radiation or high-light stress or drought. A stress-induced decline in leaf physiology and photosynthetic quantum conversion can be monitored by non-invasive measurements of the red + far-red chlorophyll fluorescence and the blue-green fluorescence.

## References


Alscher, R. G. and J. R. Cumming (eds.): Stress Responses in Plants: Adaptation and Acclimation as Mechanism. J. Wiley-Liss, New York, 1990.

Alscher, R. G. and A. R. Wellburn: Plant Responses to the Gaseous Environment. Chapman & Hall, London, 1994.

Babani, F., P. Richter, and H. K. Lichtenthaler: Changes in different chlorophyll fluorescence signatures during greening of etiolated barley seedlings as measured with the CCD-OMA fluorometer. J. Plant Physiol. *148*, 471–477 (1996).

Demming-Adams, B. and W. W. Adams III: Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. *43*, 599–626 (1993).

Devine, M. D., S. O. Duke, and C. Fedtke: Biochemistry and Physiology of Herbicide Action, pp. 95–112. Springer Verlag, Berlin, 1993.

Fowden, L., T. Mansfield, and J. Stoddart: Plant Adaptation to Environmental Stress. Chapman & Hall, London, 1993.

Konopka, C., R. Hollinderbäumer, V. Elbert, H. Wietoska, and D. Godde: Imbalances of D1 protein turnover during stress induced chlorosis of a declining spruce tree. J. Plant Physiol. *148* (1996).

Hák, R., H. K. Lichtenthaler, and U. Rinderle: Decrease of the fluorescence ratio F690/F730 during greening and development of leaves. Radiat. Environ. Biophys. *29*, 329–336 (1990).

Hock, B., C. Fedtke, and R. R. Schmidt: Herbizide. G. Thieme Verlag, Stuttgart, 1995.

Jackson, M. B. and C. Black: Interacting Stresses on Plants in a Changing Climate. NATO Advanced Research Workshop, Springer Verlag, Berlin, 1993.

Kautsky, H. and A. Hirsch: Neue Versuche zur Kohlensäureassimilation. Naturwiss. *19*, 964 (1931).

Krause, G. H. and E. Weis: Chlorophyll fluorescence and photosynthesis: The basics. Annu. Rev. Plant Physiol. Plant Mol. Biol. *42*, 313–349 (1991).

Lang, M., F. Stober, and H. K. Lichtenthaler: Fluorescence emission spectra of plant leaves and plant constituents. Radiat. Environ. Biophys. *30*, 333–347 (1991).

Lang, M., H. K. Lichtenthaler, M. Sowinska, P. Summ, and F. Heisel: Blue, green and red fluorescence signatures and images of tobacco leaves. Bot. Acta *107*, 230–236 (1994).

Lang, M., H. K. Lichtenthaler, M. Sowinska, F. Heisel, H. A. Miehe, and F. Tomasini: Fluorescence imaging of water and temperature stress in plant leaves. J. Plant Physiol. *148*, 613–621 (1996).

Larcher, W.: Streß bei Pflanzen. Naturwissenschaften *74*, 158–167 (1987).

Levitt, J.: Responses of Plants to Environmental Stresses. Vol. 1, Academic Press, New York, 1980.

Lichtenthaler, H. K.: Differences in morphology and chemical composition of leaves grown at different light intensities and qualitites. In: Baker, N. R., W. J. Davies, and K. C. Ong (eds.): Control of Leaf Growth, pp. 201–222. Cambridge University Press, Cambridge, 1984.

– *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. In: LICHTENTHALER, H. K. (ed.): Applications of Chlorophyll Fluorescence, pp. 129–142. Kluwer Academic Publishers, Dordrecht, 1988.
– Applications of chlorophyll fluorescence in stress physiology and Remote sensing. In: STEVEN, M. and J. A. CLARK (eds.): Applications of Remote Sensing in Agriculture, pp. 287–305. Butterworths Scientific Ltd., London, 1990.
– The Kautsky Effect: 60 years of chlorophyll fluorescenceinduction kinetics. Photosynthetica *27,* 45–55 (1992).

LICHTENTHALER, H. K. and D. MEIER: Regulation of chloroplast photomorphogenesis by light intensity and light quality. In: ELLIS, H. (ed.): Chloroplast Biogenesis, pp. 261–281. Cambridge University Press, Cambridge, 1984.

LICHTENTHALER, H. K. and C. BUSCHMANN: Das Waldsterben aus botanischer Sicht. G. Braun Verlag, Karlsruhe, 1984.

LICHTENTHALER, H. K. and U. RINDERLE: The role of chlorophyll fluorescence in the detection of stress conditions in plants. CRC Critical Reviews in Analytical Chemistry *19,* Suppl. I, 29–85 (1988).

LICHTENTHALER, H. K. and C. SCHINDLER: Studies on the photoprotective function of zeaxanthin at high-light conditions. In: MURATA, N. (ed.): Research in Photosynthesis, Vol. IV, pp. 517–520. Kluwer Academic Publishers, Dordrecht, 1992.

LICHTENTHALER, H. K., C. BUSCHMANN, M. DÖLL, H.-J. FIETZ, T. BACH, U. KOZEL, D. MEIER, and U. RAHMSDORF: Photosynthetic activity, chloroplast ultrastructure, and leaf characteristics of high-light and low-light plants and of sun and shade leaves. Photosynthesis Research *2,* 115–141 (1981).

LICHTENTHALER, H. K., C. BUSCHMANN, U. RINDERLE, and G. SCHMUCK: Applications of chlorophyll fluorescence in ecophysiology. Radiat. Environ. Biophys. *25,* 297–308 (1986).

LICHTENTHALER, H. K., R. HÁK, and U. RINDERLE: The chlorohyll fluorescence ratio F690/F730 in leaves of different chlorophyll content. Photosynth. Res. *25,* 295–298 (1990).

LICHTENTHALER, H. K., M. LANG, F. STOBER, C. SCHINDLER, H. EDNER, H. JOHANSSON, S. SVANBERG, and L. O. BJÖRN: Remote multi-colour fluorescence imaging of selected broad-leaf plants. EARSeL Advances in Remote Sensing 3 (Part 3), 2–14 (1995).

LICHTENTHALER, H. K., M. LANG, M. SOWINSKA, F. HEISEL, and J. A. MIEHE: Detection of vegetation stress via a new high resolution fluorescence imaging system. J. Plant Physiol. *106,* 1127–133 (1996).

MCKERSIE, B. D. and Y. Y. LESHEM: Stress and Stress Coping in Cultivated Plants, pp. 1–256. Kluwer Academic Publishers, Dordrecht, 1994.

MEIER, D. and H. K. LICHTENTHALER: Ultrastructural development of chloroplasts in radish seedlings grown at high and low light conditions and in the presence of the herbicide bentazon. Protoplasma *107,* 195–207 (1981).

RENNENBERG, H., C. HERSCHBACH, and A. POLLE: Consequences of air pollution on shoot-root interactions. J. Plant Physiol. *148,* 537–547 (1996).

SCHINDLER, C. and H. K. LICHTENTHALER: Is there a correlation between light-induced zeaxanthin accumulation and quenching of variable chlorophyll *a* fluorescence? Plant Physiol. Biochem. *32,* 813–823 (1994).

SCHINDLER, C. and H. K. LICHTENTHALER: Photosynthetic $CO_2$-assimilation, chlorophyll fluorescence and zeaxanthin accumulation in fied grown maple trees in the course of a sunny and a cloudy day. J. Plant Physiol. *148,* in press (1996).

SCHREIBER, U., U. SCHLIWA, and W. BILGER: Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. *10,* 51–62 (1986).

SCHWEIGER, J., M. LANG, and H. K. LICHTENTHALER: Differences in fluorescence excitation spectra of leaves between stressed and non-stressed plants. J. Plant Physiol. *148,* 537–547 (1996).

SELYE, H.: A syndrome produced by various nocuous agents. Nature *138,* 32–34 (1936).
– The stress of Life. McGraw Hill, New York, 1956.

SIEDOW, J. N.: Public affairs. ASPP Newsletter (American Soc. Plant Physiologists) *22,* No. 2, 6–9 (1995).

SMIRNOFF, N.: Environment and Plant Metabolism: Flexibility and Acclimation. BIOS Scientific Publishers Ltd., Oxford, 1995.

STOBER, F. and H. K. LICHTENTHALER: Studies on the constancy of the blue and green fluorescence yield during the chlorophyll fluorescence induction kinetics (Kautsky effect). Radiat. Environ. Biophys. *32,* 357–365 (1993 a).

STOBER, F. and H. K. LICHTENTHALER: Studies on the localisation and spectral characteristics of the fluorescence emission of differently pigmented wheat leaves. Bot. Acta *106,* 365–370 (1993 b).

STOBER, F., M. LANG, and H. K. LICHTENTHALER: Blue, green and red fluorescence emission signatures of green, etiolated, and white leaves. Remote Sens. Environ. *47,* 65–71 (1994).

STOCKER, O.: Probleme der pflanzlichen Dürreresistenz. Naturwissenschaften *34,* 362–371 (1947).
– Transpiration und Wasserhaushalt in verschiedenen Klimazonen. I. Untersuchungen an der arktischen Baumgrenze in Schwedisch-Lappland. Jahrb. wiss. Botanik *75,* 494 (1932).

TUBA, Z., H. K. LICHTENTHALER, Z. CZINTALAN, Z. NAGY, and K. SZENTE: Reconstitution of chlorophylls and photosynthetic $CO_2$ assimilation in the desiccated poikilochlorophyllous plant *Xerophyta scabrida* upon rehydration. Planta *192,* 414–420 (1994).

WELLBURN, A.: Air Pollution and Climate change: The Biological Impact. 2nd edition. Longman Scientific & Technical, New York, 1994.