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Stress Tolerance and Stress-Induced Injury in Crop Plants Measured by Chlorophyll Fluorescence *In Vivo*[1]

CHILLING, FREEZING, ICE COVER, HEAT, AND HIGH LIGHT

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ABSTRACT

The proposition is examined that measurements of chlorophyll fluorescence *in vivo* can be used to monitor cellular injury caused by environmental stresses rapidly and nondestructively and to determine the relative stress tolerances of different species. Stress responses of leaf tissue were measured by F_R, the maximal rate of the induced rise in chlorophyll fluorescence. The time taken for F_R to decrease by 50% in leaves at 0°C was used as a measure of chilling tolerance. This value was 4.3 hours for chilling-sensitive cucumber. In contrast, F_R decreased very slowly in cucumber leaves at 10°C or in chilling-tolerant cabbage leaves at 0°C. Long-term changes in F_R of barley, wheat, and rye leaves kept at 0°C were different in frost-hardened and unhardened material and in the latter appeared to be correlated to plant frost tolerance. To simulate damage caused by a thick ice cover, wheat leaves were placed at 0°C under N_2. Kharkov wheat, a variety tolerant of ice encapsulation, showed a slower decrease in F_R than Gatcher, a spring wheat. Relative heat tolerance was also indicated by the decrease in F_R in heated leaves while changes *in vivo* resulting from photoinhibition, ultraviolet radiation, and photobleaching can also be measured.

Damage to crop plants and reductions in yields are often brought about by unfavorable environmental conditions; these include extremes of temperature, lack or surfeit of H_2O, high solar UV radiation, excess soil salinity, and the presence of toxic pollutants. Looking to the future, however, it seems that some environmental stresses will inevitably intensify, and this, coupled with such practices as utilizing land marginally suited to agriculture and growing desirable crop plants in climates for which the plants are ill adapted, *e.g.* maize in high latitudes (6) and potatoes in the semitropics (3), are likely to create an increasing need for new stress-tolerant varieties. Presently, the incidence of salt contamination of soil is increasing (10) and there also is concern that temperatures worldwide may rise because of increasing concentrations of CO_2 and methane in the atmosphere, and solar UV radiation reaching the earth may intensify (22) because of depletion of ozone in the stratosphere by fluorocarbons and nitric oxides.

To combat excess salinity which already affects one-third of the agricultural lands under irrigation, Epstein *et al.* (10) advocate the urgent development of salt-tolerant varieties. This approach, which is equally applicable to other major stresses, requires reliable screening methods to enable the selection of stress-tolerant plants. Similarly, suitable methods to follow the development of cellular damage are a prerequisite for many physiological investigations of stress-induced injuries to plants.

Although methods are generally available to define and measure the actual stress, quantifying the effect of stress on the plant is usually more difficult. Visible symptoms are often late manifestations of stress injury and are difficult to measure and while biochemical analyses may suffice in studies involving only a few samples, they are likely to be too time consuming to be used in screening applications. Ideally, a method for assessing the level of stress injury should be rapid, sensitive, and nondestructive to the tissue, and in searching for such a technique for use in physiological and plant breeding applications, the potential of Chl as a naturally occurring internal fluorescence probe sensitive to stress-induced cellular injury was investigated. Long used as a probe for photosynthesis, the Chl in the membranes of chloroplasts emits a red fluorescence, of which a part, the induced or variable Chl fluorescence, is responsive to changes in PSII activity (17). From this it follows that any stress applied to green plant tissue which directly or indirectly affects photosynthetic metabolism is likely to change the yield of this fluorescence. The yield is lowest when the immediate electron acceptors of PSII are able to extract energy from the active center, *i.e.* when the acceptors are in the oxidized state, for example, immediately upon illumination of dark-adapted photosynthetic tissue. Hence, an inhibition on the photooxidizing side of PSII will quench the yield of Chl fluorescence, whereas an inhibition on the photoreducing side of PSII will enhance it (17).

In this paper, the effects of chilling, freezing, anaerobiosis at 0°C, heat, and high light intensity stresses on Chl fluorescence kinetics are examined. Changes in Chl fluorescence in response to excess salinity are described in another paper (28). Where possible, plants with known and different tolerances towards each of the investigated environmental stresses were used as experimental material so as to evaluate the potential of the method. While the primary aim of these papers is to demonstrate that Chl fluorescence measurements can be used to detect and quantify rapidly plant response to major environmental stresses, the results at the same time provide information on mechanisms involved in the onset and development of the stress injury including the localization of sensitive sites in the photosynthetic electron transfer system.

MATERIALS AND METHODS

Chilling Stress. Bean (*Phaseolus vulgaris* L. cv Canadian Wonder) and cucumber (*Cucumis sativus* L. cv Palomar) were grown

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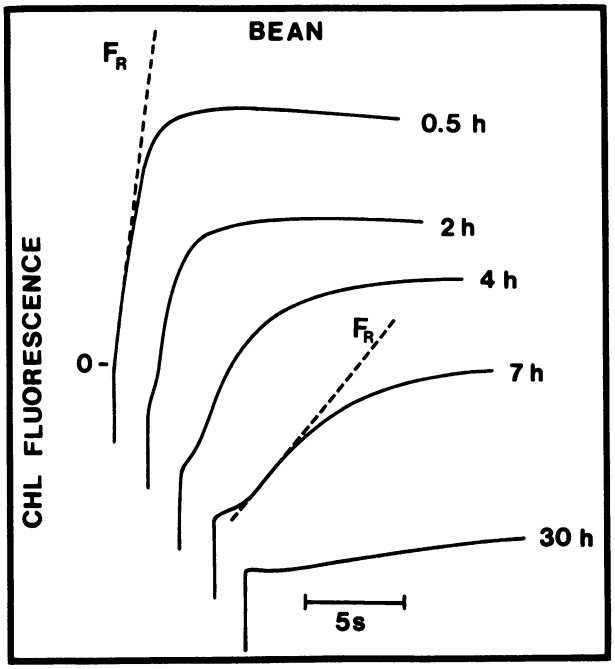

FIG. 1. Changes in the kinetics of Chl fluorescence of an expanded trifoliate leaflet of bean kept at 0°C in darkness. The first measurement of relative Chl fluorescence was made after allowing 30 min at 0°C for temperature equilibration of the leaflet. The measurement was repeated on the same area of the same leaflet at the times indicated on the figure. The results shown are typical of measurements made on several different fully expanded bean leaflets.

in pots in a glass-house (temperature controlled to 18°C minimum, 28°C maximum). Cabbage (*Brassica oleracea* L. cv *capitata*) was obtained at a market. Leaves of kumquat (*Fortunella japonica* (Thunb.) Swing.), mandarin (*Citrus reticulata* Blanco cv Allen 'Unshii'), grapefruit (*Citrus paradisi* Macf. cv Thompson), and Sebora lime (*Citrus aurantifolia* (Christm.) Swing.) were taken from mature trees growing in a citrus arboretum. All leaf material was dark adapted for 1 h before use.

To measure changes in Chl fluorescence at 0°C, leaves were cut and immediately placed, abaxial surface down, on moist filter paper spread on an aluminum plate (30 × 45 × 0.3 cm) and covered with a thin plastic film permeable to air but not to water. The leaves (up to 96 samples) and film were held in place with an over-laying sheet of solid plastic containing guide holes (3.3 cm diameter, one centered over each leaf hr) for the measuring probe of the fluorometer. The plate was placed on melting ice in a darkened insulated box and after temperature equilibration the induced Chl fluorescence was recorded for each leaf in turn and at intervals thereafter. Control plates were stored in darkness in a room maintained at 10°C. During measurements of Chl fluorescence, leaf temperature was maintained at either 0°C or 10°C (controls). The handling of dark-adapted material and the measurements of Chl fluorescence were done under dim green light.

Chl Fluorescence Measurements on Leaves of Spring and Winter Cereals Stored at 0°C. Cold-hardened and unhardened seedlings of spring barley (*Hordeum vulgare* L. cv Abyssinian) and wheat (*Triticum aestivum* L. cv Gatcher), and winter barley (cv Tennessee), wheat (cv Kharkov), and rye (*Secale cereale* L. cv Frontier) were used. Unhardened plants were grown for 18 d in a growth cabinet (25°C day, 19°C night; 12-h day at 170 μE m^{-2} s^{-1}, 400–700 nm). Plants to be cold-hardened were grown in the same way for 14 d, transferred to 5°C for 1 d, and then to 1°C for 18 d (12-h d at 50 μE m^{-2} s^{-1}, 400–700 nm). To measure the effect of a prolonged stress at 0°C on the leaves of these plants, the top half of the secondary leaf was excised and placed on moist filter paper on a metal plate. Sixteen seedlings, eight unhardened and eight cold-hardened, of each cultivar were used. The leaves were covered with thin plastic to prevent water loss and the metal plate embedded in melting ice contained in an insulated, darkened box. The box was stored in a cold room (1°C) and the induced Chl fluorescence rise recorded on each leaf periodically. Fixing a hard plastic sheet containing guide holes for the sensor of the fluorometer over the metal plate enabled successive measurements to be made on the same part of each leaf. Each value for F_R[2] given in the text is the mean of readings made on eight individual leaves.

Simulated Ice Cover. To approximate conditions under ice cover, cultivars of spring wheat (*T. aestivum* L. cv Gatcher) and winter wheat (cv Kharkov) were cold-hardened and leaves then detached and kept at 0°C under N_2. Seedlings were grown in pots containing a mixture of peat, vermiculite, and perlite (1:1:2, v:v:v) at 25°C/19°C (day/night) temperatures and a 12-h d at 170 μE m^{-2} s^{-1}, 400–700 nm. When 14 d old, the plants were then transferred to a room maintained at 1°C (12-h day at 50 μE m^{-2} s^{-1}, 400–700 nm) for 2 months. Leaves were then cut from the plants and placed on moist filter paper in an air-tight, dark chamber embedded in ice. The chamber was flushed with humidified N_2, precooled to 0°C, for 1 h/d, and then sealed. Chl fluorescence was recorded at intervals during storage of the leaves under N_2 at 0°C; successive measurements on each leaf were always made on the same area of the leaf. During readings of Chl fluorescence, air entered the chamber for about 10 min but leaf temperature was maintained at 0°C. The chamber was immediately flushed with N_2 after each reading. The values for F_R given in the text are the mean ± SE of readings made on secondary leaves taken from 12 individual plants of each cultivar.

Heat Stress. The following plants were used: pea (*Pisum sativum* L. cv Greenfeast), snow pea (*Pisum sativum* L.), pigeon pea (*Cajanus cajan* L.), wheat (*T. aestivum* L. cv Gatcher), peanut (*Arachis hypogaea* L.), pearl millet (*Penniserum typhoides* Stapf.), and papaya (*Carica papaya* L.). All plants, except those of papaya which were grown outside in garden soil, were grown for 30 d in pots containing a mixture of peat, vermiculite, and perlite (1:1:2, v:v:v) in a controlled environmental room (26°C, 21°C night, 12 h day at a photon flux of 300 μE m^{-2} s^{-1}). The most recent fully expanded leaf was harvested from each plant and the fluorescence measurements were always made on the center of the left-hand side of the leaf. Each value of F_R given is the mean of readings made on eight leaves, each from a different plant (eight leaves from two plants in the case of papaya). Two species of wild potato were also used. The plants were grown in the same plot in a field from tubers collected from plants of each species found growing naturally in the wild. *Solanum bukasovi* Juz. was found at an altitude of 3900 m in La Oroya, Junin, Peru, and *Solanum tarijense* Hawkes at 1950 m near Taripa, Bolivia. To measure heat tolerance, fully expanded leaves were arranged on an aluminum plate as described above in the section on chilling stress. After dark-adapting the leaves for 1 h, Chl fluorescence was recorded on each leaf (4 s duration at 15 μE m^{-2} s^{-1}). The plate was then immersed in a water bath at 44 ± 0.05°C (41 ± 0.05°C for potato) for 10 min, removed, and cooled by immersion in water at 22°C and, after drying the plate, the Chl fluorescence of each leaf was again recorded. In practice, leaves were precooled to 0°C for 20 min before measuring Chl fluorescence as this virtually eliminated the slow quenching of Chl fluorescence (see section below on chilling stress).

Chl Fluorescence Measurements. The kinetics of the induced

[2] Abbreviations: F_R, the maximal rate of the induced rise in Chl fluorescence (the I to P rise, in the terminology adopted by Papageorgiou [17]) upon exposing dark-adapted green plant tissue to light; LD_{50}, 50% inhibiting dose.

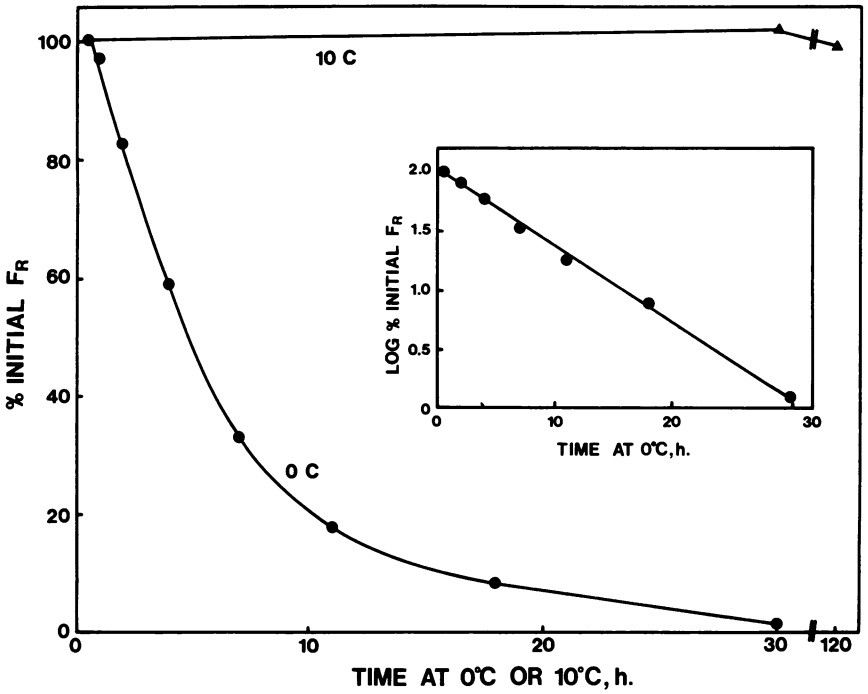

FIG. 2. Time course of changes in F_R in fully expanded leaves of cucumber kept at either 0°C or 10°C. The values for F_R are expressed as a percentage of the first reading (0.5 h on ice or at 10°C). The inset shows a logarithmic plot of the same values. Experimental conditions are described in the text.

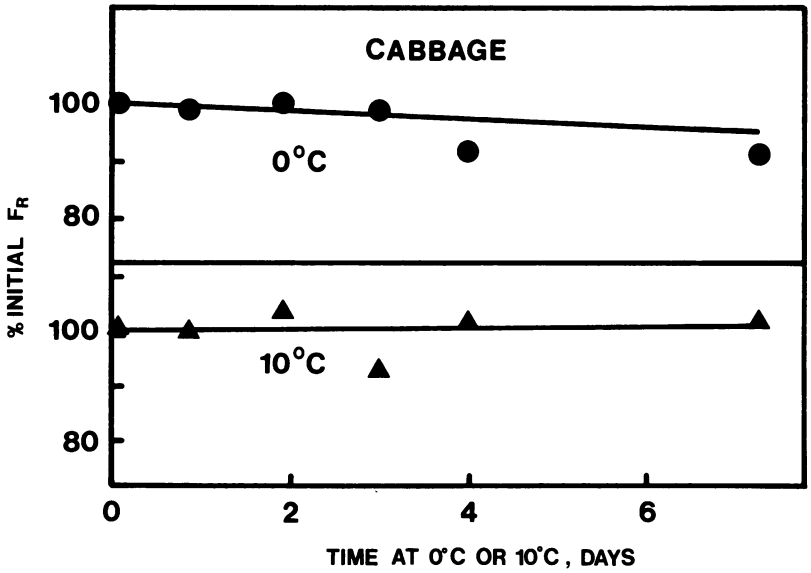

FIG. 3. Time course of changes in F_R in leaves of cabbage kept at either 0°C or 10°C. Experimental conditions are described in the text.

Chl fluorescence rise were recorded as previously described (11) using a portable fluorometer, model SF-10 (Richard Brancker Research Ltd., Ottawa). The measuring sensor of the fluorometer, which can be placed directly on the leaf surface, contains both a light-emitting diode to irradiate the leaf surface (red light, 11.2 $\mu E\ m^{-2}\ s^{-1}$ unless stated otherwise) and a photodiode to detect Chl fluorescence emission.

Photon fluence rates were measured with a LI-185A Quantum Meter (LI-COR, Lincoln, NE).

RESULTS AND DISCUSSION

Chilling Stress. Plants adapted to lowland tropical and semi-tropical climates are usually sensitive to chilling temperatures; growth rates decrease rapidly below about 20°C, growth ceases around 8 to 14°C, and still lower temperatures can result in permanent physiological disorders (chilling injury). Severe chilling injury produces visible symptoms; but, before these are evident, numerous changes in cell metabolism can be detected including altered physical properties of cell membranes, cessation of protoplasmic streaming, uncontrolled electrolyte movement across membranes, and anomolous respiratory activity (15). Although it is apparent from the literature that physiological changes resulting from chilling injury are both numerous and diverse, none appear to be suitable for rapid quantification of chilling injury, and screening methods to select for chilling tolerance are lacking (7).

Leaves of the chilling-sensitive plant bean rapidly lost Hill activity when stored at 0°C (16). Subsequently, it was shown that

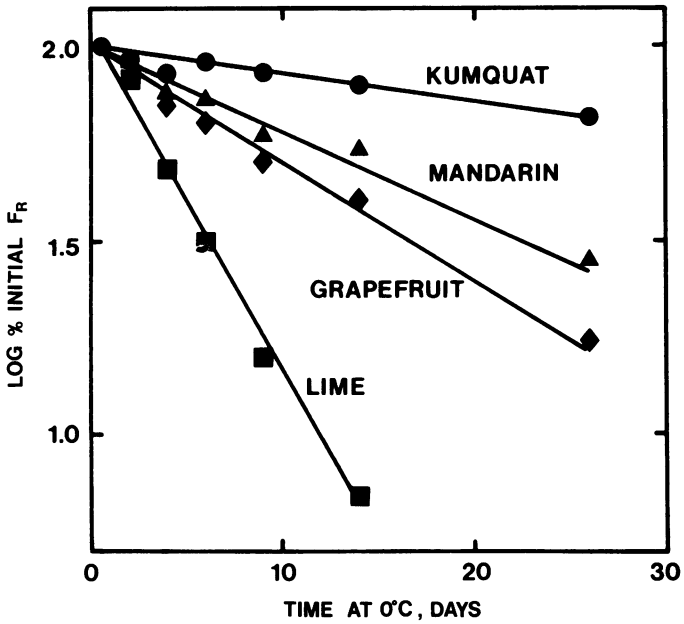

FIG. 4. Changes in log F_R during chilling at 0°C of fully expanded leaves of *Citrus* species. The values plotted for F_R are the logarithm of the mean, expressed as the percentage of the first reading, made on eight individual mature leaves. (●), Kumquat; (▲), mandarin; (◆), grapefruit; (■), Sebora lime.

the extent of the decrease in Hill activity in chloroplasts isolated from chilled leaves of different species of tomato (27) and passionfruit (23) is related to plant chilling tolerance. Inasmuch as the inhibition develops on the photooxidizing side of PSII (16, 27), which therefore results in a decrease in the yield of the induced Chl fluorescence (17), it should be feasible to use measurements of Chl fluorescence to follow the time course of chilling injury and determine the relative susceptibility of different species to chilling. Preliminary evidence supporting this suggestion has been published (23).

Figure 1 shows that the induced Chl fluorescence of bean leaves stored in darkness at 0°C was progressively lost. Upon irradiating a chilled but still undamaged leaf (0.5 h on ice), the Chl fluorescence rose quickly to the 0 level (17). The fluorescence then continued to rise and, after a small delay or 'dip' (seen best in the leaves stored at 0°C for 7 and 30 h), rose to a maximum. When measurements were made at room temperature on unchilled, dark-adapted leaves, Chl fluorescence subsequently declined slowly, but at 0°C this competing quenching of Chl fluorescence which arises mainly from the reoxidation of PSII acceptors was largely repressed. F_R, the maximal rate of the rise in Chl fluorescence after the delay or dip decreased with increasing time of chilling. F_R was easily determined from measurements made at 0°C, as the rate of the Chl fluorescence rise is almost linear for a considerable portion of the rise. This is not always obvious in all of the figures shown in this paper as the time scales were chosen to show other features of the induction curves. Where the primary aim was to determine F_R, an appropriate time scale was chosen (*e.g.* 1 s) and measurements were recorded using an oscilloscope or fast recorder. Similarly, when values of F_o were required, shorter time scales (100 ms or less; see Ref. 11) were employed. At 20°C and higher, the fluorescence rise may become more curved because of increasing competition from the slow quenching of Chl fluorescence and in some plant species measurements of changes in F_R resulting from other stresses, *e.g.* heat, may be better resolved by cooling the leaf material to 0°C before recording the Chl fluorescence rise.

In bean leaves stored at 0°C, the magnitude of the induced Chl fluorescence rise also showed a time-dependent decrease (Fig. 1); however, the extent to which this value declined was not a reliable guide of chilling tolerance. In some species in which F_R had been reduced to a very low value as the result of chilling injury to the leaves, with prolonged irradiation the induced Chl fluorescence continued to rise very slowly and reached almost the same level as recorded in unstressed control leaves. The decrease in both the induced Chl fluorescence yield and F_R can be attributed to the effects of the chilling temperature on the leaves as neither decreased in bean leaves kept at 10°C for up to 7 d. The F_o-Chl

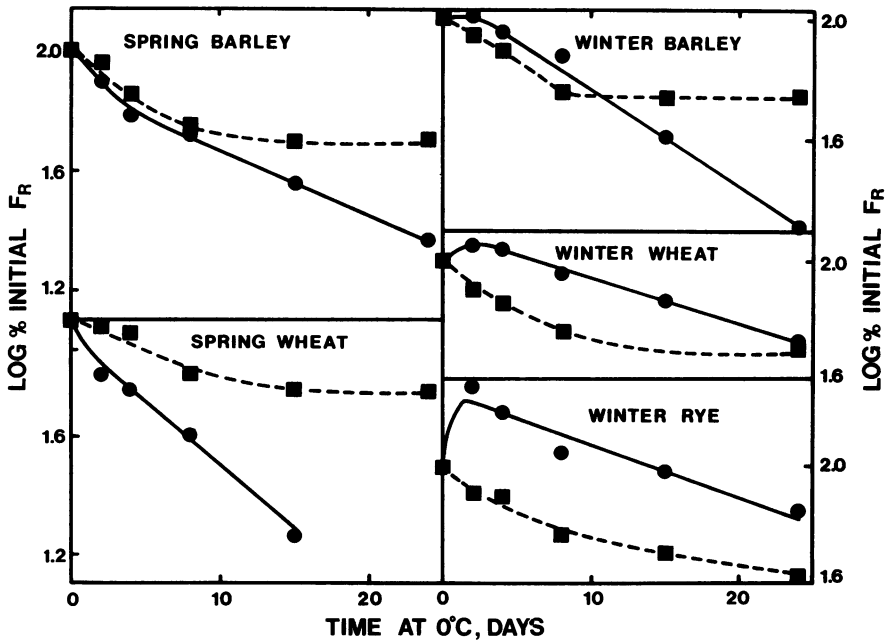

FIG. 5. Changes in log F_R of leaves kept at 0°C of Abyssinian spring barley, Gatcher spring wheat, Tennessee winter barley (LD_{50}: −10°C), Kharkov winter wheat (LD_{50}: −22°C), and Frontier winter rye (LD_{50}: −30°C). The experimental procedure is described in "Materials and Met hods." (●), Unhardened leaves; (■), cold-hardened leaves.

Table I. *Comparison of the Time to 50% Decrease in F_R with Freezing Tolerance*

Unhardened leaves of the five cereals were stored at 0°C and F_R determined at intervals as described in "Materials and Methods."

Cultivar	Type (and LD_{50})	Time to 50% decrease in F_R at 0°C
		d
Abyssinian	Spring barley	8 ± 0.5
Gatcher	Spring wheat	6 ± 0.4
Tennessee	Winter barley (−10°C)	12 ± 0.9
Kharkov	Winter wheat (−22°C)	26 ± 2.2
Frontier	Winter rye (−30°C)	31 ± 4.6

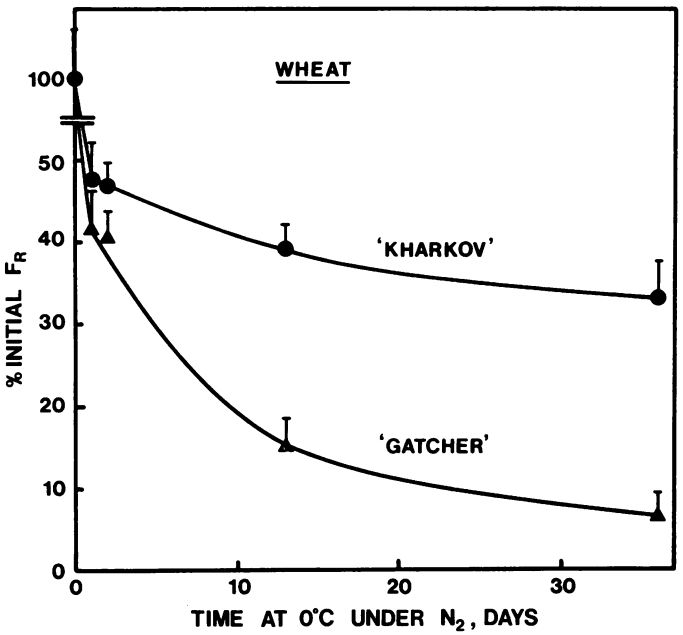

FIG. 6. The effect of chilling and anaerobic conditions on the Chl fluorescence of leaves of two cultivars of wheat, Kharkov and Gatcher. Leaves of cold-hardened plants were detached and kept at 0°C under N_2 and F_R (expressed as a percentage of the first reading) determined at various intervals. The vertical bars each indicate +SE. Details of the cold hardening and experimental procedure are given in "Materials and Methods."

Table II. *Relative Heat Tolerance as Estimated by the Heat-Induced Decrease in F_R*

The mean % F_R remaining after heating leaves (n = 8) at 44°C for 10 min was determined. Low values indicate low heat tolerance.

Plant	F_R Remaining after Heating
	%
Pea	7.3 ± 1.6
Snow pea	11.7 ± 1.8
Pigeon pea	22.0 ± 2.3
Wheat	26.8 ± 2.8
Peanut	33.6 ± 2.7
Pearl millet	68.0 ± 3.7
Papaya	80.6 ± 5.6

fluorescence was not changed in leaves stored at either 0°C or 10°C.

The kinetics of the chilling-induced decrease in F_R are shown in Figure 2 for another chilling-sensitive plant, cucumber. The decrease in F_R was exponential, the time for 50% decrease in F_R being 4.3 h. As with bean, there was no decrease in F_R in the leaves kept at 10°C. Hence the changes in Chl fluorescence at 0°C cannot be attributed to natural or detachment-induced senescence or to the way in which the leaves were stored and handled during the course of the experiment, as the effects of any of these on Chl fluorescence should have been greater at 10°C than at 0°C. The absence of any physiological effect of leaf detachment on chilling tolerance was also demonstrated in experiments with maize not reported in this paper. The time to 50% decrease in F_R of maize leaves chilled at 0°C was the same (about 8 h) whether the leaves were detached or left attached to the plants during chilling. In the experiment with cucumber (Fig. 2), leaf to leaf variability was avoided by confining the fluorescence measurements recorded during chilling to the same region of a single leaf held in place on a metal plate as described in "Materials and Methods." (The SE for measurements of F_R made on different cucumber leaves was ±5.3% where n = 16; all leaves showed a decline in F_R at 0°C, as shown in Fig. 2). A linear decrease in log F_R with time of chilling was found using several other chilling-sensitive plants including *Capsicum*, maize, peanut, and tomato. At 10°C, F_R declined slightly in the leaves of these plants, or not at all.

In contrast, there was little change in F_R in leaves of a chilling-tolerant plant, cabbage, kept at 0°C (Fig. 3). The experiment was carried out as described for cucumber. For measurements of F_R made on different leaves, the SE was ±7.4% where n = 16. Nor was there any significant decrease in F_R of cabbage leaves kept at 10°C for 7 d. Similar results were obtained using pea leaves.

A comparison of the results shown in Figures 2 and 3 show that by measuring Chl fluorescence changes in chilled leaves, plants susceptible to chilling injury can be distinguished from those which are not susceptible. Figure 4 illustrates that it is also possible to use this method to compare the chilling tolerances of closely related species. Citrus species are sufficiently alike that they can be hybridized with each other and, with the closely related kumquats, yet display a wide range of chilling tolerance. As a group they are much less sensitive than the other chilling-sensitive plants used in this study, such as bean and cucumber, and additionally show a moderate degree of frost resistance. For the four species shown in Figure 4, the order of decreasing frost tenderness is lime, grapefruit, mandarin, and kumquat (5). The same ranking was obtained for chilling tolerance as measured by changes in Chl fluorescence of leaves at 0°C. The most sensitive species, lime, showed a 50% decrease in F_R in 3.5 ± 0.4 d. This compared with 9.5 ± 1.2 d for grapefruit, 13 ± 1.2 d for mandarin, and >26 d for kumquat, which was quite chilling tolerant.

The results presented demonstrate the potential utility of measurements of Chl fluorescence *in vivo* for rapid, nondestructive estimations of chilling tolerance. The use of the Chl fluorescence parameter F_R means that the fluorescence rise of each leaf sample can be recorded within a few seconds and because the signals can be stored and processed by computer when a large number of leaves are to be sampled, the method appears to be suitable for development as a screening test for chilling tolerance for both plant physiological and plant breeding applications. The further development and use of the Chl fluorescence method to measure the chilling tolerances of different populations of maize has been described (13).

Freezing Stress. The freezing tolerance of crop plants is usually determined as the temperature at which 50% of the plants survive a freeze-thaw treatment, but independent methods to measure freezing tolerance and to follow the process of cold acclimation would be valuable. One experimental approach, the continuous monitoring of steady-state Chl fluorescence during temperature lowering, has been used to study frost tolerance in potato (29). As an alternative approach, changes in Chl fluorescence associated with cold acclimation are examined in this paper. During the

course of the work on chilling-sensitive plants, it was found that even leaves of many plants considered to be relatively chilling tolerant, including potato, blueberry, and barley (also the kumquat in Fig. 4), showed a steady decline in F_R at 0°C, albeit much more slowly than leaves of chilling-sensitive plants. Barley can be grown at 1°C, but eventually shows symptoms resembling those of chilling injury such as abnormal growth and inhibition of Chl synthesis (24). At 0°C, the F_R of detached barley leaves was found to decrease over a period of 2 to 4 weeks. Here the question is addressed as to whether a relationship exists in barley and other cereals between the rate of this decrease in F_R in either cold-hardened or unhardened plants and the freezing tolerance of the plants.

Figure 5 shows the changes in log F_R in leaves at 0°C of unhardened and cold-hardened spring barley and wheat, and winter barley, wheat, and rye. The exposure at 0°C was on detached leaves and it is not known if attached leaves show the same changes. Nevertheless, using detached leaves some consistent changes in F_R with time at 0°C were obtained that appeared to be correlated with the expected freezing tolerance of the plant. As seen in Figure 5, measurements of F_R clearly distinguished between leaves unhardened to cold (grown at 19°C/25°C) and leaves cold-hardened by exposure to 1°C. When cold-hardened leaves were held at 0°C, leaf F_R decreased during the first 10 to 20 d and then stabilized. The rate and extent of the decrease in F_R for all five cold-hardened cultivars were very similar and hence were not correlated with differences in freezing tolerance. The initial values for F_R in the cold-hardened plants were 2 or 3 times higher than those of the unhardened plants (data not shown), but again no correlation was observed between the magnitude of this increase and freezing tolerance. In contrast, the F_R of leaves from unhardened plants steadily decreased after the first 2 to 3 d at 0°C (the winter cereals showed an initial increase during the first few days—see below) and, although not shown in Figure 5, F_R eventually declined to zero after several weeks at 0°C. When the experiment was repeated, the same pattern of changes were observed.

The rate of decrease in F_R of the unhardened leaves at 0°C appeared to reflect the expected freezing tolerance of the cereal. The two spring cultivars showed a slightly accelerated decrease in F_R during the first few days at 0°C, whereas the winter cultivars all showed a characteristic initial stimulation of F_R, the more tolerant the cultivar the greater the stimulation. From the data in the figure, the time to 50% decrease in F_R at 0°C was determined and it can be seen in Table I that this value was correlated with freezing tolerance, the lower the plant freezing tolerance the longer it took for F_R to decrease. The estimates of freezing tolerance, LD_{50} (defined as the temperature at which 50% of seedlings did not survive exposure for 1 h) were determined by Dr K. Pomeroy (Research Branch, Agriculture Canada, Ottawa). The LD_{50} for the spring barley and wheat were not available, but both would be expected to be around −4°C to −6°C. These results suggest that, in addition to its use as a convenient means of following changes in photosynthetic activity during freezing and thawing, the Chl fluorescence technique can detect differences between cold-hardened and unhardened plants and indicate degrees of freezing tolerance.

Ice Cover. Damage to winter cereals is a recurring problem in regions where the snow cover on fields partially thaws during brief periods of warmer weather and then refreezes to form thick layers of ice encasing the plants. The ice cover insulates against freezing temperatures but leads to depletion of O_2. This condition can be simulated in part by storing cold-hardened leaves under N_2 at 0°C. Figure 6 shows that measurements of F_R can be used to compare the tolerance of leaves to anaerobic conditions at 0°C. Two cultivars of wheat were compared, Kharkov winter wheat which has a high tolerance to ice encasement (2) and Gatcher spring wheat. During the first day under N_2 at 0°C, the F_R of the leaves of both cultivars decreased by more than 50% and thereafter decreased much more slowly. The initial decrease can be attributed to the effects of anaerobiosis as a decrease in F_R did not occur in leaves treated similarly but under aerobic conditions. Andrews and Pomeroy (2) showed that the anaerobic metabolite lactic acid increased during the 1st d of ice encasement and then remained constant.

Between day 1 and day 36 at 0°C under N_2, the F_R of Kharkov leaves decreased by 32%, whereas F_R decreased in Gatcher leaves by 85%. Damage to plants during ice encasement has been attributed to a combination of the build up of CO_2 and anaerobic metabolites, especially ethanol (2). CO_2 is unlikely to be an important factor under the experimental conditions used here and the changes observed in F_R suggest that Gatcher is far more susceptible than Kharkov to anaerobic toxins.

Heat Stress. Plants inhabit environments spanning a wide range of temperatures, from alpine meadows to hot deserts and their resistance to heat varies accordingly (1, 14). Many crop plants are not well adapted for growth in hot climates and both pre- and postharvest wastage during heat-wave conditions is not uncommon. In spite of this screening, methods for selecting for heat tolerance are generally unavailable. The development of methods to measure heat hardening in crop plants also has proven to be difficult (30). In the past, progressive heat damage to plant tissues has been followed by plasmolysis, electrolyte leakage, and by changes in vital staining, protoplasmic streaming, respiration, photosynthesis, and protein turnover (1, 4, 14). An advance in measuring heat damage in photosynthetic tissue came with the introduction of measurements of Chl F_o-fluorescence (21). Not responsive to changes in metabolism at ordinary physiological temperatures, F_o-fluorescence rises as green cells become heat damaged above about 45°C. The temperature at which the rise begins in leaves heated at a rate of 1°C/min gives a measure of heat resistance (21) and has been used to compare the heat tolerances of desert plants (4, 12, 21) and various alpine, temperate, and tropical species (26). More recently, heat-induced changes in the variable Chl fluorescence were used to measure heat hardening in spinach (20) and barley (25) and to compare the heat tolerances of different species using a procedure in which the leaf temperature was raised at a constant rate (25). In this last study, the potential advantages of measurements of the variable Chl fluorescence over those of the F_o-Chl fluorescence for the development of a screening test for heat tolerance were pointed out and it was suggested that the relative heat tolerances of closely related species might be assessed from the extent of the decrease in F_R after a standardized heat treatment of the leaf tissue. The results shown in Table II support this suggestion. In leaves of various species heated at 44°C for 10 min, the heat-induced decrease in F_R was greatest in those species, such as pea, adapted to cool temperature climates and least in those, such as papaya, adapted to warm climates. As in the case of measurements of chilling tolerance using Chl fluorescence, there is a need to establish that the size of the decrease in F_R induced by a heat treatment of leaves of different species is indeed related to the relative heat tolerance of the species. This would require the testing of a wide range of plant material showing different heat tolerances. This has been partially done in a separate study in which different species of wild potato found growing naturally in the Andean region of Peru and Bolivia at altitudes ranging from 450 to 4200 m were tested for heat tolerance (R. M. Smillie, S. E. Hetherington, C. Ochoa, and P. Malagamba, unpublished experiments). In the different species examined, the decrease in F_R in leaves heated at 41°C for 10 min was correlated with the altitude of collection, the higher the altitude at which the species was found growing the greater the decrease in F_R. For instance, the mean ± SE of the per cent decrease in F_R after heat-treating eight mature leaves of each species was 78.5 ± 4.4 for *S.*

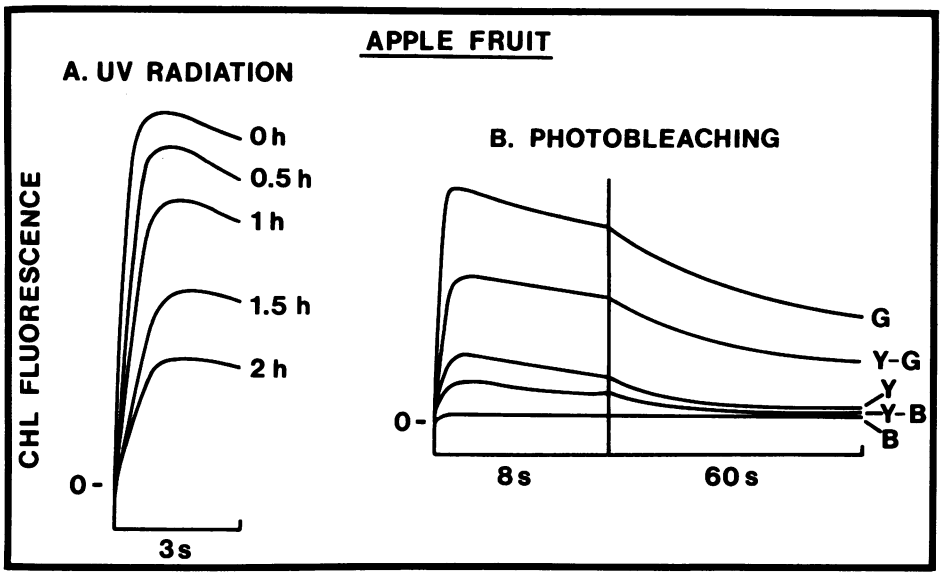

FIG. 7. Chl fluorescence of Granny Smith apples (*Pyrus malus* L.) after: A, irradiation with long-wave UV; and B, in fruit variously affected by sunburn in the field. For the UV irradiations, mature green apples were exposed for the times indicated at a distance of 6 cm from a Westinghouse FS-40 sunlamp. The Chl fluorescence (3-s duration at 15 μE m^{-2} s^{-1}) of the irradiated area of the peel was recorded after a further 30 min in darkness. Sunburnt fruit showing different degrees of photobleaching were harvested from trees in an orchard. The letters indicate the appearance of the area of peel of individual fruit on which measurements of Chl fluorescence were made. (G), Green; (Y-G), yellow-green; (Y), yellow; (Y-B), yellow-brown; (B), brown. A split time scale is used to record fast and slow kinetics of Chl fluorescence.

bukasovi (3900 m) and 25.8 ± 2.8 for *S. tarijense* (1950 m). Whereas the previous study (25) had indicated that changes in the induced Chl fluorescence of heated leaves can be used to measure differences in heat tolerances between distantly related species, these results suggest a wider use for Chl fluorescence in the detection of finer differences in heat tolerance between closely related species.

UV Radiation Damage, Photoinhibition, and Photobleaching. Plants unless adapted to high levels of solar radiation can be damaged by both the UV and visible wavelengths in sunlight. A wide range of plants are sensitive to UV radiation (8) and photosynthesis in plants grown at low light intensities is inhibited (photoinhibition) by exposure to high intensities of visible light (18). The loss of Chl (photobleaching) often follows a severe UV or photoinhibitory stress. The damaging effect of high solar radiation is commonly seen in fruit-growing areas with infrequent cloud cover where sunburn or sunscald of fruit can result in wastage of apples, pears, tomatoes, and other fruit. Visible light and heat are involved in sunburn of tomato fruit (19), but UV light is another possible contributing factor. The use of Chl fluorescence *in vivo* to detect photoinhibitory damage has already been described (9), and Figure 7 shows that it can be used to assess effects of irradiating apples with UV light under laboratory conditions and to follow photobleaching resulting from sunburning of apples in the orchard. While the measurements on sunscalded apples (Fig. 7B) do not identify the primary cause of the injury they do provide a means of measuring its effect. Identification of the actual injurious wave length bands or combination of these contributing to sunscald should be amenable to study by Chl fluorescence techniques and it should also be possible to detect early stress injuries, *e.g.* photoinhibition, which precede the loss of Chl. In the case of UV radiation of apples, the decline in log F_R was linearly related to the time of irradiation and it seems feasible to use fluorescence *in vivo* to screen for resistance and adaption to UV light, and also to intense visible light, in a way similar to that developed to screen for chilling tolerance (13).

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