

# Determining Chilling Injury Induction in Green Peppers Using Nondestructive Pulse Amplitude Modulated (PAM) Fluorometry

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**Abstract.** Storing 'Maor' green bell peppers (*Capsicum annuum* L.) for 3 weeks at 2°C resulted in the development of chilling injury (CI) evidenced as surface pitting. Fruit held at 8°C did not develop any CI symptoms, but, after 3 weeks of storage, the fruit began to change color from green to red. PAM fluorometry was used to measure changes in photosynthetic competency in whole green bell peppers. Three photosynthetic characteristics could be measured by this method: quantum yield (Fm/Fo), photochemical quenching (Qp), and nonphotochemical quenching (Qnp). Fm/Fo decreased 90% during the first week of storage at 2°C and remained low thereafter, while Qnp decreased after 2 weeks at 2°C, just before the peppers began to develop CL. Qp was similar at both storage temperatures. Potassium leakage as a CI measurement also increased in excised pepper discs after 2 weeks at 2°C. The results indicate that PAM fluorometry can measure CI nondestructively before tissue damage is visible in green peppers.

Remarkable progress has been made recently in the understanding and practical use of chlorophyll fluorescence in plant science, spurred by the need for quantitative, noninvasive, rapid methods to assess photosynthesis in intact leaves (van Kooten and Snel, 1990). In addition, the potential of chlorophyll fluorescence as a naturally occurring internal probe sensitive to stress-related cellular injury has been investigated (Smillie and Hetherington, 1983). When illuminated, chlorophyll emits a red fluorescence, of which part—the variable fluorescence—is responsive to changes in photosystem II activity (Papageorgiou, 1975). Therefore, any stress, which directly or indirectly affects photosynthetic metabolism, is likely to change the yield of this fluorescence when applied to green plant tissue.

Most studies of the effects of environmental stress on fluorescence have measured only from the initial (Fo) to the maximal (Fm) level, i.e., quantum yield (Fm/Fo), whether on leaves or fruit (Abbott et al., 1987; Smillie and Hetherington, 1983; Walker et al., 1990). PAM fluorometry measures additional characteristics of photosynthesis. In addition to measuring Fm/Fo, this technique enables the researcher to determine features of photochemical quenching (Qp), due to electron transport, and nonphotochemical quenching (Qnp), the proton pumping of ATPase and the pH gradient across the thylakoid membrane. Thus, this procedure gives more information about the state of the photosynthetic units than that derived from normal fluorescence measurements (Schreiber, 1986). We have used PAM fluorometry to follow the development of chilling injury (CI) in whole green peppers, correlating it with K<sup>+</sup> leakage from fruit discs and the development of visible CI symptoms.

## Materials and Methods

Field-harvested 'Maor' green peppers were placed directly in storage at either 8 or 2°C at 90% relative humidity (RH). At harvest

and after each week of storage for 4 weeks, peppers were placed at 20°C, 85% RH, for 5 days. At each observation time, three replications of 10 fruit from each storage temperature were examined for color changes and CI, as evidenced by surface pitting. Five fruit from each treatment were examined by PAM fluorometry. The fruit were brought to 20°C and dark-adapted for at least 20 min before being measured. Initially, the warming of fruit removed from 2 or 8°C was followed by a thermistor in the pericarp; 2 h was sufficient to bring the peppers to 20°C. Discs were prepared from the same five fruit after PAM measurement and incubated for K<sup>+</sup> leakage.

**PAM fluorometry.** Fluorescence was measured using a PAM fluorometer (Walz, Effeltrich, Germany) equipped with a fiber-optic probe (1 cm in diameter) that was placed on the fruit surface. Depending on light conditions, four fluorescent states could be distinguished (Fig. 1), with Qp and Qnp varying between 0 and 1. In dark-adapted tissue, exposing fruit to a weak modulating measuring light of 1.6 kHz at 650 nm activated Fo in state 1. In this state, all photosystem II reaction centers were open while the photosynthetic membrane was in the nonenergized state (Qp = 1 and Qnp = 0). Then a saturating light pulse of 7400 μmol·m<sup>-2</sup>·s<sup>-1</sup> for 600 msec was given to excite Fm and move the system into state 2. In this state, all photosystem II reaction centers were closed (Qp = 0) while the photosynthetic membrane was still in a nonactivated state (Qnp = 0). After the saturating pulse, an actinic red light of 75 μmol·m<sup>-2</sup>·s<sup>-1</sup> of 650 nm was turned on, while pulses of saturating light continued to be given every 5 sec. The system oscillated between state 3 (actinic light) and state 4 (actinic light plus saturating pulse). Qp and Qnp designate the relative decrease in variable fluorescence yield due to photosynthetic and nonphotosynthetic processes, respectively, derived from van Kooten and Snel (1990).

$$Q_p = F'_m - F/F'_m - F_o$$

$$Q_{np} = F_m - F'_m/F_m - F_o$$

**Potassium leakage.** Two 1-cm discs from each of five peppers were cut by a cork borer and each pair was incubated in 5 ml of 0.35 M sorbitol. At the end of 3 h, the discs were removed from solution,

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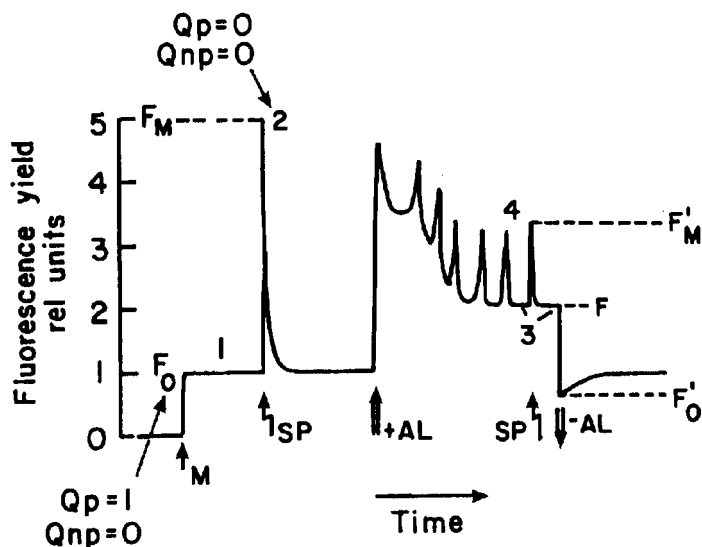


Fig. 1. Fluorescence yield and quenching analysis as measured by pulse amplitude modulation fluorometry. A weak modulated measuring light (M) gives abase level fluorescence ( $F_0$ ). This is state 1 fluorescence, where all photosystem II reaction centers are open. Then a saturating light pulse (SP) is given to close the photosystem II reaction sites (state 2), and maximal fluorescence ( $F_m$ ) is measured. A continuous actinic light (AL) is turned on and saturating light pulses continue to be given, thus alternating the system between states 3 ( $F'_m$ ) and 4 ( $F$ ). (Adapted from van Kooten and Snel, 1990).

frozen, thawed, and boiled for 20 min. The incubating solution and the boiled tissue were examined for  $K^+$  using a flame photometer, and the  $K^+$  present in the solution after 3 h was expressed as percentage of total  $K^+$ .

## Results

$F_m/F_0$  of the peppers stored at 2C was almost 10-fold lower than that of peppers held at 8C after 1 week of storage and remained low during storage (Fig. 2). When peppers were removed from 2C at weekly intervals and then held at 20C for 5 days,  $F_m/F_0$  after shelf life increased 4-fold over that of storage. After 1 week at 2C,  $F_m/F_0$  recovered to 65% and after 2 weeks to 45% of that of peppers stored at 8C. CI symptoms appeared after 3 weeks at 2C (Fig. 3, top), while no CI developed on peppers stored at 8C. Peppers stored at 8C had a decreased  $F_m/F_0$  after 3 and 4 weeks of storage. This decrease corresponded to chlorophyll disappearance as the peppers continued to mature at this temperature and began to change color (Fig. 3, bottom). Peppers held at 2C showed no color change even when moved to 20C.

$Q_p$  decreased gradually in peppers stored at 8 or 2C, with no significant difference between the two storage temperatures (Fig. 4). There was no change in this coefficient during holding at 20C. In contrast,  $Q_{np}$  was lower in fruit stored at 2C than in those at 8C for 2 weeks or longer (Fig. 5). This characteristic increased when the fruit were removed from 8 and then held at 20C for 5 days. In peppers moved from 2 to 20C,  $Q_{np}$  recovered after 1 week of storage, but not after 2 or more weeks.

Potassium leakage from discs of freshly harvested peppers was 20% after 3 h of incubation (Fig. 6). Leakage increased as the fruit were stored longer at either 8 or 2C. Peppers stored at 8C had increased leakage during storage. Fruit at 2C had a dramatic rise in leakage between 1 and 2 weeks of storage. By 4 weeks, there was a decline in leakage from discs of fruit stored at 2C so that leakage from fruit at both storage temperatures was similar.

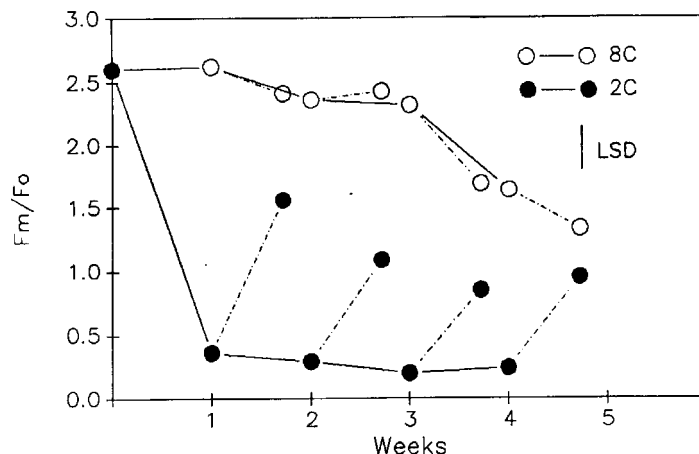


Fig. 2. The change in  $F_m/F_0$  of peppers stored for 1 to 4 weeks at 8 or 2C and then held for 5 days at 20C. Transfer from storage to 20C each week is shown by a dotted line.  $LSD_{0.05}$  is indicated.

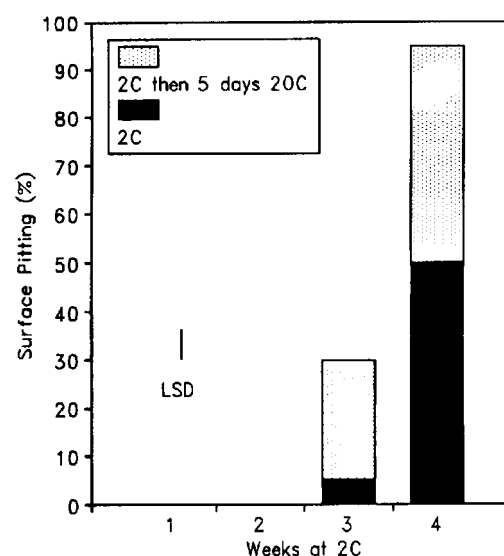
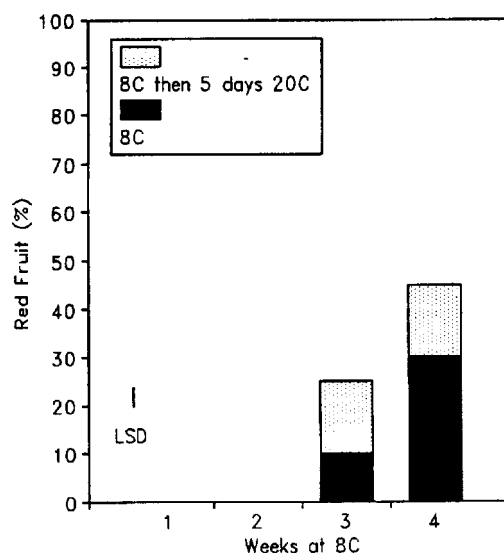


Fig. 3. (A) Surface pitting in green peppers held at 2C for 1 to 4 weeks and then at 20C for 5 days. (B) Color change in green peppers held at 8C for 1 to 4 weeks and then at 20C for 5 days.  $LSD_{0.05}$  is indicated.

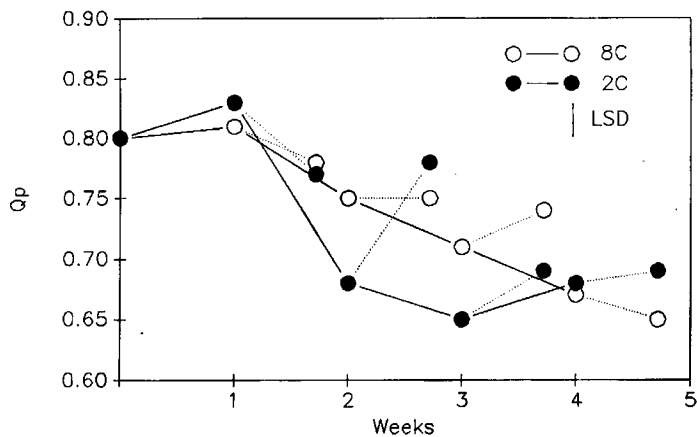


Fig. 4. The changes in Qp of peppers during storage and after 5 days at 20C following storage for 1 to 4 weeks at 2 or 8C. Transfer from storage to 20C each week is shown by a dotted line. LSD<sub>0.05</sub> is indicated.

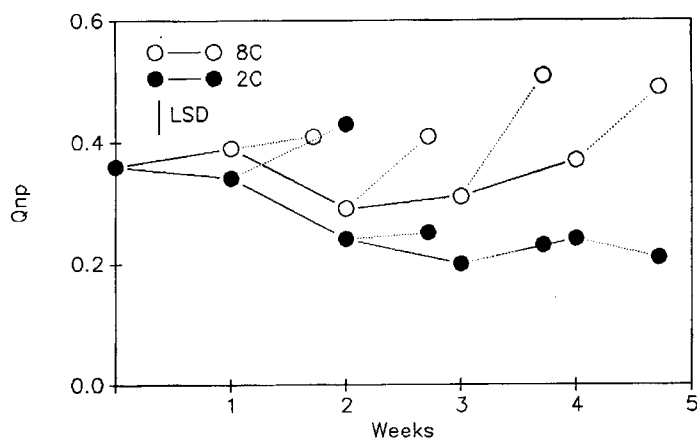


Fig. 5. The changes in Qnp of peppers during storage and after 5 days at 20C following storage for 1 to 4 weeks at 2 or 8C. Transfer from storage to 20C each week is shown by a dotted line. LSD<sub>0.05</sub> is indicated.

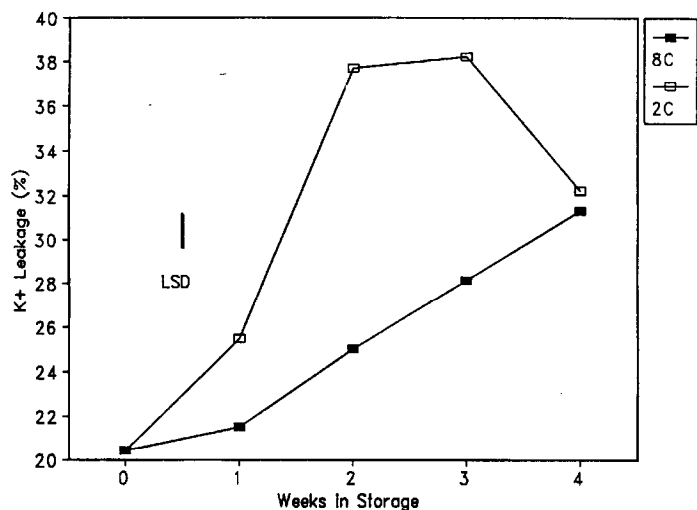


Fig. 6. Potassium leakage from discs of peppers stored at 8 or 2C for 1 to 4 weeks. LSD<sub>0.05</sub> is indicated.

## Discussion

Fm/Fo and Qnp decreased in green peppers stored at 2C before the appearance of visible CI signs. However, Fm/Fo responded rapidly to low temperature, even before the tissue became susceptible to CI, whereas Qnp decreased significantly only after 2 weeks at 2C, concomitant with the increase in K<sup>+</sup> leakage from fruit discs. Moreover, during storage, Fm/Fo recovered when the fruit were transferred to 20C, while Qnp recovered after 1 week at 2C but not thereafter. Therefore, the response of Qnp to low temperature correlated more closely than Fm/Fo to the visible CI symptoms and other physiological expressions of injury, such as leakage.

Leakage increased when plant tissues develop CI (Lafuente et al., 1991; Lurie and Klein, 1991) and has been used to indicate its development (Lafuente et al., 1991). In this study, there was a good correlation between increased K<sup>+</sup> leakage and CI development on the peppers after 3 weeks of storage. However, for some fruit sensitive to low-temperature storage, leakage either does not increase (Fuchs et al., 1989) or increases only after CI has developed (McCollum and McDonald, 1991) and, therefore, is of limited value as an early CI indicator. Moreover, leakage is a destructive measurement, while PAM is nondestructive and can measure many fruit in a short time.

Severe CI produces visible symptoms, but, before these are evident, changes in cell metabolism can be detected, including altered physical properties of cell membranes, anomalous respiratory activity, and electrolyte movement across membranes (Lyons, 1973). In addition, photosynthesis is affected in chilling-sensitive plants. Inhibition develops on the photooxidizing side of photosystem II (Margulies and Jagendorf, 1960; Smillie and Nott, 1979; Terashima et al., 1989) and, therefore, results in a decreased Fm/Fo (Papageorgiou, 1975), as was found in the peppers. This inhibition was partially reversible at early stages of low-temperature storage. The recovery of photosystem II in chilling-sensitive plants after some days at 0C depends on removing the plants to a higher temperature and illuminating the leaves (Kaniug et al., 1978). We did not determine whether holding peppers at 20C in darkness would have prevented the recovery of Fm/Fo seen after storage at 2C.

Although the photooxidizing side of photosystem II was inhibited by low temperature and Fm/Fo was reduced in peppers held at 2C, Qp (which reflects photosynthetic electron transport) was unaffected by low-temperature storage. This response has also been found in cucumber (*Cucumis sativus* L.) leaves and isolated chloroplasts, in which chilling treatments did not alter electron transport rates (Peeler and Naylor, 1988; Terashima et al., 1989). Qnp, which is a measure of the energizing of the thylakoid membranes leading to ATP formation, decreased in peppers stored at 2C. Moll and Steinbach (1986) found that chilling *Oryza sativa* L. led to the disruption of ATP formation earlier than inhibition of the electron transport reactions of photosynthesis. In chilled cucumber leaves, electron transport rates in thylakoids were not inhibited, but thylakoids were totally permeable to protons and, therefore, uncoupled from ATP formation (Peeler and Naylor, 1988). Apparently, the chloroplasts of green peppers respond to low temperature identically to chloroplasts from chilling-sensitive leaves.

Most fluorescent measurements of plant tissue in response to environmental stress have been on leaves. Many fruit at harvest contain chloroplasts in their peel and sometimes in their flesh, and these have been shown to be active photosynthetically, although contributing only minimally to the carbon balance of the fruit (Blanke and Lenz, 1989; Smillie, 1992). For fruit that are sensitive

to low-temperature storage, a nondestructive, rapid method of measuring CI would be useful. The results presented here demonstrate the potential of PAM fluorometry to estimate rapidly and nondestructively the chilling tolerance of detached organs, such as chloroplast-containing fruit. With this method, each sample can be measured in a few seconds and the signals of many samples can be stored and processed by computer.

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