Ciência Rural
ISSN: 0103-8478
cienciarural@mail.ufsm.br
Universidade Federal de Santa Maria
Brasil

Saquet Arriel, Adriano; Streif, Josef
Chlorophyll fluorescence as a predictive method for detection of browning disorders in ´conference´ pears and ´Jonagold´ apples during controlled atmosphere storage
Ciência Rural, vol. 32, núm. 4, julho-agosto, 2002, pp. 571-576
Universidade Federal de Santa Maria
Santa Maria, Brasil

Ciência Rural, Santa Maria, v.32, n.4, p.571-576, 2002
ISSN 0103-8478

CHLOROPHYLL FLUORESCENCE AS A PREDICTIVE METHOD FOR DETECTION OF BROWNING DISORDERS IN 'CONFERENCE' PEARS AND 'JONAGOLD' APPLES DURING CONTROLLED ATMOSPHERE STORAGE

FLUORESCÊNCIA DE CLOROFILAS COMO MÉTODO PREDICTIVO PARA DETECÇÃO DE DESORDENS FISIOLÓGICAS EM PÊRAS 'CONFERENCE' E MAÇÃS 'JONAGOLD' DURANTE O ARMAZANAMENTO EM ATMOSFERA CONTROLADA

Adriano Arriel Saquet[1] **Josef Streif**[2]

SUMMARY

The chlorophyll fluorescence technique was evaluated as a possible predictive and nondestructive method to detect low-O_2 and/or high-CO_2 injuries in 'Conference' pears and 'Jonagold' apples stored in controlled atmosphere (CA). The fruits were kept at 0°C in air, 1% CO_2 + 2% O_2 or 3% CO_2 + 1% O_2 during five months. Fluorescence parameters of minimal fluorescence (Fo), maximal fluorescence (Fm), and potential quantum yield [(Fm-Fo):Fm, also denoted as Fv:Fm] as well as the incidence of browning disorders were evaluated at several times during storage. No incidence of browning disorders was observed in 'Jonagold' apples, however, they showed a decrease in Fv:Fm during storage time with no differences between the CA-conditions. Air-stored apples showed a higher decrease in Fv:Fm. On the other hand, 'Conference' pears kept in 3% CO_2 + 1% O_2 developed a lot of browning injuries such as core flush, flesh browning and cavities. Under this CA-condition, a pronounced decrease in the quotient Fv:Fm was observed already in the first 15 days of storage prior to the development of browning, and this behaviour remained during the whole storage period. The air-stored pears showed a similar behaviour as of the air-stored apples with a pronounced decrease in the Fv:Fm at the end of the storage period. The present results indicate that chlorophyll fluorescence is a promising technique to detect browning injuries in 'Conference' pears prior to their development.

Key words: *fruits, quality assessment, physiological disorders.*

RESUMO

A técnica da fluorescência de clorofilas foi avaliada como um possível método não destrutivo e indicador para detecção de danos causados por baixas concentrações de oxigênio e/ou altas concentrações de gás carbônico em pêras 'Conference' e maçãs 'Jonagold' durante o armazenamento em atmosfera controlada (AC). Os frutos foram mantidos durante cinco meses à 0°C em armazenamento refrigerado (armazenamento somente em frio) e em condições de atmosfera controlada de 1% de CO_2 + 2% de O_2 ou 3% de CO_2 + 1% de O_2. Em vários intervalos durante o armazenamento, foram avaliadas a emissão mínima (Fo) e máxima (Fm) de fluorescência, bem como, o rendimento potencial quântico (Fm-Fo):Fm, também indicado como Fv:Fm. Relacionada às variações nestes parâmetros foi analisada então a incidência de desordens fisiológicas nos frutos. Durante todo o período de armazenamento não foi observada incidência de escurecimento da polpa nas maçãs 'Jonagold', porém, foi verificada uma diminuição gradativa no quociente Fv:Fm ao longo do armazenamento, não apresentando, entretanto, diferenças significativas entre as condições de AC. Maçãs armazenadas somente em frio apresentaram uma redução mais acentuada nos valores de Fv:Fm. Por outro lado, pêras 'Conference', mantidas em 3% de CO_2 + 1% de O_2, desenvolveram alta incidência de escurecimento da polpa caracterizado separadamente como o escurecimento do miolo, escurecimento da polpa e, também, cavernas. O armazenamento das pêras nesta condição de AC causou uma pronunciada queda no quociente Fv:Fm já nos primeiros 15 dias de armazenamento, antes do surgimento das desordens internas, sendo que este comportamento permaneceu

[1]PhD-Student, University of Hohenheim, Stuttgart, Germany. Institute of Fruit Science Bavendorf, D-88213 Ravensburg, Germany. Fellowship of CAPES. E-mail: adrianosaquet@hotmail.com. Author for correspondence.
[2]PhD, Researcher, University of Hohenheim, Institute of Fruit Science Bavendorf.
Received for publication:25.08.00. Aproved: 05.09.01

durante todo o período de armazenamento. As pêras conservadas em armazenamento refrigerado tiveram um comportamento muito similar àquele observado nas maçãs apresentando uma maior diminuição no quociente Fv:Fm durante o armazenamento. Os resultados indicam que o uso da fluorescência de clorofilas é promissor como um indicador para detectar a ocorrência de escurescimento da polpa em pêras 'Conference' durante o armazenamento em AC antes do surgimento das desordens.

Palavras-chave: *frutos, avaliação da qualidade, desordens fisiológicas.*

INTRODUCTION

'Conference' pears are very sensitive to low-O_2 and/or to high-CO_2 concentrations used during controlled atmosphere (CA) storage resulting often in high incidence of brown heart and quality losses (GARCIA & STREIF, 1993; SAQUET ***et al***., 2000). Several researches have been carried out in order to determine and understand the biochemical and physiological basis of the development of internal browning. A great progress would be, however, to detect the damage in its early stages or before the development of symptoms in order to avoid subsequent losses. Internal symptoms can only be detected when the fruits are cut and exposed to visual evaluation. Fruits, even with low incidence of disorders, are not suitable for comsumption, and an additional problem is that the consumers are not able to distinguish, by external examination, affected fruits from healthy fruits.

During the last years, special attention has been given to the practical application of chlorophyll fluorescence as a rapid, nondestructive and predictive method to detect stress in plants. Stress or injury in plant tissues, that disrupts photosynthesis, changes the characteristic fluorescence pattern of cells and tissues (SCHREIBER ***et al***., 1994; DeELL ***et al***., 1995, SCHREIBER, 1997). Chlorophyll fluorescence results from the reactions of deexcitation of excited chlorophyll molecules (Figure 1). Under ideal conditions most of the energy from excited chlorophyll molecules is trapped as chemical energy and used primarily to assimilate carbon. The more efficient the energy transfer, the lower is the fluorescence yield. This reduction in fluorescence yield is designated as chlorophyll fluorescence quenching (SCHREIBER ***et al***., 1986).

Many results have given indications that changes in chlorophyll fluorescence can detect low-O_2 or high-CO_2 stress in 'McIntosh', 'Delicious' and 'Golden Delicious' apples prior to the development of associated disorders (DeELL ***et al***., 1998). By using the chlorophyll fluorescence technique SMILLIE ***et al***. (1987) detected chilling injury in banana and mango fruits, while VAN KOOTEN ***et al***. (1992) and LURIE ***et al***. (1994) detected chilling injury in cucumbers and green bell pepers, respectively. Thus, the use of chlorophyll fluorescence as a predictive indicator for screening atmospheric stress tolerance in apples during CA-storage has been considered (DeELL ***et al***., 1998). Such technique would offer to the storage operator the opportunity to market fruit well in advantage of the occurrence of irreversible low-O_2 or high-CO_2 injuries.

This research was undertaken aiming to examine the changes in chlorophyll fluorescence during five months of storage of 'Conference' pears and 'Jonagold' apples under CA-storage conditions which might induce flesh browning and to determine whether this technique can detect the damage before its development.

MATERIAL AND METHODS

Preclimacteric 'Conference' pears and 'Jonagold' apples were picked at the optimum harvest date for long-term storage, selected for uniformity and immediately stored at 0°C (±0.5°C). For CA-storage, fruits were placed in 560-liter containers and the CO_2 and O_2 concentrations continuously monitored by gas analysers connected to a process computer. The pulldown of O_2 at the beginning of storage was done with N_2 flushing. Excess of CO_2 in the CA containers was scrubbed by potassium hydroxide. Storage conditions were: air control, 1% CO_2 + 2% O_2 and 3% CO_2 + 1% O_2. During the 5-months storage period, 60 fruits of each cultivar and treatment were removed from the storage containers at several time intervals and used for the measurements of chlorophyll fluorescence and immediately scored for incidence of browning disorders.

Changes in chlorophyll fluorescence emitted by fruits were measured using a PAM (pulse amplitude modulation) fluorometer (Walz, Effeltrich, Germany) equipped with an optic fiber probe (1cm in diameter). The fruits were adapted to a dark room temperature for three hours before assessments. The values of minimal flurescence (Fo), maximal fluorescence (Fm) and the potential quantum yield [(Fm-Fo):Fm, also denoted as Fv:Fm] were determined placing the optic fiber at a distance of 1cm from the fruit surface. Fo and Fm are defined as minimal and maximal fluorescence yields of the dark-adapted sample, with all PSII reaction centers fully opened or closed, respectively. The Fv:Fm-parameter was calculated from the given Fo- and

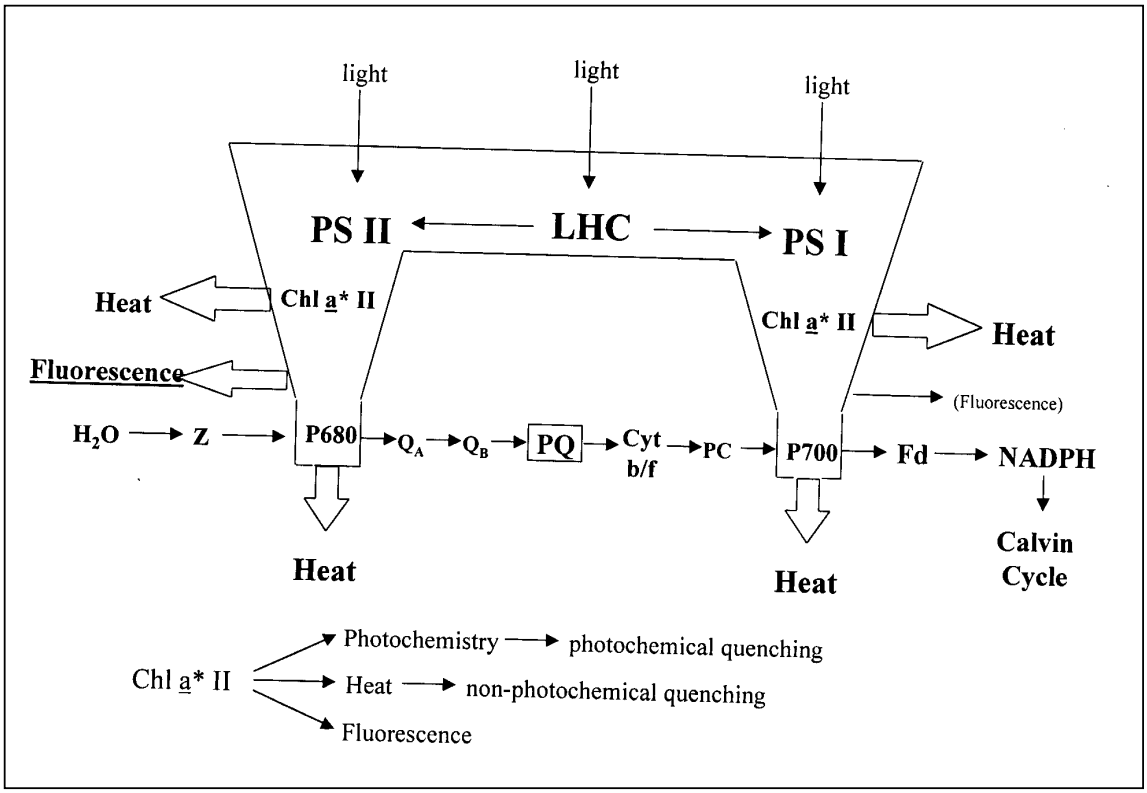

Figure 1 - Schematic illustration of primary energy conversion in photosynthesis which governs *in vivo* chlorophyll fluorescence yield. Variable fluorescence originates almost exclusively from PSII. Maximal fluorescence yield is lowered by photochemical charge separation and heat dissipation (SCHREIBER ***et al***., 1994).

Fm-values using the equation Fv:Fm = (Fm-Fo):Fm. The quotient Fv:Fm gives information on the potential quantum yield of PSII (SCHREIBER, 1997).

Physiological disorders were monitored from fruits cut open at the equatorial area, longitudinally and transversally, and scored for three types of browning disorders. Flesh browning was characterized by chocolate-coloured tissue in the cortex of fruits, while cavities were found in the area near the core and often in the cortex. Brown heart or core flush was a disorder of the core and often advanced to the cortex region. The disorders were expressed by an index which varied from 0 to 100, according to the severity of the damage.

RESULTS AND DISCUSSION

The studied apple and pear cultivars exhibited different sensitivities for CA-induced flesh browning disorders. 'Jonagold' apples were tolerant to all employed CA-storage conditions and no incidence of physiological disorders was detected during storage for up to five months. GOFFINGS & HERREGODS (1994) had already indicated similar behaviour of 'Jonagold' apples after storage under very low oxygen concentrations (0.7%). SAQUET ***et al.*** (2000) working with very extreme CA-conditions like 6% CO_2 + 0.5% O_2, also did not find any browning problems with this apple cultivar. A decrease in the potential quantum yield (Fv:Fm) in this cultivar (Figure 2) was observed during the storage time in all storage conditions, however without any difference between the CA-treatments. Only the fruits kept in air control showed a stronger decrease in Fv:Fm after four months of storage, which might be probably related to the accelerated ripening and senescence processes with higher chlorophyll breakdown of fruits stored in air compared to CA.

'Conference' pears developed high incidence of browning disorders under 3% CO_2 + 1% O_2 (Figure 3). The sensitivity of this pear cultivar to low-O_2 and/or high-CO_2 concentrations was also reported by GARCIA & STREIF (1993) and SAQUET ***et al***. (2000). Visualization of injuries was possible after one month of storage and was correlated to a stronger decrease in the Fv:Fm

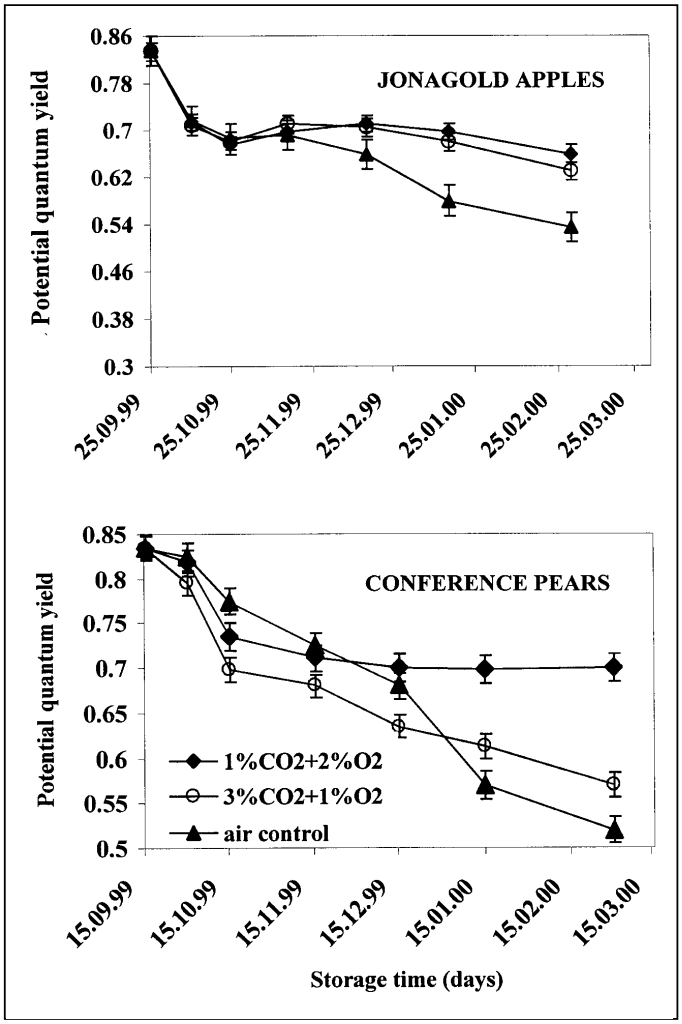

Figure 2 - Changes in chlorophyll fluorescence of 'Jonagold' apples and 'Conference' pears kept under different storage conditions at 0°C. Vertical bars indicate SE between treatment means.

(Figure 2), starting at 0.7 value and decreasing until the end of storage time following the development of flesh browning, core browning and cavities (Figure 4). These results indicate that such technique is promising to detect low-O_2 and/or high CO_2-injuries in 'Conference' pears, what has been also reported by other researchers working with different apple cultivars such as 'McIntosh' (DeELL ***et al***., 1995; MIR ***et al***., 1997), 'Elstar' (PRANGE ***et al***., 1997) and 'Red Delicious' (MIR ***et al***., 1997). A decrease in Fv:Fm in air control was also detected in 'Conference' pears (Figure 2). SONG ***et al***. (1997) evaluating changes in chlorophyll fluorescence of apples during maturation, ripening and senescence observed a decrese in Fv:Fm during the course of fruit ripening and senescence. These authors attributed this drop in Fv:Fm during ripening and senescence to the breakdown of chlorophyll molecules denoted by the yellowing of the fruit skin. This supposition is consistent with the findings of SMILIE ***et al***. (1987), who reported fluorescence during ripening of banana fruit at 20°C as a result of loss in chlorophyll content and a decrease in photosynthetic competency per chlorophyll unit.

PRANGE ***et al***. (1997) proposed two possible explanations for the decrease in Fv:Fm of fruits stored under low-O_2 with continuous high N_2 treatment: (1) the distance between the light harvesting complex (LHC) and the reaction center (RC) of photosystem II (PSII) in the thylakoid membrane increases in the presence of N_2 (as this increases, the probability of energy transfer decreases and the energy absorbed in the LHC has a higher probability to be fluoresced, increasing the Fo value and decreasing the Fv:Fm value) or; (2) the N_2 treatment results in insufficient oxygen molecules in the cytosol where they can act as electron acceptors, leading to a reduced state in the cytosol and by equilibrium (non-photochemical means) Q_A in PSII also becomes partialy reduced. The reduction of Q_A blocks the electron flow, decreasing Fm and Fv:Fm, and subsequently increasing Fo.

In the present work a static controlled atmosphere system was used, but in this case, the concentration of N_2 is also very high and these explanations can be probably applied to this storage system. It is very difficult to determine the basis of the mechanism by which these changes in chlorophyll fluorescence are induced during CA-storage. In this work there are not only low-O_2, but also a combination of low-O_2 with high-CO_2, conditions which induced browning in 'Conference' pears but not in 'Jonagold' apples.

CONCLUSION

Chlorophyll flurescence seems to be a promising nondestructive technique to detect browning disorders in ‘Conference’ pears prior to their development.

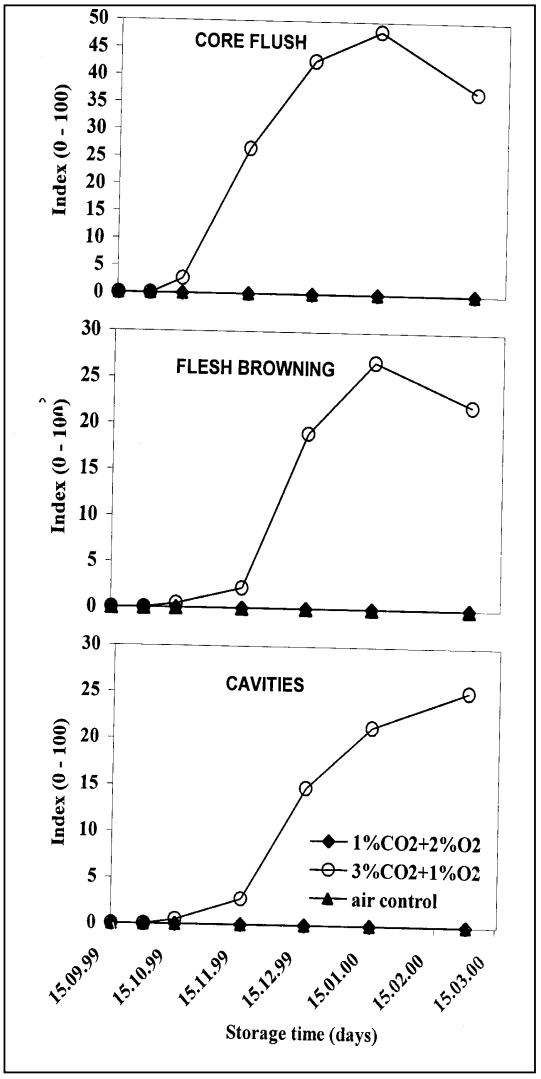

Figure 3 - Incidence of physiological disorders in 'Conference' pears kept under different storage conditions for up to five months at 0°C.

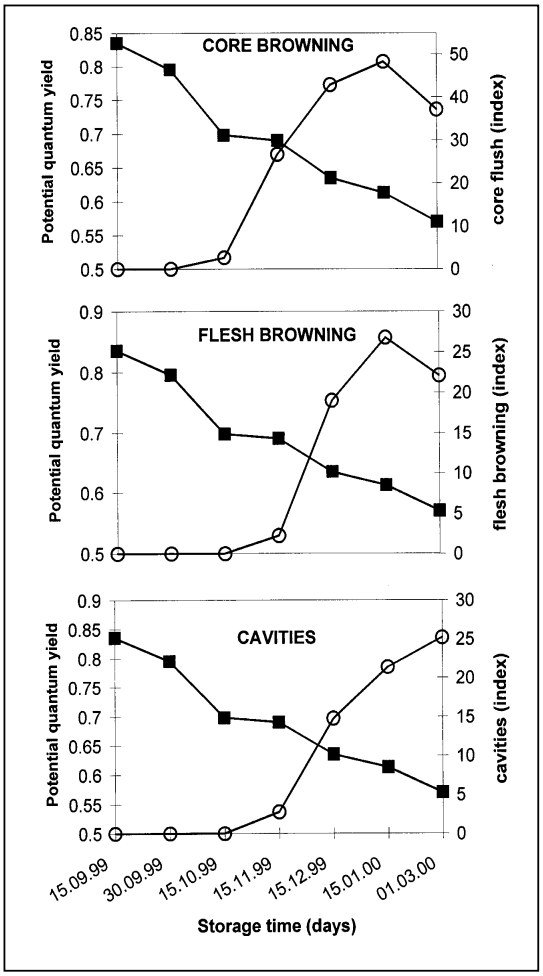

Figure 4 – Behaviour of chlorophyll flourecence related to the incidence of physiological disorders in 'Conference' pears during five months of storage at 0°C and 3% CO_2 + 1% O_2. (○) disorders; (■) Fv: Fm.

REFERENCES

DeELL, J.R., PRANGE, R.K., MURR, DP. Chlorophyll fluorescence as a potential indicator of controlled-atmosphere disorders in Marshall McIntosh apples. **HortScience**, v.30, p.1084-1085, 1995.

DeELL, J.R., PRANGE, R.K., MURR, D.P. Chlorophyll fluorescence technique to detect atmospheric stress in stored apples. **Acta Horticulturae**, n.464, p.127-131, 1998.

GARCIA, J.M., STREIF, J. Qualität und Haltbarkeit von Birnen. I. Einfluß von CA- bzw. ULO-Lagerbedingungen. **Gartenbauwissenschaft**, v.58, p.36-41, 1993.

GOFFINGS, G., HERREGODS, M. The influence of the storage conditions on some quality parameters of Jonagold apples. **Acta Horticulturae**, n.368, p.37-42, 1994.

LURIE, S., RONEN, R. MEIER, S. Determining chilling injury induction in greeen peppers using nondestructive pulse amplitude modulated (PAM) fluorometry. **Journal of the American Society for Horticultural Science**, v.119, p.59-62, 1994.

MIR, N., WENDORF, M., PEREZ, R., ***et al.*** Variable fluorescence quenching in apple during storage is a function of O_2. **Proceedings of the Seventh International Controlled Atmosphere Research Conference**, Davis, California, v.2, p.162-167, 1997.

PRANGE, R.K., SCHOUTEN, S.P., VAN KOOTEN, O. Chlorophyll fluorescence detects low oxygen stress in Elstar apples. **Proceedings of the Seventh International Controlled Atmosphere Research Conference**, Davis, California, v.2, p.57-64, 1997.

SAQUET, A.A., STREIF, J., BANGERTH, F. Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. **Journal of Horticultural Science & Biotechnology**, v.75, p.243-249, 2000.

SCHREIBER, U., SCHLIWA, U., BILGER, W. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new modulation fluorometer. **Photosynthesis Research**, v.10, p.51-62, 1986.

SCHREIBER, U., BILGER, W., NEUBAUER, C. Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: SCHULZE, E.D., CALDWELL, M.M. (eds). **Ecophysiology of photosynthesis**. Berlin : Springer, 1994. V.100, p.49-70.

SCHREIBER, U. **Chlorophyllfluoreszenz und photosynthetische Energieumwandlung**. Einfache einführende Experimente mit dem Teaching-PAM Chlorophyll Fluorometer. 1. Ausgabe : Heinz Walz GmbH, 1997. 79p.

SMILLIE, R.M, HETHERINGTON, S.E., NOTT, R., ***et al.*** Applications of chlorophyll fluorescence to the postharvest physiology and storage of mango and banana fruit and the chilling tolerance of mango cultivars. **Asean Food Journal**, v.3, p.55-59, 1987.

SONG, J., DENG, W., BEAUDRY, R.M., ***et al.*** Changes in chlorophyll fluorescence of apple fruit during maturation, ripening and senescence. **HortScience**, v.32, p.891-896, 1997.

VAN KOOTEN, O., MENSINK, M.G.J., OTMA, E.C., ***et al.*** Chilling damage of dark stored cucumbers (***Cucumis sativus* L.**) affects the maximum quantum yield of photosystem 2. In: MURATA, N. (ed.). **Progress in photosynthesis research**. The Netherlands : Kluver Academic, 1992. p.161-164.