PHYSIOLOGIA PLANTARUM 87: 515–521. 1993

Use of lysophosphatidylethanolamine, a natural lipid, tomato leaf and fruit senescence

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Farag, K. M. and Palta, J. P. 1993. Use of lysophosphatic lipid, to retard tomato leaf and fruit senescence. – Physic

We studied the influence of lysophosphatidylethanolamine rate of ethylene production and respiration of tomato (*L.* H7155) leaflets and fruit. Leaflets that had been senesci climacteric-like rise in ethylene production but not in r creased continuously with leaf age. Detached leaflets had a respiration whether they were incubated in complete dark leaflets incubated in the dark had higher rates of ethy evolution than did light-incubated leaves. There was no ethylene production or CO_2 evolution as a result of L LPE-treated attached and detached leaflets had consist evolution. The reduction in CO_2 evolution by LPE was climacteric-like peak of the detached leaves. LPE-treat chlorophyll content and fresh weight and lower electrolyte LPE-treated fruits had lower rates of ethylene and CO control. LPE-treated fruits also had higher pericarp firm leakage than the control. The results of the present study is able to retard senescence of attached leaves and detache Several recent studies suggest that lysolipids can act in a sp regulators. Our results suggest a specific role of lysolip nescence.

Key words – Aging, fruit senescence, leaf senescence, lysolipid, lysophosphophatidylethanolamine, natural lipid

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Introduction

Senescence in plant tissue is accompanied by several biochemical and biophysical changes. In the leaves, the most prominent visible change is loss of chlorophyll. During the course of senescence, enhanced ion leakage, which is a reflection of membrane perturbation, has also been observed in leaf and fruit tissues (Suttle and Kende 1980). In general, a decrease in the rate of ethy- have been observed (McGlasso and Lieberman 1979). Fruits su climacteric rise in respiration, ethylene production during ri (Burg and Burg 1965, Pratt and evidence that growth promoter rillins and cytokinins retard s plant tissues (Varga and Bruins 1979, Thimann 1980, Garrison

en found to affect the activity of
ase, phospholipases and kinases
1990, Palmgren and Sommarin
, Bille et al. 1992). It has also
can stimulate both in vivo and in
lysolipids such as lysophosphati-
) and lysophosphatidylcholine
ndre 1989).
that a postharvest dip in LPE
uit firmness and that the foliar
igated the defoliating action of
releasing compound) on tomato
1991a,b). In the present study,
dicating a specific role of LPE in
nescence. We studied the chang-
ontent, ion leakage, CO_2 evolu-
uction and pericarp firmness as

phosphatidylcholine; LPE, lysophos-
, phosphatidylcholine; PE, phospha-

ducted on attached leaves and
uits of tomato, *Lycopersicon es-*
155. One-month old seedlings
1:1 (v/v) peat and vermiculite
o, IL, USA) in 20-l pots. Plants
ths in a growth chamber main-
18-h photoperiod with 400 μmol
ically active radiation from cool
ps (Sylvania fluorescent, GTE
rs, MA, USA).

f attached leaflets

he point that solution started to
an LPE (100 mg l^{-1}) solution
water containing 1% (v/v) etha-
e sprayed with 1% ethanol (v/v).
the main shoot (counting from
, 4, 6 and 12 days, respectively,
minimize variations due to leaf
various plants was sampled on a
pair of basal leaflets from each
with distilled water and kept in
n to dissipate wound ethylene.

with a 3-ml syringe to determine CO_2 and ethylene production by the leaflets. Two separate plants were used as two replications for each treatment.

Treatment and sampling of detached leaflets

Two leaflets were selected randomly from a pool of leaflets of similar age obtained from several plants. The basal pair of leaflets of leaf number 1 on the main shoot of 3-month old plants was used. Three replications were used for each treatment. Petioles of the excised leaflets were cut back to about 2 cm under tap water and kept in jars for 3 h to dissipate wound ethylene. The leaflets were surface sterilized as above and allowed to take up LPE through the cut petiole for 5 h. This was done in a laminar flow hood with 25 μmol m^{-2} s^{-1} of cool white fluorescent light at the leaflet level, keeping the end of the cut petiole in LPE (100 mg l^{-1}) solution or in autoclaved tap water. At the end of 5 h, the petiole end was dried by blotting and leaflets were incubated in the dark in sealed side arm flasks for the determination of ethylene production and CO_2 evolution using the same procedure as described for attached leaflets. These measurements were repeated on the same leaflet pairs at 1, 2, 4, 6 and 8 days after the uptake of LPE. During the 8 days of the experiment, leaflets were kept under sterile conditions in the laminar flow hood under 25 μmol m^{-2} s^{-1} of cool white fluorescent light. Fresh autoclaved tap water was added to the jars every day to ensure moist conditions.

In another experiment, a similar procedure was followed except that leaflets were incubated in sealed jars after 4 h of water uptake through their petioles. The initial rate of ethylene production and CO_2 evolution was determined in the head space and this was designated as 100%. Leaflets were then treated with autoclaved tap water or LPE (100 mg l^{-1}) using the above procedure. Ethylene production and CO_2 were again measured after 1, 2, 4, 6 and 8 days. The experiment with detached leaflets was repeated with similar age leaflets except that the leaflets were incubated under sterile condition in the dark to accelerate senescence. CO_2 and ethylene measurements were made at 1, 2, and 4 days after the uptake of LPE by the leaflets.

Chlorophyll determination

Chlorophyll was extracted with absolute ethanol and then measured spectrophotometrically at 654 μm (Wintermans and Demots 1965). For the detached leaf ex-

Tab 1. Influence of LPE spray on the chlorophyll contents of leaves during senescence. Attached leaves were sprayed with different solutions and left to senesce on the plant. Measurements were taken by excising all leaves of the plant 12 days after the plants were sprayed. Values are means of 2 samples ± SE.

Treatments	Chlorophyll content [mg (g DW)$^{-1}$]
Control (1% ethanol)	8.3±0.3
LPE (100 mg l^{-1} with 1% ethanol)	10.3±0.5

for chlorophyll determination. The rest of the leaflet tissue was used for the determination of fresh/dry weight. Data presented are for total chlorophylls *a* and *b*.

Postharvest treatment of tomato fruits

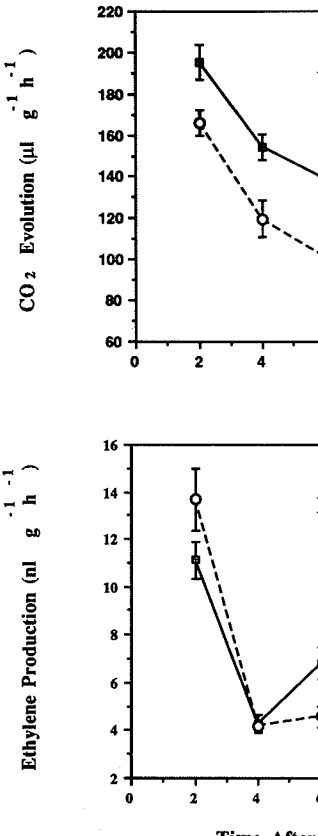
Tomato fruits at the red stage of physiological maturity were harvested. Fruits were detached with their pedicles which were dipped in tap water for 2 h to dissipate wound ethylene. After being washed with tap and distilled water, fruits were surface sterilized for 0.5 min with sodium hypochlorite (0.5%, v/v), washed again with autoclaved tap water, blotted dry, placed in wide mouth jars, flushed with sterile air and then left for a 3-h incubation. Each fruit was separately incubated in a jar. A gas sample was taken to detect their initial rate of ethylene production and respiration rate using gas chromatography. After the initial incubation, fruits were taken out and a fresh cut was made on the pedicle under sterilized tap water. Fruits were then allowed to take up either LPE (50 mg l^{-1}) or sterilized tap water from open glass vials via the pedicle cut surface. The solutions were replaced with fresh solutions every day. Fruits were incubated at room temperature in a laminar flow hood under 25 µmol m^{-2} s^{-1} of fluorescent light. After 5 and 8 days of incubation, fruits were temporarily removed and incubated for 4 h for the measurement of CO_2 and ethylene production. Each fruit was separately incubated in a sealed jar.

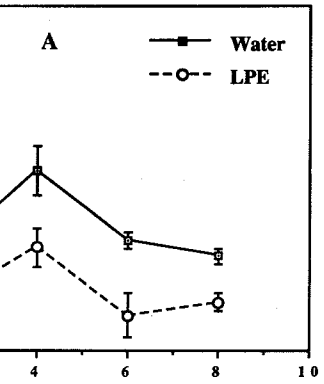
A disc 3.6 cm in diameter was taken from the pericarp and used for measuring the flesh firmness from inside to outside using an Effegi firmness tester with 1.1 cm plunger tip (McCormick Fruit, Inc., Yakima, WA, USA). The rest of the fruit was cut into 5 pieces and placed in a glass jar with 200 ml of deionized water for the measurement of electrolyte leakage.

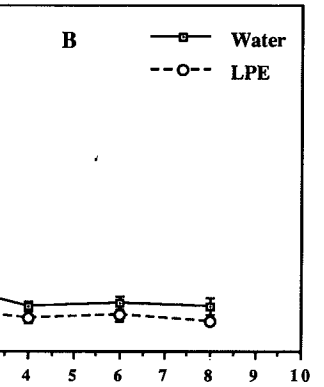
ductivity of the incubating solu
a conductivity meter (YSI m
Instrument, Inc., Yellow Sprin
solution was autoclaved for 20
temperature, the conductivity
electrolyte leakage was calcula
total (autoclaved tissue). A si
for the fruit tissue.

Measurement of ethylene and CO

A gas chromatograph equippe
detector and methanizer (Sh
Corporation, Kyoto, Japan) w
lene and CO_2 production, r
temperature was 40°C while th
tor temperatures were 150°C.
min^{-1}. A 1.2 m metal 80/100
lumn was used (Supleco, Inc.







ter Treatment (Days)

ethylene production and respira-
to leaflets during senescence at
eaflets were allowed to take up
petiole for 5 h. Leaflets were in-
he 8-day period except during the
means of 3 replications ± SE.

aflets

ent (12 days after spray appli-
ves had a higher chlorophyll
Tab. 1). Treated plants looked
ous than control plants. LPE-
ly had a lower rate of respira-
s of measurements (Fig. 1A).
to 15, 22 and 27.5% after 2, 4
pectively. There was a steady

Tab. 2. Chlorophyll content of excised tomato leaves as influenced by LPE treatment. Excised leaves were treated by dipping the petiole in various solutions for 5 h. Leaves were incubated in light. Measurements were made after 8 days of treatment. Values are means of 3 replications ± SE.

Treatments	Chlorophyll content [mg (g DW)$^{-1}$]
Control (water)	6.9±0.7
LPE (100 mg l^{-1})	11.6±0.7

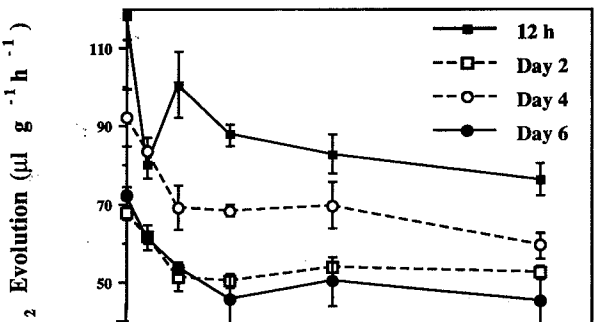
had a higher rate of ethylene production than did LPE treated leaves after 6 and 12 days.

Experiment with detached leaflets in light

The detached leaves incubated in light showed a climacteric-like pattern for respiration during the 8 days of measurements (Fig. 2A). The respiration rate declined for 2 days followed by a peak at 4 days after excision. The LPE-treated leaflets consistently maintained a lower rate of CO_2 evolution compared to the control; however, the pattern of CO_2 production was similar in the two treatments (Fig. 2A).

The climacteric-like pattern was not found with ethylene production (Fig. 2B). The rate declined rapidly after 1 day of treatment and then remained stable over 8 days. LPE-treated leaves had a lower rate of ethylene production only at 12 h after treatment. LPE-treated leaves had higher amounts of chlorophyll than did the control (Tab. 2).

The reduction in the rate of CO_2 evolution by LPE was concentration dependent (Fig. 3). In general, there was a sharp decrease in the rate at LPE concentrations between 0 to 50 mg l^{-1} except at the 12-h measurement time. During the early period of incubation (12 h) and the period of climacteric-like rise (4 days), there was a general decrease in the rate of CO_2 evolution with in-



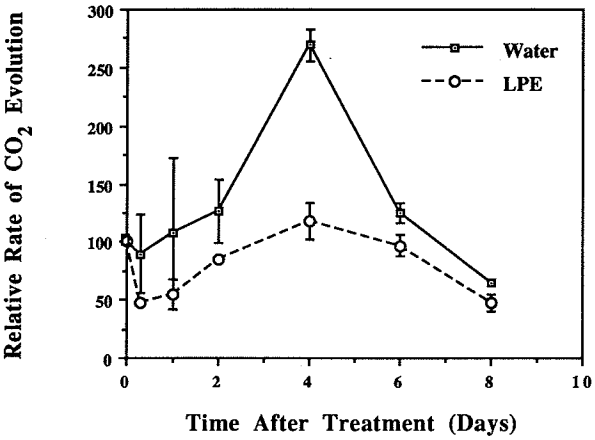


Fig. 4. Influence of LPE on the relative CO_2 evolution of tomato leaflets during senescence at 23 ± 2°C in light. Petioles of excised leaflets were first dipped in autoclaved tap water for 4 h, then enclosed in a side arm flask for the determination of their pretreatment respiration rate. Leaflets were allowed to take up autoclaved tap water or LPE through the petiole cut for 5 h. After the treatment, excised leaflets were allowed to senesce under conditions similar to those described in Fig. 2. Data are expressed relative to the pretreatment rate. All values are means of 3 replications ± SE.

creasing concentration of LPE up to 200 mg l^{-1}. At 2 and 6 days after treatment, the decrease in the rate of CO_2 evolution with LPE levelled off at about 50 mg l^{-1} (Fig. 3). In a separate experiment, the rate of CO_2 evolution was measured prior to treatment with LPE. The data on CO_2 evolution with and without LPE treatment, during the 8 days of incubation, are shown in Fig. 4. Throughout the measurement period of 8 days, LPE-treated leaflets maintained a lower rate of CO_2 evolution. The most dramatic reduction was during the climacteric-like rise with a maximum reduction at 4 days after incubation (Fig. 4). No ethylene could be detected in any of the treatments for 1–2 h of incubation period.

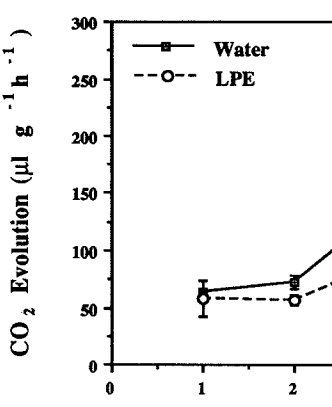
Experiment with detached leaflets in darkness

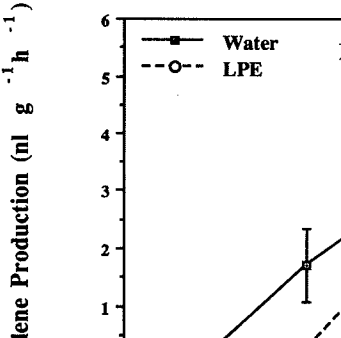
Dark-incubated detached leaflets showed a much faster rate of senescence than did the light-incubated leaflets. After 4 days of incubation, there was a 25% loss in fresh weight and 79% electrolyte leakage in the control leaflets incubated in the dark. (Tab. 3). The LPE-treated leaflets had lower loss in fresh weight (17%) and much lower electrolyte leakage (22.7% leaflets (Tab. 3). The respiration leaflets was lower than that of th especially at 4 days after incubat pared to water-treated leaflets, the LPE-treated leaflets was lo cubation and similar at 1 and 4 (Fig. 5B).

Tab. 3. Electrolyte leakage and loss in fresh weight of detached tomato leaves as influenced by LPE. Excised leaves were treated by dipping the petiole in various solutions for 5 h. Leaves were incubated in the dark. Measurements were made

Experiment with detached fruits

The relative rate of ethylene p declined dramatically during the ment from the plant (Tab. 4). T more pronounced in the fruits su compared to the fruits supplied v lar differences existed between days of incubation (Tab. 4). The by the fruits increased at 8 da tached from the plant (Tab. 4). treatment differences, LPE-tre slightly lower rates of CO_2 ev treated with water. At the end





nse of red tomato fruits to a continuous supply of LPE through the fruit pedicle. Values of ethylene tion are relative to initial reading (before the treatment began). Initial rates of ethylene production were r control and LPE, respectively. Initial rates for CO_2 evolution were 9.3 ± 0.7 and 10.4 ± 0.8 for control l values are means of 8 replications ± SE.

Ethylene production			CO_2 evolution			Pericarp firmness (N)	Electrolyte leakage (% of total)
Days of incubation							
0	5	8	0	5	8		
00	37.3±3.7	32.0±6.4	100	128.7±14.5	167.3±14.5	36.0±1.3	42.1±2.0
00	10.1±3.8	13.8±7.0	100	114.3± 7.4	145.3± 9.4	43.6±1.8	32.0±1.7

higher pericarp firmness and
age compared to fruits treated

nescence observed in our study
h has been reported by others in
bean leaves (McGlasson et al.
berman 1979). Attached leaves
uous decline in respiration dur-
cence and at the same time show
ved by a climacteric-like rise in
he detached leaves, on the other
decline in both respiration and
lowed by a climacteric-like rise.
ght-incubated detached leaflets
s in senescence than the dark-
se results are also consistent with
d Laetsch (1967), and Thimann

sent study show that LPE is able
attached leaves, detached leaves
tomato. Changes in respiration
ntent of leaf tissue are known to
ence (Tetley and Thimann 1974,
an 1979). LPE-treated leaflets
respiration rates (Figs 1A, 2A, 4
chlorophyll content (Tabs 1 and
ise in CO_2 and ethylene produc-
be associated with senescence in
asson et al. 1975). In the present
ly reduced the climacteric-like
n (Figs 4 and 5A). Leakage of
d to be associated with leaf se-
, 1959). This leakage has been
own of membrane lipids during
7, Draper and Simon 1971). Re-
te specific loss in membrane lip-

and senescence. We found that LPE-treated fruits had higher pericarp firmness (Tab. 4). Thus the results of our study provide evidence that LPE treatment can retard leaf and fruit senescence.

We don't know the mechanism by which LPE retards leaf and fruit senescence. Several reports (Thimann 1980, Garrison et al. 1984) have documented the role of cytokinins in retardation of senescence. In general, cytokinins have been found to reduce the rate of respiration and eliminate the climacteric-like rise of respiration. Thus cytokinins have been suggested to alter the senescence pattern in leaves (Tetley and Thimann 1974). However, LPE did not alter the pattern of senescence of attached or detached leaves under dark or light conditions. It appears, therefore, that the mechanism of retardation of senescence by LPE is not similar to that of cytokinins.

Lipids have been shown to be a target of degradation during plant aging and senescence. Extensive catabolism of membrane phospholipids during senescence has been reported in senescing flower petals (Sacher 1957). During aging and senescence, membranes are subjected to the increased action of some enzymes which leads to the release of linoleic and linolenic acids (Brown et al. 1987). It was also found that a parallel decrease in the amounts of PC, PE and phosphatidylglycerol and phosphatidic acid occurs during the postclimacteric stage of tomatoes (Guclu et al. 1989). PE, however, was the only phospholipid that showed a sharp reduction after the climacteric peak, while its proportion (% of total) did not vary greatly before or during the postclimacteric peak (Guclu et al. 1989). A reduction in PE (Guclu et al. 1989) and a release of free fatty acids (Brown et al. 1987) suggest an accumulation of LPE during senescence. It is possible that the presence of LPE can retard senescence by feed-back inhibition of some key enzymatic reactions associated with senescence. The inhibition of several mitochondrial enzymes by the lysolipid LPC supports this idea (Kalous et al. 1992).

manner. The results of the present study suggest a specific role of lysolipid LPE in aging and senescence.

Tomato fruit softening during senescence has been associated with the rise in the activity of polygalacturonase and pectinmethylesterase (Buescher et al. 1976, Tucker et al. 1982, Fisher and Bennett 1991). Our recent studies indicate that LPE can inhibit polygalacturonase activity extracted from tomato pericarp (Farag and Palta 1992). Thus, LPE could be retarding softening of tomato fruit by its impact on cell wall hydrolases. Further research is needed to demonstrate the specific role of LPE in senescence and fruit ripening.

Acknowledgements – This work was supported by the College of Agricultural and Life Sciences and by a grant from the Graduate School of the University of Wisconsin, Madison.

References

Aharoni, N. & Lieberman, M. 1979. Patterns of ethylene production in senescing leaves. – Plant. Physiol. 64: 796–800.

Bille, J., Weiser, T. & Bentrup, F. W. 1992. The lysolipid sphingosine modulates pyrophosphatase activity in tonoplast vesicles and isolated vacuoles from a heterotropic cell suspension culture of *Chenopodium rubrum*. – Physiol. Plant. 84: 250–254.

Brown, J. H., Lynch, D. V. & Thompson, J. E. 1987. Molecular species specificity of phospholipid breakdown in microsomal membranes of senescing carnation flowers. – Plant Physiol. 85: 679–683.

Buescher, R. W., Sistrunk, W. E., Tigchelaar, E. L. & Ng, T. J. 1976. Softening, pectolytic activity and storage life of *rin* and *nor* tomato hybrids. – HortScience 11: 603–604.

Burg, S. P. & Burg, E. A. 1965. Ethylene action and the ripening of fruits. – Science 148: 1190–1196.

Draper, S. R. & Simon, E. W. 1971. Changes in the free fatty acid content and respiratory activity during the senescence of cotyledons of cucumber. – J Exp. Bot. 22: 481–486.

Farag, K. M. & Palta, J. P. 1991a. Enhancing ripening and keeping quality of apple and cranberry fruits using lysophosphatidylethanolamine, a natural lipid. – HortScience 26: 67.

– & Palta, J. P. 1991b. Improving postharvest keeping quality of vine-ripened tomato fruits with a natural lipid. – HortScience 26: 162.

– & Palta, J. P. 1992. Evidence for a specific inhibition of the activity of polygalacturonase by lysophosphatidylethanolamine in tomato fruit tissue: Implication for enhancing storage stability and reducing abscission of the fruit. – Plant Physiol. (Suppl.) 99: 54.

Fisher, R. L. & Bennett, A. B. 1991. Role of cell wall hydrolases in fruit ripening. – Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 675–703.

Garrison, F. R., Brinker, A. M. & Noodén, L. D. 1984. Relative activities of xylem supplied cytokinins in retarding soybean leaf senescence and sustaining pod development. – Plant Cell Physiol. 25: 213–224.

lipids during ripening and sen
(*Lycopersicon esculentum*): Rel
ylene increases. – Physiol. Plan

Kalous, M., Rauchova, H. & Drah
lysophosphatidylcholine on the
chondrial enzymes. – Biochem.
171.

McGlasson, W. B., Poovaiah, B.
Ethylene production and respira
and in discs of fruit tissue of nor
Plant Physiol. 56: 547–549.

Noodén, L. D., Kahanak, G. M. &
tion of monocarpic senescence
cytokinin: an antidote for self-
841–843.

Oishi, K., Zheng, B. & Kuo, J. F.
potassium-ATPase and sodium
regulators sphingosine, lysopho
acid. – J. Biol. Chem. 265: 70–

Palmgren, M. G. & Sommarin, M
choline stimulates ATP depend
isolated oat root plasma memb
siol. 90: 1009–1014.

– , Sommarin, M., Ulvskov, P.
Modulation of plasma membr
roots by lysophosphatidylcholin
pholipase A_2. – Physiol. Plant.

– , Sommarin, M., Ulvskov, P. &
detergents on the proton-ATPas
right-side-out plant plasma mem
Biophys. Acta 1021: 133–140.

Pratt, H. K. & Goeschl, J. D. 1969
lene in plants. – Annu. Rev. Pl

Sacher, J. A. 1957. Relationship be
integrity in tissue senescence an
1199–1200.

– 1959. Studies of auxin-membra
fruit and leaf tissues. – Plant Pl

Scherer, G. & Andre, B. 1989. A
hormone: Auxin stimulates pho
in vitro. – Biochem. Biophys. R

Spencer, P. W. & Titus, J. S. 1972.
changes in apple leaf tissue dur
Plant Physiol. 49: 746–750.

Suttle, J. C. & Kende, H. C. 1980.
membrane integrity during pet
tia. – Plant Physiol. 65: 1067–1

Tetley, R. M. & Thimann, K. V. 19
leaves during senescence. I. Res
tabolism, and the action of cyto
294–303.

Thimann, K. V. 1980. The senesc
cence in Plants (K.V. Thiman
Press, Boca Raton, FL. ISBN

– , Tetley, R. M. & Krivak, B. M
leaves during senescence. V. S
Physiol. 59: 448–454.

Tucker, G. A., Robertson, N. G. &
fication and changes in activitie
isoenzymes. – J. Sci. Food Agr

Varga, A. & Bruinsma, J. 1974. T
tomato fruits at different levels
J. Hortic. Sci. 49: 135–142.