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Peroxidative Activity of Apple Peel in Relation to Development of Poststorage Disorders

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Lipid peroxidation is a factor in the development of plant tissue senescence (Thompson et al., 1991), and in responses to physiological stresses such as chilling (Parkin and Kuo, 1989). Apple (*Malus domestica* Borkh.) fruit are commonly stored for long periods at low temperature, and during this time a variety of disorders can develop, only some of which appear to be uniquely associated with senescence. Many apple cultivars are chilling sensitive, although several months near 0°C are required for symptoms to develop (Bramlage and Meir, 1990). During this time, both senescence and chilling injury can occur, and an interaction between these conditions could contribute to the development of certain disorders.

Superficial scald develops on the surface of certain apple cultivars following long-term storage near 0°C. The scald results from peroxidation of the sesquiterpene α -farnesene in fruit peel, producing conjugated trienes that presumably perturb membrane lipids under certain conditions, causing disruption, discoloration, and death of surface cells (Anet, 1974; Huelin and Coggiola, 1970a). The disorder is usually controlled by applying the antioxidant diphenylamine (DPA) to fruit before placing them in storage. DPA inhibits the oxidation of α -farnesene (Huelin and Coggiola, 1970b).

We recently presented evidence that only a portion of the conjugated trienes are positively correlated with scald development, while another portion is negatively correlated with it (Du and Bramlage, 1993), and that ethylene is fundamentally involved in development of conditions that help determine scald development (Du and Bramlage, 1994). Ethylene production is mediated by free radicals (Gardner and Newton, 1987), and ethylene regulates many aspects of fruit ripening and senescence (Abeles et al., 1992). Also, DPA treatment affects ethylene production, respiration, and enzyme activity (Baker, 1963; Lurie et al., 1989), as well as reducing α -farnesene oxidation in apple peel. Since lipid peroxidation is a prominent factor in apple senescence (Feys, 1985; Feys et al., 1980), we proposed that lipid peroxidation products may contribute to conditions that determine scald development, and possibly to symptom expression itself. To investigate this hypothesis, we sampled apple fruit under a variety of conditions related to scald development, and assayed them for some general peroxidation products, and for activities of enzymes that are important in controlling the accumulation of peroxidation products in plant tissues. We now detail this work as part of this workshop.

Plant materials

Investigations occurred over 3 years using fruit grown under normal orchard conditions at the Univ. of Massachusetts Horticultural Research Center, Belchertown.

On 26 Sept. and 10 Oct. 1990, $\approx$ 200 'Cortland' and 'Delicious' apples, respectively, were harvested from each of three single-tree replications at commercial maturity. Before storage, half of the harvested fruit of each cultivar was dipped in fungicide [227 g of 50% methyl 1-(butyl carbamoyl)-2-benzimidazocarbamate (benlate) and 454 g of 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1-*H*-isoindole-1,3(2*H*)-dione (captan) per 400 liters of water] as control, and the other half was dipped in 2 g DPA/liter plus fungicide. All fruit then were stored at 0°C in air. Ten-fruit samples were taken from each replicate at harvest and at every 4 weeks during storage, up to a maximum of 16 weeks. Sampled fruit were peeled, and the peel was cut into small pieces, mixed, frozen immediately in dry ice, and stored at -25°C for subsequent analyses. Scald incidence on the remaining fruit was evaluated visually after 16 weeks at 0°C plus 7 days at 20°C. No scald had developed on 'Delicious' at this time, so fruit were returned to storage for an additional 8 weeks plus 7 days at 20°C for a second scald evaluation.

On 30 Sept. 1991, $\approx$ 400 'Empire' fruit were harvested from each of four single-tree replications and stored at 0°C in air for 24 weeks. Ten-fruit samples were taken at 4-week intervals, peeled, and frozen for analyses as described above. After 24 weeks at 0°C, senescent breakdown was present in some fruit. Ten fruit with the disorder and 10 fruit free of it were selected from each replication. Peel was taken from disorder-free fruit as above, but diseased fruit peel was taken only from areas with symptoms. Peel was frozen for assay as above.

On 9 Sept. 1992, $\approx$ 200 'Cortland' fruit were harvested from each of four single-tree replications and stored at 0°C for 20 weeks. Stored fruit then were transferred to 20°C for 7 days and evaluated for scald development. Fruit within each replication then were sorted into three groups according to their scald intensity, i.e., no scald; 1% to 33% of fruit surface; and $>33\%$ of fruit surface affected with scald. Ten fruit without senescent breakdown were selected randomly from each group, peeled, and frozen for analyses.

Assays of lipid peroxidation products

Extraction. Ten grams of frozen peel were homogenized in a blender with 50 ml of cold acetone for 3 min and filtered through Whatman no. 4 paper. The filtrate was brought to 75 ml with water and kept at 4°C under refrigeration for measurements.

Measurements. Two groups of products of lipid peroxidation were measured in the present study. The thiobarbituric acid-reactive substances (TBARS) were measured using the thiobarbituric acid assay modified to adjust for the high sugar content of fruit [expressed as nmols of malondialdehyde (MDA) per gram of fresh weight; Du and Bramlage (1992)]. Total peroxides were determined according to Brennan and Frenkel (1977), modified as follows: 1 ml of properly diluted extract and 4 ml of water were added to 1 ml of titanium reagent (20% titanous tetrachloride in concentrated HCl, v/v). After mixing and

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standing for 5 min, the absorbance was measured at 415 nm against a blank that contained all components except the extract. A standard curve was made from commercial hydrogen peroxide (H_2O_2), and it gave good linearity for concentrations <2 mM. The total peroxides were expressed as nmoles of H_2O_2 per gram of fresh weight.

Assays of enzyme activities

Extraction. Two grams of frozen tissue was ground with a mortar and pestle in 10 ml of 50 mM phosphate buffer (containing 0.2% insoluble polyvinylpyrrolidone, 0.1 mM EDTA, and 3 mM MgCl_2 , pH 7.0), and a small amount of washed sand. The homogenate was centrifuged at $15,000 \times g$ for 15 min, and the supernatant obtained was used for assays of enzyme activities. Extract preparation was carried out at 0 to 4°C. Enzyme activities were expressed as units per gram of fresh weight.

Assay of catalase. Catalase activity was assayed by the ability to decompose H_2O_2 . Four milliliters of assay mixture (50 mM Tris buffer, pH 6.8, containing 5 mM H_2O_2) was added to 0.5 ml of extract. After 10 min at 20°C, the reaction was stopped by adding 0.5 ml of titanium reagent, as described above. The resulting solution was appropriately diluted and the residual H_2O_2 was determined as described above for total peroxides. A control, in which the enzyme activity was stopped at "zero" time, was run at the same time. One unit of catalase activity is defined as the amount of enzyme that decomposed 0.01 μmol of H_2O_2 per minute under the assay conditions.

Assays of peroxidase (POD) and polyphenoloxidase (PPO). POD and PPO activities were assayed essentially according to Kar and Mishra (1976). One unit of activity of POD or PPO is defined as the amount of the enzyme that caused an increase of 0.1 in the absorbance at 420 nm by the purpurogallin formed in the assay.

Assays of superoxide dismutase (SOD). SOD activity was measured according to Beauchamp and Fridovich (1971) and Dhindsa et al. (1981) by measuring the ability of SOD to inhibit the photochemical reduction of nitro blue tetrazolium (NBT). The assay mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 2 μM riboflavin, 0.1 mM EDTA, and 100 μl of enzyme extract. Three milliliters of the assay mixture in uniform, transparent tubes was shaken and placed 50 cm below a lightbank consisting of eight 15-W fluorescent lamps. The reaction was started by switching on the lights and was allowed to proceed for 10 min. The reaction then was stopped by switching off the lights, and the absorbance by the assay mixture at 560 nm was read. The diffuse room light had no measurable effect. The nonirradiated assay mixture did not develop color and served as control, and the assay mixture lacking enzyme extract developed maximum color, which decreased with increasing amount of extract added. One unit of SOD activity is defined as the amount of enzyme that inhibited the NBT photoreduction by 50% under the assay conditions. However, the percent inhibition was not linear with SOD concentration. Thus, units of SOD activity were calculated from transformed data as described by Asada et al. (1974) and Giannopolitis and Ries (1977). SOD units per milliliter = $(V_0/V - 1) \times (\text{dilution factor})$, where V_0 is the slope of the change in absorbance in the absence, and V in the presence, of enzyme extract. The transformation resulted in a linear correlation between the SOD activity and the amount of the extract used in this study [$V_0/V = 0.989 + 0.48 (\mu\text{l})$, $R = 0.951^{***}$].

Data were subjected to analyses of variance. Least significant difference or Duncan's new multiple range test was used to separate means when applicable.

Findings

Peroxidative characteristics in fruit peel during storage at 0°C were compared among three apple cultivars with different scald susceptibilities (Table 1). 'Cortland' and 'Delicious' are scald susceptible, while 'Empire' is scald resistant. 'Empire' fruit peel accumulated significantly more TBARS and peroxides during storage than did either 'Cortland' or 'Delicious' fruit peel, and 'Delicious' accumulated somewhat higher concentrations than did 'Cortland'. 'Empire' peel exhibited lower catalase, POD, and PPO activities than that of the

Table 1. Comparisons among cultivars of lipid peroxidation products and enzyme activities in apple peel and development of superficial scald after storage.^z

Measurement ^y	Cultivar		
	Cortland	Delicious	Empire
TBARS (nmol·g ⁻¹ fresh wt)	55 a ^x	79 b	102 c
Peroxides (nmol·g ⁻¹ fresh wt)	20 a	24 b	31 c
Catalase (unit/g fresh wt)	58 b	58 b	56 a
POD (unit/g fresh wt)	53 c	25 b	15 a
Total SOD (unit/g fresh wt)	248 c	51 a	157 b
PPO (unit/g fresh wt)	18 b	21 b	10 a
Scald (%)	23 b	20 b ^w	0 a

^z'Cortland' and 'Delicious' were harvested on 26 Sept. and 10 Oct. 1990, respectively, and stored for 16 weeks at 0°C. 'Empire' was harvested on 27 Sept. 1991 and stored for 24 weeks at 0°C. Each cultivar was sampled at 4-week intervals and data are means of all sample times.

^yTBARS = thiobarbituric acid-reactive substances; POD = peroxidase; SOD = superoxide dismutase; PPO = polyphenoloxidase.

^xMean separation within rows by Duncan's new multiple range test, $P \leq 0.05$.

^w'Delicious' did not scald following 16 weeks at 0°C and was returned to storage for an additional 6 weeks. Scald developed during subsequent 7 days at 20°C.

Fig. 1. Changes in (A) concentrations of peroxidation products and in (B) activities of certain enzymes in 'Delicious' apple peel during storage at 0°C. ns, **, ***Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively, for linear regressions over time.

Table 2. Effects of diphenylamine (DPA) applied at 2 g-liter⁻¹ before storage on accumulation of peroxidation products, enzyme activity, and superficial scald development on apples after storage.^z

Measurement ^y	Cortland		Delicious	
	-DPA	+DPA	-DPA	+DPA
TBARS (nmol·g ⁻¹ fresh wt)	55	54	78	80
Peroxides (nmol·g ⁻¹ fresh wt)	22	17	24	20
Catalase (unit/g fresh wt)	83	39	70	45
POD (unit/g fresh wt)	55	32	27	22
Total SOD (unit/g fresh wt)	248	267	51	45
PPO (unit/g fresh wt)	18	18	24	18
Scald (%)	20	1	20	5

^z'Cortland' and 'Delicious' were harvested on 26 Sept. and 10 Oct., respectively, and stored for 16 weeks at 0°C. Fruit were sampled every 4 weeks, and data are the means of all sample times.

^yTBARS = thiobarbituric acid-reactive substances; POD = peroxidase; SOD = superoxide dismutase; PPO = polyphenoloxidase.

^xScald did not develop on 'Delicious' after 16 weeks at 0°C. Fruit were returned to 0°C for an additional 6 weeks, and scald developed during the subsequent 7 days at 20°C.

ns, **, ***Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively. Significance of DPA treatment effect for a cultivar and measurement.

other cultivars, and 'Empire' and 'Delicious' peel had lower SOD activity than did that of 'Cortland'. Despite the indications of higher peroxidative activity in 'Empire' peel during storage, these fruit developed no scald after storage, while the disorder occurred on the other two cultivars.

During storage of 'Delicious' at 0C, slow but significant linear increases in TBARS and peroxides occurred over time (Fig. 1). Catalase, POD, and PPO activities also increased significantly over time, but SOD activity did not change significantly (Fig. 1). Similar results were recorded for 'Cortland', except that SOD activity declined 75% during 16 weeks at 0C (data not shown). In 'Empire', only TBARS and catalase increased significantly during storage, while SOD activity decreased slowly (data not shown).

DPA applied before storage slightly reduced peroxide concentrations in peel of both 'Cortland' and 'Delicious' apples during storage but had no effect on TBARS concentrations (Table 2). DPA also markedly reduced catalase activity in both cultivars, slightly reduced POD and PPO activities in 'Delicious', but had no effect on SOD activity in either cultivar. Treatment reduced scald development in both cultivars.

'Cortland' apples at 20C developed no scald, but after 24 weeks of storage at 0C, about one-third of the fruit had developed the disorder (Table 3). At 20C, TBARS and peroxides were higher after 1 week than at harvest, but after 2 weeks, they were at the same levels as at harvest. This transient increase is associated with the onset of ripening (Du, 1993), but it was not accompanied by significant changes in activity of any of the measured enzymes. At 0C, no significant change in either TBARS or peroxides was evident after 12 or 24 weeks, while catalase and POD activities increased during storage. Overall, temperature had no significant effect on peroxidative characteristics other than inducing higher POD activity at 0C than at 20C.

Peel from 'Cortland' apples with different amounts of superficial scald on their surfaces had similar peroxidative activity after storage for 20 weeks at 0C (Table 4). There was no significant difference in peroxidative activity of peel between scald-free and scalded fruit.

During storage of 'Empire' apples, some fruit developed symptoms of senescent breakdown: soft, brown areas with dark vascular strands developing just beneath the peel, primarily near the calyx end

of the fruit. When peel from above these areas ("senescent") was compared with peel from disorder-free fruit ("healthy"), marked differences in peroxidative activity were evident (Fig. 2). Senescent tissue contained much higher concentrations of TBARS and peroxides, and higher activities of catalase, POD, and SOD, but there was no difference in PPO activity.

Implications

During storage of 'Delicious' apples at 0C, concentrations of TBARS and peroxides gradually increased (Fig. 1), and peroxidation products also increased in 'Cortland' and 'Empire' (data not shown). Since temperature had no significant effect on TBARS or peroxides (Table 3), the increases in apples during storage apparently reflected effects of fruit senescence and were not the result of chilling damage. The suggestion that lipid breakdown results from some combination of chilling and senescence (Parkin et al., 1989) also appears unlikely.

Since plant tissues possess complex systems that control buildup of oxidation products (Winston, 1990), these assays gave limited insight into cellular processes. Catalase, POD, and SOD activities were assayed to see if substantial changes occurred in protecting tissues from peroxide accumulation. Catalase activity increased substantially in fruit peel during storage (Table 3, Fig. 1); the only indications of a marked loss of protection were a 75% reduction in SOD activity in 'Cortland' peel during 16 weeks at 0C (data not shown), and a possible reduction of SOD activity in 'Empire' (Table 3). Nevertheless, the TBARS and peroxide data suggest that control systems in apple peel largely kept peroxidative activities in check during prolonged storage of the fruit, and that overall changes were remarkably slow.

Superficial scald is induced soon after harvest, but its symptoms are not expressed until fruit have remained in storage for 3 or more months (Bramlage and Meir, 1990). During this extended storage time, conjugated trienes usually increase in fruit peel, and scald development is positively correlated with maximum conjugated triene concentration (Huelin and Coggiola, 1968). Our hypothesis was that lipid peroxidation products in tissue increase over time, to a point where they contribute to development of scald symptoms. No general relationship existed between scald susceptibility and concentration of peroxidation prod-

Table 3. Effects of temperature on accumulation of lipid peroxidation products, enzyme activity, and superficial scald development on 'Cortland' apples.

Time of measurement	TBARS ^z (nmol·g ⁻¹ fresh wt)	Peroxides (nmol·g ⁻¹ fresh wt)	Catalase (units/g fresh wt)	POD ^z (units/g fresh wt)	Total SOD ^z (units/g fresh wt)	PPO ^z (units/g fresh wt)	Scald (%)
At harvest	53	19	29	41	155	25	---
20C, 1 week	75	27	51	37	108	23	---
20C, 2 weeks	51	19	65	40	100	22	2 ^y
0C, 12 weeks	52	20	58	54	98	23	---
0C, 24 weeks	52	17	77	60	68	27	34
LSD _{0.05}	20	8	48	14	89	9	16
0C vs. 20C	NS ^s	NS	NS	*	NS	NS	***

^zTBARS = thiobarbituric acid-reactive substances; POD = peroxidase; SOD = superoxide dismutase; PPO = polyphenoloxidase.

^ySlight scald-like symptoms were present on a few fruit at harvest, but did not become more prevalent during the postharvest period.

^sThis comparison indicates only the main effects of temperature.

NS, *, *** Nonsignificant or significant at $P \leq 0.05$ or 0.001, respectively.

Table 4. Concentrations of lipid peroxidation products and activities of certain enzymes in 'Cortland' apple peel affected with different intensities of superficial scald.^z

Surface affected with scald (%)	TBARS ^y (nmol·g ⁻¹ fresh wt)	Peroxides (nmol·g ⁻¹ fresh wt)	Catalase (units/g fresh wt)	POD ^y (units/g fresh wt)	Total SOD ^y (units/g fresh wt)	PPO ^y (units/g fresh wt)
None	62	22	35	27	36	11
1 to 33	57	22	21	24	40	12
34 to 100	55	22	21	26	35	12
Significance	NS	NS	NS	NS	NS	NS

^zFruit were harvested on 9 Sept. 1992, and stored at 0C for 20 weeks, plus 1 week at 20C.

^yTBARS = thiobarbituric acid-reactive substances; POD = peroxidase; SOD = superoxide dismutase; PPO = polyphenoloxidase.

NS Nonsignificant.

Fig. 2. Comparisons of concentrations of lipid peroxidation products and enzyme activities between peel of 'Empire' apples above areas with senescent breakdown ("senescent") and peel from fruit free of senescent breakdown ("healthy") after storage at 0C for 24 weeks. Mean separation within pairs at $P \leq 0.05$.

ucts. Scald-resistant 'Empire' apples contained higher TBARS and peroxide concentrations than scald-susceptible 'Cortland' and 'Delicious' (Table 1). In addition, there was no difference in TBARS or peroxide concentrations in 'Cortland' at scald-inducing and noninducing temperatures (Table 3), and no sudden increase occurred as 'Cortland' apples became liable to scald development at removal from storage (data not shown). However, the increase in peroxidation products (Fig. 1) may have been associated with the increase of conjugated trienes (Huelin and Coggiola, 1968), or with changes in cellular conditions that result in symptom development.

When the lipid-soluble antioxidant DPA was applied to apples at harvest, it reduced the peroxide concentration in apple peel during storage, despite reducing catalase and peroxidase activities (Table 2). This might have resulted from significantly reduced ethylene production (Du and Bramlage, 1994), but it also may indicate that DPA significantly supplemented the endogenous antioxidant concentrations in the peel. Barden and Bramlage (1994b) found that water-soluble antioxidants in apple peel decreased during fruit storage, and that among different lots of fruit, differences in lipid-soluble antioxidants at harvest usually persisted as levels increased during storage. These at-harvest differences among fruit lots were negatively correlated with scald development (Barden and Bramlage, 1994a). Applied DPA may reduce scald by contributing directly, as a lipid-soluble antioxidant, to cellular resistance to initiation of scald symptoms, perhaps by inhibiting fruit senescence (Lurie et al., 1989) or α -farnesene oxidation to conjugated trienes (Huelin and Coggiola, 1970a).

Our results provide no insight into events during scald symptom development. Tissue with different levels of symptom expression contained TBARS and peroxide concentrations equal to those in scald-free tissue, and equal enzyme activities (Table 4). PPO activity was equal in peel regardless of symptom development (Table 4), and no difference existed in PPO activity among cultivars (Table 1) or between temperatures (Table 3). Lurie et al. (1989) suggested that DPA may reduce scald symptom development by maintaining low PPO activity. We found only slight increases of PPO activity during fruit senescence (Fig. 1), and slight reduction of PPO by DPA (Table 2).

Peel over tissue with senescent breakdown had markedly different peroxidative characteristics than that from breakdown-free fruit

(Fig. 2). These results also suggest that development of scald symptoms is distinct from general cell breakdown and discoloration.

In summary, our work provides no evidence that marked changes in peroxidative activity of apple peel are related to scald development in apples. However, it does not preclude the possibility that lipid peroxidation is related to scald development in more subtle ways, especially in the susceptibility of cell membranes to perturbation by conjugated trienes, which may produce cellular disorganization and symptom expression.

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Release of Fluorescent Peroxidized Lipids from Membranes in Senescing Tissue by Blebbing of Lipid-Protein Particles

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Several lines of evidence indicate that peroxidation of membrane lipids is an inherent feature of ripening and senescence of plant tissues. Dhindsa and Dhindsa (1981), for example, demonstrated a correlation between lipid peroxidation and increased membrane permeability in senescing leaves. Levels of H_2O_2 and lipid hydroperoxides also increase in ripening fruit, and Frenkel (1978) has reported that ripening can be delayed by inhibiting the formation of H_2O_2 . Pear (*Pyrus communis* L. var. Bartlett) and banana (*Musa cavendishii* L. var. Valery) fruit contain fluorescent products of lipid peroxidation that increase during natural or ethylene-induced ripening (Maguire and Haard, 1975). Senescing leaves and cotyledons also accumulate fluorescent peroxidation products (Wilhelm and Wilhelms, 1981), and evidence shows that they are localized in membrane lipid bilayers (Pauls and Thompson, 1984).

Fluorescent peroxidized lipids are a family of compounds with a characteristic Schiff base structure ($-N=C-C=C-N$) that is formed by the reaction of aldehydes from peroxidized lipids with free amino groups (Tappel, 1975). Schiff bases formed from malondialdehyde and amino compounds have characteristic fluorescence spectra that feature an excitation maximum at ≈ 360 nm and an emission maximum at ≈ 430 nm (Fig. 1), and are thus similar to the lipofuscins known to accumulate in animal tissues during aging (Tappel, 1975). Fluorescent peroxidized lipids appear to be formed in membrane bilayers (Pauls and Thompson, 1984), and if they were to accumulate therein, they could be expected to disrupt membrane structural integrity. Indeed, the accumulation of peroxidized lipids in senescing membranes may contribute to loss of membrane function as tissues age (Thompson et al., 1987).

In this report, we describe evidence indicating that fluorescent peroxidized lipids are released from membranes by blebbing (pinching of surface protrusions) of lipid-protein particles from the membrane surface. The data presented have been obtained from studies with senescing bean (*Phaseolus vulgaris* L.) cotyledons grown under etiolation conditions and senescing carnation (*Dianthus caryophyllus* L.) flower petals. These lipid-protein particles have been partially characterized previously (Yao and Thompson, 1993; Yao et al., 1991a, 1991b, 1993a), are present in plant and animal tissue (Yao et al., 1991a, 1993b), and have been tentatively termed deteriosomes to denote their putative role in removing lipid and protein catabolites from membranes (Yao et al., 1991a). Lipid and protein catabolism is a normal feature of membrane turnover, and any significant accumulation of these catabolites in the bilayer could have a disruptive effect on membrane structure. Recent evidence suggests that these catabolites

are selectively removed by blebbing of lipid-protein particles from the membrane surface (Yao and Thompson, 1993; Yao et al., 1991a, 1991b, 1993a). Impairment of this blebbing process with advancing senescence appears to result in an accumulation of lipid catabolites in senescing membranes that causes bilayer destabilization and loss of membrane function (Yao et al., 1991b).

Peroxidized lipid accumulation in senescing membranes

Fluorescence spectra of lipid extracts from microsomal membranes of cotyledons at various stages of senescence all show excitation maxima at 370 nm and emission maxima at 430 nm (Fig. 1). The

Fig. 1. Excitation (A) and emission (B) spectra for lipid extracts of microsomal membranes isolated from senescing bean cotyledons (age in days). (●) young, 2 days old; (▲) 4; (■) 7; (solid hexagon) 9. At 4 days, the cotyledons are beginning to show visible manifestations of senescence, and by 9 days, they are extensively senescent (data are from Pauls and Thompson, 1984). Inset: corresponding excitation spectra for lipid extracts of microsomal membranes from the petals of carnation flowers. (●) Young, stage 2 flowers (petals fully expanded and flowers fully open with yellow-tinted centers); (○) senescing stage 4 flowers (petals showing pronounced inrolling).

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