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Age-Related Changes in Petal Membranes from Attached and Detached Rose Flowers

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ABSTRACT

Changes in petal membrane properties during aging were studied in cut and in attached rose flowers (*Rosa hybrida* L., cv Mercedes). Both cut and attached flowers exhibited a growth phase characterized by an increase in fresh weight and an accumulation of membrane components. The growth phase, which was more pronounced in the attached than in the cut flowers, was followed by a senescence phase, characterized by a decrease in fresh weight and a decline in membrane components. In cut flowers, both the growth and the senescence phases were accompanied by a decrease in membrane fluidity and in the ratio of unsaturated to saturated fatty acids, but the ratio of sterol to phospholipid increased. In attached flowers, while both the membrane fluidity and the sterol-to-phospholipid ratio remained unchanged during the growth phase, the senescence phase was accompanied (as in cut flowers) by a decrease in membrane fluidity and an increase in the sterol-to-phospholipid ratio. Unlike in cut flowers, however, the age-related changes in the ratio of unsaturation of fatty acids were not correlated with those of fluidity. Changes in the saturation of phospholipid acyl chains are commonly thought to influence membrane fluidity. Our observations question this view and suggest instead that the ratio of sterol to phospholipid may play the major role in maintaining membrane lipid fluidity.

The development and senescence of both cut and attached rose flowers have been extensively studied (3). Flower aging is accompanied by typical changes in petal color, shape, fresh weight, and metabolic processes. The gradual loss in fresh weight is a characteristic symptom of flower senescence, finally leading to wilting (3, 8). Age-related alterations in the water content of the flower are widely thought to be a result of changes in the ability of the membranes to retain solutes within the cell (3, 8, 14).

In all plant tissues studied so far, aging cell membranes exhibit gradual changes in the physical properties of their component lipids, including a decrease in their fluidity (3) and an increasing proportion of lipid domains in the gel phase (6, 14). These changes precede the loss in the capacity of the membranes to act as a hydrophobic barrier (6).

During aging of flowers, changes in the physical properties of the membranes reflect changes in their composition as a result of alterations in metabolic processes (3). Senescence is accompanied by a decrease in the membrane phospholipid content, leading, in turn, to an increase in the sterol-to-phospholipid ratio (1, 3). A concomitant increase in saturation of the phospholipid acyl chains has also been reported (7, 12). The above changes have been found to correlate well with the age-related decrease in membrane lipid fluidity (3).

In the present work, the aging processes in petal membranes were studied using both cut and attached rose flowers, which are known to age at different rates (8). In particular, the composition of the membranes during the life-span of the flower was analyzed in detail.

MATERIALS AND METHODS

Plant Material

Rose flowers (*Rosa hybrida* L., cv Mercedes) at developmental stage A (stage C in ref. 5) were either picked or tagged and left on plants grown at 26/18°C. After 2.9 ± 0.1 d on average, when the outer petals had turned bright red-orange and were reflexed at 90° to the flower stem, the flowers were considered to be at stage B. At stage C (5.3 ± 0.3 d after stage A), all the petals were rolled outwards, petal discoloration could be detected, and yellow anthers were visible. Stage D, at which the petals showed advanced discoloration and the yellow center had turned white, occurred 15.9 ± 0.7 d after stage A.

Cut flowers were trimmed to 6 cm and were held in deionized water at 22 ± 1°C, RH 55 ± 5% under continuous light from a fluorescent lamp (cool white) at 1.4 W × m^{-2}.

Membrane Preparation

Microsomal membranes were isolated from flower petals essentially as described previously (11), except that a chilled mortar and pestle were used instead of a blender. The buffer was as described by Yoshida (16) but without BSA and $K_2S_2O_5$. The membrane pellet was resuspended in 10 mM Mops-KOH, pH 7.6. Total membrane fraction was prepared under the same conditions, but centrifugation was according to Fobel *et al.* (7). Protein was determined according to the method of Bradford (4).

Fluidity Measurements

Microsomal membranes (25 μg protein per ml) were labeled as described previously with the lipophylic fluorescent probe, 1,6-diphenylhexatriene, at a final concentration of 1 μM (1, 2). The fluorescence polarization of the 1,6-diphenylhexatriene-labeled membranes was measured using a spectrofluorimeter (SLM model 4800) at room temperature. Excitation was at 350 nm and emission was measured with cutoff filters at 420 nm.

Lipid Extraction and Analysis

Lipids were extracted from membrane suspensions using chloroform:methanol, and liposomes were prepared from the lipid extracts as described previously (2). Phospholipids in the extract were determined following perchlorate oxidation using Fiske-Subbarow reagent (2). Fatty acids in the lipid extract were methylated for 30 min at 90°C using trimethylanilinium hydroxide in methanol (MethElute reagent, Pierce) and analyzed by gas chromatography using a mixture of known fatty acids as a standard. The sterol content was measured by the cholesterol oxidase method (Boehringer Mannheim) using cholesterol as a standard (10). Sterols were quantitatively analyzed by gas chromatography following silylation with pyridine and *N-O*-bis(trimethylsilyl)trifluoroacetamide (Pierce) at room temperature for 12 h, using a mixture of known sterols as a standard.

Experimental

The curves represent the mean values obtained from the sum of six to eight (Fig. 1) or two (Figs. 2 and 4–6) separate experiments.

RESULTS AND DISCUSSION

Flower Morphology

As the petals of the attached flowers developed and enlarged, they reflexed outward, so that the whole flower increased in size. Concomitant with this, their color gradually faded from bright red-orange to paler orange shades. In cut flowers following harvest, the changes occurring as the flowers opened were more pronounced in their shape than in their size. There was only limited enlargement of the petals, especially of the younger inner petals, so that the open flower was characteristically cup-shaped. As was clearly indicated, in terms of retarded growth, the younger petals are more affected by detachment of the flower than are the outer, more mature petals. The petals slowly faded to a bluish color. The attached flowers lasted for much longer than the cut flowers (*ca.* 16 d as compared with *ca.* 8 d in the spring; their longevities may vary with the growing season).

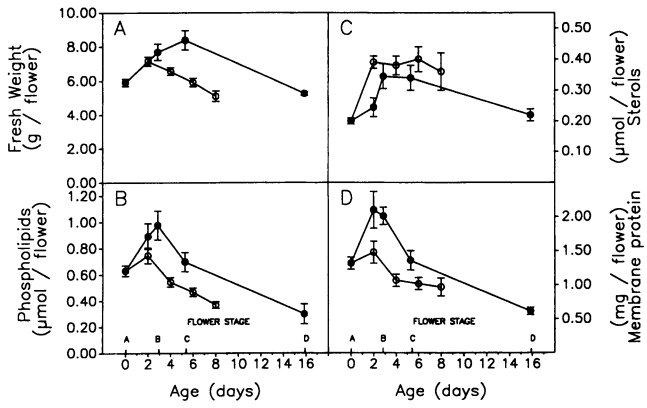

Figure 1. Fresh weight of petals (A), phospholipid (B), sterols (C), and protein (D) content of microsomal membranes isolated from petals of attached (●) and cut (○) Mercedes roses at different developmental stages. Bars, 2 SE (n = 12).

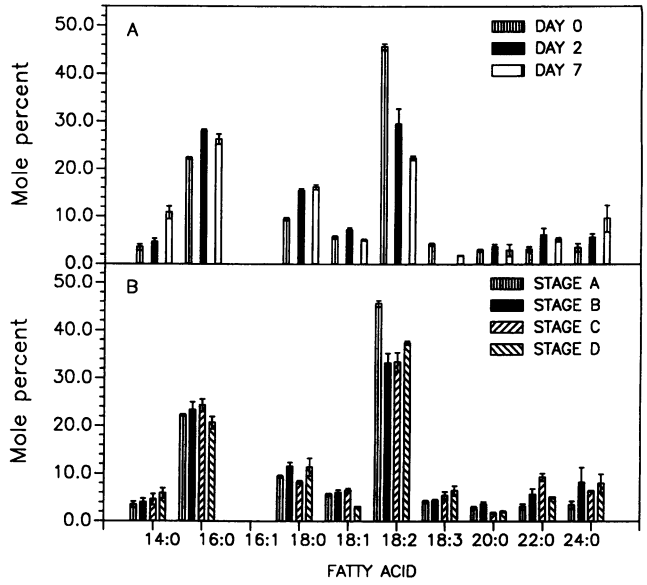

Figure 2. Phospholipid fatty acids of microsomal membranes isolated from petals of cut (A) or attached (B) Mercedes roses at different developmental stages. Bars, SE (n = 3).

Changes Related to Development

Senescence in flowers is usually timed from harvest or the closed bud stage (stage A in the present study) until the appearance of advanced symptoms of aging. In the case of the attached flowers, analysis of the data revealed two distinct phases. The first was characterized by an increase in fresh weight and in the content of phospholipids, sterols, and membrane proteins (Fig. 1, A–D), clearly indicating growth. This was followed by a second phase in which these parameters gradually declined. It thus seems that the onset of senescence follows a growth phase. Cut flowers showed similar trends, except that the sterol content remained constant during the second phase (Fig. 1C). The membrane parameters presented so far were obtained through analysis of microsomal membrane fraction. This fraction includes everything that pellets upon centrifugation between 10,000 and 100,000*g*, thus it is heterogeneous. The measured changes have been questioned on occasion as reflecting changes in composition of the microsomal fraction with age. However, this prospect was ruled out previously in a study that demonstrated comparable changes in fluidity with advancing senescence in a total membrane fraction (the membranes obtained by centrifuging petal homogenates at 131,000*g* for 3 h) as well as in a microsomal fraction (7). In the present study we have used the same strategy and found that as the flowers aged the mole ratio of sterols to phospholipids in total membranes increased from 0.117 for stage A flowers to 0.189 in flowers aged for 2 d. Additional calculations indicated a rise in the ratio of sterols to membrane protein and a decline in the ratio of phospholipids to membrane proteins in the microsomal mem-

branes as well as in the total membrane fraction during the senescence of the cut flowers.

On the basis of these findings we suggest that the postharvest period during which flower senescence is evaluated should be treated as two separate phases rather than one as in many studies (3, 8).

Fatty Acid Composition

During the initial growth phase in both cut (0–2 d) and attached (stage A–stage B) flowers, an increase was observed in the phospholipid content (Fig. 1B). This most likely reflects an increase in the associated fatty acids. During this phase there was an increase in the mole percent of all fatty acids except linoleic acid, which showed a reduction (Fig. 2). Thus, the data also point to the occurrence of an overall net saturation process in the phospholipid fatty acids during the growth phase. In contrast, during the senescence phase, different trends were observed in cut flowers (between 2 and 7 d) and in attached flowers (between stages B and D). In cut flowers, the proportion of unsaturated fatty acid content (18:1, 18:2) gradually declined (Fig. 2), while there was a limited increase in the mole percent of saturated fatty acids. Since the senescence phase was characterized by a marked reduction in phospholipids, it seems likely that the relative saturation effect resulted from a selective degradation of unsaturated fatty acids, probably as an outcome of the action of lipoxygenase (7, 14). In attached flowers, although the phospholipid content also showed a decline, the mole percent of either saturated or unsaturated remained unchanged (Fig. 2). This suggests that the pathways of phospholipid degradation in cut and in attached rose flowers are different.

In both cut and attached flowers, the relative proportion of long-chain fatty acids (20:0, 22:0, 24:0) increased with age from *ca.* 12% to *ca.* 20% of the total fatty acid content (Fig. 2). The occurrence of these fatty acids in flower petals has not been previously reported. Long-chain fatty acids require relatively more energy in order to exist in the crystal-liquid state, and might therefore rigidify the membranes (9). This would be consistent with the decrease in membrane fluidity found to occur during senescence.

As in carnation petals (15), GLC analysis revealed that sitosterol was the major sterol component, accounting for more than 95% of the sterols at all stages analyzed in both cut and attached flowers except for a temporary increase of campsterol in attached flowers at stage C (data not shown).

Lipid Fluidity

Changes in membrane composition determine the physical properties of plant membrane lipids, including fluidity (3, 14). The latter is known to be correlated with various membrane functions (3, 13). Another difference between attached and cut flowers was evident in their patterns of change in membrane fluidity. In microsomal membranes isolated from cut flower petals the fluidity gradually decreased with age (Fig. 3), whereas in membranes from attached flowers the fluidity remained constant during the first 2 d and then began to decrease, reaching the same values at wilting (*ca.* 0.22) as those obtained in cut flowers. Similar changes of fluidity were obtained with preparation of total membranes (data not shown).

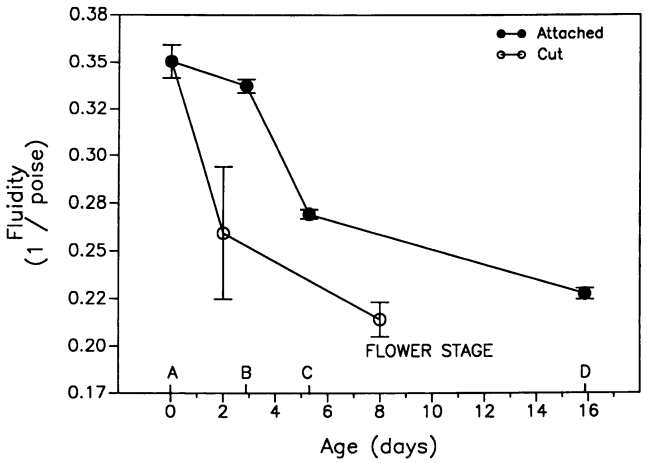

Figure 3. Fluidity of microsomal membranes isolated from petals of attached and cut Mercedes roses at different developmental stages. Bars, 2 SE (n = 3).

The decline in membrane fluidity either as a regulatory factor or as a symptom has been described in many studies of flower senescence (3). In cut flowers we found that the decline in fluidity spanned both phases, namely growth and senescence. In attached flowers, however, where growth processes are apparently more intensive, fluidity remained constant during the growth phase and declined only as the flowers continued to develop and senesce (Fig. 3). This suggests that the decrease in fluidity may signify the termination of growth processes and the onset of senescence. The two sets of processes may overlap, in the sense that as the one tails off the other emerges.

A number of possible factors might explain the observed decrease in membrane fluidity (13). The increase in the protein-to-lipid ratio does not appear to play a major role, since the fluidity of liposomes prepared from lipids extracted from membranes isolated from petals of different ages showed the same age-related changes as the membranes (Table I).

Analysis of the changes in fatty acids species revealed that in cut flowers the ratio of unsaturated to saturated fatty acids decreased with the decline in fluidity (Fig. 4). This would appear to support the notion that processes leading to saturation of fatty acids, also related to senescence (13, 14), may affect the pattern of change in fluidity (7). In attached flowers, however, the decrease in the ratio of unsaturated to saturated

Table I. *Fluidity of Liposomes*

Liposomes were prepared from lipids extracted from membranes isolated from petals of cut Mercedes roses, which were held in water for different time periods (n = 3).

Flower age	Liposome fluidity (1/poise ± SE)
Time zero	0.33 ± 0.03
2 d	0.24 ± 0.01
7 d	0.20 ± 0.01

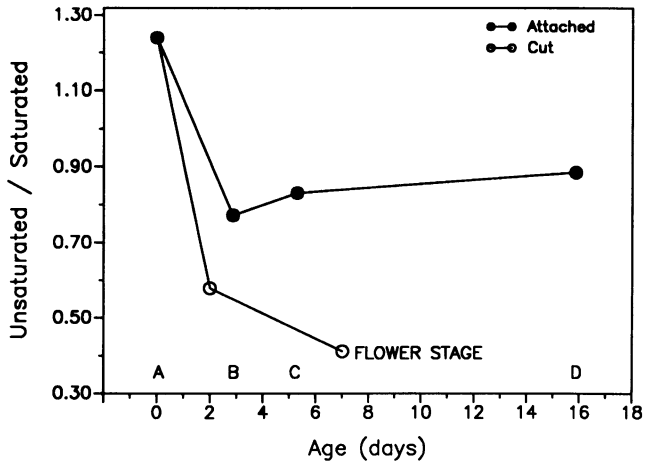

Figure 4. Ratio of unsaturation to saturation in phospholipid fatty acids of microsomal membranes isolated from petals of attached and cut Mercedes roses at different developmental stages.

fatty acids occurred between stage A and stage B, during which time the fluidity remained constant (Fig. 3). With the further development of the flower, while fluidity continued to decrease the ratio of unsaturated to saturated fatty acids remained constant. It follows that at least during senescence of attached flowers, *i.e.* during natural senescence, the ratio of unsaturated to saturated fatty acids is not an influential factor.

In both cut and attached flowers, an increase followed by a decrease in the content of sterols and of phospholipids was observed. However, while in cut flowers this ratio of sterols to phospholipids increased, as also reported earlier (1, 2), it remained almost unchanged in attached flowers between stages A and B and started to increase only later (Fig. 5). This

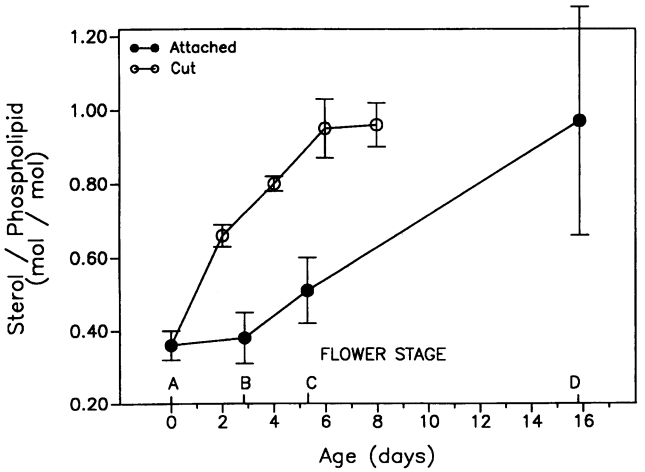

Figure 5. Mole ratio of sterol to phospholipid in microsomal membranes isolated from petals of attached or cut Mercedes roses at different developmental stages. Bars, 2 SE (n = 4).

increase in the ratio of sterols to phospholipids was also observed in total membrane fraction as outlined above. These results clearly point to the involvement of the sterol-to-phospholipid ratio in influencing fluidity.

Our observations question the widely held view that saturation of the phospholipid acyl chains is a general regulatory mechanism of the membrane fluidity during flower aging. Instead, they suggest that the sterol-to-phospholipid ratio may play the major role in this mechanism.

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