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Physiological Changes Accompanying Senescence in the Ephemeral Daylily Flower

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ABSTRACT

The daylily flower, *Hemerocallis* hybrid cv Cradle Song, develops from the opening bud to full senescence in 36 hours. Unlike other ephemeral flowers studied to date, it does not respond to ethylene, but other senescence phenomena are similar. There was a small respiration climacteric coinciding with early flower senescence, and it was also observed in isolated petals and petal slices. Cycloheximide abolished the climacteric and delayed senescence in all three systems. Petal apparent free space increased from 30% at bud opening to 38% at the onset of senescence, and sugar efflux increased from 0.2 to 2.8 milligrams per gram of fresh weight per hour during the same period. A sharp increase in ion efflux from 0.8 to 4.0 micromoles of NaCl equivalents per gram of fresh weight per hour, coinciding with the climacteric, was abolished by cycloheximide. Uptake of radiolabeled inorganic phosphate by petal slices from 100 micromolar solution increased during onset of senescence from 6 to 10 nmoles per gram of fresh weight per hour. Half was esterified; of this, 14% went into ATP, and the cellular energy charge remained high at 0.86 during senescence. The proportion incorporated into phospholipid (2.2%) did not change during senescence, but the proportion in phosphatidyl choline increased and in phosphatidyl glycerol decreased during senescence. The general phosphate ester pattern in presenescent slices closely resembled that in other plant tissues except that phospholipid precursors were more prominent (approximately 20% of total organic ^{32}P *versus* 5%). In senescent slices, the proportion of hexose phosphates decreased from 40 to 15% of total organic ^{32}P and that of phospholipid precursors increased to approximately 50%, suggesting that phospholipid synthesis was blocked early in senescence.

There has been much research interest in plant senescence during the past 30 years, as is appropriate to one of the most fundamental of plant processes (15, 23, 25). Most effort has concentrated on leaf senescence and postharvest behavior of fruits. In their 1980 review, Mayak and Halevy (17) noted that comparatively little had been published about the physiology of flower senescence; yet, it offers a model that is readily manipulated and still has much in common with the more familiar fruit ripening and leaf senescence. The process often occurs in well-defined stages, without requiring artificial triggering treatments, and without a background of generalized aging behavior resulting from continued deposition of structural, storage, and waste materials, and exposure to weather. In the 10 years since the review by Mayak and Halevy, nearly all of the physiological studies of flower senescence have concentrated on five species: carnation, rose, morning glory (*Ipomoea*), hibiscus, and *Tradescantia*, the first four being dicotyledons and the last three ephemerals. Ephemeral flower senescence in particular has been an attractive and productive model system because events happen so quickly. Some features characterizing the process are ethylene production, increased respiration, induction of catabolic enzymes, a decrease in protein, polysaccharide and nucleic acid content, and a loss of membrane function. Perhaps the most central question to emerge has concerned the extent to which ethylene triggers petal senescence and is the driving force behind it or is simply one of its manifestations (8).

Hemerocallis, the daylily, is also an ephemeral flower, in the Liliaceae, and one of an important group of cut flowers that has received little research attention. The feature of particular interest to us is that ethylene appears not to be involved in its senescence (16; M. Lay-Yee, A.D. Stead, and M.S. Reid, manuscript in preparation). Therefore, it may prove useful for studying non-ethylene-mediated senescence, particularly in relation to the nature of various membrane-related phenomena. The hypothesis addressed here is that the rapid wilting and senescence of daylily flowers is associated with loss of membrane function; to test it, we have studied uptake, efflux, and respiration and phospholipid behavior of whole flowers and petal slices.

MATERIALS AND METHODS

Plant Material

Hemerocallis hybrid cv Cradle Song plants were grown in a greenhouse under normal production conditions (day/night temperatures 25/20°C, with natural lighting extended by low-intensity incandescent light to an 18-h day). Unpollinated flowers were cut with a 5-mm pedicel attached and held in vials containing DI[1]. When intact petals and sepals were to be studied, they were cut from the flower at the point where the corolla segments joined to form a tube. Senescence changes in sepals lagged slightly behind those in petals; therefore, the two were not mixed, most studies being confined to petal tissues. To prepare tissue slices, the midrib and the top and bottom one-fifth of the petal were cut off and discarded; the lamina pieces obtained were sliced laterally into approximately 10-mm-wide strips and then longitudinally into approximately 5-mm-wide pieces, and the resulting segments

[1] Abbreviations: DI, deionized water; AFS, apparent free space.

were aerated in DI for 10 to 20 min to wash and bring them to a consistent state of turgor. Normal blotting methods caused damage; therefore, the harvested slices were flipped onto heavy filter paper and moved around with filter paper spills to absorb surface liquid until the pieces no longer clung together, at which point additional blotting did not further reduce tissue weight. Weighed samples were put in the experimental solutions described below (ratio 1 g fresh weight/15–40 mL). The same blotting procedure was used when samples were recovered from experimental solutions.

Respiration Studies

Plant material, 3 to 6 g fresh weight, was sealed in a 1.5-L jar in darkness at 20°C, CO_2-free air was passed through (3.09 $L \cdot h^{-1}$), and the change in CO_2 concentration in the air stream was measured using an Analytical Development Co. series 225 IRGA. In a typical experiment, a single flower with the pedicel in DI, three to six petals from one to two flowers with their bases in DI, or 4 g petal slices derived from two flowers, floating in DI, was used. When the effect of cycloheximide was to be studied, petal slices were floated in 10 μM cycloheximide, and intact petals were first pretreated by floating them on the surface of 50 μM cycloheximide for 30 min before transferring them to the jar with their bases in 20 μM cycloheximide.

Measurement of AFS and Sugar Efflux

The time course of mannitol uptake into that part of the tissue freely accessible to diffusion by solutes, AFS (23), was first established by putting petal slices in 200 mM mannitol, sampling the tissue at various times, and then aerating each sample in DI for 2 h. The mannitol concentration of the water was then measured and used to calculate the initial mannitol content of the tissue sample, expressed as a fraction of the tissue volume occupied by 200 mM solution. The time course of mannitol efflux was similarly established by first loading petal slices in 200 mM mannitol for 3 h, then transferring the tissue to aerated DI, and sampling the solution at various times for measurement of mannitol concentration, as before. After the uptake and efflux characteristics were known, AFS of various tissues was measured. Tissue slices were aerated in 200 mM mannitol for 1 h (when mannitol uptake should have been approximately 97% complete), blotted, transferred to DI, and aerated for 1 h (when efflux should have been approximately 97% complete); then the mannitol concentration of the water was measured and used to calculate the AFS of the tissue. Sugar efflux was studied by the same procedure as mannitol efflux. Mannitol and sugar concentrations in efflux media were measured by filtering each sample through a 0.22-μm cellulose acetate membrane filter and then separating and quantifying the sugars by HPLC (6).

Efflux of Ions

Ion efflux was followed by measuring conductivity of the external medium (DI or 10 μM or 20 μM cycloheximide) in which petal slices were being aerated. Slices were prewashed in treatment solutions for 45 min beforehand to remove materials released by slicing and to equilibrate the tissue.

Uptake and Incorporation of Phosphate

Flowers were harvested at various stages of development and petal slices prepared as above: 0.5-g fresh weight samples were then aerated in 10 mL 100 μM $KH_2{}^{32}PO_4$ (^{32}Pi), 3 $KBq \cdot mL^{-1}$, for 3 h at 20°C, rinsed briefly in DI, transferred to nonradioactive 1 mM Pi, and aerated for 30 min to halt ^{32}Pi uptake and wash it from the AFS. The samples were then blotted, killed in 4 mL methanol:chloroform:7 M formic acid, 12:5:3 v/v, at −25°C, extracted, and fractionated (3). The chloroform and aqueous fractions, containing phospholipids and Pi plus phosphate esters, respectively, were then assayed for radioactivity. To study incorporation of ^{32}Pi into individual compounds, tissues were prepared and allowed to take up ^{32}Pi as above, except that the specific activity supplied was 450 $Kbq \cdot mL^{-1}$. Presenescent samples (duplicate) were from flowers harvested at 9.30 AM (hour 9.5), sliced, washed, and incubated in ^{32}Pi from 10 AM until 1 PM. Senescing samples (duplicate) were from flowers harvested at 7 PM (hour 19) and incubated in ^{32}Pi from 7:30 to 10:30 PM. Samples were killed and extracted; then, phosphate esters and phospholipids were separated by two-dimensional TLC and individually identified and quantified (3).

RESULTS

Time Course of Respiration

Buds detached up to 2 d before opening developed and became senescent at the same time as they would have done on the plant: the various stages are shown in Figure 1. Timing of the various events is given in terms of bud opening, so that midnight, when the flower is partially open, is hour 0; 12 hours earlier when the bud is still closed is hour −12; the following midnight when the flowers are collapsing is hour 24 (Fig. 1). The respiration pattern was consistent from flower to flower. Immediately after the flowers were picked, there was a small peak lasting 1 to 2 h, apparently caused by

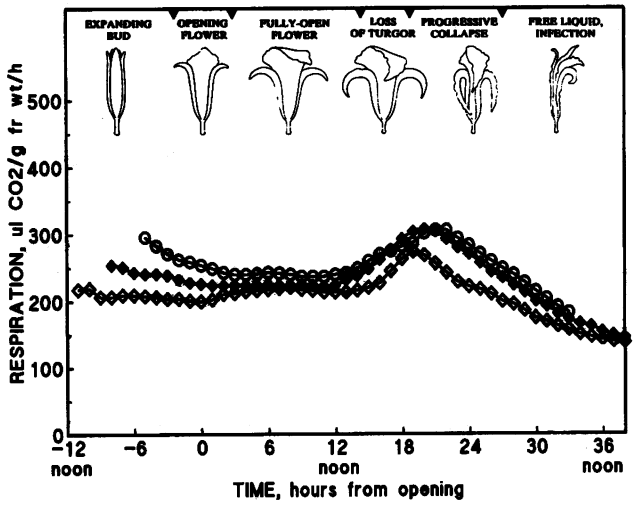

Figure 1. Respiration of daylily flowers at 20°C during opening and senescence. The three curves are for separate flowers harvested at different dates during a 3-week period.

handling. Respiration then remained steady at about 240 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$ throughout development from opening bud to fully expanded flower. At hour 16, respiration started to increase, to a peak of approximately 300 μL CO_2 at hour 20, coinciding with incipient petal autolysis. Respiration then decreased slowly to about 160 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$ by hour 32. At this stage, the flower had completely collapsed, and under conditions of high humidity (as in a respiration chamber) it glistened with free liquid released from the cells. From this point, respiration usually continued to decrease but occasionally increased to very high levels (>500 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$ by hour 38), presumably through invasion by microorganisms. Flowers picked after opening, at hour 7 to 8, showed the same time course of development and respiration (Fig. 2A).

Respiration of Flower Parts

Respiration patterns of whole flowers and various flower parts were compared (Fig. 2A). In each case, there was a plateau at about 200 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$, and respiration started to increase at about hour 17, peaked at hour 21, and then decreased. The increase and decrease was more sharply defined with petals than with the rest of the flower. Sepal respiration closely resembled petal respiration in rate, pattern, and timing. With the gynecium, there was an overall higher rate of respiration, and the size of the peak was less pronounced, but it still occurred at about the same time as in the corolla segments (Fig. 2A).

Effect of Cycloheximide on Petal Respiration

Respiration of petals detached at hour 9 was not immediately affected by cycloheximide (Fig. 2B). However, the rate slowly declined during the following 20 h, and the climacteric peak at hour 19 was completely abolished. By hour 33, the untreated petals had collapsed and become translucent, as they would have done on the plant, whereas the cycloheximide-treated ones were turgid and essentially unchanged in appearance from hour 9. Thus, cycloheximide halted both the respiration peak and the major visible changes of senescence.

Respiration of Petal Slices

The potential for using petal slices in further studies was tested by comparing their respiration pattern with that of intact petals. During the first 2 to 3 h after cutting, slice respiration was apparently affected by handling (Fig. 2C). It then stabilized at approximately 200 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$ until hour 15 to 16, increased to a peak of 250 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$ at hour 19, and then declined slowly to below 100 μL $CO_2 \cdot g$ fresh $weight^{-1} \cdot h^{-1}$ by hour 33. Cycloheximide completely abolished the respiration peak at hour 19. By hour 33, untreated slices had collapsed, whereas the cycloheximide-treated slices were turgid, with limited water injection and little or no tissue breakdown. Occasionally, the respiration rate increased markedly after hour 28 to 30, apparently through invasion by microorganisms. Thus, the behavior of petal slices closely matched that of the intact petals and the whole flower.

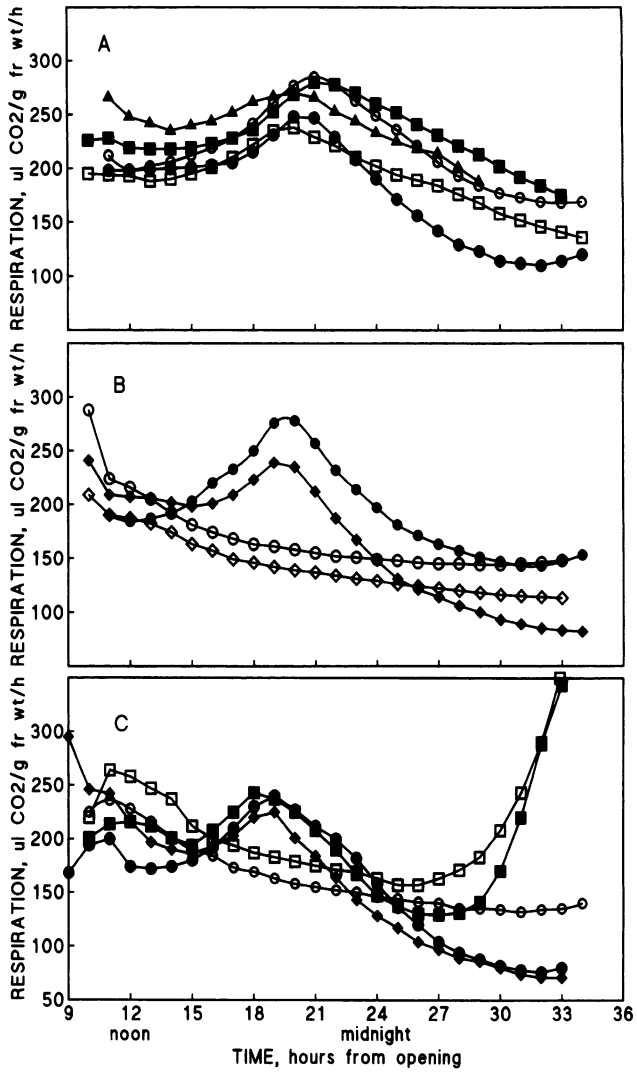

Figure 2. Respiration patterns at 20°C in tissues from daylily flowers. A, Respiration of the separate flower organs. ■, Intact flower; ●, isolated petals; □, flower minus petals; ○, isolated sepals; ▲, isolated gynecia. B, Effect of cycloheximide on respiration of isolated petals. ●, ◆, Petals held in DI water (two separate experiments); ○, ◇, petals held in 20 μM cycloheximide (two separate experiments). C, Respiration of petal slices, and the effect of cycloheximide on it. ●, ■, ◆, Slices in DI water (three separate experiments); ○, □, slices in 10 μM cycloheximide (two separate experiments).

Apparent Free Space

Unlike the storage tissue slices used in most AFS studies, petal slices have their two major surfaces covered with cuticle rather than cut cells and, therefore, should take longer to reach diffusive equilibrium with surrounding solutions. Consequently, the time course of mannitol entry into petal slices was tested: it was half-way to equilibrium after approximately 3 min and 95% complete after 23 min (data not shown). Mannitol efflux from preloaded petal slices was also studied: it was half complete after approximately 2.3 min and 95% complete after 57 min. Thus, it takes approximately 60 min

for daylily petal slices to approach diffusive equilibrium with surrounding solutions.

Petal and sepal slices (six replicates) were prepared from flowers harvested at four stages of development (fully developed bud, hour −9; opening flower, hour −1; fully open flower, hour 7; and flower in early senescence, hour 15), and their AFS was measured. Tissue from the lower half of the petal had a significantly lower AFS than that from the upper half, whereas the sepal tissue had a significantly lower AFS again (Fig. 3). The AFS in all three increased significantly as the flower approached senescence, particularly so in the upper petal (Fig. 3).

Efflux of Sugars

Flowers were harvested at hour 12 (mature blooms) or hour −12 (opening buds, immature), and efflux measurements on petal slices were started at hour 13 (mature) or hour −11 (immature). Sugar efflux was markedly greater from mature petal slices than immature ones and increased during the monitoring period (Fig. 4). Fructose was the major efflux sugar (approximately 75% of total,) with less glucose (approximately 25%) and no detectable sucrose (data not shown). The same conclusions were drawn when the experiment was repeated.

Efflux of Ions

Flowers were harvested at hour 8.5, and efflux measurements on slices started at hour 10. Slices from upper and lower halves of the petal behaved identically. During the first 30 min, there was a brief release of ions, due to the time taken

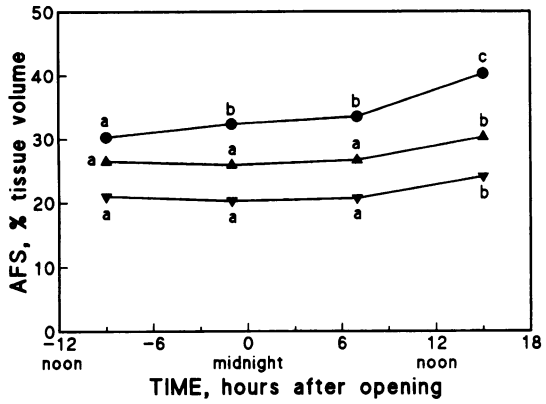

Figure 3. Changes in AFS of daylily flower tissue slices with development of the flower (six samples per time point). Stages of flower development are shown in Figure 1. ●, Slices from the distal half of the petals; ▲, slices from the proximal half of the petals; ▼, slices from the sepals. AFS differences between tissue types have a combined standard error of difference of 0.621; thus, upper petal samples have a significantly higher AFS than lower petal or sepal samples ($P < 0.001$), and lower petal samples have a significantly higher AFS than sepal samples ($P < 0.001$). AFS differences with time have a combined standard error of difference of 1.241; thus, there was a significant increase of AFS with time in all samples ($P = 0.002$). For each tissue type, points showing a different letter are significantly different at $P < 0.05$.

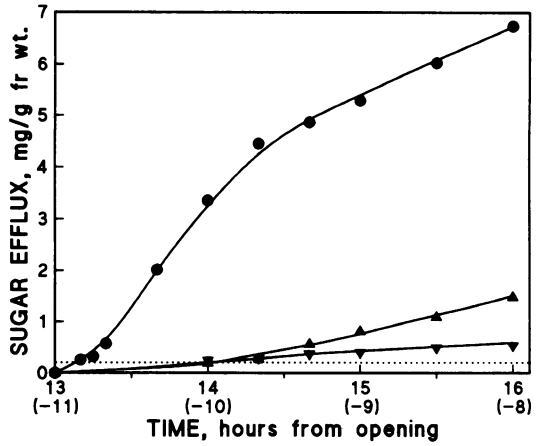

Figure 4. Efflux of sugar (fructose and glucose) from daylily flower tissue slices. Efflux from flower petal and sepal slices (flowers harvested at hour 12) was studied during the period of early senescence (*x* axis, figures not in parentheses). Efflux from bud petal slices (buds harvested at hour −12) was studied during the period of early petal expansion (*x* axis, figures in parentheses). Dotted line, Limits of detection of sugar with the method used. ●, Petal slices from hour 12 flowers; ▲, sepal slices from hour 12 flowers; ▼, petal slices from opening buds (hour −12 flowers).

to blot and weigh tissues before starting efflux measurements. After that, efflux was steady at 0.85 to 0.95 μmol NaCl-equivalents·g fresh weight^{-1}·h^{-1} until hour 17 (upper petal) or hour 18 (lower petal) when the rate increased sharply to 3.8 to 4.3 μmol NaCl-equivalents·g fresh weight^{-1}·h^{-1} (Fig. 5). In the presence of cycloheximide, the initial rate of ion efflux was rather higher (1.85–2.15 μmol NaCl-equivalents·g fresh weight^{-1}·h^{-1}), but there was no increase at approximately hour 18 as in the control samples; therefore, the cumulative efflux by hour 24 was lower. At that time, control slices were collapsing and becoming translucent, whereas cycloheximide-treated slices were turgid, with little or no tissue breakdown. In other experiments, the same conclusions were drawn; the rate of ion efflux from sepal slices was found to be about 40% of that from petal slices, with the sharp increase occurring about 1 h later, at hour 19 (data not shown).

Phosphate Uptake and Metabolism

The rate of Pi uptake from 100 μM solution increased slightly from 6 nmol·g fresh weight^{-1}·h^{-1} in slices from petals harvested at hour 8 to 8 nmol·g fresh weight^{-1}·h^{-1} in those harvested at hour 16.5. It increased further to 12 nmol·g fresh weight^{-1}·h^{-1} at hour 18 to 21, after onset of the respiratory climacteric (Fig. 6A). The proportion of ^{32}P metabolized to phospholipid was nearly constant at about 2.2% throughout (Fig. 6B).

Incorporation of ^{32}Pi into individual compounds was then studied. In presenescent samples (hour 9.5 to 13), the proportions of total extractable ^{32}P in phospholipid, phosphate ester, and Pi were 2.3, 52.6, and 45.1% respectively. In the senescent samples (hour 19 to 22.5), they were 3.1, 41.1, and 55.8%, respectively. The pattern of phosphate esters in presenescent

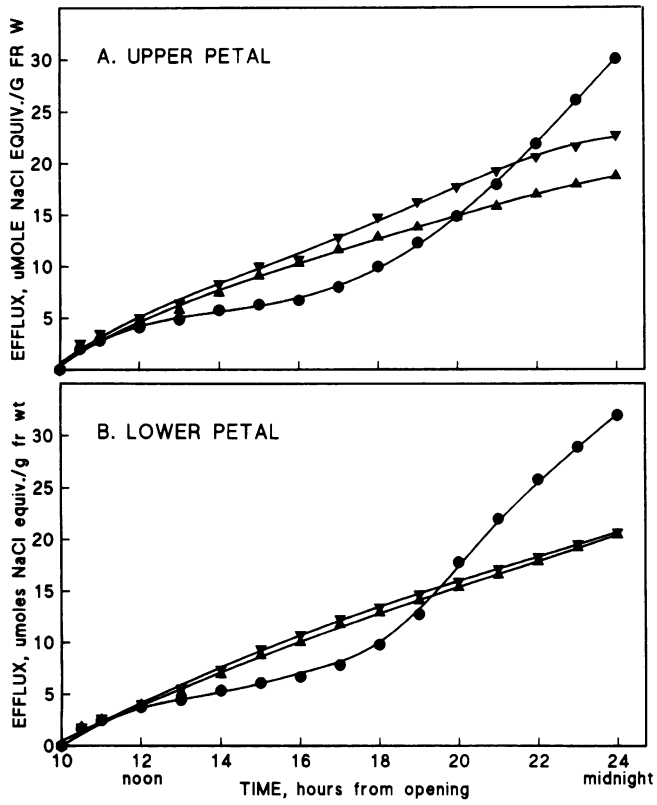

Figure 5. Efflux of ions from daylily petal slices during onset of senescence, in the absence or presence of cycloheximide. A, Slices from the distal half of the petal; B, slices from the proximal half of the petal. ●, Slices in DI water; ▲, slices in 10 μM cycloheximide; ▼, slices in 20 μM cycloheximide.

tissues was similar to that seen in other plant tissues except that the proportions of activity in phosphocholine and phosphoethanolamine were higher than normal (Table I). The ^{32}P phospholipid pattern in presenescent tissues showed more phosphatidyl glycerol and less phosphatidyl choline than is usual for nonphotosynthetic tissues (Table II). The pattern changed with the onset of senescence, the proportions of phosphatidyl glycerol decreasing and those of phosphatidyl choline and origin-bound material increasing. There was less ^{32}P activity in the phospholipids, phosphatidyl choline and phosphatidyl ethanolamine, than in the corresponding precursors, phosphocholine and phosphoethanolamine (Tables I and II).

DISCUSSION

There are many similarities between the ripening of fruits and the senescence of flowers, one of which is respiration behavior. Thus, daylily flowers showed a small but consistent and well-defined respiratory climacteric, coinciding with the first visible signs of senescence as the petal tips became limp (Fig. 1). By the time the climacteric had passed (hour 24), the whole flower had wilted and the ends of the petal had started to fray with onset of autolysis, a sequence of events resembling those seen in carnation and other flowers (1, 18). Such a climacteric has often been interpreted as one of the physiological responses to autocatalytic ethylene production (15, 18). But unlike carnation, the daylily flower produces little ethylene during senescence, and its senescence is not affected by ethylene or ethylene inhibitors (16; M. Lay-Yee, personal communication), resembling some leaves in this respect (25). Thus, the respiration climacteric and ethylene climacteric are best envisaged as independent events, sharing a common trigger and having a capacity to interact with each other.

The next point of interest is that the climacteric took place simultaneously in all parts of the flower, with the possible exception of the gynecium, even though they were isolated from each other 10 h before the event (Fig. 2A). We agree with Mor *et al.* (19) that petal senescence is not directly influenced by the ovary or its fertilization. Ethylene is an obvious candidate as controller of the timing of senescence in most flowers (8, 15), but in daylily the synchrony must arise in some other way. The data are consistent with there being a series of genetically programmed events, timed to take place in all parts of the flower at the same time and having an increase in respiration as one of the consequences. Thus, cycloheximide, an inhibitor of RNA-directed protein synthesis, completely inhibited the progress of senescence and also abolished the respiration climacteric.

Petal slices paralleled the flower behavior by showing the same climacteric, the same progress of autolysis, the same timing, and the same inhibition of these events by cycloheximide (Fig. 2, B and C). This allowed us to use petal slices to study a number of other phenomena that might be involved

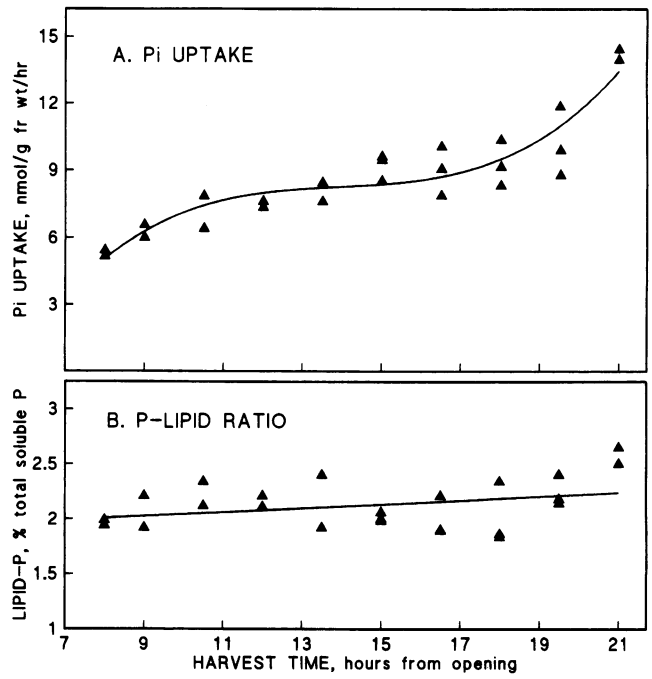

Figure 6. Change in ability of daylily petal slices to accumulate and metabolize phosphate, as a function of stage of development of the flower from which petals were taken. A, Uptake of [^{32}P]phosphate; B, fraction of extractable ^{32}P metabolized into phospholipid. Each point represents one uptake sample.

Table I. *Distribution of ^{32}P in Phosphate Esters from Daylily Petal Slices Compared with a Model Tissue (Defined in Ref. 4)*

Morning (presenescent) and evening (senescent) samples metabolized ^{32}Pi during hour 10 to 13 and hour 19.5 to 22.5, respectively. Values are means ± SE.

Ester	Morning	Evening	Model
	% total ester		
Glucose-6-phosphate[a]	19.5 ± 0.57	7.7 ± 1.25	20.9
Fructose-6-phosphate[a]	5.9 ± 0.37	2.3 ± 0.19	6.3
Mannose-6-phosphate[a]	2.4 ± 0.08	1.6 ± 0.15	4.2
Glucose-1-phosphate[a]	5.8 ± 0.01	2.1 ± 0.23	1.6
Sugar diphosphate[a]	3.0 ± 0.27	0.8 ± 0.04	2.9
6-Phosphogluconate	1.4 ± 0.01	0.4 ± 0.10	0.5
Total sugar phosphate[a]	(38.0 ± 1.27)	(14.9 ± 1.94)	(36.4)
Phosphocholine[a]	13.8 ± 0.33	37.8 ± 1.59	2.1
Phosphoethanolamine[a]	4.6 ± 0.01	10.3 ± 0.45	2.1
1-Phosphoglycerol[a]	2.5 ± 0.07	0.8 ± 0.07	1.0
Total lipid precursor[a]	(20.9 ± 0.38)	(48.9 ± 1.95)	(5.2)
ATP	13.5 ± 0.91	13.8 ± 0.19	10.5
ADP	2.7 ± 0.01	2.6 ± 0.57	3.2
AMP	1.2 ± 0.04	1.1 ± 0.07	0.8
UTP	2.9 ± 0.06	2.6 ± 0.30	4.2
UDP	0.6 ± 0.01	0.7 ± 0.28	5.2
UMP	1.1 ± 0.11	0.9 ± 0.15	2.1
CTP + GTP	2.1 ± 0.23	1.8 ± 0.03	1.5
CDP + GDP	0.9 ± 0.05	0.6 ± 0.01	0.6
ADPG	0.9 ± 0.03	0.8 ± 0.03	2.6
UDPG	5.2 ± 0.01	4.4 ± 0.33	8.4
3-Phosphoglycerate	2.8 ± 0.12	0.8 ± 0.11	8.9
Phosphoenolpyruvate	0.9 ± 0.29	0.4 ± 0.10	1.6
Other compounds	6.3 ± 0.16	6.4 ± 0.57	8.7

[a] Morning and evening values differ significantly ($P < 0.001$).

in petal senescence. For example, ripening fruits show an increase in AFS which accompanies the respiration climacteric (23); a similar AFS increase in carnation petals occurs somewhat after the climacteric and coincides with changes in phospholipase activity (9). In daylily, we found a significant AFS increase in the distal part of the petal, coinciding with the onset of the respiratory climacteric and with wilting (Figs. 1 and 3). Neither the base of the petal nor the sepals, which had the same climacteric but which lost turgor several hours later, showed such a pronounced AFS change. Thus, increase in AFS seems more closely related to loss of cell water, presumably through increased permeability of the plasmalemma, than to the climacteric. The sugar efflux pattern resembled the AFS changes. Thus, slices from immature petals taken at hour −11 had a very low rate of sugar loss, whereas slices of petals approaching senescence, at hour 13, had a 15 times higher efflux rate (Fig. 4), equivalent to approximately 7% of the total tissue sugar content lost per hour. Sepal tissues showed a much lower rate of sugar efflux than corresponding petal tissues. Again, the results are consistent with there being an increase in cell permeability as the tissues enter senescence, occurring in the petals before the sepals. Similar findings have been reported for *Ipomoea* (14) and carnation (27). An alternative that has not been tested is that the cells begin actively secreting sugars, as a step in remobilizing petal solutes back to the developing ovary or scape. It is not a far-fetched notion, because the base of the petal (the nectary) has been demonstrably secreting sugars 12 h earlier.

Ion efflux can be followed much more accurately than sugar efflux. Several studies with petal segments have shown a pronounced increase, coinciding with other senescence phenomena: wilting in *Ipomoea* (14) and carnation (18, 27), loss of lipid phosphorus in *Tradescantia* (24), onset of lipid peroxidation in carnation (21), increased sugar efflux in *Ipomoea* (14) and carnation (18), and increased ethylene production in carnation (11) and rose (12). Treatment with ethylene hastened the increase and advanced the associated senescence events in *Ipomoea* (14), carnation (18), and *Tradescantia* (24) flowers. Although senescence in daylily is independent of ethylene action, there was a similar sharp increase in ion efflux coincident with the onset of wilting and the respiratory climacteric (Figs. 2C and 5). There were two features of interest in relation to the published studies. First, although the initial rate of efflux was increased slightly by cycloheximide, the sharp increase at hour 18 was totally inhibited by it. Second, the increase took place much more abruptly than in all other flowers studied but *Ipomoea*: this is in keeping with the ephemeral nature of the daylily flower. Somewhat unexpectedly, upper and lower halves of the petal showed approximately the same time course in ion efflux behavior, unlike the AFS changes. Ion efflux from sepal slices matched that from the lower half of the petal (data not shown). Thus, ion efflux seems more closely coupled to biochemical changes in the cell (respiration) than does the AFS volume.

Phosphate transport in apple fruit slices (29) and sugar transport in carnation petal slices (1) both declined markedly after the climacteric was reached. Daylily slices showed a different behavior: phosphate uptake increased at the time of the respiration climacteric (Fig. 6A). The response did not arise as an artifact through adsorption of ^{32}P to binding sites exposed by increased cell permeability, because most or all of the ^{32}P must have entered the metabolic pool (Table I; refs. 4

Table II. *Distribution of ^{32}P in Phospholipids from Daylily Petal Slices*

Morning (presenescent) and evening (senescent) samples metabolized ^{32}Pi between hour 10 to 13 and hour 19.5 to 22.5, respectively. To directly compare ^{32}P activities in individual phospholipids with those in phosphate esters from the same tissue (Table I), multiply morning phospholipid values by 0.044 and evening values by 0.075, being the ratio of total activities in the phospholipid and phosphate ester fractions for the two treatments (see text). Values are means ± SE.

Phospholipid	Morning	Evening
	% total phospholipid ^{32}P activity	
Origin-fixed material[a]	6.9 ± 0.10	15.7 ± 0.94
Phosphatidyl choline[a]	18.9 ± 1.73	33.6 ± 0.01
Phosphatidyl ethanolamine	23.5 ± 0.39	19.6 ± 0.78
Phosphatidyl glycerol[a]	20.8 ± 1.13	8.1 ± 0.12
Phosphatidyl inositol	19.8 ± 0.07	14.9 ± 0.09
Phosphatidyl serine	1.5 ± 0.35	1.3 ± 0.02
Phosphatidic acid	1.6 ± 0.22	1.1 ± 0.07
Other compounds	7.0 ± 0.39	5.7 ± 0.07

[a] Morning and evening values differ significantly ($P < 0.001$).

and 5). The pattern of ^{32}P activity in the phosphate esters is informative. Most phosphate esters in plant tissues reach equilibrium labeling within 10 to 30 min (5); therefore, in Table I, the amount of each should be approximately proportional to its radioactivity. After reviewing existing literature, Bieleski (4) noted that "there has been a remarkable unanimity in results obtained, so that it is possible to write down a model P ester pattern ... any marked departure [from it] is likely to be significant". Compared to that model (Table I), the daylily petal phosphate esters showed similarities in the nucleotide phosphate patterns and large differences in the phospholipid precursors. It is significant that the proportion of ^{32}P in the nucleotide triphosphates remained high and unchanged during the climacteric (Table I). If we assume that equilibrium labeling of the α-P, β-P, and γ-P in the adenine nucleotides had been reached (5), the relative amount of each nucleotide and thence the energy charge of the cell can be calculated. It was about 0.86 for both the presenescent and the senescent tissues and in the range to be expected for a tightly coupled, actively metabolizing nongreen tissue (22). Our results support the finding for carnation petals that the energy charge stays high until petal senescence is almost complete, indicating that respiration remains tightly coupled until then (28).

In contrast to the similarity of the nucleotide phosphate patterns, the proportion of ^{32}P in phospholipid precursors (1-phosphoglycerol, phosphocholine plus phosphoethanolamine) was markedly higher than in the model, implying a perturbation in phospholipid synthesis. Several studies have found dramatic changes occurring in cell membrane phospholipids during petal senescence. First, there is a decrease in the amount of phospholipid in *Ipomoea* (2), *Tradescantia* (24), and rose (7), due to both reduced synthesis and enhanced degradation. Second, there is an increase in the sterol to phospholipid ratio, leading to decreased membrane fluidity in *Ipomoea* (2), carnation (1, 26), and rose (13) and to changes in the fatty acid component of the lipids (21). In our study, we looked at relative ^{32}P incorporation into individual phospholipids rather than at their amounts. We expected there to be a reduced synthesis of phospholipid accompanying senescence which would show up as reduced incorporation of ^{32}P. Instead, the proportion remained essentially constant at 2 to 2.5% (Fig. 6B), approximately one-third to one-fifth the value normally obtained in plant tissues after 3 h of labeling (4). One possibility was that the main decline in phospholipid synthesis had already occurred by the time the presenescent samples were taken, 9 h before senescence was visible. This would be in line with findings for *Ipomoea* (2), *Tradescantia* (24), and rose (13) in which a decrease in the amount of phospholipid and its rate of synthesis can precede by several hours the ethylene climacteric and loss of turgor.

Although the rate of incorporation of ^{32}P into the phospholipid fraction did not change with onset of senescence, a significantly lower proportion entered phosphatidylglycerol, implying a decline in the proportion of that phospholipid in the senescing petals (Table II). This finding is at odds with results for senescing *Ipomoea* (2), *Tradescantia* (24), and rose (7), in which phospholipid composition did not change, although it matches that for carnation cv Ember (10). The explanation is probably to be found in the form of pigmentation rather than in any difference between ethylene-sensitive and -insensitive flowers. Daylily cv Cradle Song is bright yellow and contains abundant chromoplasts, which are derived from chloroplasts (17). Breakdown of the phosphatidyl glycerol-rich chloroplasts is one of the early stages in leaf senescence (25), and a parallel behavior of chromoplasts in senescing daylily petals could produce the pattern we observed.

Direct evidence that phospholipid synthesis was affected early in daylily senescence comes from the phosphate ester data (Table I). In all tissues we have studied in the past, the phospholipid precursors phosphocholine, phosphoethanolamine, and 1-phosphoglycerol together have contained <5% of ester ^{32}P (4, 5). The presenescent daylily samples had 20.9%, whereas the proportion in senescent tissues was significantly higher again at 48.9% and higher than in the sugar phosphates (Table I). We take this as evidence that phospholipid synthesis was already perturbed by the time the presenescent samples were taken and that passage of ^{32}P from precursors to phospholipid was already restricted. As senescence continued, the pool of precursors increased to the point at which it dominated the phosphate esters, significantly reducing the proportion of hexose phosphates (to 14.9% in the senescent tissue), tying up phosphate, and perhaps increasing the demand for external phosphate. We do not favor two alternative explanations. The first is that phospholipase caused a breakdown of phospholipid to the corresponding ester during tissue extraction; however, the techniques used have been designed to prevent this postmortem action (3). The second is that endogenous phospholipase action in the living tissue caused phospholipid breakdown during senescence and before extraction. However, because ^{32}P activity in the ester, phosphocholine, exceeded that in the putative phospholipase substrate, phosphoryl choline, by 10 times (Tables I and II), nearly all the phospholipid would need to have been hydrolyzed and specifically by phospholipase C rather than other phospholipases which give other end products such as phosphatidic acid, which was barely detected. Again, this seems unlikely. The simplest explanation is that phospholipid synthesis became blocked and feedback mechanisms failed to halt precursor synthesis. Thus, from a different line of evidence we have come to the same conclusion as Paliyath and Thompson (20) and others cited above: that a very early event in petal senescence, beginning soon after the flower has opened, is a reduction in phospholipid synthesis (even though some phospholipid synthesis continues well past the onset of the climacteric) and that this is as true for daylily as the ethylene-sensitive flowers.

On the surface, some of these results appear mutually inconsistent. From the AFS and efflux data, we have deduced that petal cells begin losing their semipermeable properties at the onset of the climacteric (hour 18). Yet, the ^{32}P results show conclusively that the tissues as a whole remain metabolically competent well past that time, by being able to maintain active transport of Pi, synthesis of phosphate esters, a high energy charge, and synthesis of phospholipids (albeit with changes in phospholipid/precursor patterns). We are forced to conclude that tissues that are wilting and well into senescence, judged by some criteria, retain competent cells with functional, if leaky, membranes. One explanation could

be that some of the cells remain substantially normal while most are collapsing: if so, one would have to propose that these survivors markedly increase their accumulating and synthesizing activity during the period of senescence to account for the increased Pi uptake rate for the tissue as a whole. The alternative explanation, which we favor, is that most or all the petal cells, although becoming leaky, retain metabolic competence for much longer during senescence than has generally been presumed. The explanation that the climacteric is a type of uncoupled respiration resulting from cellular disorganization has never been fully satisfactory, and our data support the alternative, that climacteric respiration is coupled and is a consequence of an increased metabolic demand by functional cells that are carrying out an active, controlled dismantling process.

In the last 10 years, many physiological studies of flower senescence have focused on the behavior of ethylene, with other aspects of senescence being considered mainly in relation to it, leading Borochov and Woodson (8) to write, "the phytohormone ethylene plays a critical role in the regulation and coordination of flower petal senescence". Yet, as we show, most of the major events modulated in most flowers by ethylene also take place in the ethylene-unresponsive daylily: these are an early decline in phospholipid synthesis; an increase in cell permeability, leading to increased efflux of sugars and ions; an increase in AFS; a respiration climacteric; and early wilting and then autolysis of petal tissues. Here, an apparently identical course of flower senescence occurs in the absence of ethylene modulation. The daylily offers a valuable model for study of flower senescence, by allowing us to get away from the apparent supremacy of ethylene as a controller, to explore other paths and mechanisms that may be operating.

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