Is Petal Senescence Due to Sugar Starvation?

Wouter G. van Doorn*

Wageningen University and Research Centre, P.O. Box 17, 6700 AA Wageningen, The Netherlands

Senescence occurs at every stage of plant development. Shriveling of the cotyledons in young plants and the seasonal recurrence of leaf yellowing are obvious examples. Similarly, after pollination, petals have fulfilled their biological role and become senescent. Despite this ubiquity, however, the molecular events that initiate senescence have thus far remained a mystery.

Thimann et al. (1977) hypothesized that sugar starvation is the direct cause of leaf senescence. Later work with Arabidopsis plants, grown in the light, showed that leaves exhibit reduced expression of photosynthetic genes, after a fixed time span. The decrease in photosynthesis was followed by expression of senescence-associated genes, apparently induced by sugar starvation. If Arabidopsis leaves were held in darkness, the ensuing low carbohydrate levels also induced expression of senescence-associated genes (Hensel et al., 1993; Quirino et al., 2000; Lam et al., 2001). However, other arguments may favor the opposite hypothesis: An increase rather than a decrease in sugar levels induces leaf senescence. Arabidopsis and tomato (*Lycopersicon esculentum*) plants in which hexokinase (which acts as a sugar sensor) was overexpressed, exhibited accelerated leaf senescence, and transgenic Arabidopsis plants expressing antisense hexokinase showed delayed senescence. Additionally, sugar levels were highest in tobacco (*Nicotiana tabacum*) leaves that were about to senesce, compared with younger and older leaves on the same plant, and sugar treatment hastened senescence of tobacco leaf discs (Masclaux et al., 2000; Yoshida, 2003).

Petal senescence may also be due to sugar starvation or sugar accumulation. Sugar starvation may be involved because application of sugars to cut flowers generally delays visible senescence. A role for sugar starvation is also suggested by the similarities between starvation-induced changes in cell physiology and those observed before cell death during senescence. However, sugar concentrations are still high when petals show the first visible senescence symptoms. What then is the role of sugars, if any, in petal senescence?

Three alternative models about the cause of petal senescence are shown in Figure 1. Degradation of polysaccharides, proteins, lipids, and nucleic acids results in mobilization of sugars and nitrogenous compounds, before visible senescence. These mobile molecules are transported, through the phloem, to other plant parts. Mobilization is common to all three models. There are at least three conceivable signals for mobilization: maturation, starvation, and sugar accumulation. According to the standard model (Fig. 1A), the maturation and starvation signals act independently. According to Thimann's model (Fig. 1B), the maturation signal results in starvation, and starvation causes expression of genes involved in mobilization. Finally, if sugar accumulation were a signal for senescence (Fig. 1C), it may also act on genes that induce mobilization. A maturation signal may precede sugar accumulation.

Advanced senescence symptoms are accompanied by cell death. At least two types of cell death are described in plants. The first, of which the hypersensitive response to invading micro-organisms is an example, is limited to a relatively low number of cells and exhibits a short time between external stimulus and death. In the hypersensitive response, rapid cell death is required as the dead cells pose a barrier to the intruding organism. The second type, of which leaf and petal senescence are examples, is characterized by export of valuable materials and takes considerably more time.

This *Update* article discusses how mobilization and cell death are related and examines the possible role of sugars as a signal for petal cell death. It will emphasize the close similarities between starvation-induced changes in cell physiology and those observed upon senescence. It will nonetheless be concluded that there is, at present, no good reason to accept the hypothesis for either sugar starvation or sugar accumulation as a general signal for petal cell death, in flowers of intact plants. The discussion is mostly relevant to petal senescence but also draws some parallels with leaf senescence and may have a bearing to senescence-related cell death in general.

COMPARISON OF MOBILIZATION IN STARVING AND SENESCENT CELLS

One argument for the idea that senescence may be triggered by starvation is that mobilization is induced both by senescence and starvation. Mobilization and starvation show similarity at the level of cell structure, biochemistry, and gene expression.

* E-mail wouter.vandoorn@wur.nl; fax 31–317–475347.
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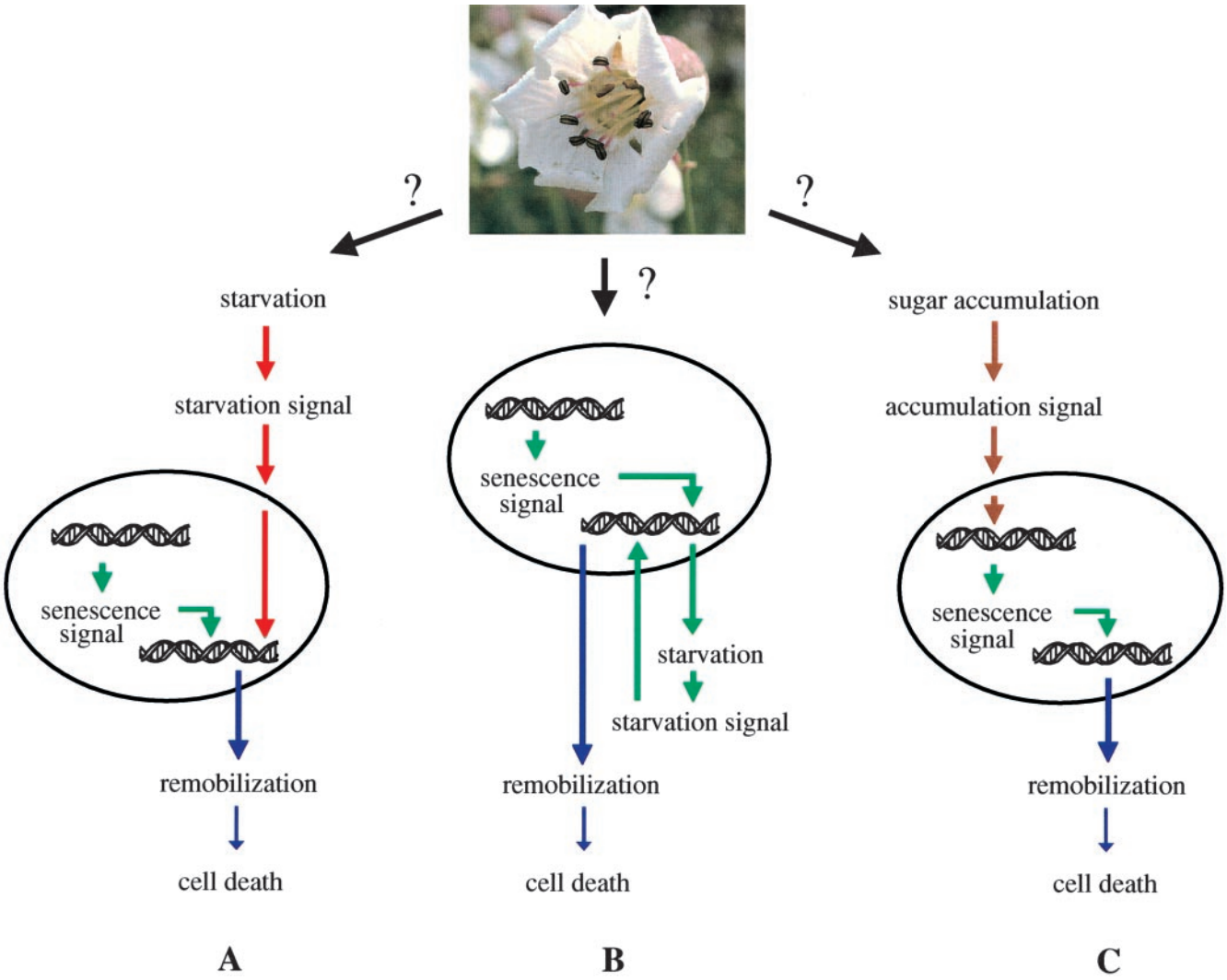

Figure 1. Hypothetical schemes of the relationship between maturation, sugar starvation, mobilization, and cell death during petal senescence. In these schemes, cell death is a result of mobilization of proteins, carbohydrates, and lipids. In A and B, a maturation signal precedes the signal for senescence; in C, the accumulation of sugars may be due to a maturation signal. A, A maturity signal results in mobilization and cell death. Starvation may result in premature senescence, independent of a maturation signal. B, According to Thimann's hypothesis, a maturation signal results in starvation, which results in mobilization and cell death (Thimann et al., 1977). If starvation occurs for other reasons than maturity, it is superimposed on the starvation that results from senescence. This is the same as the red lines in A, but has not been included. C, Sugar accumulation results in mobilization by inducing the senescence signal. Starvation effects can also be superimposed, as in A. The flower is *Silene alba*, a species in which petal senescence is regulated by ethylene, showing the first senescence symptoms (inward rolling of the petals).

Ultrastructure

Before visible petal senescence, the initially small vacuoles fuse into a single large organelle. A decrease in endoplasmatic reticulum is also observed at an early stage. Several organelles in the cytoplasm subsequently disappear, but the nuclei and mitochondria are maintained until late. Membrane fragments and whole organelles have been observed in the vacuole, suggesting autophagy. Similar changes have been observed in carbohydrate-starved plant cells. In rice (*Oryza sativa*) suspension cells, for example, the vacuoles engulf various organelles and portions of the cytoplasm enclosed in vesicles. The membranes remain visible for some time but are eventually digested (Matile and Winkenbach, 1971; Yu, 1999).

Metabolism

Glc starvation in maize (*Zea mays*) root tips resulted in rapid depletion of sugars and proteins. Addition of cycloheximide to the sugar-depleted medium prevented the increase in proteolytic activity, suggesting that new proteases were synthesized. Asn and Ser accumulated during protein degradation, until 45 h

of starvation. Thereafter, nitrogen from protein breakdown was released from the cells as ammonia. Lipids were also continuously degraded, lipids and proteins being the only substrates for respiration after the first 20 h of starvation. Cell death was no longer reversible by sugar application after 90 to 100 h of starvation (Brouquisse et al., 1998).

This is very similar to the changes observed in senescent petals, at least in petals of cut flowers. Extensive degradation of lipids and protein has been observed. In senescent petals, Suc is the main transport carbohydrate, whereas the amides Asn and Gln are usually the main organic compounds in which nitrogen is translocated in the phloem. Ammonia accumulation has also been observed in cells of some cut flowers. In several species, the time to petal wilting can be postponed by application of cycloheximide, which prevents the increase in protease activity. Sugars or cycloheximide often cannot postpone senescence if they are applied after a fixed period from the onset of flower opening. Figure 2 shows the time line of a number of processes, both during starvation and during petal senescence (Wiemken et al., 1976; Brown et al., 1987; Stephenson and Rubinstein, 1998).

Gene Expression

Upon starvation, expression of genes involved in the breakdown of starch, proteins, and lipids is enhanced. Examples are α-amylase, proteases, and genes involved in synthesis of amino acids and amides. After only 18 to 24 h of starvation, Asn synthetase was maximally expressed in maize root cells. In senescing petals, protease genes and a gene for Asn synthetase also showed increased expression (Valpuesta et al., 1995; Yu, 1999; Eason et al., 2000).

Both during starvation and senescence, lipids are usually degraded by lipases, and the fatty acids are further degraded by β-oxidation, yielding acetyl CoA. Lipid degradation is often accompanied by the induction of a new biochemical pathway, the glyoxylate cycle, which converts acetyl CoA to Suc. Two key enzymes in this pathway become synchronously expressed: malate synthase and isocitrate lyase. The pathway is found both in sugar-starved and in senescing tissues, including the petals of cucumber (*Cucumis sativum*; Graham et al., 1994).

Sugars suppress both starvation-induced genes and senescence-associated genes. For example, sugar addition repressed expression of Asn synthetase in starving asparagus (*Asparagus officinalis*) cells. Application of sugars delayed the expression of malate synthase and isocitrate lyase in senescing petals of *Cucumis sativa* and delayed, by 2 d, bulk protein degradation in *Sandersonia* sp. petals. SDS-PAGE showed that some polypeptides in petals of control *Sandersonia* sp. petals shortly after cutting (d 3) were not found in Suc-treated flowers. The expression of a protease gene in *Sandersonia* sp. petals was also delayed by application of exogenous sugars (Graham et al., 1994; Eason et al., 1997; J.R. Eason, personal communication).

Similar mobilization processes are thus observed, using various means of analysis, in senescent petals and in starving cells. In both types of tissue, sugars repress the expression of several genes involved in mobilization. However, it is not clear whether these similarities simply reflect a similar syndrome or point to a causal relationship between carbohydrate starvation and senescence.

MOBILIZATION AND CELL DEATH: ROLE OF MEMBRANE PERMEABILITY

What is the cause of cellular death during senescence? Data on starving cells may again give some

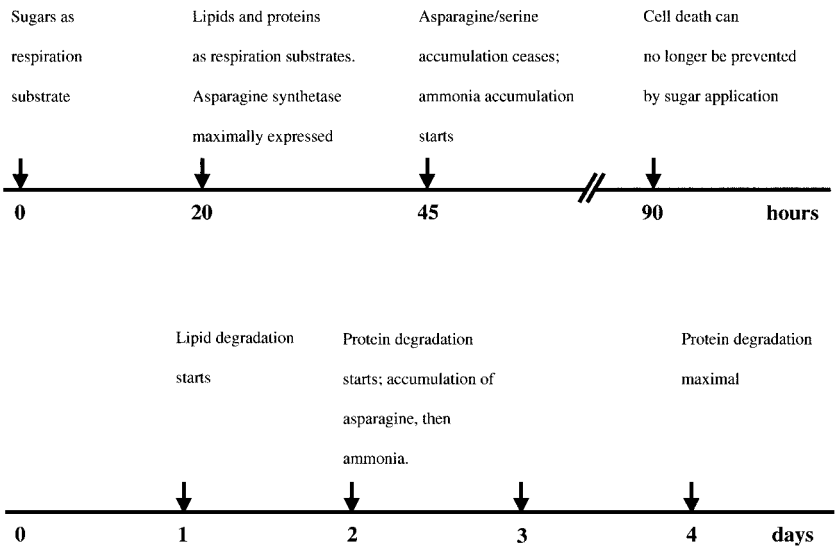

Figure 2. Time line of similar processes during sugar starvation (top line) and petal senescence (bottom line). Sugar starvation data from maize root tips (Brouquisse et al., 1998). Petal senescence data from *Iris* × *hollandica* cv Blue Magic (W.G. van Doorn, unpublished data). *Iris* sp. flowers are almost completely open by the end of d 1 and show the first visible senescence symptoms at d 4.

clues. In starving root cells, a salvage program apparently starts to produce adequate metabolic energy. In maize root tips, cell death was prevented by sugar application, although only during the first 80 to 90 h of starvation. Respiration in maize root cells was based on degradation of protein and lipid, from 20 h of starvation onward. Cell death, therefore, was prevented even after degradation of considerable quantities of lipid and protein (Brouquisse et al., 1991). Because sugars can delay petal senescence, similar processes may occur during senescence.

Membrane Degradation

In maize root cells, intracellular osmolarity fell sharply at the time when starvation could no longer be reversed by sugar application. The decrease of osmolarity apparently reflected loss of selective permeability in the plasma membrane. In other words, cell death could no longer be prevented when the cells had become very leaky for ions. Membrane degradation was apparently reversible as long as the residual proteins and lipids were able to maintain membrane structure. As soon as plasma membrane structure was considerably disrupted, charged compounds were released from the cell. The large increase in leakiness apparently reflected cell death (Brouquisse et al., 1991).

Similar changes have been observed before visible petal senescence. Senescent petals showed increased leakage of anthocyanins and electrolytes. As the anthocyanins are located in the vacuole, their release indicates loss of selective permeability of both the tonoplast and the plasma membrane (Matile, 1997). It is as yet unknown how this loss of permeability comes about, but the mechanism may be similar to that proposed for cell death in starving maize root cells. During mobilization, intrinsic membrane proteins and lipids may be selectively degraded until structural integrity is lost. In addition, the tonoplast becomes considerably larger as the vacuole volume increases. Cessation of lipid synthesis, in the face of enlargement of the tonoplast surface and increased turnover, may result in rupture. Alternatively, the cell may insert a compound in the tonoplast whereby it becomes leaky. Whatever the mechanism, loss of tonoplast selective permeability will result in contact between the numerous vacuolar lytic enzymes and the cytoplasm. These enzymes will degrade the remaining membranes, including the plasma membrane. The literature often suggests that leakage of anthocyanins and electrolytes occurs before cell death. It is thereby assumed that most cells are at the same stage of development. This seems incorrect. Leakage may rather be an indicator of cell death and its increase a measure of the number of dead cells.

EFFECT OF APPLIED SUGARS ON PETAL SENESCENCE

Exogenous sugars delay senescence in many cut flowers. This may be taken as an argument for a role of low sugar levels in activation of the salvage pathways and in cell death. However, it is often not clear to what extent applied sugars serve to improve petal water relations by increasing the level of osmotic solutes or delay cell death. Additionally, sugars may act indirectly, on the sensitivity to ethylene.

Role of Ethylene

In many species, the onset of petal senescence is regulated by endogenous ethylene, and in many other species, there is no such ethylene control. In the first group, a sharp rise in ethylene production precedes visible wilting, and any stress that increases the rise in endogenous ethylene production hastens the time to senescence. In still other species, flower life is terminated by petal abscission, before visible senescence symptoms. In these petals, the salvage pathways may also be under way by the time the petals fall, and its onset may be regulated by ethylene (van Doorn and Stead, 1997; van Doorn, 2001).

In the group of flowers where senescence is regulated by ethylene, exogenous sugars considerably delay the large increase in ethylene production and the time to visible senescence. Sugar feeding of cut carnations (*Dianthus caryophyllus*), for example, delays the time to petal wilting from d 7 (controls) to about 15 and results in a petal lifespan similar to that of flowers left on the plant. Exogenous sugars delay the rise in ethylene production mainly by decreasing ethylene sensitivity (Nichols, 1973; Mayak and Dilley, 1976). Blocking of the ethylene receptor also considerably delays and attenuates the large rise in ethylene production and delays visible senescence. In a cDNA micro-array analysis of gene expression during carnation senescence, we found that sugar feeding delayed expression of virtually the same group of genes (several hundreds) as silver thiosulphate, which blocks the ethylene receptor. These same genes showed early up-regulation, with respect to controls, after treatment with exogenous ethylene (F.A. Hoeberichts, W.G. van Doorn, O. Vorst, R.D. Hall, M.F. Van Wordragen, unpublished data). The data indicate that sugar is an early regulator of carnation petal senescence. In Arabidopsis, the product of *GIN1* (Glc insensitive) plays a pivotal role in the Glc repression of seedling development. GIN1 is equivalent to SDR (short chain dehydrogenase/reductase), the one but last enzyme in the ABA biosynthesis pathway. Figure 3 shows a model of sugar action in this system, as envisaged by Leon and Sheen (2003). Low ABA levels antagonize the ethylene signal transduction pathway. This model may similarly apply to senescence.

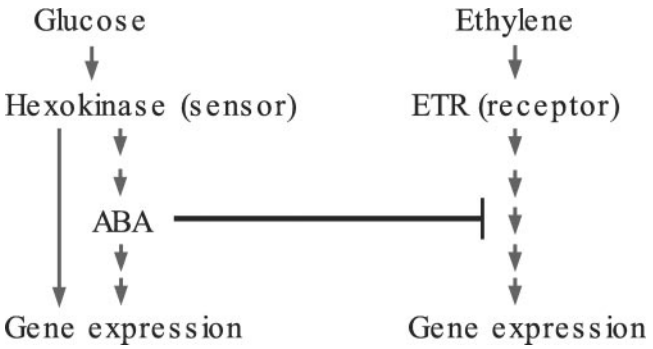

Figure 3. Model of sugar repression of ethylene sensitivity, according to Leon and Sheen (2003). Some Glc-insensitive mutants showed no ABA synthesis and increased ethylene sensitivity. Suc therefore apparently inhibits the ethylene signal transduction pathway through production of low ABA concentrations. A high ABA level tends to have an opposite effect. The model was developed for seedling growth, but may also apply to senescent cells.

Exogenous sugars also delay the time to visible senescence in petals where senescence is not regulated by ethylene, but the effect is generally much smaller than in species with ethylene-regulated petal senescence. For example, sugar feeding delayed visible senescence by about 0.5 and 2 d in *Iris* sp. and *Sandersonia* sp. flowers, respectively (controls have a life span of about 4 and 7 d). Even when the increase in life span is expressed as a percentage of the untreated controls, the effect of sugar is considerably smaller than in carnation. It is not clear in the ethylene-insensitive flowers whether sugar delayed petal senescence by preventing a drop in osmotic pressure or by delaying cell death. In *Sandersonia* sp. petals, the delay in protein degradation and in up-regulation of a protease gene, after Suc feeding, indicates a relationship with mobilization and cell death (Eason et al., 1997). It follows that sugar feeding delays petal senescence of cut flowers, although the effect is considerably larger in species where petal senescence is regulated by ethylene. In this group, the sugar effect is mainly due to a reduction of ethylene sensitivity.

Endogenous Sugar Levels

Petals of many cut flowers contain considerable free carbohydrate levels when senescence symptoms are already visible (Eason et al., 1997; van der Meulen-Muisers et al., 2001). At face value, this is evidence against the hypothesis that starvation induces senescence: The petals still seem to have adequate osmotic solutes and energy reserves at the time of wilting. If this interpretation were correct, however, it is difficult to understand why exogenous sugars exert such a positive effect on the time to senescence in cut flowers. The interpretation is also difficult to reconcile with the finding that exogenous sugars delay protein degradation and the expression of genes involved in mobilization (see previous section).

The presence of considerable quantities of sugars at the time of petal wilting may be explained by a number of factors. First, the measurements may not have been detailed enough because various tissues in a petal are at different stages of senescence. Second, the cytoplasm and the vacuole may have different sugar levels. Third, at some point during senescence, sugars are formed again, due to mobilization.

Unequal Distribution of Sugars

Various tissues in a petal are at different stages of development. In petals of *Iris* × *hollandica*, for example, most mesophyll cells had died by the time of visible senescence, and their cell walls had been largely degraded. Visible wilting coincided with the loss of turgor in a few cells of the upper epidermis. The vascular bundle did not show any sign of senescence at the time of petal wilting and may be actively involved in sugar transport. Moreover, in *Iris* sp. petals, as in several other species, wilting proceeds from the distal to the basal parts (Wagstaff et al., 2002; van der Kop et al., 2003).

The discrepancy between the effect of exogenous sugars and the relatively high soluble sugar levels at the time of petal wilting may also be explained by the unequal distribution of sugars in various cellular compartments. As discussed above, increased expression of malate synthase and isocitrate lyase, key enzymes in the glyoxylate cycle, preceded lipid degradation. Interestingly, the up-regulation of both genes occurred in the presence of a relatively high overall sugar concentration. It was nonetheless concluded that the two enzymes were up-regulated because of low sugar levels in the cytoplasm. These concentrations had apparently been overestimated because the cytoplasm and the vacuoles had not been separated (Graham et al., 1994). The idea that starvation induces the senescence of cut flowers can therefore not be dismissed simply by referring to high carbohydrate levels at the time of senescence, because measurements did thus far not distinguish between the fraction that is available for respiration and the fraction that is not.

UNCUT FLOWERS

I will now examine the question whether cell death in petals on intact plants would be triggered by sugar starvation or sugar accumulation. Little information is available, however, to address this point. Petal senescence in uncut flowers has rarely been studied, and seldom has a comparison been made between senescent petals on cut flowers and senescent petals that remain attached to the plant. As far as reported, the petals of non-cut flowers contain elevated levels of Glc and Fru and low levels of Suc, by the time of

senescence. They may also contain some less common sugars, such as various inositol compounds, Xyl, and mannitol (Nichols, 1973; Collier, 1997; Ichimura et al., 2000). It was argued in the preceding section that the presence of overall elevated levels of hexoses and Suc in cut flowers cannot be taken as a decisive argument against starvation-induced death. The same arguments apply to uncut flowers.

Comparison of Cut and Uncut Flowers

Ipomoea sp. flowers open in the morning and start wilting the same day. A comparison has been made between flowers that were cut 1 or 2 d before flower opening and uncut flowers. On the day of opening, an increase in the levels of Glc, Fru, Suc, and starch was observed in petals of flowers that remained on the plant, and these levels were higher than in the cut flowers. Before flower opening, many mesophyll cells were at an advanced stage of the cell death program, judged by their ultrastructure. Cell death in uncut *Ipomoea* sp. flowers was therefore apparently initiated in the presence of (overall) high levels of starch and sugars (Winkenbach, 1970).

Uncut *Ipomoea* sp. flowers showed senescence-related mobilization. A drastic decrease was observed in the levels of starch, protein, RNA, and DNA, concomitant with a sharp rise in activities of RNase and DNase. At the same time, Suc, amino acids, and amides were formed. These compounds were apparently exported through the phloem, which remained active after most other petal cells had died. Two-thirds of the potassium ions and more than one-half of the magnesium ions were also exported from the petals. Cell walls were apparently hydrolyzed, as β-glucosidase activity drastically increased. The increase in activities of RNase and β-glucosidase in cut *Ipomoea* sp. flowers was similar to that observed in uncut flowers The results show, therefore, that mobilization in uncut flowers proceeds just as in cut ones (Winkenbach, 1970; Baumgartner et al., 1975; Wiemken et al., 1976).

These results indicate that total (overall) soluble sugar levels in petals of most uncut flowers growing under natural light levels remain high until visible senescence. This may mean that sugar starvation does not happen. However, the distribution of sugars among the various cell groups and cellular compartments has not been reported, which means that the data are not precise enough to be taken as good evidence.

SUGAR ACCUMULATION AS A CAUSE OF SENESCENCE

It was argued above that the presence of high overall sugar levels in petals of senescent flowers cannot serve as an argument against starvation-induced senescence. Conversely, these data also do not support the idea that elevated sugar levels are the cause of senescence. Again, no detailed description has been published on the sugar distribution in various tissues, cells, and cellular compartments. High sugar levels may be a result of mobilization, thus a result of senescence rather than its cause. Moreover, when cut flowers or isolated petals are fed with sugar, hastening of petal senescence has apparently not been reported, in any species. There is, therefore, at present no support for the hypothesis that high sugar levels initiate petal senescence.

Similarly, the evidence for the idea that high sugar levels are a cause of leaf senescence is rather weak. Sugar treatments do hasten leaf yellowing in cut stems of a few species such as *Lilium multiflorum* and *Alstroemeria pelegrina*, but have no effect in numerous other species. Although tobacco leaf discs show accelerated senescence after sugar treatment, rigorous tests have not been reported in which endogenous sugar levels in various cellular compartments of intact plants were compared with those in leaf discs. The experiments with transgenic plants that overexpress hexokinase, resulting in earlier leaf senescence (see the introduction) are also not conclusive. These experiments were taken to indicate that high sugar levels are a cause of senescence. However, the leaves of tomato plants with hexokinase overpression had a lower photosynthetic rate and lower sugar concentration than wild-type plants. High sugar levels are known to reduce the rate of photosynthesis, through hexokinase regulation (Yoshida, 2003). The low sugar levels in the transgenic tomato leaves, therefore, seem a result of a high hexokinase signal, which down-regulated photosynthesis. The results with these transgenic plants, therefore, argue just as much for the starvation hypothesis.

CONCLUSIONS AND PERSPECTIVES

There is, at present, no good evidence that high sugar levels would induce petal senescence. In contrast, there is considerable circumstantial evidence for starvation to be a cause of petal senescence. Changes in ultrastructure, metabolism, and gene expression in senescent petals are remarkably similar to those in sugar-starving organs. Both tissues exhibit mass degradation of starch, proteins, lipids, and nucleic acids, with Suc and amides as the main products. Cell death is apparently the direct result of loss of selective permeability in the tonoplast, both in senescent petals and in starving cells. In either case, the cell may be disassembled to a point where it collapses.

Thimann's Hypothesis Evaluated

Although starvation may be a cause of early senescence, little evidence has been found in favor of the hypothesis, first proposed by Thimann—for green

leaves—that sugar starvation is a general cause of senescence. It still needs to be demonstrated that a low sugar level in at least one cellular compartment precedes mobilization in senescent petals and also that this low level is the cause of mobilization. Circumstantial evidence indicates that starvation occurs in some cut flowers. A main argument is the delay in protein degradation and delayed expression of a number of genes involved in mobilization, after sugar feeding. Thus, low sugar levels may trigger mobilization and petal cell death in cut flowers. Nonetheless, a main effect of sugar feeding in cut flowers is the delay in the rise in ethylene production, in species where petal senescence is regulated by ethylene. Low sugar levels may therefore not induce starvation in these species, but rather allow the ethylene signal to proceed.

For uncut flowers, the data are much less conclusive. When sugars are supplied to cut flowers, the time to visible senescence is generally only postponed to the time it occurs in flowers left attached to the plant. This can be taken as an argument in favor of the idea that the effect of sugars in cut flowers is superimposed on a process that regulates the onset of senescence independently of sugar starvation. With regard to petals on intact plants, no positive evidence, therefore, has been found in favor of Thimann's hypothesis, and the circumstantial evidence tends to count against it. The present evidence thus favors the left-hand scheme of Figure 1: Senescence is triggered by developmental genes, which are expressed independent of a mechanism whereby senescence is induced by sugar starvation.

Cell death in petals (of a number of species, such as *Ipomoea* sp., *Tradescantia* sp., and *Iris* sp.) occurs earlier in the mesophyll than in the epidermis and quite late in the phloem. In some species, cells at the distal part of the petal die earlier than more proximal ones. This review argues for more precise measurements whereby cells that are at various stages in the senescence pathway are separated. More detailed assessment of carbohydrate concentrations by tissue, by the cells in each tissues, and by cellular compartment would yield more insight into the role of starvation in the processes leading to cell death.

The main themes of the present review can now be summarized. Relatively low sugar levels may be common in some cellular compartment in petals of cut flowers but perhaps do not occur in petals of uncut flowers. The low sugar levels may induce premature senescence through starvation and increased ethylene sensitivity. Mobilization may be causally related to petal cell death: According to one hypothesis, the net degradation of tonoplast components renders death unavoidable. We still do not know how the onset of mobilization in senescent cells is regulated. This review argues for more detailed experimentation to further address these themes.

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