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REVIEWS

Dedicated to the memory of Academician Mikhail Kh. Chailakhyan, on the occasion of the 110th anniversary of his birthday, for his insightful early work on the induction of flowering, the plant process preceding the senescence of the whole plant.

Towards an Integrated View of Monocarpic Plant Senescence[1]

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Abstract—After the flowering of an annual plant, the whole plant will senesce and die. For the process to go to completion, this monocarpic senescence must include three coordinated processes, which have not previously been considered as a total syndrome: (1) the arrest of growth and senescence of the shoot apical meristem; (2) senescence of the leaves; and (3) the suppression of axillary bud growth. Concurrently there is a shift in resource allocation from continued vegetative growth to reproductive growth, combined with a withdrawal of nutrients, especially nitrogen compounds, from the leaves and the transfer of these nutrients to the developing seeds. The start of the senescence process is caused by a shift, almost certainly in gene expression, very early in the reproductive phase. Continuation of the resource transfer and senescence of the vegetative plant involves hormonal regulation and continued changes in gene expression. Each of these processes is examined, especially with reference to the transfer of resources from vegetative to reproductive growth.

Keywords: Pisum sativum, Arabidopsis thaliana, Spinacia oleracea, senescence, monocarpic, whole plant, resource allocation, leaf, apical bud, axillary buds, flowering, reproduction, sugar, nitrogen.

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INTRODUCTION

After the flowering of an annual plant (and the production of seeds by a hermaphrodite or female plant), the whole plant will senesce and die [1–4]. This represents one extreme of a life cycle strategy, whereby the adult is sacrificed for maximal reproductive success, which is presumed to be an optimal lifestyle for shifting environmental conditions [1]. For the process to go to completion, this monocarpic senescence must include three coordinated processes: (1) the arrest of growth, and possibly senescence, of the shoot apical meristem(s) (SAM); (2) senescence of somatic organs and tissues such as leaves; and (3) the prevention of regrowth by suppression of axillary buds [5], as otherwise either growth will continue and/or the reserves will not be available for optimal seed production. Concurrently there is a shift in resource allocation from continued vegetative growth to reproductive growth, combined with a withdrawal of nutrients from the leaves and the transfer of these nutrients to the developing seeds. However, these processes, particularly leaf senescence, are frequently dealt in isolation with no attempt to examine the whole syndrome. Indeed, the role of axillary bud regrowth has been largely overlooked until recently. We will now attempt to integrate these various facets of monocarpic plant senescence and the relationships between them. Further we will examine the controls at the physiological and molecular levels and suggest mechanisms that underlie the entire syndrome.

GROWTH ARREST AND SENESCENCE OF THE SHOOT APICAL MERISTEM

Apical senescence has been most studied in peas (*Pisum sativum*). Impending senescence is first noticed as a slowing of apical growth: the apical bud decreases in size and often assumes a more open appearance due to the presence of numerous flower buds, at the same time as elongation growth is reduced (Fig. 1). Apical growth then ceases, and the apical tissues become chlorotic. Although the apical buds at this stage are clearly in the mid-stages of the senescence process, they are not dead and can be rejuvenated [6]. Such regrowth often occurs as the fruits and seeds mature, although in such cases the regrowth is a very brief weak flush of growth, which, upon the devel-

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Abbreviations: JA—jasmonic acid; LD—long day; SD—short day.

opment of one or two more pods, soon ceases, and the progress of senescence continues. As time progresses, further degradative processes of senescence continue in the arrested apex, so that the tissues become necrotic and die. At this stage no further apical growth can occur. Starting at about the time of apical arrest, the leaves and the rest of the plant visibly start to senesce. The completion of this leaf senescence follows the death of the apical bud. In peas, apical senescence is thus the first part of the three senescence processes in the whole plant.

A genetic line of peas, bred by G.A. Marx and named G2, was found to have indefinite growth with no senescence in a greenhouse in winter. Unlike wild-type peas, where senescence invariably follows fruiting, apical senescence in the G2 peas was found to be regulated by photoperiod. Under long days (LD), the G2 plants flower, fruit, and senesce, while under short days (SD) the plants flower and fruit, but continue vigorous growth (Fig. 1). Although dwarf (as judged by internode length), the plants can reach a height of 3 m. As the photoperiod is extended beyond 12 h, the number of nodes produced before senescence declines, reaching a minimum at 18 h [6].

The presence of fruits is needed to induce senescence in most peas, including G2 in LD. If all the flowers are removed, growth of G2 continues, even in LD, although the internodes shorten, the apical bud assumes a very open shape as distinct from the normal "clamshell" appearance of a pea apical bud, and the leaves become very dark green and convoluted. In such circumstances, the cessation of flower removal leads to a very rapid fruit growth and a more rapid senescence than would normally take place. If not all flowers are removed, then there is an effective titration between the number of fruits developing at any given time and the time prior to the development of senescence symptoms [6]. Under these circumstances, apical death does not occur, being replaced by continued weak growth or a series of growth stops and starts. The start of senescence symptoms in the apical bud occurs with the initial development of the first fruits and reaches a maximum when these fruits are fully elongated [7]. The senescence-promoting effect of the fruits seems to decline thereafter, so that with the completion of seed development the fruits no longer contribute to the senescence response.

The photoperiodic regulation of senescence in G2 peas is associated with a particular genotype dictated by the presence of two dominant alleles, *Sn* and *Hr* [8, 9]. Genotypes of pea recessive at either or both these loci undergo senescence after a period of reproduction regardless of photoperiod. Wild-type plants are *sn*, *hr*. *Sn* is the prime regulator and operates to delay flowering [10], with *Hr* amplifying the effect [11]. *Sn* appears to be responsible for the production of a graft-transmissible substance that delays flowering [12]. Both Sn and Hr could possibly be transcription factors regulating growth or negative regulators of the initiation of reproduction, resource redistribution, and senescence. Before visible senescence symptoms appear in the bud, the differences in the development of flowers and pods show a differential commitment to reproduction triggered by the different day lengths, under which the plants were growing. The flowers and pods of the pre-senescent long-day plants develop far more rapidly (Fig. 1), correlating with the greater resource allocation to reproduction, well before senescence symptoms are visible [13].

Cellular changes associated with the initiation of apical senescence in G2 peas were found soon after the start of flowering [14]. Cell death in the apical meristem, as detected by the labeling of breaks in the DNA (that is generally referred to as TUNEL assay), started soon after floral initiation in LD, and steadily increased with time up to 80 days post floral initiation, as did DNase activity and oxidative stress (as measured by MDA content). Under SD there was no cell death in the apical meristem and very little increase in MDA content or nuclease activity. Gibberellin (GA_3) application will prevent senescence in G2 plants grown under LD, and GA_3 treatment inhibited the occurrence of cell death, MDA accumulation, and nuclease activity in the apical buds of LD-grown plants. LD thus function by promoting nuclear degeneration and cell death from early on in the reproductive process, and these changes are inhibited by SD, possibly through the elevation of GA levels.

Fig. 1. Shoot tips of G2 pea plants just after the start of flowering, grown in (a) short days, in which indeterminate growth occurs, and (b) long days, in which senescence takes place after the production of a certain number of flowers and fruits. Insert (c) shows the apical bud of a LD-grown plant (x4 magnification) in a state of arrest and senescence after further growth has ceased. Note that under LD the development of flowers and fruits is more rapid than under SD, so that flower buds open closer to the stem apex, even right up amongst the developing leaves of the apical bud, causing the apical bud displays a more open appearance. This shows that the signal initiating the transfer of resources to reproductive growth and impending senescence starts very early in the reproductive phase. See [13].

Fig. 2. Male (a) and female (b) spinach (*Spinacia oleracea*) plants have very different inflorescence architecture. Male staminate flowers are a significant sink for the partitioning of fixed carbon, even in excess of the female pistillate flowers and fruits. In both male and female plants, the induction of senescence is associated with a shift in resource partitioning from vegetative growth to reproductive growth [4].

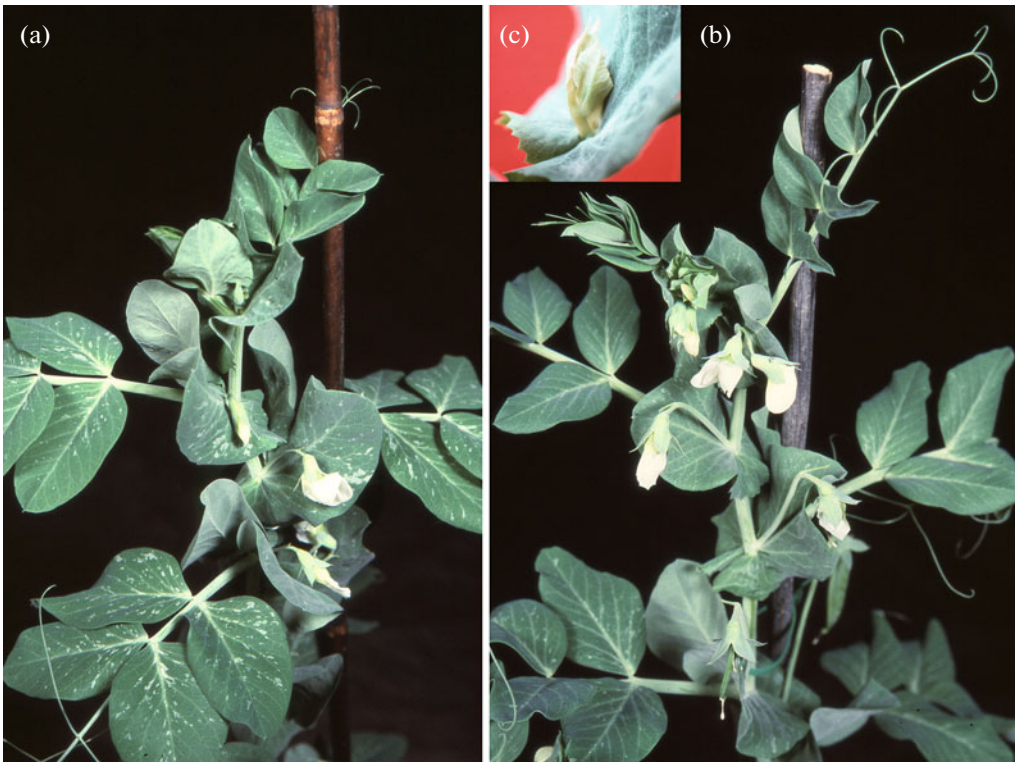

Fig. 1.

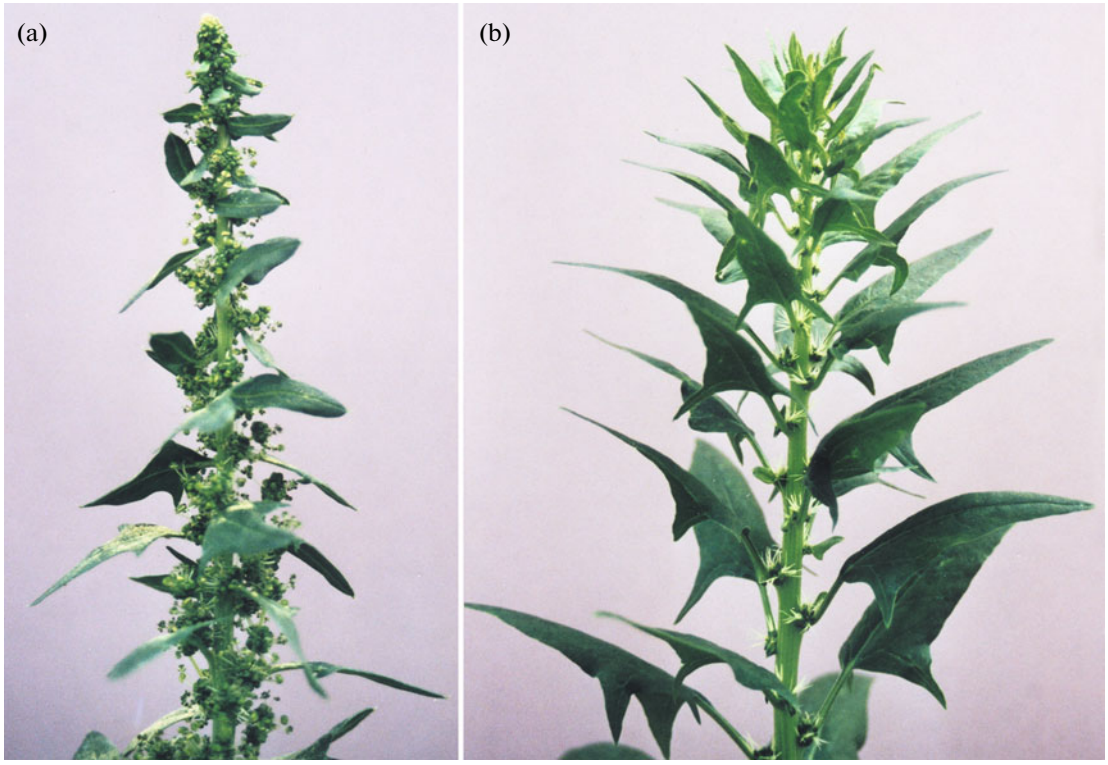

Fig. 2.

Fig. 3. (a) A tobacco plant transformed with the isopentenyltransferase gene for cytokinin biosynthesis under the control of a *SAG12* promoter that is normally involved in the initiation of the leaf senescence program. This leads to cytokinin production as the senescence program starts, so inhibiting further progression of senescence and extending the longevity of the plant. At the time when the *ipt*-transformed plant appears quite vigorous a wild-type plant (b) has lost most of its leaves to senescence [41, 42].

Fig. 4. Twelve-week-old wild-type (a) and *atmyb2* null Arabidopsis plants (b).
Whereas the wild-type plant has senesced because it no longer produces axillary branches, the plant, in which *AtMYB2* expression is inhibited, continues to branch profusely, so delaying senescence. The *atmyb2* null plant has a higher bud cytokinin level, which is implicated in the enhanced branching and senescence delay [5].

THE ROLE OF REPRODUCTIVE GROWTH IN MONOCARPIC SENESCENCE

The Timing of Senescence

The initiation of the senescence program appears to occur very early in the flowering period, and, in most cases, senescence can only be delayed, not prevented, by surgery or hormone application. In G2 peas the change in apical bud morphology under senescence-inducing conditions is visible from the start of flowering [13]. Sterile and other mutants of Arabidopsis (*Arabidopsis thaliana*) with delayed senescence did not exhibit prolonged leaf life, but did show an extended production of leaves and flowering stalks [15]. Following soybean pod removal, the leaves did not show the dramatic visual yellowing associated with senescence, but function, as measured in terms of the rate of photosynthesis or the activity of Rubisco, was inhibited[16–18]. Delayed senescence in "stay-green" genotypes of sorghum did appear to result in prolonged photosynthetic capability, but this seemed to be most closely correlated with greater nitrogen assimilation during the grain-filling period [19].

Resource Redistribution and Whole Plant Senescence

The explanation for whole plant senescence that best fits the evidence currently available is that a physiological transition is initiated at flowering and results in a resource allocation that is detrimental to the maintenance of vegetative tissues in comparison to the uninduced state. Many studies have demonstrated that alterations in the source–sink relations of vegetative and reproductive tissues can affect the course of senescence [1, 2, 20]. Senescence can be prevented in pea genotypes with a slower rate of reproductive growth that enables continued resource allocation to vegetative growth [13, 21]. The endogenous auxin and gibberellin content of floral and vegetative tissues within the apical buds of these peas correlated with this resource allocation [22]: a higher gibberellin content of the apical vegetative tissues within the apical bud was associated with vigorous vegetative growth, slower floral development, and continued growth, whereas the greater rate of floral bud growth, which precedes senescence, was associated with a higher indoleacetic acid content in the floral buds. Senescence of soybean was also delayed by the mechanical prevention of pod expansion [23, 24]. Such physical restrictions to soybean seed growth did not alter the time of initiation of senescence but decreased the rate of leaf senescence as judged by changes in photosynthesis [23], indicating that senescence was initiated at the same time regardless of sink size but that the rate of senescence was modulated by sink size. Restricting soybean pod growth did not affect total plant dry matter and N accumulation during seed-fill because there were proportional increases in partitioning of assimilates into stems and leaves [24]. Likewise pea plants bearing the alleles *ar* and *n*, which have smaller seeds and lower total seed yield, showed a resurgence of growth after apical growth had initially stopped, which is when normal plants undergo senescence. Only after further growth did the plants undergo full senescence [25]. Recessive alleles *ar* and *n* were proposed to impose a lower metabolic drain per reproductive node as a consequence of their restrictive effects on hilum anatomy and pod morphology, respectively, leading to a reduction in sink capacity. As a consequence, the developing seed crop fails to cause plant senescence and death at the usual developmental time. Similarly, in maize, crop manipulations leading to kernel set restrictions enhanced post-flowering assimilate availability and reduced leaf senescence [26], whereas the acceleration of senescence by the imposition of water stress increased the rate of grain filling in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) [27–29].

G2 peas allocate less photosynthate to their vegetative buds in LD, when they senesce after flowering, than in SD, when the plants continue to flower without senescing, showing the importance of resource partitioning in the mediation of senescence phenomena [21]. Before visible senescence symptoms appear in the bud, the differences in the development of flowers and pods show a differential commitment to reproduction triggered by the different day lengths, under which the plants were growing. The flowers and pods of the pre-senescent LD plants develop far more rapidly, correlating with the greater resource allocation to reproduction, well before senescence symptoms are visible [13] while SD plants allocate less photosynthate to the flowers, leading to slower development, and more to the vegetative tissue of the apical bud. The fact that these differences appear early in both the development of individual flowers and in the flowering stage of the whole plant emphasize the early timing of the initiation of the senescence program.

Resource Partitioning in Dioecious Plants

In dioecious spinach (*Spinacia oleracea*), senescence takes place following flowering in both female and male plants and yet only female plants produce seeds. The prior assumption was that the utilization of carbohydrate resources by staminate spinach flowers is negligible and would have no effect on senescence regulated by nutrient "exhaustion" [30]. Sklensky and Davies [4] investigated photosynthate allocation in both male (staminate) and female (pistillate) spinach plants, as an indication of overall phloem-derived resource allocation, in order to determine whether the nutrient demand by staminate spinach flowers is insignificant as has been previously assumed. Contrary to previous assumptions, staminate flowers have a nutrient demand exceeding the rate of import of pistillate flowers, especially early in the flowering period, and thus could be a determining factor in bringing about monocarpic whole-plant senescence, even in male

plants. During pollen development there is a strong diversion of phloem resources to the male flowers, which display a high rate of respiration that possibly accounts for the resource allocation. By contrast, during the early flowering stage in female plants, more resources go to the continued development of new leaves within the inflorescence, so that female plants retain more source tissue for a longer period before senescence (Fig. 2). At the time of senescence in both male and female spinach plants, there was substantial nonstructural carbohydrate in the leaves directly, strongly suggesting that the lack of metabolizable carbohydrate is not a cause of leaf senescence.

The work of Leopold et al. [30], who examined the effect of flower removal on the senescence of male spinach plants, has been consistently misinterpreted as a counter-example to the nutrient drain hypothesis. The different morphology, timing of senescence, and the high rate of respiratory activity of male, as compared to female, spinach plants explain how the excision of pollen-producing flowers results in the loss of a very significant sink for photosynthetic carbon and thus a sink for any other phloem-transported resource such as nitrogen or hormonal compound(s) [4]. In unpollinated plants, senescence did proceed even though it was delayed [30]. The removal of unpollinated female flowers delayed senescence and the removal of flowers at a younger stage resulted in a greater delay of senescence. Young staminate flowers draw large allocations of photoassimilate, whereas, by contrast, young pistillate flowers are a relatively small sink, but, because of their size, they are active sinks on a per gram basis [4]. The lack of the fruits as a large sink in the unpollinated or de-flowered plants examined by Leopold et al. [30] may have caused an alteration in the pattern of resource allocation, but the early shift to support the reproductive process was apparently sufficient to lead to eventual senescence.

Causes of Nutrient Diversion

During senescence phloem-transported compounds are diverted from vegetative to reproductive sinks. This indicates that there are likely changes in these respective sinks and also in source leaves as they transition from being a source of photosynthates to that of catabolites that are to be remobilized. Sinks have profound effects on the photosynthesis in source tissue [31]. In addition to affecting partitioning enzymes, sugars also play a key role in the transcription regulation of such key photosynthetic genes as *rbcS, cab*, and *atpD* (nuclear genes encoding the small subunit of Rubisco, the chlorophyll *a/b* binding protein, and the D subunit of a thylakoid ATPase, respectively) [32]. Extracellular invertases are important for apoplastic phloem unloading and are key enzymes in determining sink strength [33]. The sink activity for pollen development is because of an anther-specific extracellular invertase activity that supplies the developing microspores with carbohydrates. Engelke et al. [34] were able to cause male sterility through the antisense repression of the anther-specific cell wall invertase, or interference with this invertase activity by the expression of an inhibitor. They then achieved the restoration of fertility by replacing the down-regulated endogenous plant invertase with a localized yeast invertase.

While the allocation of carbohydrate resources is undoubtedly influential in the process of whole plant senescence, other changes in the metabolism of the plant must be directing that allocation. Different structures of spinach receive different amounts of photosynthate, and the amount does not depend on size alone, as shown by the analysis of partitioning data, taking into account the weight of the sink tissue [4]. The distribution of assimilate must be directed by mediating factors. Strong candidates for such mediating factors are the known plant hormones, which regulate the sink capacity of various tissues [22], although other factors are possible. An essential link between phytohormone action and sink strength is that numerous hormones affect the expression of extracellular invertase [33]. Amongst the latter could be a signaling role for sugars [35]. Partitioning enzymes in sink tissue, such as sucrose phosphate synthase, showed both short- and long-term regulation by sugar concentrations [36]. Interactions between sugar and hormone signaling also play a role in the induction of senescence, especially in response to stress [37].

In G2 pea, there is a strong correlation between hormone contents and the allocation of fixed carbon. The vegetative tissues of apical buds of LD-grown plants, which are destined to senesce, experience a drop in concentrations of gibberellins, while the same tissues of SD-grown plants maintain the high levels of hormone and remain vigorous. By contrast IAA is higher in the rapidly-growing flower buds of LD plants than in the slower-growing SD flower buds [22]. This correlates strongly with the partitioning to these structures [21], suggesting that the combination of IAA and GA direct the allocation of phloem-transported resources, resulting in differential growth and maintenance.

The discovery of hormonal correlations with variations in senescence patterns will help to elucidate the nature of the signals that regulate the shift in nutrient partitioning from the vegetative to the reproductive tissues. However, the cause of the variations in hormone levels, such as changing micro-RNA expression as occurs during the shift from juvenile to adult forms must also be clarified, leading one step closer to the initiation of senescence. Resource allocation is involved in the process, and the fact that these shifts occur early in the flowering period suggests that the timing of initiation of senescence in monocarpic plants may be coincident with the initiation of flowering.

LEAF SENESCENCE AS PART OF THE SENESCENCE OF THE WHOLE PLANT

Leaf senescence is a developmental process involving active degeneration of cells and recycling of released nutrients to seeds of monocarpic plants. Changes include the breakdown of the chloroplast and the catabolism of chlorophyll and macromolecules, such as proteins, nucleic acids, and membrane lipids. The chloroplasts contain up to 70% of the leaf protein [38]; so their degradation makes fixed nitrogen available for use in other locations. Cellular materials accumulated during the growth phase of the leaf are thus converted into exportable nutrients that are supplied to developing seeds or to other growing organs. As floral initiation and leaf senescence of Arabidopsis accessions are linked [39], it is possible that photoperiod controls leaf senescence through its effect on floral initiation. However, the nature of the relationship between floral initiation and leaf senescence remains unresolved.

Genetic and Hormonal Control of Leaf Senescence

Leaf senescence is a genetically controlled developmental phenomenon involving numerous regulatory elements [40]. The senescence process is also regulated by various plant hormones, including ABA, ethylene, jasmonic acid (JA), and salicylic acid, which promote senescence, and cytokinins and auxin, which delay or prevent senescence [20, 38, 41]. For example, genetically altering the cytokinin content of tobacco leaves by attaching cytokinin-production genes to the promoter of the *SAG12* (senescence-associated) gene, which is up-regulated in senescence, results in a delay of leaf senescence and the concomitant senescence of the whole plant (Fig. 3) [42].

DNA microarray analysis of gene expression patterns during leaf senescence have identified more than 800 Senescence Associated Genes (*SAG*s), illustrating the dramatic alteration of cellular physiology that takes place during leaf senescence [38]. Among the senescence up-regulated genes are numerous transcription factors, including those of the WRKY and NAC families. *WRKY53* is up-regulated at a very early stage of leaf senescence but decreases again at later stages, so WRKY53 might play a regulatory role in the early events of leaf senescence. A knockout line of the *WRKY53* gene showed delayed leaf senescence, whereas inducible overexpression caused precocious senescence, showing that WRKYs function as positive regulators of leaf senescence. One-fifth of the members of the NAC superfamily of plant-specific transcription-factor genes in Arabidopsis are up-regulated during senescence [43]. An Arabidopsis NAC gene, *AtNAP*, plays an important role in leaf senescence as leaf senescence is considerably delayed in knockout lines, while inducible overexpression of *AtNAP* in young leaves causes precocious senescence [44].

The Role of Micro-RNAs in Leaf Senescence

Two miRNAs have been shown to regulate different mechanisms involved in leaf ageing and senescence, as well as earlier stages of leaf development, so enabling an orderly transition into the nutrient remobilization phases of leaf senescence [45]. miR319/miRJAW targets leaf senescence via TCP transcription factors that regulate the transcription of genes involved in JA biosynthesis [46]. Schommer et al. [46] suggested that in parallel to JA biosynthesis regulation, miR319/TCPs might also regulate other genes such as *WRKY53*, which is an important positive regulator of senescence, thus possibly providing a more general role for TCPs in leaf ageing and senescence.

WHY DOES REALLOCATION TO REPRODUCTIVE SINKS CAUSE SENESCENCE?

There is now considerable evidence that senescence may be induced by carbohydrate accumulation and not by starvation [39, 47]. In Arabidopsis, senescence was delayed in a hexokinase mutant that did not accumulate hexoses, and the induction of senescence by externally supplied glucose was partially abolished in this mutant, indicating that delayed senescence was a consequence of decreased sugar sensitivity [48].

Nitrogen Remobilization from Leaves to Seeds during Leaf Senescence

In the reallocation of phloem-transported materials towards reproductive development, we may be dealing with the supply of nitrogen-containing compounds, which are clearly needed for sustained growth. ^{15}N taken up by the roots of rice was initially distributed between the various plant organs depending on their demand for nitrogen during the period of absorption, but later transfers of ^{15}N occurred between organs, in particular from the leaves to the developing rice inflorescence. About 30% of the total nitrogen in the grain was acquired before panicle initiation [49]. Sugar-induced senescence of source leaves may be a signal of low nitrogen availability [50], and high leaf-carbohydrate to nitrogen (C : N) ratios have been implicated in the induction or acceleration of the senescence process. Paul and Foyer [31] suggested that the C : N ratio and hormonal balances of the plant regulate photosynthesis, the development of leaves, and the whole plant nitrogen distribution, leading to the sink regulation of photosynthesis as well as senescence. Glucose has been shown to cause the induction of the senescence-specific gene *SAG12* by over 900-fold, and two MYB transcription factor genes induced by glucose in turn induced genes for nitrogen remobilization [48]. Several proteases were induced by high carbohydrate level in barley (*Hordeum vulgare*) leaves at the same time that senescence was accelerated [51, 52]. Agüera et al. [53] examined sunflower (*Helianthus annuus*)

grown at different levels of supplied nitrate. Plants grown with low N showed more pronounced senescence symptoms and a greater decline in photosynthetic activity than with high N. Soluble sugars increased with aging, while starch content decreased. Hexose to sucrose ratio increased, starting at the beginning of senescence, and this rise was higher in N– plants than in N+ plants, indicating that the sugar regulation of senescence is dependent on nitrogen. The enzyme pyruvate orthophosphate dikinase (PPDK), which is up-regulated in naturally senescing leaves, functions in a pathway that generates the phloem-transported amino acid glutamine. In Arabidopsis, overexpression of PPDK during senescence can significantly accelerate nitrogen remobilization from leaves, and thereby increase rosette growth rate and the weight and nitrogen content of seeds [54]. The drop in the ratio of (glucose + aspartate)/(glutamine + asparagine) as sunflower leaves aged suggested a greater nitrogen mobilization out of the leaves. This ratio declined earlier and more rapidly in N– plants than in N+ plants, suggesting that the N– remobilization rate correlates with leaf senescence severity [53].

The influence of the reproductive sink on the induction of monocarpic senescence has been suggested to be due to its ability to stimulate the nitrogen mobilization process from the source tissue through high levels of reactive oxygen species (ROS) in the source tissue leading to protein breakdown prior to mobilization [55]. To determine the relationship between leaf senescence and whole-plant nitrogen reallocation, Jukanti et al. [56] conducted a transcriptome analysis of barley lines and showed an association of several genes regulating grain protein content with the senescence process. These include the up-regulation of genes coding for both plastidial and extra-plastidial proteases in lines with accelerated leaf senescence. Further manipulation of the C : N ratio obtained by blocking phloem transport from mature barley leaves at various supplied nitrate levels found that the transcription of a C1A cysteine protease, located in a lytic vacuolar compartment, was strongly induced by high C : N ratios during leaf senescence, so is most likely to participate in bulk protein degradation during barley leaf senescence [57].

What Triggers Resource Reallocation?

We suggest that the initiation of senescence associated with a reallocation of resources to reproductive growth in monocarpic plants is under genetic control and occurs coincident with the initiation of flowering. The transfer of a single chromosome from a perennial relative was able to confer a polycarpic growth habit to monocarpic wheat, leading to a second phase of tiller initiation after the initial flowering and fruiting was complete [58], so that the genetic regulation of senescence most likely relies on only a few loci. A QTL for whole rosette senescence in Arabidopsis co-localized with *FRI*, a major determinant of flowering, and interacted epistatically with a QTL where the floral repressor *FLC* localizes [59]. Vernalization accelerated senescence in late-flowering lines with functional *FRI* and *FLC* alleles, and rapid rosette senescence on a glucose-containing medium was correlated with early flowering and high sugar content. Not surprisingly a correlation was found between the expression of flowering- and senescence-associated genes. An additional QTL was linked to nitrogen-use efficiency. The results show that whole-rosette senescence is genetically linked to the vernalization-dependent control of flowering, but is also controlled by flowering-independent pathways. A grain protein content (GPC) locus in barley strongly influences the timing of post-anthesis flag leaf senescence, with high-GPC germplasm not only senescing early but also showing an accelerated pre-anthesis plant development [60]. This locus appears to be the ortholog of Arabidopsis *AtNAP*, and as *AtNAP* it does not directly control protein and other nutrient contents; instead, the delay in leaf senescence somehow impairs the efficient nutrient recycling from senescing leaves during the grain-filling period.

In Arabidopsis the developing reproductive structures appeared to cause the death of the plant by preventing the regeneration of leaves and the development of additional reproductive structures [61]. All this is in agreement with the ideas expressed by Kelly and Davies [1] and Sklensky and Davies [2] that senescence is triggered by a physiological change very early in the reproductive period. Lacerenza et al. [60] suggest that one of the *GPC* genes associated with flag leaf senescence in barley may be a functional homologue of the Arabidopsis gene for glycine-rich RNA-binding protein 7, which has previously been implicated in the promotion of flowering. We may therefore be on the verge of a more detailed analysis of the interactions between the physiological and molecular networks controlling monocarpic senescence.

THE REGULATION OF AXILLARY BUD REGROWTH

During vegetative growth, axillary bud outgrowth may be prevented by auxin and strigolactones [62]. As size increases, a plant may become bushy through an increased distance from the source of auxin, a decrease in strigolactones, or an increase in cytokinins, which overcome the growth inhibition. In Arabidopsis, *AtMYB2* is expressed at late developmental stages in the compressed basal internodes where it inhibits the outgrowth of axillary buds as part of the whole-plant senescence program after the normal apical dominance mechanism has terminated following the arrest of further apical bud growth and when all leaves have started senescing [5]. *AtMYB2* acts by suppressing the production of cytokinins, and thus axil-

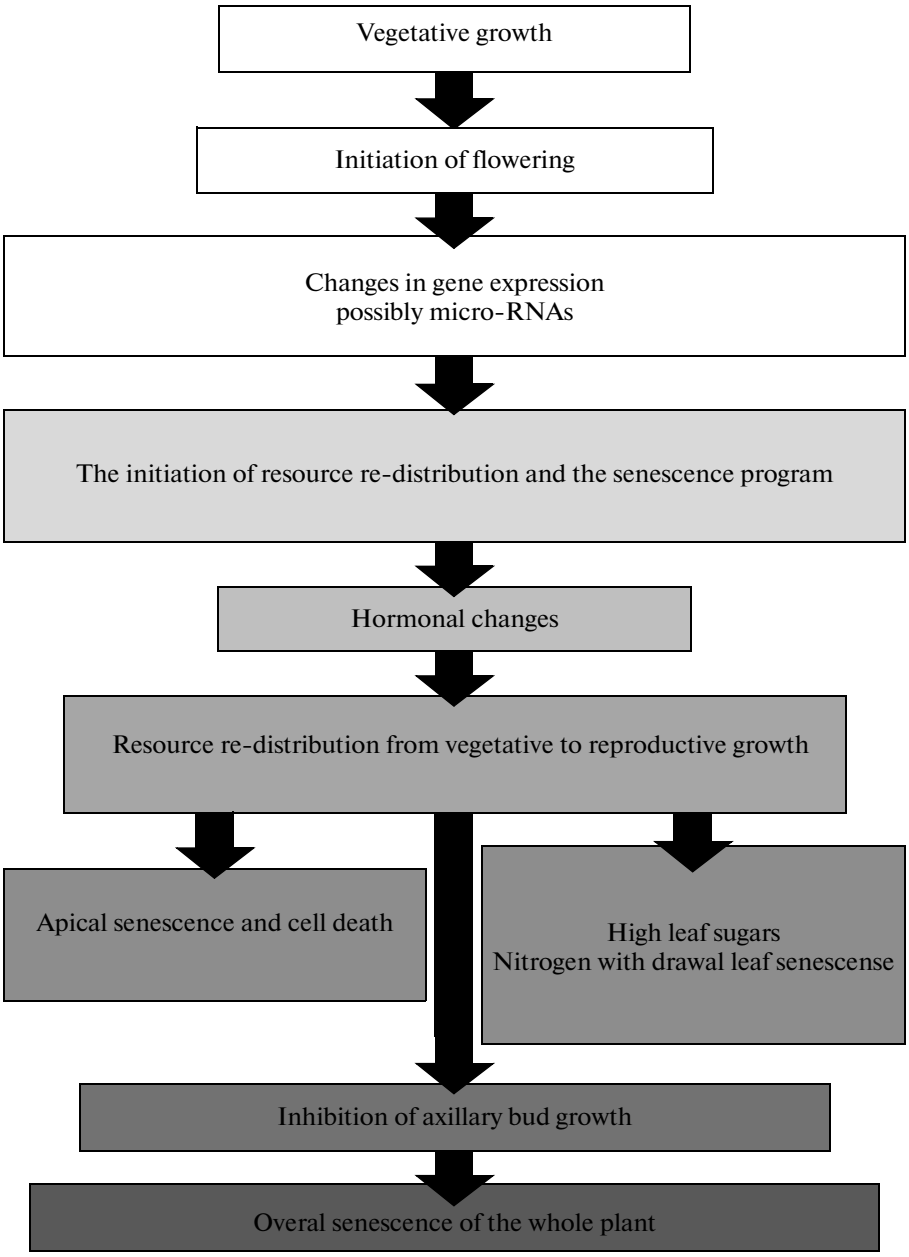

Fig. 5. A scheme of the sequential activities involved in the senescence of a monocarpic plant from the initiation of the resource redistribution to the final demise of the whole plant.

lary bud outgrowth. By contrast *atmyb2* T-DNA insertion lines have enhanced expression of cytokinin-synthesizing isopentenyltransferase genes, contain higher levels of cytokinins, and display a bushy phenotype at late stages of development. As a result of the continuous generation of new shoots, *atmyb2* plants have a prolonged life span (Fig. 4). As a further confirmation of this role, the presence of *AtMYB2* promoter-directed cytokinin oxidase 1 gene in the T-DNA insertion lines reduced the endogenous cytokinin levels and restores the bushy phenotype to the wild type.

RESOURCE ALLOCATION AND SENESCENCE IN PERENNIALS

A similar mechanism of senescence induction as in annual plants almost certainly applies to biennial or perennial plants that flower only once in their life cycle, except that the shift from vegetative to reproductive growth is delayed. In polycarpic perennials that flower many times without the induction of senescence, we suggest that the resource reallocation is so much weaker that overall plant senescence is not induced, although we know relatively little of this reproductive lifestyle [63]. Indeed, reproduction-

associated senescence can be modified in a heritable fashion by introducing genes for perenniality into an annual background. This has been demonstrated in "stay-green" genotypes of sorghum (*Sorghum bicolor*) that have arisen by introgressing genes from perennial landraces into monocarpic cultivars. Stay-green genotypes retain more green leaf area than do genotypes not possessing this trait, and they also continue to fill grain normally under drought conditions [64, 65]. The nature of what might be happening is demonstrated in rice, where, towards maturity, a competition for nitrogen developed between the panicles and the next generation of developing tillers [49], so that rice displays some weak perennial tendencies, even though it is an annual. This is thus indicative that the same sort of resource redistribution occurs in perennial as in annual plants, but to a reduced extent. An intermediate status can be seen in alternate bearing fruit trees, where flowering and fruiting result in decreased growth, leading to reduced flowering the following year. Resource reallocation in alternate bearing trees has received little attention, but there is some indication that nitrogen allocation to fruits in high fruiting years leads to a reduced N retention by leaves or allocation to young shoots [66], which might then reduce flower bud formation for the subsequent year. Recent results show that fruit inhibits flowering by repressing the expression of flowering-associated genes in leaves of alternate-bearing citrus [67], which clearly has to be regulated by some form of communication, such as resource redistribution between the fruit and leaves.

THE UNDERLYING MECHANISMS TRIGGERING MONOCARPIC PLANT SENESCENCE

Our work in peas and spinach [4, 21] clearly shows an early reallocation of phloem-transported fixed carbon to reproductive development; so the resource diversion can account for the induction of senescence in vegetative tissues (Fig. 5). However, the crucial compound clearly is not carbohydrate as carbohydrates are available in leaves and stem late in flowering. Rather than senescence resulting from voracious reproductive sinks stripping energy reserves from vegetative tissues, which then starve to death, a more likely mechanism involves a global shift in the hormonal and/or nutrient balance, likely including nitrogen, resulting from flowering. This would then lead to changes in gene expression associated with the cessation of growth and the development of the senescence syndrome in the vegetative tissues. In retrospect this is not surprising, as not only are carbohydrates not deficient at the time of whole plant senescence, but a low carbohydrate level alone would be expected to result in slower growth rather than senescence.

The observed diminution of the leaves in the inflorescence of spinach [4], and in the apical senescence in peas [21, 22] can be explained by this shift. The changes in allocation, including the proximity of the apical meristem to floral sinks, which may equal or exceed the apical meristem in sink strength, must affect the meristem itself. The meristem may then decline in size (and thus produce smaller leaves) and eventually often either senesces or converts to a flower primordium. The loss of the apical bud leads to a number of physiological changes, and the inevitable senescence of the whole plant, due to the inability to produce new organs. When the balance of carbohydrates is again altered by cessation of development in the floral sinks, the resultant feed-back inhibition could cause not only a repression of photosynthesis, but leaf senescence. Thus, it is not the drain to the reproductive sinks per se, but the permanent diversion away from the development of further new vegetative sinks that may be responsible for some of the observed phenomena in whole-plant senescence. Included in the overall diversion to reproductive growth is the prevention of the growth of axillary buds. As noted by Leopold et al. [30], once flowering is initiated, even if flowers are removed or remain unpollinated, senescence will surely follow.

In 1938 Molisch [68] suggested that plant senescence came about through nutrient exhaustion. He was right in that there is a diversion of nutritional resources from vegetative to reproductive growth. But he was wrong in that senescence does not occur simply because the vegetative tissues run out of nutrients. Possibly nitrogen becomes deficient, thought probably not lethally deficient, but certainly not carbohydrates. What happens is a directed change in gene expression triggering the cessation of vegetative growth, the breakdown of cellular components in the leaves, and the transfer of these components to the developing pollen, seeds, and fruits, and the inhibition of the growth of any axillary buds. We still do not know the nature of this master regulator that is activated (or inhibited) at the start of the reproductive phase, but with the continued identification of earlier and earlier changes in gene expression, including micro-RNAs, the answer is likely to be soon forthcoming.

REFERENCES

1. Kelly, M.O. and Davies, P.J., The Control of Whole Plant Senescence, *Crit. Rev. Plant Sci.*, 1988, vol. 7, pp. 139–173.
2. Sklensky, D.E. and Davies, P.J., Whole Plant Senescence: Reproduction and Nutrient Partitioning, *Hort. Rev.*, 1993, vol. 15, pp. 335–366.
3. Gan, S., *Senescence Processes in Plants*, Oxford: Blackwell, 2007.
4. Sklensky, D.E. and Davies, P.J., Resource Partitioning to Male and Female Flowers of *Spinacia oleracea* L. in Relation to Whole-Plant Monocarpic Senescence, *J. Exp. Bot.*, 2011, vol. 62, pp. 4323–4336.
5. Guo, Y. and Gan, S., *AtMYB2* Regulates Whole Plant Senescence by Inhibiting Cytokinin-Mediated Branching at Late Stages of Development in Arabidopsis, *Plant Physiol.*, 2011, vol. 156, pp. 1612–1619.

6. Proebsting, W.M., Davies, P.J., and Marx, G.A., Photoperiodic Control of Apical Senescence in a Genetic Line of Peas, *Plant Physiol.,* 1976, vol. 58, pp. 800–802.
7. Davies, P.J., Proebsting, W.M., and Gianfagna, T.J., Hormonal Relationships in Whole Plant Senescence, *Plant Growth Regulation*, Pilet, P.E, Ed., Heidelberg: Springer-Verlag, 1977, pp. 273–280.
8. Murfet, I.C. and Marx, G.A., Flowering in *Pisum:* Comparison of the Genewa and Hobart Systems of Phenotypic Classification, *Pisum Newslett.*, 1976, vol. 8, pp. 46–48.
9. Proebsting, W.M., Davies, P.J., and Marx, G.A., Photoperiod-Induced Changes in Gibberellin Metabolism in Relation to Apical Growth and Senescence in a Genetic Line of Peas (*Pisum sativum* L.), *Planta,* 1978, vol. 141, pp. 231–238.
10. Murfet, I.C., Flowering in *Pisum*: Reciprocal Grafts between Known Genotypes, *Aust. J. Biol. Sci.,* 1971, vol. 24, pp. 1089–1101.
11. Murfet, I.C., Flowering in *Pisum: Hr,* a Gene for High Response to Photoperiod, *Heredity*, 1973, vol. 31, pp. 157–164.
12. Murfet, I.C. and Reid, J.B., Flowering in *Pisum*: Evidence That Gene *Sn* Controls a Graft-Transmissible Inhibitor, *Aust. J. Biol. Sci.,* 1973, vol. 26, pp. 675–677.
13. Kelly, M.O. and Davies, P.J., Genetic and Photoperiodic Control of the Relative Rates of Reproductive and Vegetative Development in Peas, *Ann. Bot.*, 1986, vol. 58, pp. 13–21.
14. Wang, D.Y., Li, Q., Cui, K.M., and Zhu, Y.X., Gibberellin Is Involved in the Regulation of Cell Death-Mediated Apical Senescence in G2 Pea, *J. Int. Plant Biol.*, 2007, vol. 49, pp. 1627–1633.
15. Hensel, L.L., Grbic, V., Baumgarten, D.A., and Bleecker, A.B., Developmental and Age-Related Processes That Influence the Longevity and Senescence of Photosynthetic Tissues in Arabidopsis, *Plant Cell,* 1993, vol. 5, pp. 553–564.
16. Mondal, M.H., Brun, W.A., and Brenner, M.L., Effects of Sink Removal on Photosynthesis and Senescence in Leaves of Soybean (*Glycine max* L.) Plants, *Plant Physiol.,* 1978, vol. 61, pp. 394–397.
17. Wittenbach, V.A., Effects of Pod Removal on Leaf Senescence in Soybeans, *Plant Physiol.*, 1982, vol. 70, pp. 1544–1548.
18. Wittenbach, V.A., Effect of Pod Removal on Leaf Senescence and Soluble Protein Composition of Field-Grown Soybeans, *Plant Physiol.,* 1983, vol. 73, pp. 121–124.
19. Borrell, A., Hammer, G., and van Oosterom, E., Stay-Green: A Consequence of the Balance between Supply and Demand for Nitrogen during Grain Filling? *Ann. Appl. Biol.*, 2001, vol. 138, pp. 91–95.
20. Guiboileau, A., Sormani, R., Meyer, C., and Masclaux-Daubresse, C., Senescence and Death of Plant Organs: Nutrient Recycling and Developmental Regulation, *C. R. Acad. Sci., Ser. Biol.,* 2010, vol. 333, pp. 382–391.
21. Kelly, M.O. and Davies, P.J., Photoperiodic and Genetic Control of Carbon Partitioning in Peas and Its Relationship to Apical Senescence, *Plant Physiol.,* 1988, vol. 86, pp. 978–982.
22. Zhu, Y.-X. and Davies, P.J., The Control of Apical Bud Growth and Senescence by Auxin and Gibberellin in Genetic Lines of Peas, *Plant Physiol.*, 1997, vol. 113, pp. 631–637.
23. Crafts-Brandner, S.J. and Egli, D.B., Modification of Seed Growth in Soybean by Physical Restraint Effect on Leaf Senescence, *J. Exp. Bot.*, 1987, vol. 38, pp. 2043–2049.
24. Miceli, F., Crafts-Brandner, S.J., and Egli, D.B., Physical Restriction of Pod Growth Alters Development of Soybean Plants, *Crop Sci.*, 1995, vol. 35, pp. 1080–1085.
25. Murfet, I.C., The Influence of Genes *Ar* and *Sn* on Senescence in *Pisum sativum* L., *Ann. Bot.*, 1985, vol. 55, pp. 675–684.
26. Borras, L., Maddonni, G.A., and Otegui, M.E., Leaf Senescence in Maize Hybrids: Plant Population, Row Spacing and Kernel Set Effects, *Field Crops Res.*, 2003, vol. 82, pp. 13–26.
27. Yang, J., Zhang, J., Wang, Z., Zhu, Q., and Liu, L., Water Deficit-Induced Senescence and Its Relationship to the Remobilization of Pre-Stored Carbon in Wheat during Grain Filling, *Agron. J.*, 2001, vol. 93, pp. 196–206.
28. Yang, J., Zhang, J., Wang, Z., Zhu, Q., and Wang, W., Hormonal Changes in the Grains of Rice Subjected to Water Stress during Grain Filling, *Plant Physiol.*, 2001, vol. 127, pp. 315–323.
29. Yang, J., Zhang, J., Wang, Z., Liu, L., and Zhu, Q., Postanthesis Water Deficits Enhance Grain Filling in Two-Line Hybrid Rice, *Crop Sci.*, 2003, vol. 43, pp. 2099–2108.
30. Leopold, A.C., Niedergang-Kamien, E., and Janick, J., Experimental Modification of Plant Senescence, *Plant Physiol.*, 1959, vol. 34, pp. 570–573.
31. Paul, M.J. and Foyer, C.H., Sink Regulation of Photosynthesis, *J. Exp. Bot.*, 2001, vol. 52, pp. 1383–1400.
32. Krapp, A. and Stitt, M., An Evaluation of Direct and Indirect Mechanisms for the "Sink-Regulation" of Photosynthesis in Spinach: Changes in Gas Exchange, Carbohydrates, Metabolites, Enzyme Activities and Steady-State Transcript Levels after Cold-Girdling Source Leaves, *Planta*, 1995, vol. 195, pp. 313–323.
33. Roitsch, T., Balibrea, M., Hofmann, M., Proels, R., and Sinha, A., Extracellular Invertase: Key Metabolic Enzyme and PR Protein, *J. Exp. Bot.,* 2003, vol. 54, pp. 513–524.
34. Engelke, T., Hirsche, J., and Roitsch, T., Anther-Specific Carbohydrate Supply and Restoration of Metabolically Engineered Male Sterility, *J. Exp. Bot.*, 2010, vol. 61, pp. 2693–2706.
35. Koch, K.E., Carbohydrate-Modulated Gene Expression in Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1996, vol. 47, pp. 509–540.
36. Huber, S.C. and Huber, J.L., Role and Regulation of Sucrose-Phosphate Synthase in Higher Plants, *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 1996, vol. 47, pp. 431–444.
37. Wingler, A. and Roitsch, T., Metabolic Regulation of Leaf Senescence: Interactions of Sugar Signalling with Biotic and Abiotic Stress Responses, *Plant Biol.*, 2008, vol. 10, pp. 50–62.
38. Lim, P.O., Kim, H.J., and Nam, H.G., Leaf Senescence, *Annu. Rev. Plant Biol.*, 2007, vol. 58, pp. 115–136.
39. Levey, S. and Wingler, A., Natural Variation in the Regulation of Leaf Senescence and Relation to Other Traits in Arabidopsis, *Plant Cell Environ.*, 2005, vol. 28, pp. 223–231.

40. Lim, P.O. and Nam, H.G., The Molecular and Genetic Control of Leaf Senescence and Longevity in Arabidopsis, *Curr. Top. Dev. Biol.,* 2005, vol. 67, pp. 49–83.
41. Gan, S., The Hormonal Regulation of Senescence, *Plant Hormones: Biosynthesis, Signal Transduction, Action!* Davies, P.J, Ed., Dordrecht: Springer-Verlag, 2010, pp. 597–617.
42. Gan, S. and Amasino, R.M., Inhibition of Leaf Senescence by Autoregulated Production of Cytokinin, *Science*, 1995, vol. 270, pp. 1986–1988.
43. Guo, Y., Cai, Z., and Gan, S., Transcriptome of Arabidopsis Leaf Senescence, *Plant Cell Environ.*, 2004, vol. 27, pp. 521–549.
44. Guo, Y. and Gan, S., AtNAP, a NAC Family Transcription Factor, Has an Important Role in Leaf Senescence, *Plant J.,* 2006, vol. 46, pp. 601–612.
45. Pulido, A. and Laufs, P., Co-Ordination of Developmental Processes by Small RNAs during Leaf Development, *J. Exp. Bot.*, 2010, vol. 61, pp. 1277–1291.
46. Schommer, C., Palatnik, J.F., Aggarwal, P., Chetelat, A., Cubas, P., Farmer, E.E., Nath, U., and Weigel, D., Control of Jasmonate Biosynthesis and Senescence by MiR319 Targets, *PLoS Biol.*, 2008, vol. 6, pp. 1991–2001.
47. Van Doorn, W.G., Is the Onset of Senescence in Leaf Cells of Intact Plants Due to Low or High Sugar Levels? *J. Exp. Bot.*, 2008, vol. 59, pp. 1963–1972.
48. Pourtau, N., Jennings, R., Pelzer, E., Pallas, J., and Wingler, A., Effect of Sugar-Induced Senescence on Gene Expression and Implications for the Regulation of Senescence in Arabidopsis, *Planta*, 2006, vol. 224, pp. 556–568.
49. Sheehy, J., Mnzava, M., Cassman, K., Mitchell, P., Pablico, P., Robles, R., Samonte, H., Lales, J., and Ferrer, A., Temporal Origin of Nitrogen in the Grain of Irrigated Rice in the Dry Season: The Outcome of Uptake, Cycling, Senescence and Competition Studied Using a ^{15}N-Point Placement Technique, *Field Crops Res.,* 2004, vol. 89, pp. 337–348.
50. Wingler, A., Purdy, S., MacLean, J.A., and Pourtau, N., The Role of Sugars in Integrating Environmental Signals during the Regulation of Leaf Senescence, *J. Exp. Bot.*, 2006, vol. 57, pp. 391–399.
51. Parrott, D., Yang, L., Shama, L., and Fischer, A.M., Senescence Is Accelerated, and Several Proteases Are Induced by Carbon "Feast" Conditions in Barley (*Hordeum vulgare* L.) Leaves, *Planta*, 2005, vol. 222, pp. 989–1000.
52. Parrott, D.L., McInnerney, K., Feller, U., and Fischer, A.M., Steam-Girdling of Barley (*Hordeum vulgare*) Leaves Leads to Carbohydrate Accumulation and Accelerated Leaf Senescence, Facilitating Transcriptomic Analysis of Senescence-Associated Genes, *New Phytol.*, 2007, vol. 176, pp. 56–69.
53. Agüera, E., Cabello, P., and de la Haba, P., Induction of Leaf Senescence by Low Nitrogen Nutrition in Sunflower (*Helianthus annuus*) Plants, *Physiol. Plant.*, 2010, vol. 138, pp. 256–267.
54. Taylor, L., Nunes-Nesi, A., Parsley, K., Leiss, A., Leach, G., Coates, S., Wingler, A., Fernie, A.R., and Hibberd, J.M., Cytosolic Pyruvate, Orthophosphate Dikinase Functions in Nitrogen Remobilization during Leaf Senescence and Limits Individual Seed Growth and Nitrogen Content, *Plant J.*, 2010, vol. 62, pp. 641–652.
55. Srivalli, B. and Khanna-Chopra, R., The Developing Reproductive 'Sink' Induces Oxidative Stress to Mediate Nitrogen Mobilization during Monocarpic Senescence in Wheat, *Biochem. Biophys. Res. Commun.*, 2004, vol. 325, pp. 198–202.
56. Jukanti, A.K., Heidlebaugh, N.M., Parrott, D.L., Fischer, I.A., McInnerney, K., and Fischer, A.M., Comparative Transcriptome Profiling of Near-Isogenic Barley (*Hordeum vulgare*) Lines Differing in the Allelic State of a Major Grain Protein Content Locus Identifies Genes with Possible Roles in Leaf Senescence and Nitrogen Reallocation, *New Phytol.*, 2008, vol. 177, pp. 333–349.
57. Parrott, D.L., Martin, J.M., and Fischer, A.M., Analysis of Barley (*Hordeum vulgare*) Leaf Senescence and Protease Gene Expression: A Family C1A Cysteine Protease Is Specifically Induced under Conditions Characterized by High Carbohydrate, but Low to Moderate Nitrogen Levels, *New Phytol.*, 2010, vol. 187, pp. 313–331.
58. Lammer, D., Cai, X., Arterburn, M., Chatelain, J., Murray, T., and Jones, S., A Single Chromosome Addition from *Thinopyrum elongatum* Confers a Polycarpic, Perennial Habit to Annual Wheat, *J. Exp. Bot.,* 2004, vol. 55, pp. 1715–1720.
59. Wingler, A., Purdy, S.J., Edwards, S.A., Chardon, F., and Masclaux-Daubresse, C., QTL Analysis for Sugar-Regulated Leaf Senescence Supports Flowering-Dependent and -Independent Senescence Pathways, *New Phytol.*, 2010, vol. 185, pp. 420–433.
60. Lacerenza, J.A., Parrott, D.L., and Fischer, A.M., A Major Grain Protein Content Locus on Barley (*Hordeum vulgare* L.) Chromosome 6 Influences Flowering Time and Sequential Leaf Senescence, *J. Exp. Bot.*, 2010, vol. 61, pp. 3137–3149.
61. Nooden, L.D. and Penney, J.P., Correlative Controls of Senescence and Plant Death in *Arabidopsis thaliana* (Brassicaceae), *J. Exp. Bot.,* 2001, vol. 52, pp. 2151–2159.
62. Beveridge, C.A. and Rameau, C., Strigolactones: The New Class of Branching Hormones, *Plant Hormones: Biosynthesis, Signal Transduction, Action!* Davies, P.J. Ed., Dordrecht: Springer-Verlag, 2010, pp. 507–518.
63. Munne-Bosch, S., Aging in Perennials, *Crit. Rev. Plant Sci.*, 2007, vol. 26, pp. 123–138.
64. Thomas, H. and Howarth, C.J., Five Ways to Stay Green, *J. Exp. Bot.*, 2000, vol. 51, pp. 329–337.
65. Kassahun, B., Bidinger, F., Hash, C., and Kuruvinashetti, M., Stay-Green Expression in Early Generation Sorghum [*Sorghum bicolor* (L.) Moench] QTL Introgression Lines, *Euphytica*, 2010, vol. 172, pp. 351–362.
66. Baninasab, B., Rahemi, M., and Shariatmadari, H., Seasonal Changes in Mineral Content of Different Organs in the Alternate Bearing of Pistachio Trees, *Commun. Soil Sci. Plant Anal.*, 2007, vol. 38, pp. 241–258.
67. Munoz-Fambuena, N., Mesejo, C., Gonzalez-Mas, C.M., Primo-Millo, E., Agusti, M., and Iglesias, D.J., Fruit Regulates Seasonal Expression of Flowering Genes in Alternate-Bearing 'Moncada' Mandarin, *Ann. Bot.*, 2011, vol. 108, pp. 511–519.
68. Molisch, H., *The Longevity of Plants (Der Lebensdauer der Pflanze)*, New York: E.H. Fulling, 1938.