Ann. appl. Biol. (1993), **123**, 193–219
Printed in Great Britain

Crops that stay green[1]

By HOWARD THOMAS and CATHERINE M SMART

Cell Biology Department, Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3EB, UK

(Accepted 2 February 1993)

Summary

Genetic variation exists for foliar senescence and has, usually incidentally or empirically, been exploited for crop improvement. We review the incidence of delayed or inoperative senescence in maize, sorghum, oats, rice, wheat, fescue, soybean, french bean, fruit crops, trees and other species. The insights such variants give into the genetic control of leaf senescence, and the practical implications of improved understanding of the *stay-green* phenomenon are discussed.

Key words: Leaf senescence, chlorophyll, genes, mutations, cereals, forage grasses, legumes, fruit crops, trees, evergreens, leaf duration, crop yield

Introduction

Use of greenness in plant breeding

By a fortunate coincidence, the energy band of light absorbed by photosynthetic pigments is squarely within the range of wavelengths to which human vision is receptive. This means that throughout most of the history of plant domestication the eye was able to measure, non-destructively, a plant's physiological status with a sophistication that has become possible with electronic instrumentation only very recently. It is clear that such visual assessments have been important influences on the direction taken by progressive improvements in crop plants from the earliest period of human agricultural activity, and they retain empirical significance to this day. In general, the most useful modern crop species and varieties are greener, establish green tissues faster, retain them longer and are visibly more responsive to agricultural inputs, such as fertiliser, pesticides and irrigation, than their forebears.

Chlorophyll is unstable in the light

It is ironic that the word *green* has become a benign symbol of all that is environmentally friendly, wholesome and beneficial to living organisms. In reality chlorophyll, which is responsible for all this greenness, can be a most dangerous chemical compound. The combination of chlorophyll, light and an oxygen atmosphere can be extremely damaging to living cells; and not just to the cells of green plant tissue. It is fortunate that sheep and vegetarians are opaque. A transparent herbivore such as the planktonic animal *Calanus*, is at risk from sunlight when its gut contains chlorophylls from the algae it feeds on. So it

[1]Dedicated to Professor Philippe Matile on the occasion of his sixtieth birthday

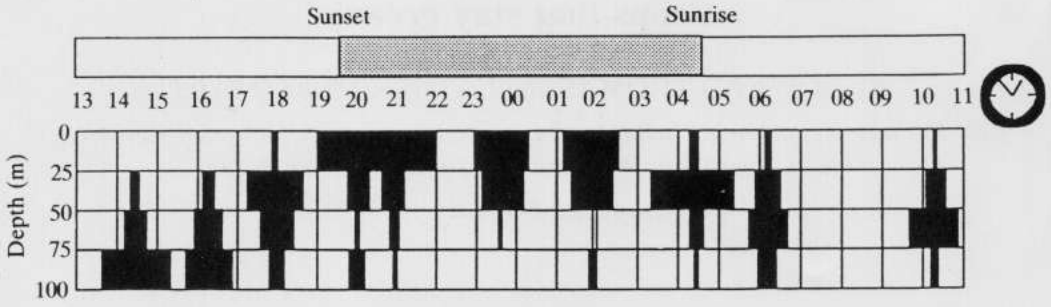

Fig. 1. Vertical distribution of female *Calanus finmarchius* at a station in the Atlantic Ocean south of Ireland on 11–12 August 1932 (after Farran, 1947).

migrates vertically in the sea on a daily basis – down at dawn, up in the evening (Fig. 1 – see also Isaacs, Tont & Wick, 1974; Omori, 1974). If it did not do this it would photodestroy like a weed treated with paraquat. Albino rats fed with a high chlorophyll diet become seriously photosensitised and suffer from all kinds of skin lesions (Tapper, Lohrey, Hove & Allison, 1975). In fact, loading up tumours with chlorophyll-like porphyrin derivatives and exposing them to high intensity laser light is being actively developed as a cancer treatment (Moreno, Pottier & Truscott, 1988).

Is senescence triggered by autodestruction of chlorophyll?

During senescence, chlorophyll disappears and the ultimate products of catabolism seem not to be pigmented. The striking colours of autumn leaves are due to carotenoids, anthocyanins, phenols and a whole range of secondary compounds metabolically unrelated to chlorophyll (Matile *et al.*, 1989). Let us suppose that built-in processes which defend against autodestruction begin to fail in older cells and as they decline, so senescence becomes apparent and the visible and biochemically measurable symptoms of the syndrome are exhibited (Leshem, 1988; Strother, 1988). Plants with highly heritable *stay-green* phenotypes present a serious challenge to this hypothesis. An explanation may be that such variants possess an abnormally high level of resistance to photodamage and therefore take longer to reach the threshold below which autodestruction occurs, or else the defence is abnormally retained in old leaves. However, where this hypothesis has been tested, the evidence indicates that *stay-green* plants are no better than normal genotypes at withstanding chlorophyll-mediated photodestruction and, conversely, that mutants with high intrinsic immunity to photodamage, such as transgenics with elevated superoxide dismutase (Bowler *et al.*, 1991), do not also exhibit *stay-green*ness.

There is evidence that mechanisms defending against chlorophyll-mediated photodamage do fail during senescence. Using a green mutant of *Festuca pratensis*, Thomas & Matile (1988) demonstrated the gradual intrusion of susceptibility to photobleaching in the final stages of senescence (Fig. 2). Pigment eventually fades from the very oldest, desiccated leaves of intact mutant *Festuca* plants. However this is a later stage than the yellowing process which occurs in the senescence of living tissue, and we believe it takes place after the loss of vital functions and the collapse of viability.

Senescence as a two-stage process

Much of the confusion about the metabolic basis of senescence seems to arise from a failure to appreciate that the term has been used to describe two quite different processes – *pre-* and *post-mortem*. As a leaf passes the peak of assimilatory capacity, mesophyll tissue

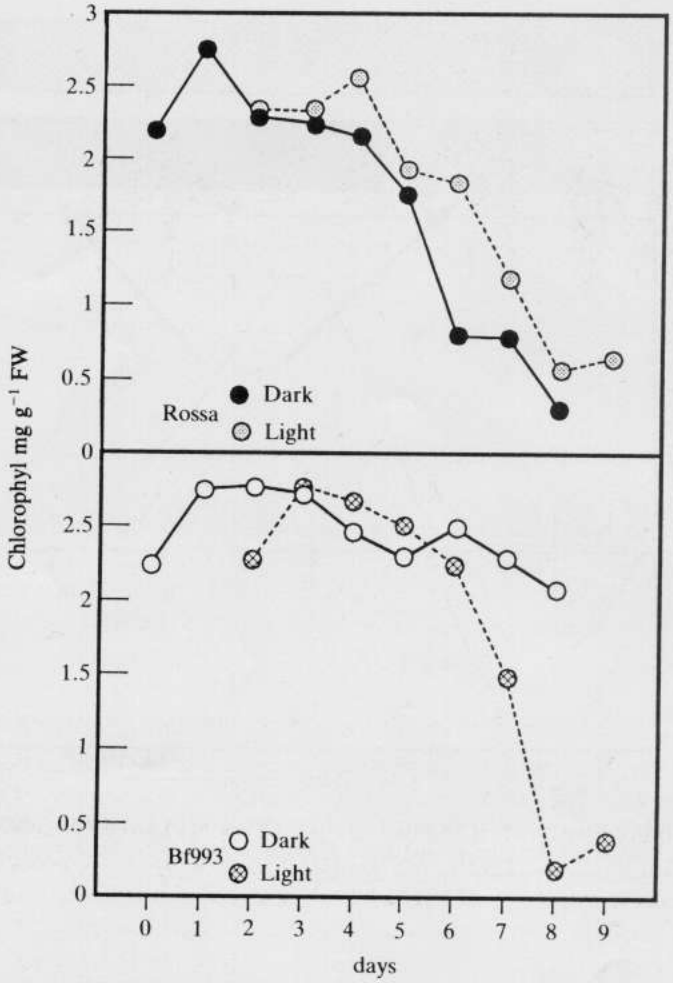

Fig. 2. Chlorophyll stability in isolated leaf tissue of *Festuca pratensis* in light and darkness. Rossa, normally yellowing genotype; Bf993, *stay-green* mutant. Redrawn from Thomas & Matile (1988).

ns to yellow and the fabric of the photosynthetic apparatus is dismantled and exported to young growing tissues or sites of reserve deposition. An important characteristic of cells undergoing these changes is that *they remain viable* – indeed, viability, tight metabolic regulation and close coordination at tissue and organ levels are prerequisites for the normal progression of the first phase of senescence (Thomas & Stoddart, 1980; Stoddart & Thomas, 1982; Matile, 1992*a*; Thomas, 1992*a*). Thereafter the tissue rapidly loses viability and undergoes deteriorative modifications which are essentially post-mortem changes. In our opinion the free radical, active oxygen or photodynamic models of senescence are relevant to the second, terminal phase but shed little light on the behaviour of viable cells and tissues.

If we consider foliar development in terms of its supply and demand relations with the rest of the plant we see a clear sequence. Fig. 3 shows how young leaves are net heterotrophs, subsisting on assimilate imported from mature foliage in positive carbon balance. Subsequently photosynthetic competence is asserted and the organ becomes a net contributor to the carbon budget of the whole plant. Then assimilation declines as the leaf yellows. In Fig. 3 the curve for nitrogen is shown along with that for carbon (phosphorus, sulphur

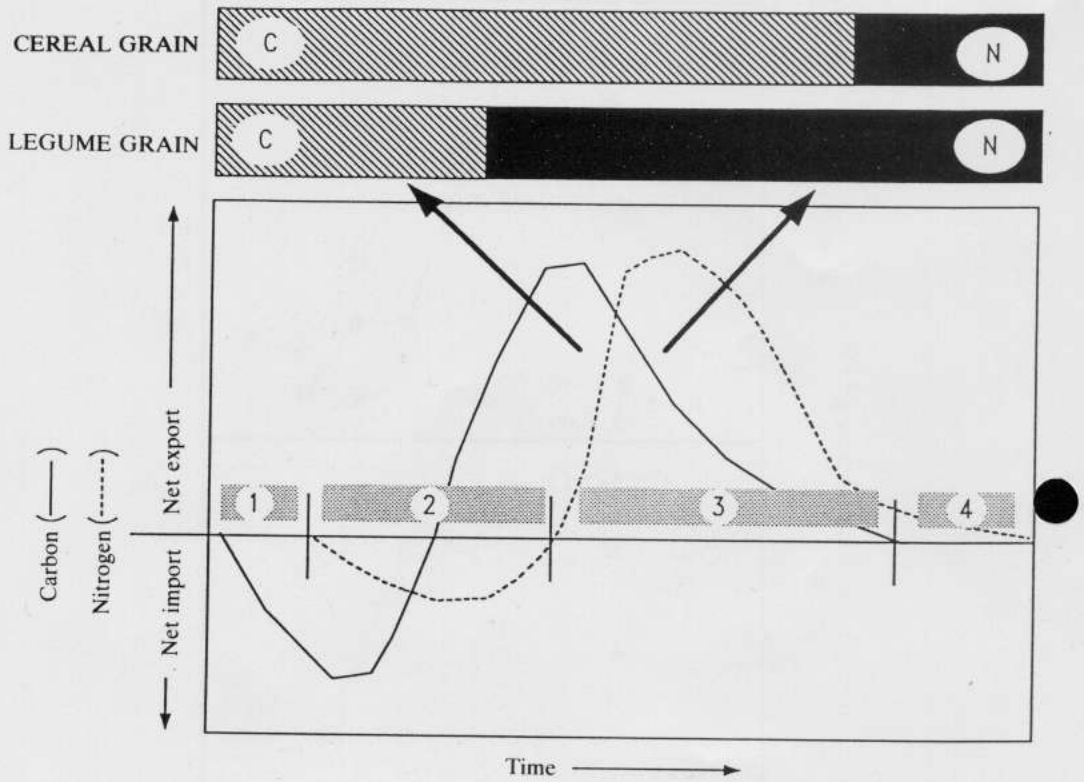

Fig. 3. Supply/demand relations of an idealised leaf from initiation to senescence, and a high C:N ratio (cereal grain) or high N:C (legume grain) sink. Periods 1–4 identify the juvenile, photosynthetic, senescence I and senescence II phases in leaf development (see text).

and other mobile nutrients behave similarly). It seems that acquisition of photosynthetic competence and the subsequent decline of carbon fixation capacity are directly linked to, and possibly even driven by, the rate of input and export respectively of nitrogen and other mobile elements. This is a complex issue beyond the scope of the present discussion. The point we wish to make is that leaf development may be divided into segments, each delimited by a condition of net nitrogen (or phosphorus, or sulphur) balance. *Phase 1* (Fig. 3) is the juvenile or immature state; *2* is the period of active photosynthesis; *3* is the first phase of senescence in which physiological integrity is maintained; Period *4* is the second, terminal phase of senescence. Juvenile → C export → NPS remobilisation (senescence stage I) → breakdown of integrity (senescence stage II) summarises the career of a normal leaf. It is emphasised that the transition from *Phase 2* to *Phase 3* (the initiation of senescence) is essentially a *change*, rather than a *loss*, of function and is approachable in terms of genetics, biochemistry and the analytical procedures of modern developmental biology.

Senescence-related genes

Fig. 4 classifies genes with functions in senescence according to their patterns of expression during leaf development. Five broad categories are recognised.

1. Genes controlling the primary metabolic activities of viable cells, e.g. respiratory components, ribosomal RNA synthesis.

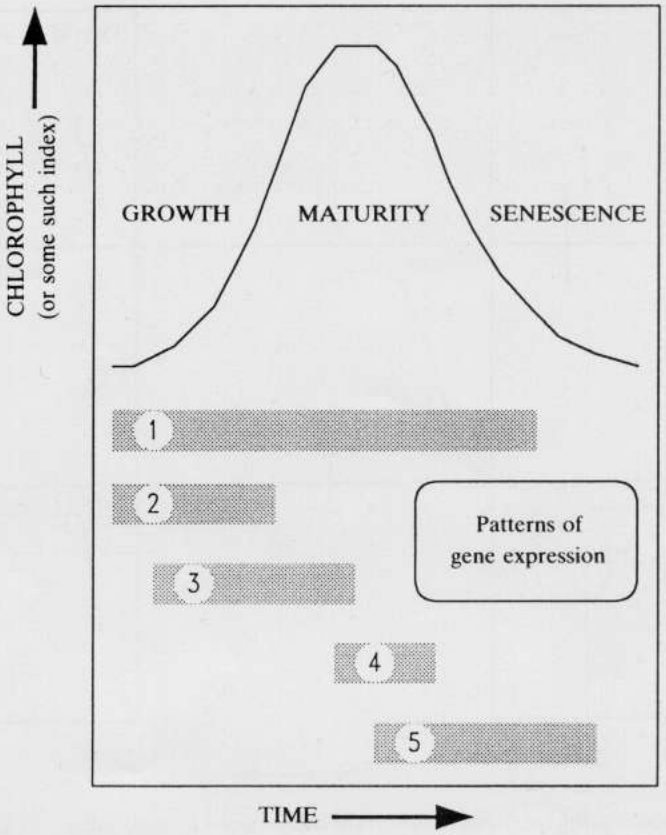

Fig. 4. Expression during leaf development of different classes of gene with functions in senescence. See text for explanation.

2. Genes directing installation in the developing mesophyll cell of latent metabolic machinery which becomes active later in the life of the leaf, e.g. vacuolar enzymes, zymogens. Transcripts of some genes may also be accumulated in an untranslated form at this stage, to become active in protein synthesis later on.
3. Genes which encode growth or carbon assimilation components and which contribute to the progress of senescence by switching off, e.g. nuclear and plastid genes for Calvin cycle enzymes and thylakoid proteins.
4. Genes specifically turned on at the initiation of senescence; the point of convergence of all the various transduction pathways through which environmental and internal cues invoke the syndrome.
5. Genes encoding senescence-related activities (e.g. catabolic enzymes) induced de novo or showing increased expression during remobilisation.

Different types of stay-green

Alterations within each class of senescence-related genes, such as the timing of a gene's expression in the life-cycle, may cause a change in the greenness phenotype. It is important to emphasise that the *stay-green* character in one genetic line may have only a superficial resemblance to the character in another and may arise from quite different underlying physiological and biochemical modifications. In most cases of extended greenness in crop species there has been little or no biochemical investigation of the character. Nevertheless,

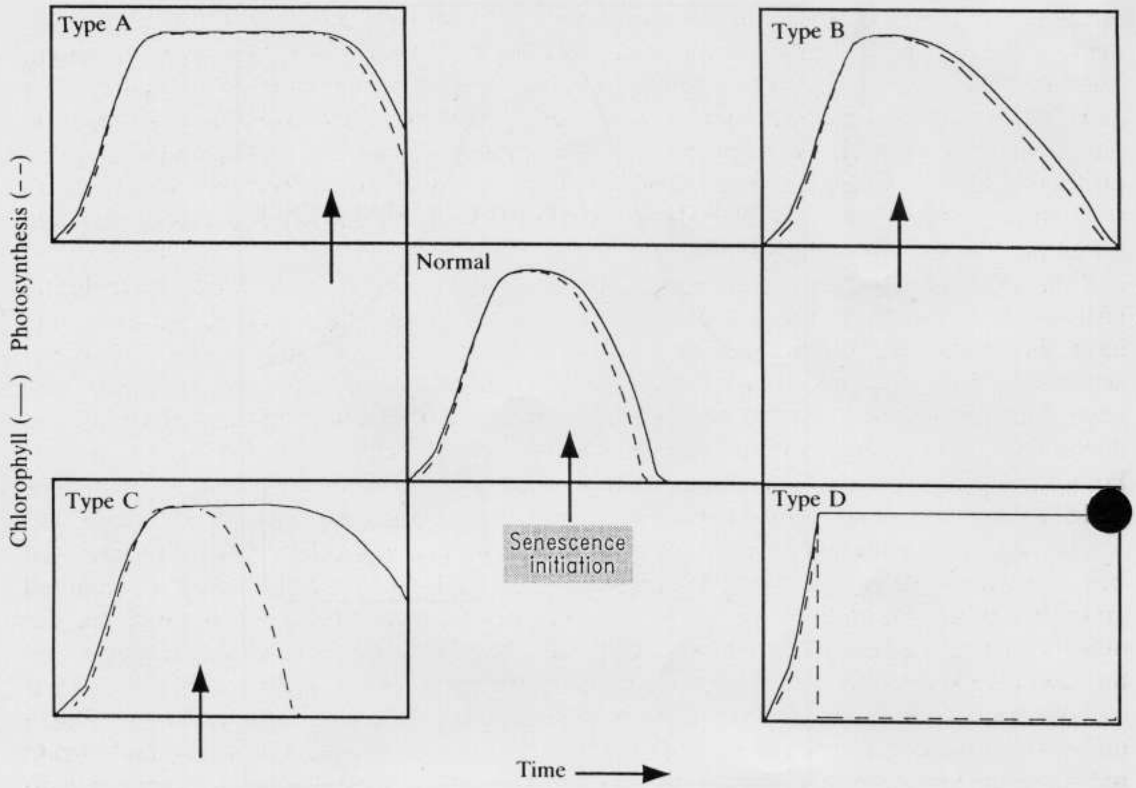

Fig. 5. Classification of different forms of *stay-green* behaviour in foliage. *Stay-green* can arise in several alternative ways. Initiation of the entire senescence syndrome may be delayed (*Type A*); the syndrome may start on time but may proceed at a reduced rate (*Type B*); the syndrome may begin and proceed on schedule but one or more of the constituent metabolic processes may be disabled (*Type C*); combinations of types *A*, *B* and *C*. These describe the behaviour of *viable* tissue. *Type D* represents the case of plant material such as herbarium specimens or frozen foods which retain greenness because they are rapidly killed at harvest.

in the specific examples of *stay-green* behaviour discussed, where possible we try to classify each case according to the type of *stay-green* involved.

Fig. 5 illustrates four possible types of *stay-green* behaviour. Types A and B are functionally *stay-green* and may arise after alteration of genes involved, respectively, in the timing of the initiation of senescence and the regulation of its rate of progress. Since these *stay-green* types continue to photosynthesise for longer than normal, they might be expected to show a higher yield in crops for which carbohydrate is a major component of the harvest. In contrast, types C and D look green but lack photosynthetic competence, either due to the majority of the senescence syndrome occurring as usual (type C) or premature death, such as that caused by harvesting for food (type D). Type C *stay-green* plants may arise by alteration of genes which regulate chlorophyll catabolism. Genes involved in the generation of type A *stay-green* lines are likely to come from group 4 of our classification of senescence-related genes, while genes affected in types B and C are more likely to be from group 5.

Stay-green cereals

Maize

A study of 10 short-season maize hybrids by Tollenaar & Daynard (1978) showed a

positive correlation between leaf area duration (**D**) and yield (see Fig. 6). A number of maize varieties which have a *stay-green* phenotype have been investigated in more detail. The inbred line Lo*876o2* exhibits delayed senescence and is characterised by higher water and chlorophyll contents in the leaves at maturity, high stalk sucrose content during grain filling, more water, sucrose and proteins than usual in the husks and cobs and a higher grain protein content (Gentinetta *et al.*, 1986). This variety also shows increased resistance to stalk-rotting pathogens, a feature which it shares with slow-senescing mutants of sorghum (Ambler, Morgan & Jordan, 1987).

Removal of the ear of maize plants has been reported to delay (Moss, 1962) or accelerate (Allison & Weinmann, 1970) leaf senescence. Christensen, Below & Hageman (1981) have shown that when ear removal does accelerate senescence, photosynthesis and the accumulation of reduced nitrogen decrease markedly. The accumulation of sucrose has been detected in the leaves of such varieties and genetic analysis suggests that a single dominant gene is responsible for this genotype (Ceppi *et al.*, 1987). Crafts-Brandner & Poneleit (1987*b*) report that ear removal may increase or decrease the rate of senescence that both rapid and delayed senescence are dominant traits transmissible to F_1 hybrids.

One *stay-green* variety which has been studied in some detail is FS854, which is credited with the world record yield for non-irrigated maize. After ear removal, FS854 remained green but otherwise showed an increase in leaf dry weight and sugar content as for the other varieties studied (Crafts-Brandner *et al.*, 1984*b*). The increase was transient for the other varieties but continuous throughout the grain-filling period for FS854. Thus carbohydrate accumulation alone does not appear to accelerate leaf senescence. FS854 differs from the other varieties in retaining in its leaves more reduced nitrogen and nitrate reductase activity, as well as carboxylating enzymes and chlorophyll. Crafts-Brandner *et al.*

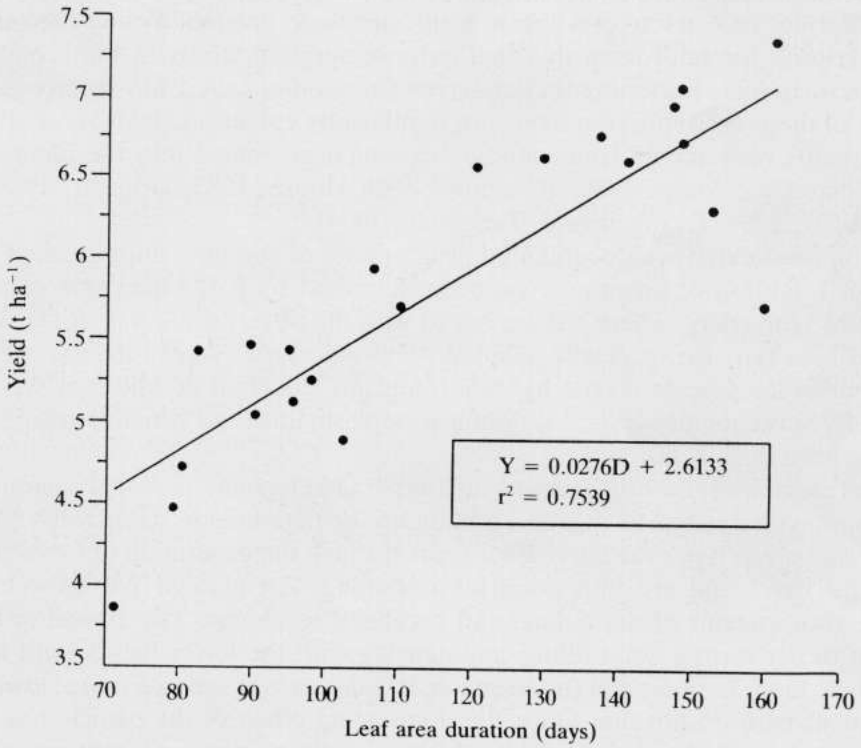

Fig. 6. The relationship between leaf area duration (D) and grain yield (Y) in 10 *Zea mays* hybrid genotypes over two growing seasons (data of Tollenaar & Daynard, 1978).

(1984*b*) speculate that the rate of flux of nitrogen into the leaf may be important in regulating the rate of senescence in the different varieties. Study of the whole plant suggests that the roots may play a role in ensuring the continuation of the supply of nitrogen to the leaves (Crafts-Brandner *et al.*, 1984*a*). In intact FS854 plants, the levels of both chlorophyll and PEP carboxylase start to decline on schedule but the rate of decrease is reduced compared with other varieties, which suggests that FS854 may be a type B *stay-green*. In contrast, a *stay-green* line with which we work retains chlorophyll while the level of PEP carboxylase declines as usual – this is type C *stay-green* behaviour.

In agronomic terms, *stay-green* maize varieties, with longer **D** than normal, have the advantage of a larger kernel weight. On the other hand, varieties with shorter **D** and effective filling period (EFP) accumulate grain dry matter at a faster rate due to more efficient leaf nitrogen remobilisation (Crafts-Brandner & Poneleit, 1987*a*). The benefits of increased yield and disease resistance provided by *stay-green* varieties have been exploited by maize breeders (Duvick, 1984).

Sorghum

Sorghum, one of the most important cereals in the semi-arid tropics, is a multifunctional crop. All parts of the plant are used for food, feed, biomass and fabrication. The economic value of the crop residues sometimes exceeds that of the grain. In India the kharif, or rainy season, crop produces a large quantity of dry stover which is harvested to feed bullocks and buffalo, the farmers' power source, and is also sold in town for the dairy buffalo herds. The post-rainy season rabi crop provides both grain and forage needed to survive the subsequent dry season until it rains again. As the rabi crop is dependent on residual moisture, drought, charcoal stem rot and lodging can be a problem particularly near the end of the season. Disease and insect resistance, wide environmental adaptiveness, appropriate time to maturity, good tillering, reasonably good grain yield, juiciness, palatability and digestibility are the major criteria for improving the dual-purpose sorghum crops of the semi-arid zone (House, 1985). In lines exhibiting the *stay-green* (sometimes called *non-senescence*) phenotype, many of these desirable characters are significantly enhanced and it is recognised that multiple benefits may accrue from building extended greenness into the ideotype (Rao, Reddy, Williams & House, 1980; Rosenow, 1980; House, 1985; Doggett, 1988; Vietor, Cralle & Miller, 1989; Evangelista & Tangonan, 1990).

One of the most extensive documented programmes of sorghum improvement based on selection for retention of greenness has been described by F R Miller and colleagues Texas A & M University. These studies began with the observation that stover dry weight of a hybrid between two tropically-adapted "non-senescent" sorghums was greater than between temperate senescent-type hybrids (Duncan, Bockholt & Miller, 1981; Gerik & Miller, 1984). Subsequently several sorghum germplasm lines and varieties arising from this programme have been registered.

Sorghum resembles some other cereals such as oat (J M Leggett, personal communication) in that greenness is related to degree of annuality or perenniality. Generally, sorghum is annual but *stay-green* types can survive for years through the generation of fresh tillers from the old plant bases and are thus good for ratooning. Zartman & Woyedwodzic (1979) studied the root systems of the annual and perennial sorghums. The annual or senescent types begin to dry during grain filling commencing with the lower leaves until finally the whole plant is dead. In perennial (non-senescent) lines, leaves senesce more slowly and the stem and plant base do not die. Once the dominating effect of the panicle has lessened, basal buds develop. Of two hybrid cultivars with a common non-senescent parent studied, the senescent hybrid always had a greater root system than the non-senescent throughout

the season as reflected by volume of soil occupied and root density; however, at the last sampling date, 100 days after sowing, root density of the senescent hybrid had declined but the non-senescent hybrid exhibited only a minimal decrease.

In the absence of non-empirical genetic and physiological analyses of the trait in this species, it is difficult to classify sorghum *stay-green* according to the scheme in Fig. 5. There is preliminary evidence that some *stay-green* lines contain a higher level of cytokinins than normal (Ambler *et al.*, 1987). Cytokinins reduce the rate of loss of both chlorophyll and photosynthesis in senescing wheat seedlings (Wittenbach, 1977), producing a type B *stay-green* phenotype. It is therefore possible that the *stay-green* lines of Ambler *et al.* (1987) may be of type B.

Other cereals

In many cereals, relationships have been observed between grain yield and duration of the areas of the total canopy and of specific leaves, both throughout the entire period of crop growth and in the interval from flowering onward (Thomas, 1987*a*, 1992*a*). In Fig. 7 leaf area trends and derived durations are illustrated for four lines of *Avena sativa*. Leaf 7 is the flag leaf. Lines A, B and C are products of introgressing *stay-green* germplasm from *Avena sterilis* into the background of Clintford, the reference genotype (Helsel & Frey, 1978). In the year in which the experiment presented was carried out, A, B and C out-yielded Clintford by 3–15% and over the preceding three-year period by about 30%.

There are many studies of cereal development showing considerable genotypic variation in greenness and chlorophyll stability, contributing to different patterns of pigmentation change during ripening. For example, Table 1 shows chlorophyll retention profiles of "sequential" and "non-sequential" rice cultivars where the flag leaf senesces slower or faster, respectively, than the leaf preceding it (Mondal & Choudhuri, 1985). Striking variations in canopy survival occur amongst rice cultivars, particularly in relation to growth duration class. Wada & Wada (1991) compared leaf senescence during grain ripening in four short-duration (96–98 days to maturity) and four medium duration (119–126 days) rice varieties. From linear regressions of ln (leaf area index) against weeks after anthesis (r^2 values in the range 0.79 – 0.997), half-lives for the canopies may be calculated. All four medium-duration lines had half-lives of less than 3 wk (shortest 2.47, longest 2.76 wk). One of the short-duration varieties had a half-life of 2.22 wk and further resembled members of the medium-duration group in possessing a relatively large sink size. It also out-yielded the other three short-duration lines by over 25%. One of the short duration lines had a greatly extended canopy half-life of 4.59 wk. This was associated with a higher sink strength than other short-duration varieties. There was no simple relationship between yield and canopy duration in these experiments. The authors discuss the trade-off between maintenance of

Table 1. *Chlorophyll retention in leaves of rice cultivars with sequential or non-sequential patterns of senescence*

Cultivar	Plant age at anthesis	% chlorophyll remaining 21 days after anthesis		
		Flag leaf	Flag-1	Flag-2
Ratna	75 d	35.7	48.3	30.0
Jaya	87 d	42.5	49.0	34.4
Masuri	110 d	39.6	30.6	22.9
Kalojira	115 d	47.8	41.1	27.5

Data of Mondal & Choudhuri (1985).

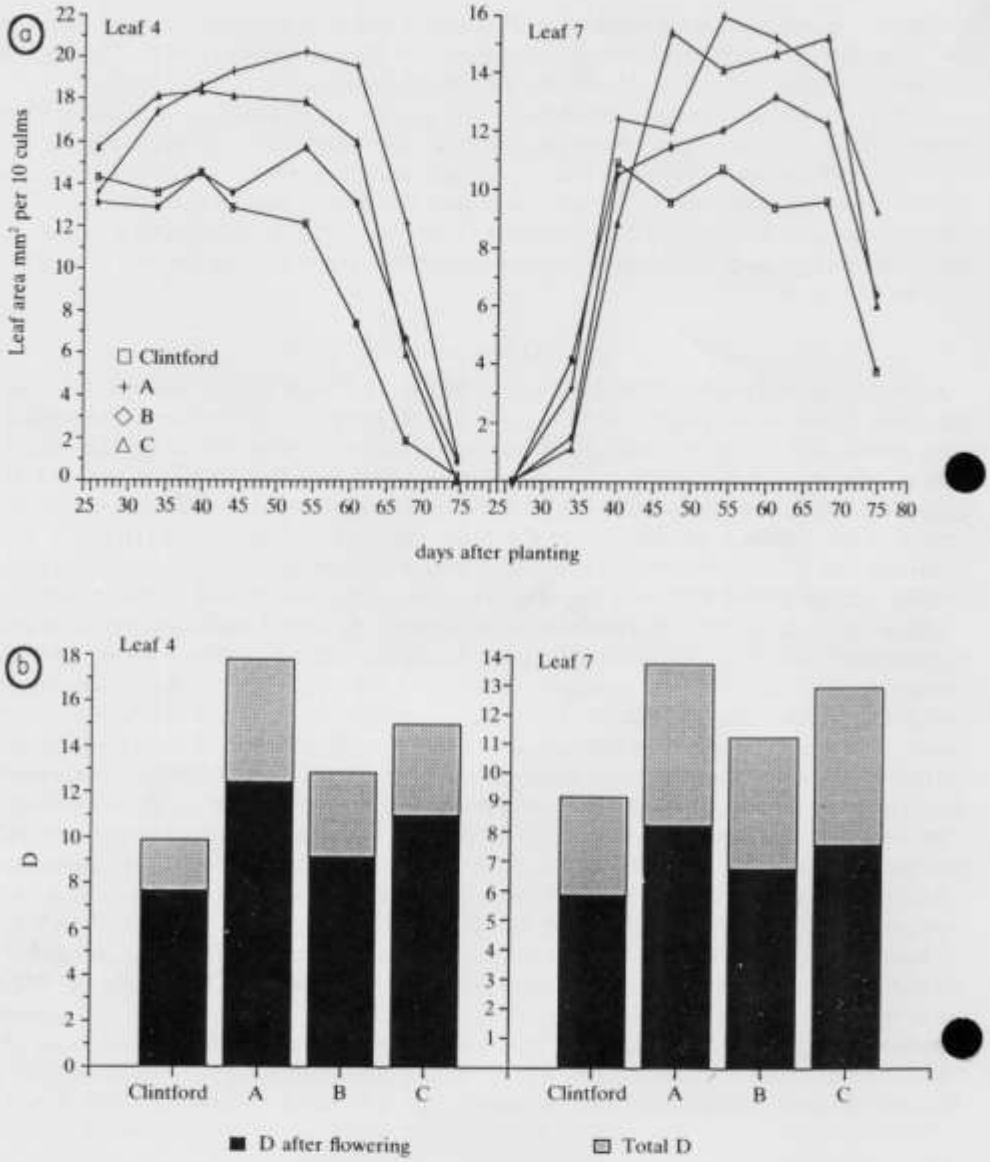

Fig. 7. Durations of leaf 4 and of the flag leaf, 7, in oat cv. Clintford and three genotypes, A, B and C, into which the *stay-green* character of *Avena sterilis* has been introgressed. (a) and (b): trends in the areas of leaves 4 and 7, respectively, with increasing crop age. (c) and (d): leaf area durations accumulated up to flowering, and to harvest (Helsel & Frey, 1978). Figure from Thomas (1992*a*), reproduced with the publisher's permission.

photosynthetic competence on the one hand and the need to remobilise nitrogen to supply the developing grain on the other and suggest that delayed senescence is a useful character provided it can be associated with good nitrogen use efficiency and optimal management of fertiliser application.

Table 2. *Inheritance of chlorophyll a stability in a partial diallel cross between four wheat varieties*

Variety	Chlorophyll stability (%) Ramona	WU29	WU30	Timgallen	Parental means
Ramona	42	46	37	16	35
WU29		46	46	13	35
WU30			45	11	37
Timgallen				20	12

Data of Boyd & Walker (1972).

A *stay-green* phenotype has also been observed on shading of mature rice (Hidema, Makino, Mae & Ojima, 1991). Exposing leaves to low light greatly extends their green duration by stabilising light-harvesting and reaction-centre chlorophyll complexes, but degradation of rubisco and other components proceeds as normal so that the leaf eventually comes to resemble a senescent *stay-green* organ of type C in physiology and cell structure. This kind of response may be a general feature of monocot foliage, since leaves of *Lolium* have been shown to react to shading in a similar way (Mae *et al.*, 1993).

A great deal of genetic variation exists for grain protein content in *Triticum*. For example a number of cultivars with up to 25% more protein than normal soft winter wheats have been derived from Atlas 66, a high protein line described by Middleton, Bode & Bayles (1954). Rao & Croy (1972) observed that three varieties of *Triticum* with Atlas 66 in their parentage exhibited higher post-anthesis levels of leaf proteolytic activity than did Triumph 64, a low nitrogen variety. In a later study, Dalling, Boland & Wilson (1976) reported that acid proteinase was highly correlated with the rate of nitrogen redistribution from senescing leaves in two *Triticum* cultivars. Genetic variation in *Triticum* leaf senescence was reported by Boyd & Walker (1972). They measured the amount of chlorophyll in segments of wheat flag leaves at the time of excision and after incubation on moist filter paper in darkness. Chlorophyll stability was expressed as the percentage of pigment remaining after 4 days. Stabilities ranging from less than 10% to more than 50% were observed in 19 varieties surveyed. The authors stated that a high level of chlorophyll in fresh leaf segments and low chlorophyll stability were characteristic of cultivars with high grain protein nitrogen. One low chlorophyll stability and three high stability varieties were selected for genetic analysis in a partial diallel cross (Table 2). "Stable" × "stable" crosses gave "stable" F_1 progeny; crosses with the "unstable" line produced progeny with reduced chlorophyll stability. These results suggest that both rapid and delayed senescence are dominant traits transmissible to the next generation, as found by Crafts-Brandner & Poneleit (1987*b*) in maize. This kind of approach looks quite promising and it is surprising that it does not seem to have been followed up further.

Temperate grasses

A mutant genotype of the pasture grass *Festuca pratensis* was described by Thomas & Stoddart (1975). It is characterised by a virtually complete disabling of the leaf yellowing process so that plants remain green throughout the life-cycle or when exposed to stresses which would normally cause chlorosis or necrosis. However, other aspects of senescence, such as degradation of rubisco, proceed according to schedule (Thomas, 1982; Hilditch, Thomas & Rogers, 1986*a*; Hilditch, Thomas, Thomas & Rogers, 1989) suggesting that the mutant is a type C *stay-green*. Inheritance studies established that the *stay-green* phenotype

is the result of a mutation at a single nuclear locus, designated *Sid* (senescence-induced degradation). The mutation is recessive so that plants homozygous for the mutation ($Sid^{g/g}$) are *stay-green* but heterozygous hybrids ($Sid^{g/y}$) exhibit the normal yellowing phenotype (Thomas, 1987*b*).

The green mutant of *Festuca* has been useful for investigating the pathway of chlorophyll breakdown, an aspect of the metabolism of senescing leaves which had defied almost seventy years of investigation by plant scientists. Thomas *et al.* (1989) established that the first step in the pathway, removal of the phytol side-chain, occurs normally in the mutant; moreover, this reaction takes place while chlorophyll is still attached to its binding protein in the photosynthetic membrane. The next steps require oxygen and ATP and do not function in mutant leaf tissue. Hence polar, dephytylated chlorophyll derivatives accumulate in the mutant as a consequence of the metabolic blockage. Further breakdown products of chlorophyll have been identified and verified as such by their absence from the mutant, and also by radiotracer analysis (Matile, Ginsburg, Schellenberg & Thomas, 1987; Matile *et al.*, 1989; Düggelin *et al.*, 1988; Peisker, Thomas, Keller & Matile, 1990). This work has led recently to the isolation of a major catabolite and establishment of its chemical structure. It is a tetrapyrrole derivative of chlorophyll *a* resulting from oxidative cleavage of the macrocycle (Kräutler *et al.*, 1991).

Other studies of the *Festuca* mutant have revealed that the increased stability of chlorophyll has consequences beyond the visible phenotype. The membrane proteins with which photosynthetic pigments are associated in leaf cells are normally degraded at the same time as chlorophyll during senescence (Thomas, 1977, 1982, 1983; Hilditch, 1986; Hilditch, Thomas & Rogers, 1986*b*; Hilditch *et al.*, 1989; Davies, Thomas & Rogers, 1990*a*; Davies, Thomas, Thomas & Rogers, 1990*b*; Nock, Rogers & Thomas, 1992). The coordinated behaviour of membrane components is preserved in the mutant so that senescent tissue retains significant amounts of protein nitrogen in the form of pigment proteolipids. This enhances the nutritional quality of otherwise low-value residual foliage and suggests that building this kind of *stay-green* character into the design of forage species, or multifunctional crops such as subtropical millet, would be beneficial.

It has been observed that the green mutant of *Festuca* synthesises a small number of additional proteins and messenger RNAs during leaf development (Fig. 8 – Thomas *et al.*, 1992). This opens the way to the use of cloning procedures for the isolation of genes, possibly including *Sid*, which correspond to these products.

Another way of producing type C *stay-green* behaviour is treatment with inhibitors of protein synthesis. The inhibitor MDMP applied to *Festuca* leaves is much more effective at preventing chlorophyll breakdown than rubisco degradation (e.g. Thomas, 1976 – Fig. 9). This may suggest that new gene expression is required only for some parts of the senescence syndrome.

Stay-green legumes

Green and yellow cotyledons were among the characters Mendel studied in his experiments on inheritance in *Pisum*. According to Darbishire (1911), Bunyard was the first to show that immature seeds of Mendel's green and yellow lines are green and that the lines differ in the presence or absence of a factor which causes the pigments to fade as the seeds mature. If we accept that cotyledons are essentially modified leaves, then pigmentation changes during seed maturation may be likened to the yellowing process in foliar senescence. The most detailed physiological analyses of altered pigmentation in legume seeds, pods and leaves have been carried out on *Glycine max* and *Phaseolus vulgaris*.

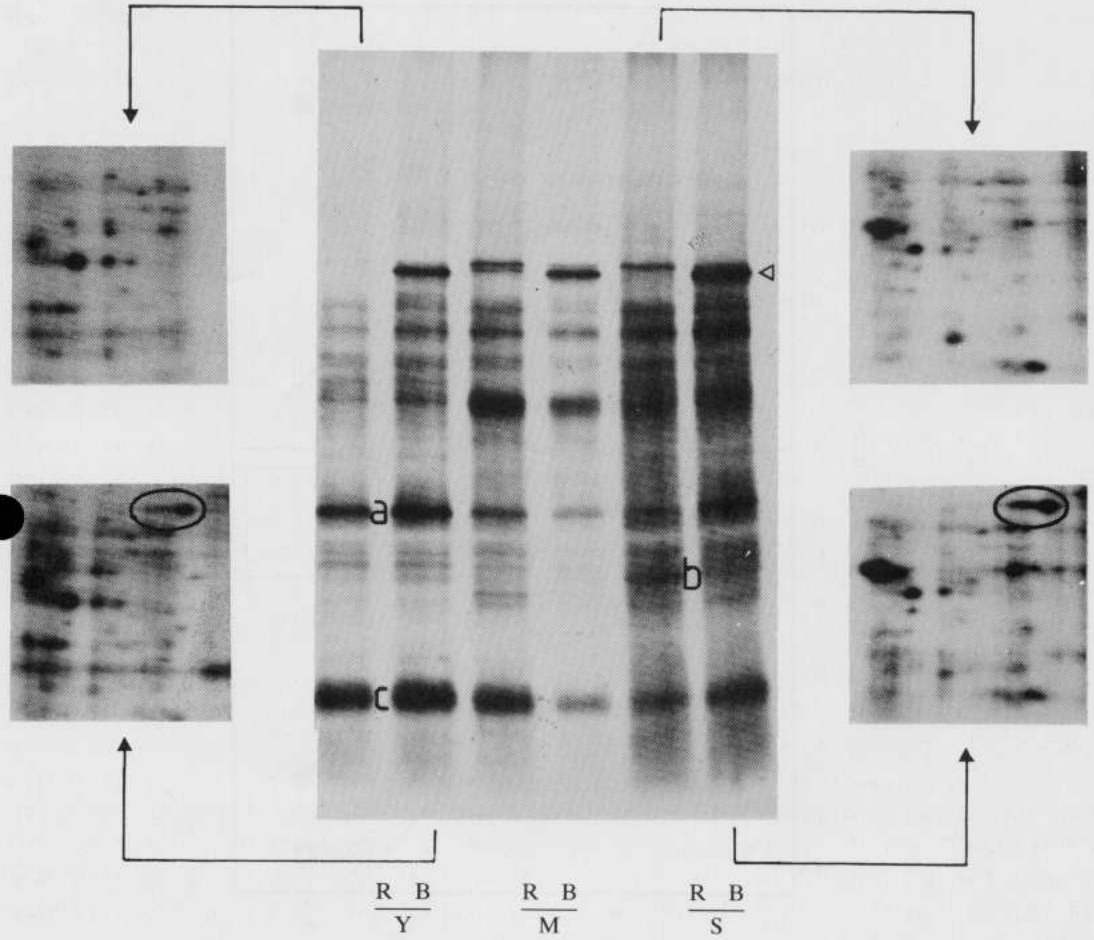

Fig. 8. *In vitro* protein synthesis products of RNA from young (Y), mature (M) and senescent (S) leaf tissue of the normal *Festuca pratensis* genotype Rossa (R) and the *stay-green* mutant Bf993. (B) Polypeptide *a*, precursor of LHCP-2; *b*, senescence-related product; *c*, precursor of rubisco small subunit. Comparable areas of 2-dimensional electrophoretograms are shown, with polypeptides uniquely expressed in the mutant outlined. Reproduced from Thomas, Ougham & Davies (1992), with permission of the publisher.

Soybean

Green and yellow cotyledon types are found in *Glycine* (Woodworth, 1921) and in some cases these characters are associated with variations in the senescence pattern of leaves (Table 3). A maternally-inherited factor, originally described by Terao (1918) and Veatch & Woodworth (1930), and now designated *cyt*G, prevents yellowing not only of embryos but also of pods and leaves. Two recessive nuclear loci d_1 and d_2 exert a similar influence over greenness in fruits and foliage (Bernard & Weiss, 1973). There are a number of nuclear and cytoplasmic genes giving rise to yellow leaves in soybeans (e.g. Shoemaker, Cody & Palmer, 1985). One of these, y_3, is expressed as early senescence in relation to pod development and is counteracted by *G*, a nuclear dominant which, in the absence of y_3, prevents yellowing of the seed coat but not of any other tissue (Bernard & Weiss, 1973).

Recently Guiamét, Schwartz, Pichersky & Noodén (1991) made electrophoretic

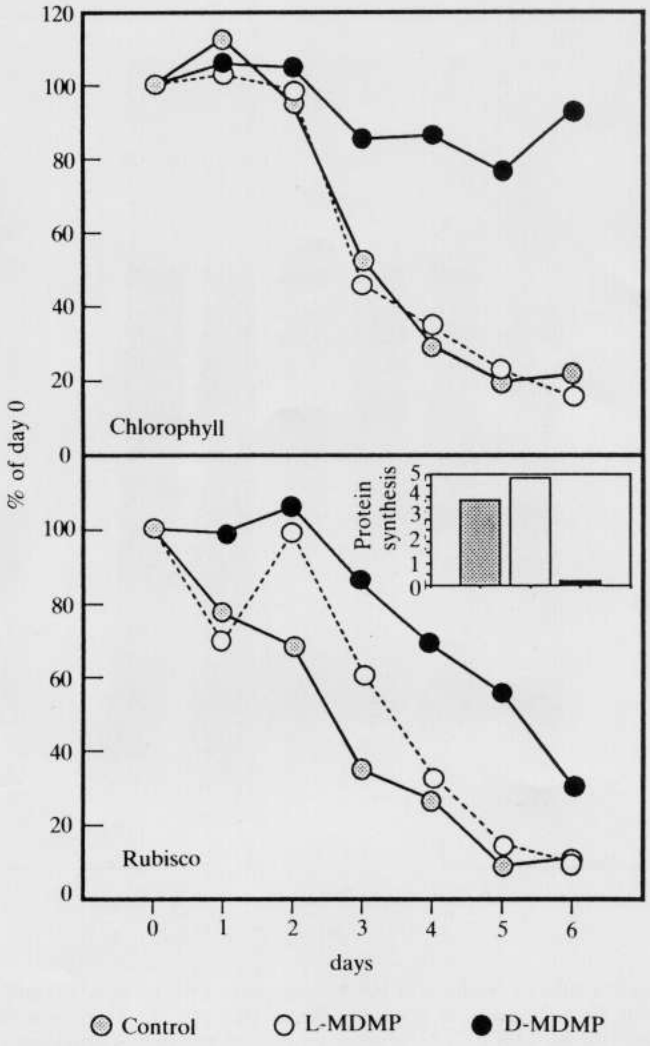

Fig. 9. Chlorophyll, ribulose-1,5-bisphosphate carboxylase and protein synthesis capacity in isolated leaf tissue of *Festuca pratensis* incubated with water (control) and with the L- and D-stereoisomers of the inhibitor MDMP. The stereospecificity of protein synthesis inhibition is illustrated in the inset, which presents data for the incorporation of [^{14}C]-leucine by 2-day old leaf tissue as a % of total uptake. From Thomas (1976).

comparisons of thylakoid membrane proteins from leaves of soybean *stay-green* lines during pod-fill. The normally-yellowing cultivar Clark was used as the wild-type reference. Its genotype with respect to genes influencing yellowing is designated $ggD_1D_1D_2D_2cyt$Y. Plants homozygous for the recessive allele at both the D_1 and D_2 loci retained chlorophyll in fully-expanded 6th-7th trifoliate leaves at levels in excess of 4 mg dm^{-2} throughout flowering and pod-fill whereas leaves of cv. Clark became almost completely yellow during this time. Retention of greenness by leaves of d_1d_2 plants is not associated with any delay or inhibition of the decline in photosynthetic rate and stomatal conductance during pod-fill, nor in subsequent abscission (Guiamét *et al.*, 1990). This suggests that such plants may be classified as type C *stay-greens*.

In combination with d_1 and d_2 the dominant allele G, which inhibits yellowing of the seed

Table 3. *Some genetic loci determining senescence behaviour in soybeans*

Loci	Mode of inheritance	Genotype	Visible phenotype				Functional phenotype			
			Foliage	Podwall	Seed coat	Embryo	Ps	Sc	Ci	Ab
*cyt*G	Cytoplasmic	*cyt*G	Stay green	Stay green	Stay pale green	Stay green	±	±	±	±
y_3	Nuclear, recessive	y_3y_3	Early yellowing	Early yellowing	Early yellowing	Early yellowing	+	+	+	−
y_3*cyt*G		*cyt*Gy_3y_3	Stay pale green	Stay pale green	Yellow	Yellow				
d_1	Nuclear, recessive	$ggd_1d_1D_2D_2$	Yellow	Yellow	Yellow	Yellow				
d_2	Nuclear, recessive	$ggD_1D_1d_2d_2$	Yellow	Yellow	Yellow	Yellow				
d_1d_2		$ggd_1d_1d_2d_2$	Stay green	Stay green	Stay green	Stay green	±	±	±	±
G	Nuclear, linked to d_1	$GGD_1D_1D_2D_2$	Yellow	Yellow	Stay green	Yellow				
Gd_1d_2		$GGd_1d_1d_2d_2$	Stay green	Stay green	Stay green	Stay green				
$dt_1dt_2e_1e_2$	Nuclear, recessive	$dt_1dt_1dt_2dt_2e_1e_1e_2e_2$	Stay green	Yellow	?	?	−	?	?	−

Ps, decline in photosynthesis during leaf senescence; Sc, decline in stomatal conductance; Ci, rise in intercellular CO_2 concentration; Ab, abscission; +, hastens; −, delays or inhibits; ±, no effect. After Thomas (1987*a*) and Guiamét, Teeri & Noodén (1990).

coat but not foliage, slightly enhanced the *stay-green* phenotype. Guiamét *et al.* (1990) also showed that the decreases in photosynthetic rate and stomatal conductance, and the associated increase in intercellular CO_2 concentration, were significantly delayed in Gd_1d_2 plants, though abscission occurred normally. The addition of the *G* allele to the genotype therefore resulted in the *stay-green* pattern becoming more similar to type A.

The cytoplasmic *stay-green* gene *cyt*G had a small effect on the stability of chlorophyll *a*, but quite a marked stabilising influence on chlorophyll *b*. Consistent with the differential retention of the two pigment types, the stability of the major chlorophyll *b*-binding thylakoid membrane complex LHC2 was much increased in *cyt*G plants, not only compared with Clark but also in contrast to the behaviour of other membrane polypeptides. The authors conclude that *cyt*G (which, like d_1 and d_2, has little effect on photosynthesis and abscission – Guiamét *et al.*, 1990) contrasts with *Sid* in *Festuca* (see below) in stabilising only peripheral light-harvesting complex rather than porphyrin-binding proteins throughout the membrane. Despite the differences in the gene products affected, both *cyt*G and *Sid* mutated plants exhibit type C *stay-green* behaviour.

A separate line of work on *stay-green* in soybean has been conducted by Phillips and co-workers. They have described in some detail the genetics, agronomy and physiology of soybean lines exhibiting a trait they call DLS – *delayed leaf senescence* (Abu-Shakra, Phillips & Huffaker, 1978; Pierce, Knowles & Phillips, 1984; Phillips *et al.*, 1984). Interest in DLS arises because of the complex interdependence of fruiting and foliar senescence and its relationship to yield in soybeans. In 1975, Sinclair & de Wit published an analysis of yield as a function of nitrogen requirement for the major seed crop species. Soybean emerged as unique in demanding a high input of nitrogen to support seed production – so high that the authors postulated that soybean plants must mobilise vegetative nitrogen during pod fill to such an extent that they "self-destruct".

In 1976, a survey of experimental soybean lines growing at the University of California, Davis was undertaken with the object of identifying individuals showing altered senescence patterns at fruiting time (Abu-Shakra *et al.*, 1978). Five plants with green leaves subtending brown pods were identified out of several thousand examined and the one with the highest nitrogen-fixation activity was used as the subject of continued study. This individual was an F_2 derivative of a cross between Lee 68 and L63–1097. Of 14 F_3 progeny produced subsequently, nine senesced normally and the remainder exhibited DLS. The DLS plants were shown to retain chlorophyll, leaf protein, ribulose bisphosphate carboxylase (rubisco) activity and nodule N fixation at high levels compared with normal siblings. This behaviour is suggestive of *stay-green* type A. Leaves of progeny from the elite F_3 plant contained 60–210% more protein and chlorophyll than those of normal F_4 plants and their root nodules fixed nitrogen over an extended period. Grafting experiments showed that the DLS trait is determined by the genotype of the shoot and not the root (Phillips *et al.*, 1984).

The analysis was continued by Pierce *et al.* (1984). Two individuals from progeny populations showing uniform DLS were selected from the F_6 generation and designated 6N3 and 8N2. F_7 plants derived from these lines were employed in crosses with a number of normal varieties of well-defined genotype. Four loci were found to be decisive in the expression of DLS. All DLS plants are of the early flowering, determinate type. Flowering time in soybean is determined by two major genes, E_1,e_1 and E_2,e_2 (Bernard, 1971) and the determinate/indeterminate growth habit by the genes Dt_1, dt_1 and Dt_2, dt_2 (Bernard, 1972). Pierce *et al.* (1984) showed that DLS was expressed only in genotypes homozygous for all four loci, that is, $dt_1dt_1dt_2dt_2e_1e_1e_2e_2$ (Table 3). They pointed out, however, that the DLS phenotype is strongly modified by environment and that analyses of F_2 and F_3 material from crosses between 6N3 and Elf (a high-yielding cultivar with $dt_1dt_1e_1e_1$ genotype) encountered variable expression of the DLS character and a quantitative mode of segregation. A number

of modifier genes appear to be at work in the DLS syndrome. The agronomic virtues of DLS remain to be demonstrated. Phillips *et al.* (1984) found the DLS trait, in two different genetic backgrounds, to be negatively correlated with yield, as the observations of Sinclair & de Wit (1975) would predict. Any practical value the character might have is likely to reflect the yield advantages of homozygosity for dt_1 and e_1, as seen in the short-stature, high-performance cultivars exemplified by Elf (Cooper, 1981).

Phaseolus

Hardwick (1979) described variation in the retention of leaves by *Phaseolus vulgaris* cultivars in relation to pod development. The proportion of foliage remaining at the time of the first dry pod ranged from less than 2% to 95%. There was no very clear relation between abscission behaviour, change in pigment content of leaves attached to the plant during pod formation or stability of chlorophyll in leaf discs incubated in darkness for 5 days (Table 4).

Inheritance studies of cotyledon greenness in several legume species have established a two-locus model comprising a gene for colour, *Gr*, and an inhibitor, *Ih*. Thus *P. vulgaris* cultivar Flageolet, in which the *stay-green* character is expressed both in the foliage and the pods, has the genotype *Gr Gr ih ih* (Honma, Bouwkamp & Stojianov, 1968). Other *stay-green Phaseolus* lines expressing variation for these genes have been described by Honma *et al.* (1968), Bouwkamp & Honma (1970) and Ronning, Bouwkamp & Solomos (1991). Some physiological observations have been made on senescence in a mutant of this kind. In both the *stay-green* genotype and the wild-type comparison there was an ethylene-stimulated climacteric in CO_2 output. Electron microscopy revealed that the accumulation of plastoglobuli, a characteristic feature of senescent plastids (gerontoplasts), is greatly restricted in the mutant. Recently proteins from the two genotypes have been compared by SDS-polyacrylamide gel electrophoresis and western blotting (H Thomas, P Matile & T Solomos, unpublished). The major chlorophyll-binding protein of thylakoids, LHCP-2, is stable during senescence of mutant leaf tissue, whereas rubisco is, if anything, rather more labile than in the wild-type. Cytochrome f is also retained to a significant extent. The general conclusion from analyses of the cellular basis of this *stay-green* variety of *Phaseolus* is that it shows type C *stay-green* behaviour.

Table 4. *Abscission and chlorophyll stability of leaves of* Phaseolus vulgaris *cultivars*

Cultivar	% Leaves retained at first dry pod	Chlorophyll (mg cm^{-2}) Day 27	Day 41	Day 41 + 5 days dark
CCT	54	6.5	5.3	2.0
196	93.5	6.4	6.2	3.5
Wintergreen	73	6.3	6.7	4.2
NEP2	1.5	6.8	5.3	2.5
Red Mexican U13	1.5	7.9	5.3	2.5
222	90.5	7.7	8.2	4.8
251	88.5	7.7	7.7	4.5
Hawksbury Wonder	95	7.2	6.8	4.4

The dark treatment was applied to excised leaf discs incubated on water *in vitro*. Data of Hardwick (1979).

Other species

Fruit crops

Delayed yellowing in crop plants is not confined to leaf tissues, as we have seen in legumes with green pods and seeds. A familiar example is the pepper *Capsicum* in which a series of blockages in the ripening syndrome gives green, yellow, red or purple fruit. An equivalent set of tomato mutants has been well characterised (Grierson, Purton, Knapp & Bathgate, 1987).

An instructive example of *stay-green* behaviour in fruit occurs in bananas. It is well known that fruit at ambient temperatures in the tropics often exhibit ripening of the pulp without yellowing of the peel (Simmonds, 1966; Peacock, 1980). In a series of publications, John and co-workers have described in detail the physiological and cellular responses underlying the inhibition of yellowing by tropical temperatures (Seymour, John & Thompson, 1987*a*; Seymour, Thompson & John, 1987*b*; Blackbourn, Jeger & John, 1990*a*; Blackbourn, Jeger, John & Thompson, 1990*b*; Blackbourn *et al.*, 1990*c*). The conclusion from this work is that heat-treated bananas behave very much like the type C *stay-green Festuca* or *Phaseolus* mutants described above, where the stability of thylakoid components associated with chlorophyll is increased during senescence as a result of a lesion in the pigment catabolism pathway.

In *stay-green* fruit the genetic lesion concerns some aspect of the transition of chloroplasts into chromoplasts. It has been argued that an homologous change occurs in foliar senescence, during which chloroplasts develop into gerontoplasts (Matile, 1992*a*,*b*). Since cryptogams possess a clear foliar senescence mechanism but lack chromoplasts, it may be speculated that the chloroplast-gerontoplast switch is the evolutionary origin of the development of colourful reproductive and dispersive structures in angiosperms (Thomas, 1992*b*). That is, petals and fruit are modified senescent leaves. The occurrence of comparable genetic blockages in all these organs is consistent with this idea.

Tobacco

At low nitrogen levels, the flue-cured tobacco variety G28 loses chlorophyll, CO_2 exchange capacity and rubisco content at a slower rate than the burley cultivar KY 14 (Crafts-Brandner, Leggett, Sutton & Sims, 1987*b*), which suggests that G28 may be showing type B *stay-green* behaviour. Flue-cured tobacco cultivars have similar yields to burley varieties despite requiring only a quarter as much nitrogen fertiliser (Crafts-Brandner, Sutton & Sims, 1987*a*). In comparison with KY 14 plants, G28 plants accumulate more lamina dry weight per unit of nitrogen fertiliser added, which may partly be explained by their higher starch concentration. This difference in nitrogen use efficiency between the two cultivars is controlled by genetic factors at two loci (Henika, 1932; Stines & Mann, 1960). The burley and flue-cured genotypes have been designated $yb_1yb_1yb_2yb_2$ and $Yb_1Yb_1Yb_2Yb_2$ respectively. Legg, Chaplin & Williamson (1977) have demonstrated by backcrossing that burley cultivars carrying the dominant *Yb* alleles produce the flue-cured type of leaf while flue-cured cultivars with recessive *yb* alleles are similar to burley cultivars.

Crafts-Brandner, Sutton & Sims (1988) have carried out leaf grafting experiments with the two cultivars. They found that grafting a KY 14 leaf onto a G28 stalk or a G28 leaf onto a KY 14 stalk did not alter the dry weight, starch or nitrogen characteristics of the grafted leaf. However, leaf senescence, as measured by the decline in chlorophyll, CO_2 exchange rate and rubisco, was delayed for KY 14 leaves grafted onto G28 stalks. Chlorophyll loss was accelerated when G28 leaves were grafted to KY 14 stalks, but declines in CO_2 exchange rate and rubisco were unaltered. They suggest that cultivar differences in dry weight and

starch accumulation may be controlled by factors within an individual leaf, while leaf senescence may be influenced by the rest of the plant.

It is possible that plant growth regulators, such as cytokinins, may play a role in controlling the timing of leaf senescence. Treatments such as disbudding, which increase cytokinin export from the roots, delay tobacco leaf senescence (Colbert & Beever, 1981). Such plants show a slower loss of leaf chlorophyll and rubisco than untreated plants (Crafts-Brandner, 1991), thus simulating type B *stay-green* behaviour. More direct evidence for the role of cytokinins in senescence has been provided by the study of tobacco plants transformed with the *Agrobacterium* gene *tmr*, under the control of a heat-shock promoter. The *tmr* gene encodes an *iso*-pentenyl transferase (Barry, Rogers, Fraley & Brand, 1984) and this enzyme catalyses what is believed to be the rate-limiting step in cytokinin synthesis (Akiyoshi *et al.*, 1984). Heat-shock of a defined area of a single leaf increases the endogenous cytokinin level and causes retention of greenness over the treated area in a transformed plant compared with an untransformed control (Smart, Scofield, Bevan & Dyer, 1991).

Arabidopsis

The obvious subject for studying the genetics and molecular biology of *stay-green* is *Arabidopsis*. A general account of leaf senescence in this species has been published (De Kok & Graham, 1989). Altered (mostly *accelerated*) senescence has been noted incidentally in studies of genotypes with modified patterns of development and growth regulator sensitivity (e.g. Bleecker, Estelle, Somerville & Kende, 1988; Chory, Nagpal & Peto, 1991). Hints of the existence of *stay-green* mutants have appeared in conference abstracts, but so far nothing has been described in detail. Emphatic, coordinated leaf senescence is a strongly-expressed characteristic of crop plants, which makes them favoured subjects for biochemical analysis; in general, non-domesticated species appear to be significantly less consistent in the way they deploy components of the syndrome in their life-cycles. This seems to be true of *Arabidopsis* and may account for the limited progress that has been made in bringing the technological advantages of this species to bear on the problem of leaf senescence and its genetic control.

Trees

In some species, such as *Alnus* and certain varieties of *Fraxinus*, leaves may be discarded without either the previous initiation of senescence or significant recovery of mobilisable nutrients (Bortlik, Gut & Matile, 1987; Neave, Dawson & DeLucia, 1989).

Evergreens

There is, of course, nothing particularly unusual about extended greenness if one looks beyond the mainstream herbaceous crop species. Evergreens are widely represented amongst angiosperms and gymnosperms. The lifespan of *Pinus longaeva* leaves has been reported to be 45 years (Ewers & Schmid, 1981) and that of the single large leaf of *Welwitschia mirabilis* is measured in decades (Molisch, 1938). An extensive literature on the ecology and population biology of evergreenness and the deciduous habit shows that long-lived leaves tend to have a number of distinctive features (Table 5). Much attention has been directed towards the contrasting photosynthetic performances of the two longevity classes. It has been suggested that extended lifespans are necessary to compensate for low assimilation rates and cost-benefit analyses have been made of the contribution of an individual leaf in terms of gross and net assimilate gain and the implications for foliar lifespan (Chabot & Hicks, 1982; Harper, 1989; Williams, Field & Mooney, 1989). Carbon

budgets alone do not fully explain the ecology of leaf lifespans. Monk (1966) proposed that the evergreen habit should be favoured in low nutrient environments because of the slower, more stringently controlled, less leaching-prone routes for nutrient cycling, occurring both internally and via the leaf-litter. This means low intrinsic turnover times and high C gain per unit nutrient. Consistent with Monk's hypothesis is the observation that fertiliser application can *decrease* long foliar lifespans (Aerts, 1989; Lajtha & Whitford, 1989).

Conclusions

Different ways of producing a stay-green *phenotype*

In herbaceous crop plants, alterations in one or a few genes may give rise to a *stay-green* phenotype. In this review we have described examples of both dominant and recessive *stay-green* genes. The degree of expression of delayed senescence in *stay-green* varieties is highly dependent on environmental conditions, and varieties showing a normal pattern of senescence may be induced to stay green by treatments such as removal of flowers or fruits, increasing the cytokinin level, heat stress, inhibition of protein synthesis or shading.

Type C *stay-green* behaviour may be caused by mutation of a single gene, as in *Festuca*, by inhibition of protein synthesis or by shading. It appears that one or a small number of activities controlling chlorophyll-protein breakdown are particularly sensitive to genetic or environmental perturbation. We speculate that the enzymes required for general proteolysis are present throughout the life of the leaf, and encoded by *type 1 or 2* genes (Fig. 4), whereas the accessibility of thylakoid proteins to this activity is regulated, at least in part, by expression of *type 5* genes for pigment catabolism. The contrasting kinetic and adaptive characteristics of such distinctively-regulated protein degradation pathways have been discussed by Hilditch, Thomas & Lucas (1989). It is not yet possible to specify the sensitive steps in pigment-proteolipid breakdown: the phytol-removing enzyme, the macrocycle-opening activity and Mg-dechelatase are the prime suspects (Thomas *et al.*, 1989; Schellenberg, Matile & Thomas, 1990; Shioi, Tatsumi & Shimokawa, 1991) and will undoubtedly become the focus of studies aimed at cloning truly senescence-specific genes. Transformation with antisense versions of such genes would be predicted to generate specific *stay-green* phenotypes.

Another way to achieve a *stay-green* phenotype would be to extend the lifespans of individual leaves, but this might be predicted to have unfavourable consequences such as reduced photosynthetic rates and responsiveness to fertiliser (see Table 3). On the other hand, increasing canopy duration while retaining high rates of leaf turnover requires extension of the period of continued recruitment of new leaves to balance those lost by senescence; the determinate nature of the reproductive apices in the major seed crops makes this a particularly difficult option. The prospect of success of either strategy depends on whether the characteristics of each of the longevity classes catalogued in Table 5 are genetically linked and if so, how easily the linkage can be dissected by classical or recombinant methods of crop improvement.

Healthy stay-green *plants produce a higher yield*

The search for physiological and biochemical determinants of crop production has been dominated by the belief that the rate of carbon assimilation is directly related to yield. But over fifty years ago (Heath & Gregory, 1938; Watson, 1952) it was realised that most of

Table 5. *Characteristics of long-lived and short-lived leaves*

Long-lived leaves	Short-lived leaves
Drought-resistant (xerophyllous)	Drought-avoiding
Long duration, low rate of photosynthesis	Short duration, high rate of photosynthesis
Able to photosynthesise when growth is limited	Photosynthesis is generally confined to periods of active growth
Construction costs relatively high	Relatively cheap to make
Herbivore resistant (sclerophyllous)	Herbivore-avoiding
Rate of nutrient cycling low	High rate of nutrient turnover
High photosynthesis per unit N	Low photosynthesis per unit N
Broad-leaved tropical and needle-leaved high altitude/latitude species	Broad-leaved and some needle-leaved temperate species
Lifespan reduced by increased fertiliser	Lifespan increased by increasing fertiliser

(From Thomas, 1992*a*).

the diversity in yield for most crops is a consequence of variation in the duration rather than the rate of photosynthetic activity.

Watson (1947, 1952) developed the concept of *leaf area duration* (**D**), a parameter which is closely correlated with production in a wide range of crops under a variety of agronomic conditions (Thomas, 1992*a*). More effective still than **D** as a correlate of yield is **G**, the *green area duration* (see, for example, Borojević, Čupina & Krsmanović, 1980; Wolfe, Henderson, Hsiao & Alvino, 1988). In some host-pathogen interactions the leaf responds to infection by undergoing rapid senescence, to which the invading organism reacts by secreting inhibitory factors which maintain a non-senescent zone around the infection point (Mothes, 1970; Scholes & Farrar, 1987; Marchoux, 1987; Kenfield *et al.*, 1989). The result of such conflicts may be to produce a state of "physiological defoliation" in the diseased crop while **D** and even **G**, measured conventionally, are maintained. Waggoner & Berger (1987) introduced the concept of *healthy area duration* (**H**), analogous to **D** or **G**, and showed that this index is tightly coupled to yield in a number of crops.

Other advantages of the stay-green *character*

Stay-green plants have increased resistance to disease and drought and possess leaves with higher nutritional quality and attractiveness to grazing animals. Their retention of chlorophyll makes them an ideal source of this pigment for the food industry and ensures that amenity and ornamental plants remain attractive over an extended period.

The future

Since it represents one of the most consistent and accessible physiological correlates with productivity, *extended greenness* might be expected to be a well-established objective in crop breeding and improvement strategies. In practice, interest in this important character has been largely empirical and incidental. We have seen that the *stay-green* phenotype has now been identified in a number of crop species. But exploitation of the *stay-green* phenomenon and understanding its consequences for crop quality and quantity are limited by the primitive state of knowledge of the biochemical and genetic basis. We hope that this survey may serve to awaken wider interest in the scientific and practical opportunities offered by this fascinating phenomenon.

Acknowledgements

The Institute of Grassland and Environmental Research is grant-aided by the Agricultural and Food Research Council. The authors' research on *stay-green* is partially supported by Zeneca plc.

References

Abu-Shakra S S, Phillips D A, Huffaker R C. 1978. Nitrogen fixation and delayed leaf senescence in soybeans. *Science* **199**:973–975.

Aerts R. 1989. The effect of increased nutrient availability on leaf turnover and aboveground productivity of two evergreen ericaceous shrubs. *Oecologia* **78**:115–120.

Akiyoshi D E, Klee H, Amasino R M, Nester E W, Gordon, M P. 1984. T-DNA of *Agrobacterium tumefaciens* encodes an enzyme of cytokinin biosynthesis. *Proceedings of the National Academy of Sciences USA* **81**:5994–5998.

Allison J C, Weinmann H. 1970. Effect of absence of developing grain on carbohydrate content and senescence of maize leaves. *Plant Physiology* **46**:435–436.

Ambler J R, Morgan P W, Jordan W R. 1987. Genetic regulation of senescence in a tropical gra In *Plant Senescence: Its Biochemistry and Physiology* pp. 43–53. Eds W W Thomson, E A Nothnagel and R C Huffaker. Rockville: American Society of Plant Physiologists.

Barry G F, Rogers S G, Fraley R T, Brand L. 1984. Identification of a cloned cytokinin biosynthetic gene. *Proceedings of the National Academy of Sciences USA* **81**:4776–4780.

Bernard R L. 1971. Two major genes for time of flowering and maturity in soybeans. *Crop Science* **11**:242–244.

Bernard R L. 1972. Two genes affecting stem termination in soybeans. *Crop Science* **12**:235–239.

Bernard R L, Weiss M G. 1973. Qualitative genetics. In *Soybeans: Improvement, Production and Uses*, pp. 117–154. Ed. B E Caldwell. Madison: American Society of Agronomy.

Blackbourn H D, Jeger M J, John P. 1990*a*. Inhibition of degreening in the peel of bananas ripened at tropical temperatures. V. Chlorophyll bleaching activity measured *in vitro*. *Annals of Applied Biology* **117**:175–186.

Blackbourn H D, Jeger M J, John P, Thompson A K. 1990*b*. Inhibition of degreening in the peel of bananas ripened at tropical temperatures. III. Changes in plastid ultrastructure and chlorophyll-protein complexes accompanying ripening in bananas and plantains. *Annals of Applied Biology* **117**:147–161.

Blackbourn H D, Jeger M J, John P, Telfer A, Barber J. 1990*c*. Inhibition of degreening in the peel of bananas ripened at tropical temperatures. IV. Photosynthetic capacity of ripening bananas and plantains in relation to changes in the lipid composition of ripening banana peel. *Annals of Applied Biology* **117**:163–174.

Bleecker A B, Estelle M A, Somerville C, Kende H. 1988. Insensitivity to ethylene conferred by dominant mutation in *Arabidopsis thaliana*. *Science* **241**:1086–1089.

Borojević S, Čupina T, Krsmanović M. 1980. Green area parameters in relation to grain yield of different wheat genotypes. *Zeitschrift für Pflanzenzüchtung* **84**:265–283.

Bortlik K, Gut H, Matile P. 1987. Yellowing and non-yellowing trees: a comparison of protein- and chlorophyll-loss in senescent leaves. *Botanica Helvetica* **97**:323–328.

Bouwkamp J C, Honma S. 1970. Physiological differences between a green and a tan dry podded line of snap bean. *HortScience* **5**:1071–1073.

Bowler C, Slooten L, Vandenbranden S, De Rycke R, Botterman J, Sybesma C, Van Montagu M, Inzé D. 1991. Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *The EMBO Journal* **10**:1723–1732.

Boyd W J R, Walker M G. 1972. Variation in chlorophyll a content and stability in wheat flag leaves. *Annals of Botany* **36**:87–92.

Ceppi D, Sala M, Gentinetta E, Verderio A, Motto M. 1987. Genotype-dependent leaf senescence in maize. *Plant Physiology* **85**:720–725.

Chabot B F, Hicks D J. 1982. The ecology of leaf life spans. *Annual Review of Ecology and Systematics* **13**:229–259.

Chory J, Nagpal P, Peto C A. 1991. Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. *The Plant Cell* **3**:445–459.

Christensen L E, Below F E, Hageman R H. 1981. The effects of ear removal on senescence and metabolism of maize. *Plant Physiology* **68**:1180–1185.

Colbert K A, Beever J E. 1981. Effect of disbudding on root cytokinin export and leaf senescence in tomato and tobacco. *Journal of Experimental Botany* **32**:121–127.

Cooper R L. 1981. Development of short-statured soybean cultivars. *Crop Science* **21**:127–131.

Crafts-Brandner S J. 1991. Nonstructural carbohydrate metabolism during leaf ageing in tobacco (*Nicotiana tabacum*). *Physiologia Plantarum* **82**:299–305.

Crafts-Brandner S J, Poneleit C G. 1987*a*. Carbon dioxide exchange rates, ribulose bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase activities, and kernel growth characteristics of maize. *Plant Physiology* **84**:255–260.

Crafts-Brandner S J, Poneleit C G. 1987*b*. Effect of ear removal on CO_2 exchange and activities of ribulose bisphosphate carboxylase/oxygenase and phosphoenolpyruvate carboxylase of maize hybrids and inbred lines. *Plant Physiology* **84**:261–265.

Crafts-Brandner S J, Sutton T G, Sims J L. 1987*a*. Root system genotype and nitrogen fertility effects on physiological differences between burley and flue-cured tobacco. II. Whole plant. *Crop Science* **27**:1219–1224.

Crafts-Brandner S J, Sutton T G, Sims J L. 1988. Influence of leaf grafting on leaf constituents and senescence characteristics of burley and flue-cured tobacco. *Crop Science* **28**:269–274.

Crafts-Brandner S J, Below F E, Harper J E, Hageman R H. 1984*a*. Differential senescence of maize hybrids following ear removal. I. Whole plant. *Plant Physiology* **74**:360–367.

Crafts-Brandner S J, Leggett J E, Sutton T G, Sims J L. 1987*b*. Effect of root system genotype and nitrogen fertility on physiological differences between burley and flue-cured tobacco. I. Single leaf measurements. *Crop Science* **27**:535–539.

Crafts-Brandner S J, Below F E, Wittenbach V A, Harper J E, Hageman R H. 1984*b*. Differential senescence of maize hybrids following ear removal. II. Selected leaf. *Plant Physiology* **74**:368–373.

Dalling M J, Boland G, Wilson J H. 1976. Relation between acid proteinase activity and redistribution of nitrogen during grain development in wheat. *Australian Journal of Plant Physiology* **3**:721–730.

Darbishire A D. 1911. *Breeding and the Mendelian Discovery*. New York: Cassell.

Davies T G E, Thomas H, Rogers L J. 1990*a*. Immunological quantification of cytochrome f in leaves of a non-yellowing senescence mutant of *Festuca pratensis*. *Photosynthesis Research* **24**:99–108.

Davies T G E, Thomas H, Thomas B J, Rogers L J. 1990*b*. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*: metabolism of cytochrome f. *Plant Physiology* **93**:588–595.

De Kok L J, Graham M. 1989. Levels of pigments, soluble proteins, amino acids and sulfhydryl compounds in foliar tissue of *Arabidopsis thaliana* during dark-induced and natural senescence. *Plant Physiology and Biochemistry* **27**:203–209.

Leggett H. 1988. *Sorghum*. 2nd edition. Harlow: Longman.

Duggelin T, Bortlik K, Gut H, Matile P, Thomas H. 1988. Leaf senescence in *Festuca pratensis*: accumulation of lipofuscin-like compounds. *Physiologia Plantarum* **74**:131–136.

Duncan R R, Bockholt A J, Miller F R. 1981. Descriptive comparison of senescent and non-senescent sorghum genotypes. *Agronomy Journal* **73**:849–853.

Duvick D N. 1984. Genetic contribution to yield gains of US hybrid maize 1930–1980. In *Genetic contributions to yield gains of five major crop plants (CSSA special publication 7)*, pp. 15–47. Ed. W R Fehr. Madison: Crop Science Society of America.

Evangelista C C, Tangonan N G. 1990. Reaction of 31 non-senescent sorghum genotypes to stalk rot complex in southern Philippines. *Tropical Pest Management* **36**:214–215.

Ewers F W, Schmid R. 1981. Longevity of needle fascicles of *Pinus longaeva* (bristlecone pine) and other North American pines. *Oecologia* **51**:107–115.

Farran G P. 1947. Vertical distribution of plankton (*Sagitta, Calanus* and *Metridia*) off the south coast of Ireland. *Proceedings of the Royal Irish Academy* **51B**:121–136.

Gentinetta E, Ceppi D, Lepori C, Perico G, Motto M, Salamini F. 1986. A major gene for delayed senescence in maize. Pattern of photosynthates accumulation and inheritance. *Plant Breeding* **97**:193–203

Gerik T J, Miller F R. 1984. Photoperiod and temperature effects on tropically and temperately adapted sorghum. *Field Crops Research* **9**:29–40.

Grierson D, Purton M E, Knapp J E, Bathgate B. 1987. Tomato ripening mutants. In *Developmental Mutants in Higher Plants*, pp. 73–94. Eds H Thomas and D Grierson. Cambridge: University Press.

Guiamét J J, Teeri J A, Noodén L D. 1990. Effects of nuclear and cytoplasmic genes altering chlorophyll loss on gas exchange during monocarpic senescence. *Plant and Cell Physiology* **31**:1123–1130.

Guiamét J J, Schwartz E, Pichersky E, Noodén L D. 1991. Characterization of cytoplasmic and nuclear mutations affecting chlorophyll and chlorophyll-binding proteins during senescence in soybean. *Plant Physiology* **96**:227–231.

Hardwick R C. 1979. Leaf abscission in varieties of *Phaseolus vulgaris* (L.) and *Glycine max* (L.) Merrill – a correlation with propensity to produce adventitious roots. *Journal of Experimental Botany* **30**:795–804.

Harper J L. 1989. The value of a leaf. *Oecologia* **80**:53–58.

Heath O V S, Gregory F G. 1938. The constancy of the mean net assimilation rate and its ecological importance. *Annals of Botany* **2**:811–818.

Helsel D B, Frey K J. 1978. Grain yield variations in oats associated with differences in leaf area duration among oat lines. *Crop Science* **18**:765–769.

Henika F S. 1932. The inheritance of the white burley character in tobacco. *Journal of Agricultural Research* **44**:477–493.

Hidema J, Makino A, Mae T, Ojima K. 1991. Photosynthetic characteristics of rice leaves aged under different irradiances from full expansion through senescence. *Plant Physiology* **97**:1287–1293.

Hilditch P I. 1986. Immunological quantification of the chlorophyll a/b binding protein in senescing leaves of *Festuca pratensis* Huds. *Plant Science* **45**:95–99.

Hilditch P I, Thomas H, Lucas K. 1989. Kinetic implications of structural modification in protein turnover. *BioSystems* **22**:241–248.

Hilditch P I, Thomas H, Rogers L J. 1986*a*. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*: photosynthesis and photosynthetic electron transport. *Planta* **167**:146–151.

Hilditch P I, Thomas H, Rogers L J. 1986*b*. Two processes for the breakdown of the QB protein of chloroplasts. *FEBS Letters* **208**:313–316.

Hilditch P I, Thomas H, Thomas B J, Rogers L J. 1989. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*: proteins of Photosystem II. *Planta* **177**:265–272.

Honma S, Bouwkamp J C, Stojianov M J. 1968. Inheritance of dry pod-color in snap beans. *Journal of Heredity* **59**:243–244.

House L R. 1985. *A Guide to Sorghum Breeding*. 2nd edition. Patancheru: ICRISAT.

Isaacs J D, Tont S A, Wick G L. 1974. Deep scattering layers: vertical migration as a tactic for finding food. *Deep Sea Research* **21**:651–656.

Kenfield D, Bunkers G, Wu Y H, Strobel G, Sugawara F, Hallock Y, Clardy J. 1989. Giganantenone, a novel sesquiterpene phytohormone mimic. *Experientia* **45**:900–902.

Kräutler B, Jann B, Bortlik K, Schellenberg M, Matile P. 1991. On the enigma of chlorophyll degradation: the constitution of a secoporphinoid catabolite. *Angewandte Chemie (International Edition, English)* **30**:1315–1318.

Lajtha K, Whitford W G. 1989. The effect of water and nitrogen amendments on photosynthesis, leaf demography, and resource-use efficiency in *Larrea tridentata*, a desert evergreen shrub. *Oecologia* **80**:341–348.

Legg P D, Chaplin J F, Williamson R E. 1977. Genetic diversity in burley and flue-cured tobacco. *Crop Science* **17**:943–947.

Leshem Y Y. 1988. Plant senescence processes and free radicals. *Free Radical Biology and Medicine* **5**:39–50.

Mae T, Thomas H, Gay A P, Makino A, Hidema J. 1993. Leaf development in *Lolium temulentum*: photosynthesis and photosynthetic proteins in leaves senescing under different irradiances. *Plant and Cell Physiology* **34**:391–399.

Marchoux G. 1987. Interactions entre virus chez un hôte commun. I. Interferences negatives, antagonisme, premunition, resistance induite. *Agronomie* **7**:149–162.

Matile P. 1992*a*. Vom Ergrünen und Vergilben der Blätter. *Neujahrsblatt von der Naturforschenden Gesellschaft*, Zürich.

Matile P. 1992*b*. Leaf senescence. In *Crop Photosynthesis: Spatial and Temporal Determinants*, pp. 413–440. Eds N Baker and H Thomas. Amsterdam: Elsevier.

Matile P, Ginsburg S, Schellenberg M, Thomas H. 1987. Catabolites of chlorophyll in senescent leaves. *Journal of Plant Physiology* **129**:219–228.

Matile P, Düggelin T, Schellenberg M, Rentsch D, Bortlik K, Peisker C, Thomas H. 1989. How and why is chlorophyll broken down in senescent leaves? *Plant Physiology and Biochemistry* **27**:595–604.

Middleton G K, Bode C E, Bayles B B. 1954. A comparison of the quantity and quality of protein in certain varieties of soft wheat. *Agronomy Journal* **46**:500–502.

Molisch H. 1938. *The Longevity of Plants*. Lancaster, Pa. USA: Science Press.

Mondal W A, Choudhuri M A. 1985. Comparison of phosphorus mobilization during monocarpic senescence in rice cultivars with sequential and non-sequential leaf senescence. *Physiologia Plantarum* **65**:221–227.

Monk C D. 1966. An ecological significance of evergreenness. *Ecology* **47**:504–505.

Moreno G, Pottier R H, Truscott T G. 1988. *Photosensitisation: Molecular, Cellular, and Medical Aspects. Nato ASI Series H. Cell Biology, Volume 15*. Berlin, New York: Springer.

Moss D N. 1962. Photosynthesis and barrenness. *Crop Science* **2**:366–367.

Mothes K. 1970. Über grüne Inseln. *Leopoldina* **15**:171–172.

Neave I A, Dawson J O, DeLucia E H. 1989. Autumnal photosynthesis is extended in nitrogen-fixing European black alder compared with white basswood: possible adaptive significance. *Canadian Journal of Forestry Research* **19**:12–17.

Nock L P, Rogers L J, Thomas H. 1992. Metabolism of protein and chlorophyll in leaf tissue of *Festuca pratensis* Huds. during chloroplast assembly and senescence. *Phytochemistry* **31**:1465–1470.

Omori M. 1974. The biology of pelagic shrimps in the ocean. *Advances in Marine Biology* **12**:233–324.

Peacock B C. 1980. Banana ripening – effect of temperature on fruit quality. *Queensland Journal of Agricultural and Animal Sciences* **37**:39–45.

Peisker C, Thomas H, Keller F, Matile P. 1990. Radiolabelling of chlorophyll for studies on catabolism. *Journal of Plant Physiology* **136**:544–549.

Phillips D A, Pierce R O, Edie S A, Foster K W, Knowles P F. 1984. Delayed senescence in soybeans. *Crop Science* **24**:518–522.

Pierce R O, Knowles P F, Phillips D A. 1984. Inheritance of delayed senescence in soybean. *Crop Science* **24**:515–517.

Rao R O, Croy L I. 1972. Protease and nitrate reductase seasonal patterns and their relation to grain protein production of "high" vs. "low" protein wheat varieties. *Journal of Agricultural and Food Chemistry* **20**:1138–1141.

Rao K N, Reddy V S, Williams R J, House L R. 1980. The ICRISAT International Crops Research Institute for Semi-Arid Tropics charcoal rot fungi, *Macrophomina phaseolina*, resistance program. In *Proceedings of the International Workshop on Sorghum Diseases*, pp. 315–321. Eds R J Williams, L K Mughogho and G D Bengtson. Patancheru: ICRISAT.

Ronning C M, Bouwkamp J C, Solomos T. 1991. Observations on the senescence of a mutant non-yellowing genotype of *Phaseolus vulgaris* L. *Journal of Experimental Botany* **42**:235–241.

Rosenow D T. 1980. Stalk rot resistance breeding in Texas. Various fungal pathogens. In *Proceedings of the International Workshop on Sorghum Diseases*, pp. 306–314. Eds R J Williams, L K Mughogho and G D Bengtson. Patancheru: ICRISAT.

Schellenberg M, Matile P, Thomas H. 1990. Breakdown of chlorophyll in chloroplasts of senescent barley leaves depends on ATP. *Journal of Plant Physiology* **136**:564–568.

Scholes J D, Farrar J F. 1987. Development of symptoms of brown rust of barley in relation to the distribution of fungal mycelium, starch accumulation and localized changes in the concentration of chlorophyll. *New Phytologist* **107**:103–117.

Seymour G B, John P, Thompson A K. 1987*a*. Inhibition of degreening in the peel of bananas ripened

at tropical temperatures. II. Role of ethylene, oxygen and carbon dioxide. *Annals of Applied Biology* **110**:153–161.

Seymour G B, Thompson A K, John P. 1987*b*. Inhibition of degreening in the peel of bananas ripened at tropical temperatures. I. Effect of high temperature on change in the pulp and peel during ripening. *Annals of Applied Biology* **110**:145–151.

Shioi Y, Tatsumi Y, Shimokawa K. 1991. Enzymatic degradation of chlorophyll in *Chenopodium album*. *Plant and Cell Physiology* **32**:87–93.

Shoemaker R C, Cody A M, Palmer R G. 1985. Characterization of a cytoplasmically-inherited yellow foliar mutant ($cytY_3$) in soybean. *Theoretical and Applied Genetics* **69**:279–284.

Simmonds N W. 1966. *Bananas*. London: Longman.

Sinclair T R, de Wit C R. 1975. Photosynthate and nitrogen requirements for seed production by various crops. *Science* **189**:565–567.

Smart C M, Scofield S R, Bevan M W, Dyer T A. 1991. Delayed leaf senescence in tobacco plants transformed with *tmr*, a gene for cytokinin production in *Agrobacterium*. *The Plant Cell* **3**:647–656.

Stines B J, Mann T J. 1960. Diploidization in *Nicotiana tabacum*. *Journal of Heredity* **51**:222–227.

Stoddart J L, Thomas H. 1982. Leaf senescence. In *Encyclopedia of Plant Physiology. New Series Volume 14A. Nucleic Acids and Proteins in Plants I.*, pp. 592–636. Eds D Boulter and B Parthier. Berlin: Springer.

Strother S. 1988. The role of free radicals in leaf senescence. *Gerontology* **34**:151–156.

Tapper B A, Lohrey E, Hove E L, Allison R M. 1975. Photosensitivity from chlorophyll-derived pigments. *Journal of the Science of Food and Agriculture* **26**:277–284.

Terao H. 1918. Maternal inheritance in the soybean. *American Naturalist* **52**:51–56.

Thomas H. 1976. Delayed senescence in leaves treated with the protein synthesis inhibitor MDMP. *Plant Science Letters* **6**:369–377.

Thomas H. 1977. Ultrastructure, polypeptide composition and photochemical activity of chloroplasts during foliar senescence of a non-yellowing mutant genotype of *Festuca pratensis*. *Planta* **137**:53–60.

Thomas H. 1982. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*. I. Chloroplast membrane polypeptides. *Planta* **154**:212–218.

Thomas H. 1983. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*. IV. Senescence in the light. *Photosynthetica* **17**:506–514.

Thomas H. 1987*a*. Foliar senescence mutants and other genetic variants. In *Developmental Mutants in Higher Plants*, pp. 245–265. Eds H Thomas and D Grierson. Cambridge: University Press.

Thomas H. 1987*b*. *Sid*: a Mendelian locus controlling thylakoid membrane disassembly in senescing leaves of *Festuca pratensis*. *Theoretical and Applied Genetics* **73**:551–555.

Thomas H. 1992*a*. Canopy survival. In *Crop Photosynthesis: Spatial and Temporal Determinants*, pp. 11–41. Eds N R Baker and H Thomas. Amsterdam: Elsevier.

Thomas H. 1992*b*. Resource rejection by higher plants. In *Resource Capture by Crops. Proceedings of the 52nd Easter School, University of Nottingham School of Agriculture* (in press).

Thomas H, Matile P. 1988. Photobleaching of chloroplast pigments in leaves of a non-yellowing mutant genotype of *Festuca pratensis*. *Phytochemistry* **27**:345–348.

Thomas H, Stoddart J L. 1975. Separation of chlorophyll degradation from other senescence processes in leaves of a mutant genotype of meadow fescue (*Festuca pratensis*). *Plant Physiology* **56**:438–441.

Thomas H, Stoddart J L. 1980. Leaf senescence. *Annual Review of Plant Physiology* **31**:83–111.

Thomas H, Ougham H J, Davies T G E. 1992. Leaf senescence in a non-yellowing mutant of *Festuca pratensis*: transcripts and translation products. *Journal of Plant Physiology* **139**:403–412.

Thomas H, Bortlik K, Rentsch D, Schellenberg M, Matile P. 1989. Catabolism of chlorophyll in vivo: significance of polar chlorophyll catabolites in a non-yellowing senescence mutant of *Festuca pratensis*. *New Phytologist* **111**:3–8.

Tollenaar M, Daynard T B. 1978. Leaf senescence in short-season maize hybrids. *Canadian Journal of Plant Science* **58**:869–874.

Veatch C, Woodworth C M. 1930. Genetic relations of cotyledon color types of soybeans. *Journal of the American Society of Agronomy* **22**:700–702.

Vietor D M, Cralle H T, Miller F R. 1989. Partitioning of ^{14}C-photosynthate and biomass in relation to senescence characteristics of sorghum. *Crop Science* **29**:1049–1053.

Wada Y, Wada G. 1991. Varietal difference in leaf senescence during ripening period of advanced indica rice. *Japanese Journal of Crop Science* **60**:529–536.

Waggoner P E, Berger R D. 1987. Defoliation, disease, and growth. *Phytopathology* **77**:393–398.

Watson D J. 1947. Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties, and within and between years. *Annals of Botany* **11**:41–76.

Watson D J. 1952. The physiological basis of variation in yield. *Advances in Agronomy* **4**:101–145.

Williams K, Field C B, Mooney H A. 1989. Relationships among leaf construction cost, leaf longevity, and light environment in rain-forest plants of the genus *Piper*. *American Naturalist* **133**:198–211.

Wittenbach V A. 1977. Induced senescence of intact wheat seedlings and its reversibility. *Plant Physiology* **59**:1039–1042.

Wolfe D W, Henderson D W, Hsiao T C, Alvino A. 1988. Interactive water and nitrogen effects on senescence of maize. I. Leaf area duration, nitrogen distribution, and yield. *Agronomy Journal* **80**:859–864.

Woodworth C M. 1921. Inheritance of cotyledon, seed-coat, hilum and pubescence colors in soybeans. *Genetics* **6**:487–553.

Zartman R E, Woyewodzic R T. 1979. Root distribution patterns of two hybrid grain sorghums under field conditions. *Agronomy Journal* **71**:325–328.

(Received 13 October 1992)