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# Postharvest Biology and Technology of Cut Flowers and Potted Plants

*Michael S. Reid*
Department of Plant Sciences
University of California
Davis, California 95616, USA

*Cai-Zhong Jiang*
Crops Pathology and Genetic Research Unit
USDA-ARS
Davis, California 95616, USA

**ABSTRACT**

The relatively brief postharvest life of most cut flowers and potted flowering plants can be extended by a range of technologies. Studies have shown that vase life is negatively correlated with respiration after harvest, so prompt cooling to the lowest safe storage temperature is a key to long-distance transport of these perishable crops. Forced air cooling is the method of choice for cut flowers, and vacuum cooling has been shown to be very effective for cooling potted plants. In contrast to some other horticultural crops, controlled and modified atmospheres seem to have little effect on petal respiration, and these techniques have not proved commercially useful in the marketing of many cut flowers. Low temperatures are also important in managing the effect of other factors contributing to early senescence, including water loss, the effects of ethylene, leaf yellowing, and the growth of diseases, particularly caused by *Botrytis cinerea*. Ornamentals originating in the tropics and subtropics cannot be cooled below 10°C because they rapidly show the symptoms of chilling injury. Chemical strategies to improve the life of ornamentals include the application of abscisic acid to reduce water loss, particularly in potted and bedding plants, pretreatment with the volatile ethylene inhibitor 1-methylcyclopropene (1-MCP) to prevent the

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effects of endogenous or exogenous ethylene, treatment with gibberellins or cytokinins (CKs), which often delay leaf yellowing and may increase bud opening and flower life. Thidiazuron, a nonmetabolized CK, has proven particularly effective for this purpose. A new strategy for inhibiting the growth of *B. cinerea* on floral tissues is to treat them with low concentrations of hypochlorite. Floral senescence is an active process with many of the hallmarks of programmed cell death. Molecular analysis has revealed a large number of candidate genes with possible roles in senescence and remobilization. Virus-induced gene silencing has been used to evaluate the potential role of some of these genes, particularly regulatory genes such as transcription factors and kinases, although none has yet been identified as a key controller. Ornamentals are particularly suited to testing transgenic strategies for extending shelf-life, and we report results of experiments using constructs where inducible promoters are used to drive genes that extend flower life. Of particular interest is the dramatic extension of longevity resulting from silencing a component of the 26S proteasome, which indicates the importance of targeted protein degradation in control of floral senescence, and could serve as a strategy for extending the life of ethylene-insensitive ephemeral flowers. Future research will undoubtedly focus on providing better germplasm by using traditional, genomic assisted, and/or molecular breeding approaches for improving the postharvest performance of ornamentals.

**KEYWORDS:** biotechnology; gene regulation; growth regulators; temperature; water relations

## ABBREVIATIONS

| | |
|---|---|
| ABA | Abscisic acid |
| BA | Benzyl adenine |
| CA | Controlled atmosphere |
| DACP | Diazocyclopropene |
| GA | Gibberellic acid |
| IPT | Isoptentenyl transferase |
| 1-MCP | 1-Methylcyclopropene |
| MA | Modified atmosphere |
| MJ | Methyl jasmonate |
| NCED | 9-*cis*-Epoxycarotenoid dioxygenase |
| NPA | Naphthyl phthalamic acid |
| PBB2 | Proteasome beta subunit B-2 |
| PCDA | Programmed cell death |
| PIN | Auxin efflux facilitator |
| SAG | Senescence-associated gene |
| TDZ | Thidiazuron |
| WT | Wild type |

## I. INTRODUCTION

Much has changed in our understanding of the postharvest biology of floral and foliage crops since the publication, in the first and third volumes of *Horticultural Reviews*, of the two parts of Halevy and Mayak's comprehensive review of this topic (Halevy and Mayak 1979, 1981). Since then other reviews on aspects of the basic biology of flower

senescence (Borochov and Woodson 1989; van Doorn and Stead 1994; van Doorn and Woltering 2004), and on the role of ethylene (Reid and Wu 1992) have been complemented by practical handbooks on postharvest technology for flowers (Nell and Reid 2000). In addition to articles in the peer-reviewed literature, the proceedings of the quadrennial meeting of postharvest floriculture members of ISHS, published in *Acta Horticulturae*, volumes 181 (1986), 261 (1989), 298 (1991), 405 (1995), 543 (2001), 669 (2005), and 847 (2009), provide concentrated sources of information on new developments in the field, and descriptions of the postharvest behavior of a wide range of floricultural crops.

Our goal in the present review is to describe studies that have changed our understanding of the postharvest biology of floricultural crops or added to the palette of postharvest technologies since previous reviews, and to indicate current optimal technologies based on that new understanding. In particular, we have focused on recent findings in relevant areas of basic plant biology, and conclude with a discussion of the way in which molecular strategies are being, or could be deployed in the future, to extend postharvest life and reduce postharvest losses of perishable ornamental crops.

## II. THE ORNAMENTAL INDUSTRY

In the past 50 years, the cut flower market has changed dramatically, from a local market with growers located on city outskirts, to a global one; flowers and cut foliage sourced from throughout the world are sold as bunches or combined into arrangements and bouquets in the major target markets, such as North America, Japan, and the European Union. Items in a single florist arrangement are often sourced from countries in three or more continents. The high value of cut flowers has driven major increases in production in many developing countries. Production of cut flowers and foliage can be highly profitable in countries with an ideal growing environment (particularly those close to the equator where the environment is uniform throughout the year), and labor costs are low. The costs of establishing production in the field or even in plastic houses are relatively modest, and harvest may start within a few months of planting.

This reshaping of the market has occurred with little consideration for its postharvest consequences. Flowers that used to be obtained from local growers and were retailed within days of harvest may now take as long as three weeks to arrive at the retail florist or supermarket. Increased emphasis on holidays as occasions for sale of cut flowers has exacerbated this trend. The volume of flowers required to meet the

demand for the major holidays (Valentine's day, Mothers' day) has led to widespread storage. The peak in harvest of roses for Valentine's day in Central America, is three weeks prior to the holiday itself!

Because of their perishability, flowers and foliage produced in distant growing areas have traditionally been shipped by air (a transportation system whose rapidity fails to offset the disadvantages of poor temperature management and low humidities). The increasing cost of jet fuel, and the volumes of flowers being produced in countries such as Colombia and Kenya has led to many efforts to ship ornamentals in marine containers, further extending the time from harvest to market. These market and transportation changes have not been accompanied by changes in postharvest technologies to offset the time/temperature effect on the life of ornamentals. The net result, especially in North America, has been a reduction in display life of cut flowers and foliage, disenchantment with the cut flower purchase experience, documented in many surveys, and a per capita consumption of cut flowers in the United States that is less than that in almost all other developed countries (Reid and Jiang 2005).

## III. FACTORS AFFECTING THE POSTHARVEST LIFE OF ORNAMENTALS

The intersection of art, design, and horticulture represented by the ornamental plant industry has led to the use of a very wide variety of plant organs and taxa for ornamental purposes. Plants used range from the *Lycopsida* to the flowering plants, genera from *Acanthus* to *Zingiber*, and tissue types from young buds to fruits and seeds. This diversity of taxa, physiological state, and organ means that generalizations about their biology and even technology are often misleading. In this review, we focus largely on cut and potted flowers and foliage. The unique characteristics of the more unusual ornamental plant materials, and other horticultural crops properly classified as ornamentals (bulbs, corms, tubers, bedding plants, bare-root and dormant nursery materials, and the like) and their unique physiology and technology requirements will be mentioned only where recent research has provided information of interest and importance to their postharvest handling.

Some ornamentals, particularly potted and cut foliage can be extraordinarily long-lived. The *Aspidistra* of Victorian parlors have been replaced in our time by immortal *Scindapsis* (*Pothos*) plants that trail through offices and hotel lobbies everywhere. Nevertheless, the majority of the ornamentals of commerce have relatively short lives. The

delicate petals of flowers are easily damaged, and are often highly susceptible to disease. Even under optimum conditions, their biology leads to early wilting, abscission, or both. Foliage is longer lived, although the low light of the postharvest environment frequently leads to early leaf yellowing, and, in some cases, leaf abscission. As with other perishable horticultural crops, the life of ornamentals is affected by physical, environmental, and biological factors. Choice of plant material, and preharvest factors play an important role. After harvest, temperature is of over-riding importance, and affects plant water relations, growth of disease, response to physical stresses, carbohydrate status, and the interplay among endogenous and exogenous growth regulators. Much has been learnt in the past 30 years about the role of these factors and the response of ornamentals to them, and some of the research findings have led to technologies that can greatly improve marketing and postharvest quality of ornamentals.

## A. Genotype

It is common knowledge that the postharvest life of flowers varies enormously, from the ephemeral flowers of the daylily to the extremely long-lived flowers of some orchid genera. Less extreme, but still marked variations are also seen within genera and even species, and certainly this variation provides a great opportunity for breeders to develop longer lasting flowers. Color, form, productivity, and disease resistance continue to be the targets of breeding programs. This can be seen by comparing the postharvest life of different cultivars from the same breeder. In *Alstroemeria*, we showed that time of petal fall and time of leaf yellowing both showed variation of more than 100% in lines released by the same breeder. Elibox and Umaharan (2008) reported vase lives of anthurium cultivars ranging from 14 to 49 days. A simple model, based on abaxial stomatal density and flower color accurately predicted the relative vase life ranking of different cultivars, providing an excellent tool for future breeding. Variations in other important postharvest characteristics have also been reported, for example, for ethylene sensitivity in carnations (Woltering and van Doorn 1988; Wu et al. 1991; Reid and Wu 1992) and in roses (Evans and Reid 1988; Macnish et al. 2010c). In their study, Macnish et al. (2010c) demonstrated a difference in vase life of modern rose cultivars of from 5 to 19 days. Five of the 38 cultivars tested were insensitive to ethylene indicating the breeding opportunities not only for extending vase life, but also eliminating the problem of ethylene-induced senescence and abscission. Mokhtari and Reid (1995) analyzed the difference in

vase life between two rose cultivars, and noted several morphological and anatomical characteristics that correlated with improved water uptake and longer vase life.

Clements and Atkins (2001) characterized a single-gene recessive mutant ($Abs^{-}$) of *Lupinus angustifolius* L. 'Danja' in which no organs abscise in response to continuous exposure to high concentrations of ethylene. A long-lived *Delphinium* mutant (Tanase et al. 2009) also showed no ethylene-induced sepal abscission. These mutants indicate the opportunity for a genetic approach to prevent flower abscission and petal abscission that is a common postharvest problem in cut flowers and potted plants.

## B. Preharvest Factors

It seems axiomatic that preharvest factors would strongly affect the postharvest performance of cut flowers and potted plants. Certainly acclimation of potted foliage plants to low light and water stress has long been known to be important to satisfactory postharvest performance (Staby and Kofranek 1979; Nell et al. 1990), but there are relatively few demonstrations of such effects. The most comprehensive studies have been with greenhouse roses, where production in high humidities, under artificial light, and with $CO_2$ enrichment, is known to result in rapid water loss through unregulated stomata. Marissen and Benninga (2001) studied a range of pre and postharvest factors that might affect vase life of roses. Using multivariate analysis and regression techniques, they demonstrated that mean relative humidity in the greenhouse was the most important variable determining differences in vase life. The number of branchlets per square meter on the plants at harvesttime also influenced the vase life, presumably because they represent alternative sinks. In et al. (2009) used a neural network approach to predict the vase life of greenhouse-grown cut roses. They used 29 environmental, morphological, and physiological parameters as the input layer to the network, and were able to accurately predict the vase life of the cultivars that were used to train the neural network. Whether the system would predict vase life for other cultivars remains to be tested.

## C. Temperature

The marked effects of temperature on the life of cut flowers were first quantified in 1973 (for carnations) by Maxie et al. (1973). Our subsequent studies have extended their findings to a wide range of other

crops, including potted flowering plants (Cevallos and Reid 2000). Our findings have been consistent with those of Maxie and his colleagues—respiration of flowers has a very high $Q_{10}$ value, higher than that of most other perishable crops. We found the $Q_{10}$ for Narcissus, for example, to be more than 7 between 0 and 10°C (Cevallos and Reid 2001). The close link between respiration and growth and senescence in these poikilotherms means that a Narcissus flower held at 10°C may lose as much vase life in 1 day as does a similar flower held for one week at 0°C.

We used a dynamic system for measuring the effect of temperature on flower respiration, in which the effect of a chosen temperature was measured on replicate single flowers (Cevallos and Reid 2000, 2001; Celikel and Reid 2002, 2005). The temperature was then increased and respiration measured until it stabilized at the new temperature (usually within 2 h). Graphing the data obtained from these studies using the Arrhenius function resulted in highly significant straight lines (Fig. 1.1) demonstrating a logarithmic relationship between temperature and respiration.

The industry has long been aware of the importance of cool temperatures in improving long-distance marketing of ornamentals, as demonstrated by the widespread adoption of forced air precooling, and the use of coolrooms. However, temperatures in these facilities are often well

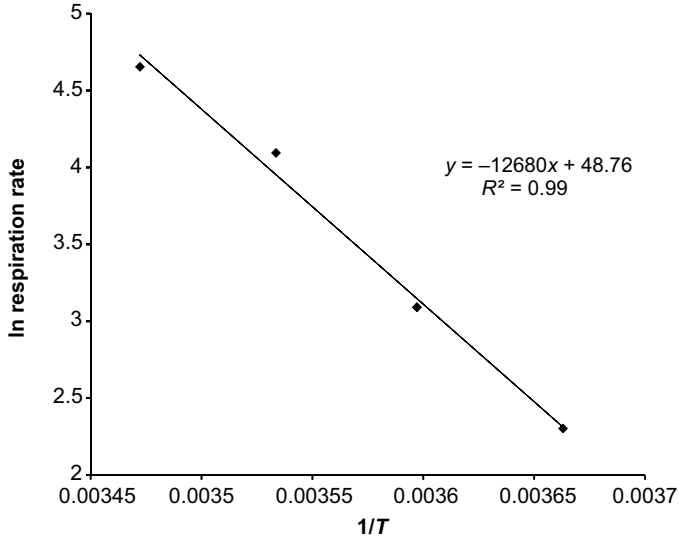


**Fig. 1.1.** Arrhenius plot of the effect of temperature on the respiration of cut carnation flowers. (J.-C. Cevallos and M. Reid, unpublished).

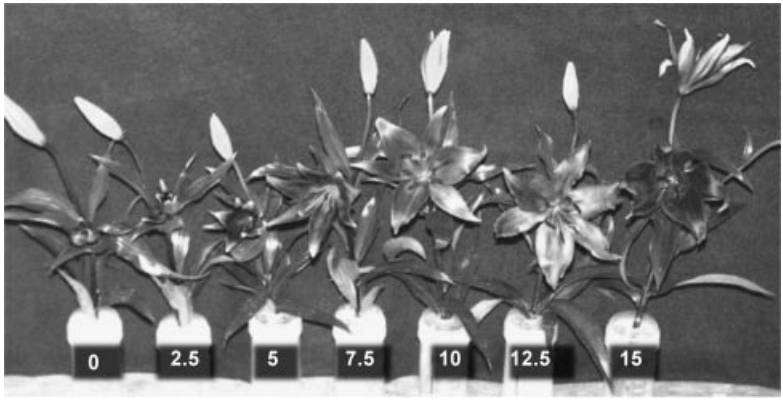


**Fig. 1.2.** Effect of storage temperature on the vase life of lily flowers. Flowers were stored for 5 days at different temperatures and then returned to room temperature for evaluation of vase life. Photograph taken after 2 days at 20°C. (J.-C. Cevallos and M. Reid, unpublished).

above the optimal (which is at or near 0°C for most flowers), suggesting that the true importance of temperature has not been adequately demonstrated. Simulated transport at temperatures above the freezing point results in accelerated opening and reduced vase life in many flowers, when they are subsequently held in the consumer environment (Fig. 1.2). In our experiments with different flower species (Cevallos and Reid 2000, 2001; Celikel and Reid 2002, 2005), we demonstrated an extremely close relationship (Fig. 1.3) between respiration during storage and residual vase life at room temperature (20°C), and were able to use such relationships to model the effects of transit temperatures on residual vase life. These mathematical models were subsequently used to program active radio frequency identification (RFID) tags that were used in pilot studies of the value of time temperature tags in flower marketing (Staby and Reid 2005) (http://www.wffsa.org/pdf/Robin/netWORK/RFIDtags.pdf).

In an analysis using our results, van Meeteren (2007), developed an equation to describe the effect of temperature on senescence rate:

$$k_s = \frac{k_{\max}}{1 + 10^{(T_{\text{half}} - T_s).\text{slope}}}$$

in which $k_s$ [day$^{-1}$] = rate of senescence at temperature $T_s$ [K], $k_{\max}$ [day$^{-1}$] = maximum rate of senescence, $T_{\text{half}}$ [K] = temperature at which

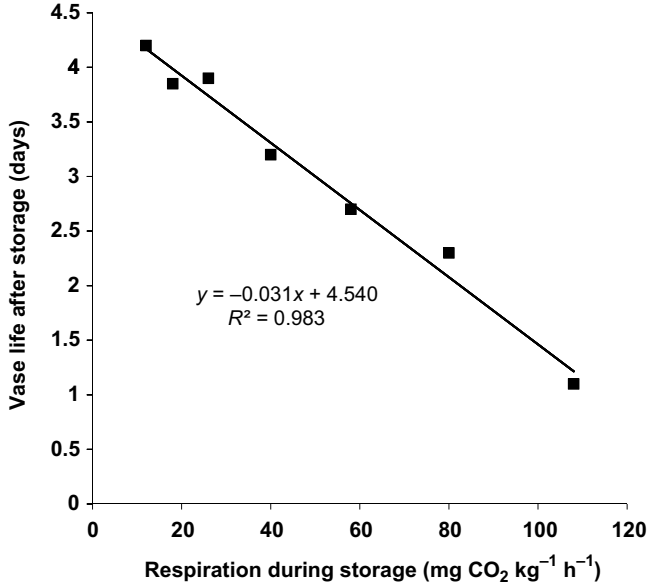


**Fig. 1.3.** Relationship between vase life and storage respiration for daffodils. Daffodil flowers were stored for 5 days at different temperatures and then held at 20°C for evaluation of vase life. (J.-C. Cevallos and M. Reid, unpublished).

the senescence rate is half of $k_{max}$, and slope [$K^{-1}$] describes the steepness of the curve. This equation could potentially be used for generalized modeling of the temperature/vase life relationships for cut flowers.

The value of time–temperature monitoring in commercial handling of cut flowers is well demonstrated by a survey experiment conducted by Staby and Reid (2005) (www.wffsa.org/pdf/Robin/netWORK/RFIDtags.pdf). Experimental active RFID tags were included in flowers shipped from producers in South America and California to U.S. wholesalers. The data demonstrate that the temperature history of the flowers varied dramatically. Some were in danger of freezing at some point during transit, others were exposed to temperatures in excess of 35°C. Modeling the expected vase life of the flowers based on our respiration/vase life regressions suggested that the vase life would be reduced in some cases by as much as 40%, and the data show that this reduction was primarily a matter of temperature during transit rather than the transit duration.

## D. Controlled and Modified Atmospheres

The close association between flower respiration during storage and vase life after storage suggests the potential usefulness of controlled (CA) or modified (MA) atmospheres, in which the $O_2$ content of the storage atmosphere is reduced, sometimes with an increase in the $CO_2$ content. In some fruits and vegetables (particularly apples, kiwifruit, and cabbage), such atmospheres are routinely used commercially to extend storage life. The beneficial effects are attributed to reduced respiration (resulting from low internal oxygen concentrations), and reduced ethylene sensitivity (attributed largely to elevated $CO_2$ levels) (Kader et al. 1989; Kader 2003). Although such atmospheres have been tested frequently with cut flowers, results have been disappointing (Reid 2001). Commercial trials have failed to demonstrate benefits, and where such benefits have been claimed, the absence of valid controls has clouded the credibility of the results. Recent studies have focused on the use of sealed packages (such as salad packages) for single flowers or small bouquets, and while the results have sometimes been promising, they only apply to specific species or even varieties of flowers, and are therefore of very little general utility.

In an attempt to examine the reason for the lack of benefit from controlled atmosphere storage, we measured respiration of flowers at different temperatures and in different concentrations of oxygen (Macnish et al. 2009b). Petals held at warm temperatures (10, 15°C) showed a fall in respiration similar to the classic curves reported by Kidd and West (1932) for apples, as $O_2$ partial pressure fell below 0.02% (2% by volume). However, the respiration of flowers held at storage temperatures (0–5°C) fell only modestly, if at all (Fig. 1.4). The reasons for this disparity have not been examined, but it seems possible that the difference in surface/volume ratio between bulky fruits and thin petals may be part of the reason for the difference. $O_2$ diffusion is likely to be limiting in bulky fruit, so that the terminal oxidases are limited for $O_2$ at much higher external $O_2$ than their actual $K_m$ would suggest. Curiously, too, the flowers failed to show the rise in respiration at very low $O_2$ levels (the Pasteur effect) that results from the onset of anaerobic respiration. Increased $CO_2$ production under anaerobic conditions has been attributed to increased glycolysis and the increased decarboxylation of phosphoenyl pyruvate. Although we have no explanation for the absence of a Pasteur effect, it is clear that low $O_2$ results in anaerobic respiration, since flowers stored under anaerobic conditions can smell alcoholic, and may collapse shortly after placing at room temperature in air (Macnish et al. 2009b).

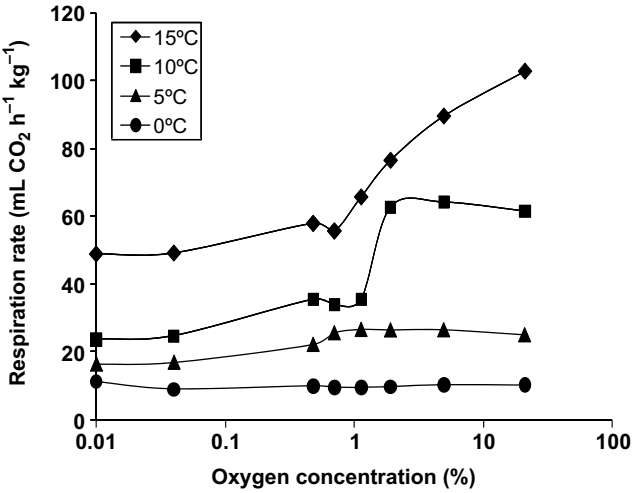


**Fig. 1.4.** Effect of temperature and oxygen tension of respiration of carnation flowers. (D. Granello and M. Reid, unpublished).

A potential benefit of CA or MA atmospheres that has been demonstrated but little employed is their use in controlling insects. In a study of a range of cut flowers, Joyce and Reid (1985) demonstrated that high levels of $CO_2$ and low levels of $O_2$, while they had no positive effect on storage life of the flowers, often had no negative effects. This implies that such atmospheres could be used in reducing disease or killing quarantine insects in cut flower shipments. The variability in the response unfortunately means that the treatment could only be applied in single-crop boxes, and that may explain why it has not become popular. In a study of the potential use of high $CO_2$ atmospheres, Hammer et al. (1990) found a significant reduction in *Botrytis* incidence both in naturally inoculated and in artificially inoculated flowers. Unfortunately, bronzing of the leaves in response to the high $CO_2$ atmospheres impaired the marketability of the flowers.

The question whether the beneficial effects of low temperature are directly attributed to respiration could be tested by examining the effect on vase life of reducing respiration by other means, such as the use of an inducible silencing system to block glycolysis or some other rate-limiting process. An alternative hypothesis is that the respiration rate is just one of a number of biochemical processes affected in a similar fashion by the reduction in temperature.

## E. Chilling Injury

Although it is recommended that most ornamentals should be stored close to the freezing point (0°C), there are well-known exceptions, including the tropical cut flowers such as anthurium, heliconias, and gingers, most foliage plants, and some important flowering plants (including Poinsettia and African violet). These tropical species must be transported and handled at temperatures above 10°C. Symptoms of exposure to chilling temperatures include wilting, necrosis, and browning of colored bracts and petals. Recent studies with a range of summer flowers suggest a more nuanced view of optimal temperatures—some of these flowers, such as Zinnia, Celosia, and Cosmos perform better when stored at temperatures above 0°C (Dole et al. 2009). Harvesttime can have a significant impact on the severity of chilling symptoms. In *Heliotropium arborescens* and *Lantana camara* cuttings, Friedman and Rot (2005) demonstrated that cuttings harvested in the morning were more sensitive to storage at chilling temperatures than those harvested at noon. This difference presumably reflects the effect of carbohydrate status of the cuttings, as demonstrated by King et al. (1988), for tomato seedlings, which show a similar periodic variation in chilling sensitivity. Despite the importance of chilling injury as a limitation to the use of near-freezing temperatures in storage of a wide range of horticultural commodities originating in the tropics and subtropics, there has been very little recent research in the underlying mechanisms of the disorder, which is still thought to be a result of impaired metabolism resulting from phase change in membranes and associated or consequent changes in activities of key enzymes and processes (Lyons 1973; Parkin et al. 1989; Raison and Orr 1990). Murata et al. (1992) transformed tobacco plants with cDNA encoding glycerol-3-phosphate acyltransferases from chilling sensitive and resistant species, and found changes in membrane fatty acid composition and chilling sensitivity of the transformed plants that supported the phase-change hypothesis. In a study of the role of intracellular calcium in the response to chilling stress, Woods et al. (1984a,b) studied cytoplasmic streaming and structure in hair cells from flowers and other organs of chilling sensitive and insensitive species. They demonstrated that the immediate cessation of streaming and loss of cytoplasmic structure resulting from exposure of sensitive cells to chilling temperatures was accompanied by a change in cytosolic calcium, and could be evoked by perturbing cytosolic calcium with a calcium ionophore. The dramatic effects of chilling temperatures on structure and cytoplasmic movement were suggested to be due to depolymerization of F-actin, all events that would certainly upset metabolic homeostasis and lead to the

accumulation of toxic metabolites that result in the visible damage to chilled tissues. Despite these interesting fundamental findings, they have not yet been deployed in the development of chilling resistant ornamentals, even in taxa where there is diversity in chilling tolerance (Patterson and Reid 1990).

## F. Water Relations

Adequate water relation in harvested pot plants and cut flowers is an obvious and important element of their postharvest management. Water balance is determined by the differential between water supply and water loss, and optimal postharvest handling includes managing both sides of this relationship. The primary tool in reducing water loss is temperature control. The water content of saturated air rises in an exponential fashion (doubling for every 11°C). Depending on the humidity, therefore, water loss can rise with temperature in a similar fashion. Sealed bags, or perforated polyethylene wraps can maintain higher humidities and thus reduce water loss after harvest, but at higher temperatures the likelihood of condensation and attendant proliferation of diseases is greatly accentuated.

**1. Cut Flowers.** Intuitively, providing adequate water to a cut flower should be an easy matter, since the vase solution has direct access to the xylem, without the need for transport from the soil and across the tissues of the root. In practice, water uptake is frequently impeded, by the desiccation that occurs during extended dry handling of the flowers, by air emboli that form when the water column in the xylem is broken, and very commonly by microbial occlusion and/or the formation of physiological plugs, tyloses, and gels (van Doorn and Reid 1995). Differences among species and even between varieties of the same species are a function of the structure of the xylem, the size of emboli and cavitations, embolism repair ability (Brodersen et al. 2010), and the likelihood of colonization of the stem by microbes.

*Desiccation.* Although floral tissues, devoid of functional stomates, lose relatively little water themselves, water loss can occur rapidly through the stomata of stems and leaves during postharvest handling. Surprisingly, the opening and vase life of flowers, at least in roses (Macnish et al. 2009a) and gypsophila (Rot and Friedman 2010), is not affected unless desiccation is in excess of 15% of the fresh weight. In their study, Rot and Friedman used the apoplastic fluorescent dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) to measure water uptake

by florets and whole stems. These dye studies verified the effects of anionic detergents (such as Triton X-100) in improving water uptake in dehydrated flowers (Jones et al. 1993).

*Emboli.* Formed immediately on cutting the stem, as tension in the xylem water column is released, emboli can result in a temporary reduction in water uptake that may become permanent if the rate of transpiration exceeds the water conductance of the embolized stem. In some taxa, such as *Heliconia* spp. that have very long vessels, the embolism can result in permanent failure of conducting elements. More often, the emboli are resorbed by the xylem (apparently by water influx from surrounding living cells, with individual droplets expanding over time, filling vessels, and forcing the dissolution of entrapped gas Brodersen et al. 2010). Detergent dips or vase solutions, low pH, and hydrostatic pressure all overcome emboli in the stem, as, of course does recutting under water—one of the very traditional practices in the floral trade. We have tested deep water treatments, in which the stem is immersed in water, containing a biocide, that is at least 50 cm as much as 1 m deep. This pretreatment improved rehydration and vase life of recalcitrant cut flowers, such as heliconia, ginger, and a range of woody species (A. MacNish, M. Reid, and J. Farragher, unpublished results).

*Microbes.* A rapidly respiring and wounded stem placed in water quickly depletes the oxygen in the vase solution, providing perfect growing conditions for microbes (yeasts and bacteria) that benefit from the cellular contents released from the cells damaged during cutting. Occlusion by microbes (Fig. 1.5) and the extracellular polysaccharides

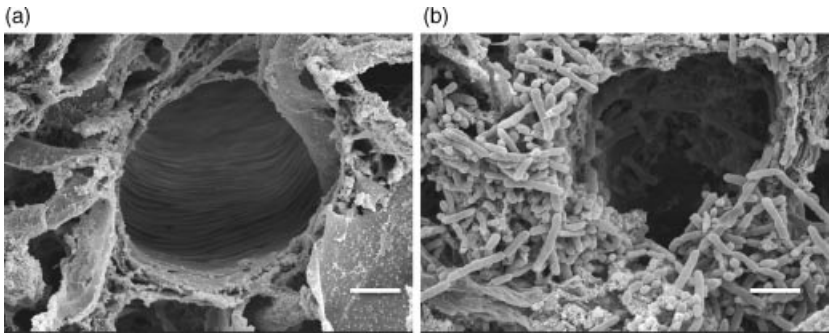


**Fig. 1.5.** Scanning electron micrographs showing the cut surfaces of *Rosa hybrida* 'Charlotte' flower stem bases after they were kept in vase water containing either 10 $\mu L\,L^{-1}$ (a) or 0 $\mu L\,L^{-1}$ (b) $ClO_2$ for 3 days at 21°C. Scale bars represent 5 $\mu$m. (Macnish et al. 2008).

that they elaborate is by far the most common cause of poor water relations in cut flowers (Macnish et al. 2008). The standard treatment for avoiding these events is the use of bactericides ($HClO_4$, $Al_2(SO_4)_3$, and quaternary ammonium compounds are among the most popular). Reduction in pH of the solution [citric acid, $Al_2(SO_4)_3$] is also helpful in reducing bacterial growth but is insufficient on its own, since acidophilic yeasts and bacteria can quickly colonize a vase or bucket solution. Not all bacteria are deleterious in the vase solution. Zagory and Reid (1986), demonstrated that some of the microbial species isolated from vase solutions have no affect (or may even augment) the life of carnations, roses, and chrysanthemums, but the potential of a biological control system for avoiding the effects of bacteria and yeasts in the vase solution has not been explored.

It has long been known that abscisic acid (ABA) plays an important role in the regulation of stomatal aperture, and is therefore a key hormone in the plant's response to water stress (Radin and Ackerson 1982; Wilkinson and Davies 2002). The regulation of gas exchange in droughted plants involves both long-distance transport and modulation of ABA concentration in the guard cells, as well as differential sensitivity of the guard cells. Plants are thought to use the ABA signaling mechanism and other chemical signals to adjust the amount of water that is lost through the stomata in response to changes in both the root and atmospheric environment. ABA therefore seems an obvious tool for reducing water stress in cut flowers, but its other hormonal effects—stimulating ethylene synthesis or enhancing the sensitivity to ethylene (Mayak and Halevy 1972; Mayak and Dilley 1976), and accelerating petal senescence—make it an undesirable choice. Kohl and Rundle (1972), for example, demonstrated that this hormone would reduce water use in roses, but also reduced their vase life.

**2. Potted Plants.** In the marketing of ornamental plants, desiccation is the most important cause of reduced quality and postharvest loss. These losses are experienced in the marketing chain (failure to water potted plants on display is a common problem in supermarkets and "club" stores), and in the consumers' homes. Bedding plants frequently fail because of wilting after planting. Produced under 'luxury' conditions, where water is freely and regularly available, they have a large leaf area and are usually root-bound. Placed in the landscape, the plants quickly use all the water in the root ball, but are frequently unable to obtain sufficient water from the surrounding soil.

Postharvest water loss in potted plants starts from the moment of the last irrigation in the greenhouse and is affected by a range of

environmental factors, including temperature, light, relative humidity, and air movement. However, temperature is the over-riding factor affecting water loss. Cooling the plants rapidly and maintaining them at the optimal transportation temperature (typically close to 0°C) is therefore a primary approach to reducing water loss. Unfortunately, cooling potted plants is difficult and time consuming, and the industry's approach typically is simply to put plants at ambient temperature into cooled trucks for transport. This may be worse than not cooling the plants at all, because it results in condensation on the (cooled) aerial parts of the plant (given that the soil mass cools much more slowly). Such condensation likely aggravates postharvest disease, particularly grey mold caused by *Botrytis cinerea*.

In the salad vegetable industry, difficulties in cooling products such as iceberg lettuce are overcome by the use of vacuum coolers, and these have become standard in that industry. We reasoned that vacuum cooling might be an effective strategy for cooling potted plants, since the soil water is freely available and would result in rapid cooling of the soil. We have shown that vacuum cooling is, in fact, an excellent means of cooling potted plants, and that vacuum cooled plants had improved shelf life after long-distance transportation (Fig. 1.6; M. Reid, C.-Z. Jiang, J. Thompson, and S. Han, unpublished results). In this test, potted

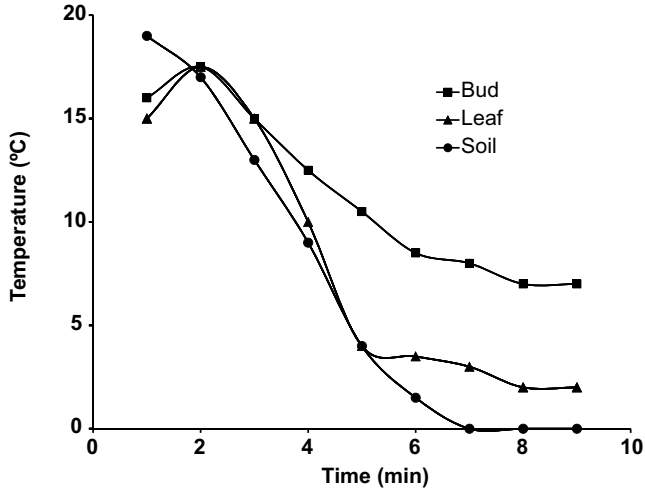


**Fig. 1.6.** Vacuum cooling of potted plants. Potted rose plants were instrumented with thermocouples, then placed in a vacuum cooler, and the pressure was rapidly lowered to ca. 4.6 Torr (610 Pa) using a rotary vacuum pump and a refrigerated condenser. (M. Reid, J. Thompson and C.-Z. Jiang, unpublished).

rose and campanula plants were cooled in a vacuum cooler, or placed uncooled in a truck. Dataloggers included in the shipment recorded temperatures during transport. Following transcontinental transport, the vacuum-cooled plants performed as well as or better than noncooled plants.

The use of ABA to close stomata seems to have considerable potential for reducing water loss during postharvest handling of potted plants. We showed that application of ABA to potted chrysanthemum plants significantly reduced their stomatal conductance, and their rate of postharvest water loss (Cornish et al. 1985), thereby extending the time until wilting of unwatered plants by 3 days (from 5 to 8 days). At that time, ABA was a prohibitively expensive biochemical, so these results were of little commercial interest. As an alternative, we tested a postproduction drench of the soil medium with saline solutions. A solution containing 100 mM NaCl resulted in an even greater extension of unwilted life (to 10 days). We hypothesized that the osmotic stress following the NaCl treatment would induce ABA biosynthesis and thereby stomatal closure. However, although the stomatal conductance of the salt-treated plants was considerably lower than the controls, there was no significant difference in the ABA content of the tissues following the salt treatment. Apparently, the stomatal closure induced in salt-stressed plants was the result of some (perhaps osmotic) mechanism. Recently, S-abscisic acid (S-ABA), the biologically active form of ABA produced through microbial fermentation, has become available at a commercially viable price, and the beneficial postharvest effects of treatment of several bedding plants with this material have recently been reported (Blanchard et al. 2007; van Iersel et al. 2009; Waterland et al. 2010). In some species such as pansy and viola, spray applications of this chemical at the recommended rates (500–1,000 ppm) resulted in phytoxic responses, suppressed shoot elongation, and decreased flower number (Blanchard et al. 2007; Waterland et al. 2010). In our experiments with roses, lavender, and impatiens, we used much lower concentrations of ABA, and obtained excellent extension of shelf life without any of these negative effects (Fig. 1.7).

## G. Ethylene and Other Hormones

It has long been known that plant hormones and plant growth regulators can have dramatic effects on floral longevity—the dramatic effects of pollination on orchid flowers (anthocyanin accumulation, wilting) have long been explained in terms of a response to plant hormones and the interplay among them (Arditti 1975). Ethylene is certainly principal

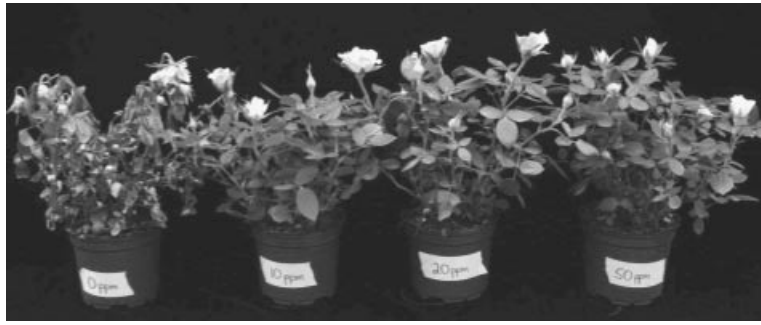


**Fig. 1.7.** Effect of postharvest spraying with water or low concentrations of abscisic acid on the postharvest performance of miniature roses. The plants were held without water for 8 days in a standard interior environment. (A. MacNish, C.-Z. Jiang, and M. Reid, unpublished).

among the hormones affecting flower longevity, but other hormones can affect sensitivity to ethylene, and a large group of flowers is insensitive to ethylene. The nature of the senescence signal in ethylene-insensitive flowers remains to be established, but there is evidence that ABA and GA may respectively play accelerating and retarding roles.

**1. Ethylene.** Sleepiness of carnations, premature wilting of petals before the flowers even open, was known to be the result of gas leaks in greenhouses long before the active principle was shown to be ethylene (Crocker 1913), and the dramatic effects of ethylene on the senescence of flowers and abscission of flowers and flower parts was well documented in the first half of the 20th century by researchers at the Boyce Thompson institute and others. The role of endogenous ethylene in triggering senescence has been well documented by a range of studies reporting the dynamics of ethylene production, changes in activity of the biosynthetic enzymes (Bufler 1984, 1986), and up-regulation of the genes encoding these enzymes (Woodson et al. 1992). The key role of ethylene has been corroborated by studies with long-lived carnation cultivars (Wu et al. 1991) and with transgenic or VIGS constructs silencing the biosynthetic pathway (Savin et al. 1995; Bovy et al. 1999; Chen et al. 2004).

The discovery that the action of ethylene could be inhibited by $Ag^{+}$ (Beyer 1976) and the subsequent development of the stable, nontoxic, yet effective silver thiosulfate complex (Veen and van de Geijn 1978) has provided an important commercial tool, still in widespread use, for preventing ethylene-mediated senescence and abscission in cut flowers

and potted plants. Other inhibitors of ethylene synthesis (aminoethoxyvinyl glycine, aminooxyacetic acid, $Co^{++}$) and action (2,5-norbornadiene) were also effective to varying degrees, but none is presently being used commercially.

One of the numerous olefins synthesized by Sisler, at North Carolina State University, 2,5 norbornadiene was an important tool in studies aimed at understanding the nature of ethylene binding (Sisler et al. 1984; Sisler and Yang 1984). Noting that 2,5-norbornadiene inhibited ethylene action in a competitive manner, Sisler reasoned that it would be possible to use a diazo derivative of this compound to identify the ethylene-binding site using activation tagging. He synthesized diazocyclopentadiene (DACP), a cyclic diolefin with an attached reactive diazo group, and found that it was very effective in inhibiting ethylene action when dissociated with UV light after being applied to the tissue (Sisler and Blankenship 1993). Curiously, the activity only required exposure to fluorescent light, not the expected shorter wavelength UV (Sisler and Blankenship 1993), and DACP treated with fluorescent light was just as active as DACP itself (Blankenship and Sisler 1993; Sisler and Lallu 1994). Examination of the mixture of breakdown products in the irradiated DACP revealed the presence of 1-MCP which these researchers found to be a potent inhibitor of ethylene action (Sisler and Blankenship 1996). This material has now become a standard treatment for ethylene-sensitive flowers and potted plants (Serek et al. 1994b, 1995a,b) applied either as a gas in an enclosed space, or through the use of sachets or nanosponges (Seglie et al. 2011) that are placed in boxes prior to transportation (Fig. 1.8). However, the volatile nature of 1-MCP restricts its application to an airtight environment. A nonvolatile 1-MCP formulation, *N*,*N*-dipropyl (1-cyclopropenylmethyl) amine (DPCA), has recently been successfully tested for improvement of postharvest quality of ornamental crops (Seglie et al. 2010). Spray application of this new formulation could provide a major advantage for handling ornamental crops, since they could be treated prior to harvest in the field or greenhouse.

As with fruits, the response of ethylene-sensitive ornamentals to treatment with 1-MCP varies widely—in many cases, the inhibitory effects are quickly lost at room temperature and wears off quite quickly. In a study of ethylene-induced petal abscission in *Pelargonium*, for example, Cameron and Reid (2001) measured the response to ethylene by determining percentage petal abscission from detached flowers after a 2-h ethylene exposure. The half-life of 1-MCP activity was determined to be 2, 3, and 6 days after 1-MCP treatment at 25, 20, and 12°C, respectively, and there was no evidence for a residual effect after


**Fig. 1.8.** Inhibition of ethylene-induced shattering by 1-MCP. Snapdragon flowers on right were pretreated with 600 ppb 1-MCP for 2 h and then both vases were exposed to 1 ppm ethylene for 2 days. (M. Reid, unpublished).

4 or 5 days at the two warmer temperatures. The effects of temperature, and perhaps differences among species in the persistence of inhibition may reflect differences in the rate of turnover of the ethylene-binding site. In studies using carnation (*Dianthus caryophyllus* L. 'White Sim') petals to determine the optimal conditions for commercial treatment, Reid and Çelikel (2008) noted some aspects of the inhibition response that were not consistent with the competitive inhibition model of 1-MCP action. They suggested an alternative model in which 1-MCP binds to a site that is exposed during the allosteric changes that accompany the enzymatic activities of the binding site in the absence of ethylene.

Using their response to exogenous ethylene, pollination, and 1-MCP, flowers have been broadly classified into two groups—ethylene-sensitive and ethylene-insensitive. However, this classification is undoubtedly too simplistic, since some flowers show an intermediate behavior. In daffodil, for example, pollinated flowers, or flowers exposed to ethylene senesce rapidly, indicating an ethylene-sensitive senescence pattern (Hunter et al. 2004a). However inhibitors of ethylene action have minimal effect on the senescence of daffodil flowers held in ethylene-free air indicating that natural senescence is initiated by

regulators other than ethylene. There is still considerable need for research to identify the role of other hormones in floral senescence.

**2. Abscisic Acid.** There is substantial published evidence implicating ABA in the regulation of perianth senescence. Not only have researchers shown a close association between petal senescence and increased petal ABA concentrations (Nowak and Veen 1982; Hanley and Bramlage 1989; Onoue et al. 2000), but exogenously applied ABA has also been shown to accelerate the senescence of a number of flowers (Arditti 1971; Arditti et al. 1971; Mayak and Halevy 1972; Mayak and Dilley 1976; Panavas et al. 1998b). Such application results in many of the same physiological, biochemical, and molecular events that occur during normal senescence (Panavas et al. 1998b).

In ethylene-sensitive flowers such as carnation flowers and roses, ABA-accelerated senescence appears to be mediated through induction of ethylene synthesis, since it is not seen in flowers that are pretreated with ethylene (Mayak and Dilley 1976; Ronen and Mayak 1981; Muller et al. 1999). This is consistent with the pattern of endogenous ABA content in rose petals, where the increase in ABA concentration occurs 2 days after the surge in ethylene production (Mayak and Halevy 1972).

Because daylilies are ethylene-insensitive (Lay-Yee et al. 1992), ABA presumably induces senescence independently of ethylene (Panavas et al. 1998b). The fact that ABA accumulates in daylily tepals before any increase in activities of hydrolytic enzymes and even before the flowers have opened was considered evidence that the hormone may coordinate early events in the transduction of the senescence signal (Panavas et al. 1998b), Application of ABA to presenescent daylily tepals resulted in a loss of differential membrane permeability, an increase in lipid peroxidation, increase in the activities of proteases and nucleases, and the accumulation of senescence-associated mRNAs (Panavas et al. 1998b).

During senescence of daffodil flowers, however, Hunter et al. (2002) reported that although ABA accumulated in the tepals as they senesced, it did not appear to play a signaling role in natural senescence. The increase in ABA concentrations in the tepals occurred *after* the induction of senescence-associated genes. They concluded that the increase in ABA content is therefore most likely a consequence of the cellular stresses that occur during senescence and suggested that the hormone does not trigger senescence, but may help drive the process to completion.

**3. Cytokinins.** The striking effects of CK in delaying senescence of leaves were known (from the effects of benzyl adenine) long before the

first isolation of zeatin. Given the homology between leaves and petals, it is perhaps not surprising that CKs were also found to delay petal senescence (Mayak and Kofranek 1976; Eisinger 1977), an effect that was shown to be associated both with reducing the sensitivity of the corolla to ethylene (Mayak and Kofranek 1976), and with delaying the onset of ethylene biosynthesis (Mor et al. 1984). Endogenous CK content shows a pattern consistent with its putative role in delaying senescence—buds and young flowers contain high CK levels, which fall as the flower ages and commences senescence (Mayak and Halevy 1970; Van Staden and Dimalla 1980; Van Staden et al. 1990). The interplay between CK content and senescence in ethylene-sensitive flowers was elegantly demonstrated by Chang et al. (2003), who transformed petunia with a SAG12-IPT construct designed to increase CK synthesis at the onset of senescence in leaves (Gan and Amasino 1995). CK content of corollas in the transformed plants increased after pollination, ethylene synthesis was delayed, and flower senescence was delayed 6–10 days. As in flowers treated with exogenous CKs, the flowers from the IPT-transformed plants were less sensitive to exogenous ethylene and required longer treatment times to induce endogenous ethylene production, and the symptoms of floral senescence.

Leaf senescence is also an important component of loss of quality in floricultural crops, particularly members of the Liliaceae, and commercial pretreatments containing CKs and/or gibberellins are recommended as a prophylaxis in sensitive genera such as *Alstroemeria* and *Lilium*. The nonmetabolized CK, thidiazuron (TDZ), has proven very useful as an amendment in tissue culture and transformation/regeneration media, and Ferrante et al. (2001) reasoned that it might be a useful tool for preventing leaf yellowing in cut flowers. Pulse treatment of cut *Alstroemeria* stems with as little as 5 μM TDZ essentially prevented leaf yellowing in flowers of the cultivar 'Diamond', where yellowing normally starts after 4–5 days (Ferrante et al. 2001). The flowers of *Alstroemeria* are ethylene-insensitive, yet the TDZ treatment had only a minor effect on *Alstroemeria* flower life, although CKs have been shown to increase the life of iris, whose natural senescence is ethylene-independent (Wang and Baker 1979; Mutui et al. 2003). In Iris, TDZ treatment at considerably higher concentrations (200–500 μM) significantly improved flower opening (including the opening of axillary flowers, if present) and flower life (Macnish et al. 2010b). The treatment was of particular value in that it reduced the loss of vase life that results from cool storage. While control iris that were held in cool storage for two weeks had only a very short display life, those pretreated with TDZ had the same vase life as freshly harvested controls (Fig. 1.9).

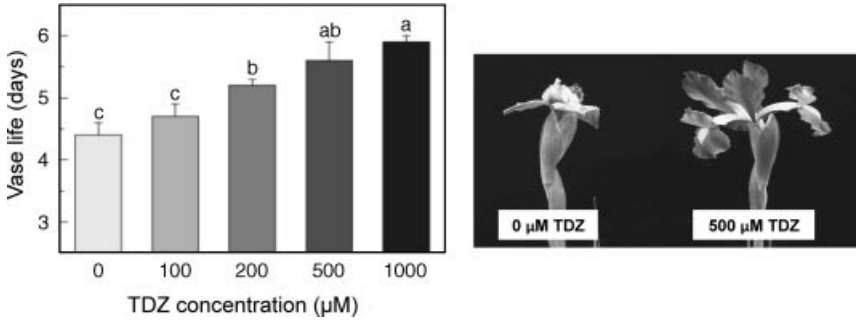


**Fig. 1.9.** Effect of thidiazuron (TDZ) on the opening and vase life of cut iris flowers. Iris buds were harvested at commercial maturity (pencil tip), pulsed for 24 h with different concentrations of TDZ, and then maintained in deionized water for vase life evaluation in standard conditions. (Macnish et al. 2010b).

Most experiments with TDZ have been conducted with flowers that are insensitive to ethylene, but in lupins and phlox, TDZ has been shown to improve flower opening and reduce ethylene-mediated flower abscission and senescence (Sankhla et al. 2003, 2005), indicating that TDZ acts like other CKs in decreasing ethylene sensitivity and that this regulator should be tested on a broader range of ornamentals.

TDZ has also proved to have remarkable effects in improving the postharvest life of potted flowering plants. Leaf yellowing is a common postharvest problem with potted flowering crops, and we have found that low concentrations of TDZ are very effective in preventing this symptom in a wide range of crops (M. Reid and C.-Z. Jiang, unpublished). The TDZ treatment appears to maintain the photosynthetic ability of the plants, since fresh and dry weights of TDZ-treated plants are much higher than those of the controls (C.-Z. Jiang and M. Reid, unpublished results). After 2 months, potted cyclamen plants treated with 5 μM TDZ maintained full display value, while control plants had almost ceased flowering and were showing obvious etiolation in response to the low light of the display environment (Fig. 1.10).

**4. Other Hormones and Regulators.** Gibberellins, auxins, and other plant hormones and regulators have also been shown to have positive and negative effects on floral longevity. For years, auxin was considered an important component of the rapid senescence response of orchids and other flowers to pollination (Arditti 1975), although this is more likely to be a response to auxin-induced ethylene biosynthesis. Saks and Staden (1993) showed an increase in longevity of carnation flowers

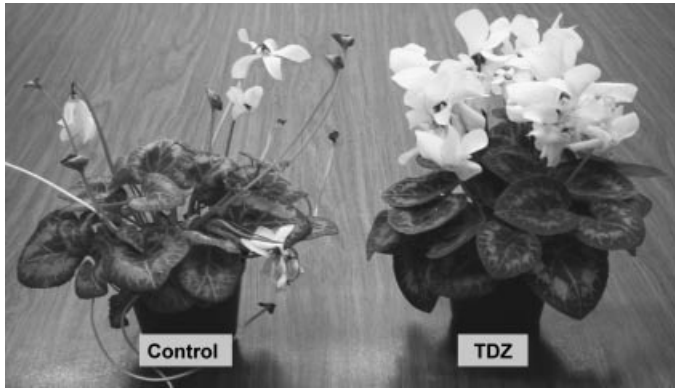


**Fig. 1.10.** Effect of thidiazuron on display life of potted cyclamen. Cyclamen plants at harvest maturity were sprayed with 5 μM TDZ and then held for 2 months in standard evaluation conditions. (C.-Z. Jiang and M. Reid, unpublished).

treated with 0.1 mM gibberellic acid (GA); Eason (2002) found a modest increase in life of *Sandersonia* pulsed with 1 mM GA, and Hunter et al. (2004b) demonstrated a similar effect on natural senescence of daffodils. Commercially, GA (sometimes in combination with BA) is used in solutions to prevent leaf yellowing in cut bulb flowers and potted flowering plants. GA treatments may have the undesirable side effect of increased stem or scape length.

Measurements of floral longevity are largely absent from studies of the powerful effects of jamonic acid, brassinosteroids, and salicylic acid on plant growth, development, and responses to biotic and abiotic stress (Ashraf et al. 2010). Similar to auxins, brassinosteroids stimulate ethylene biosynthesis, and their effects on ethylene-sensitive flowers would be expected to be negative. Jasmonic acid reduced life of petunias and dendrobiums through stimulation of ethylene production (Porat et al. 1993). The salicylic acid signaling pathway has shown to be required for up-regulation of genes required for leaf senescence (Morris et al. 2000), but the effects of down-regulating this pathway on flower senescence have not been studied.

## H. Disease

Although a detailed discussion of the postharvest pathology of cut flowers and potted plants is outside the scope of this review, completeness calls for a brief mention of the importance of postharvest disease in the global marketing of ornamentals. Improper temperature

management, including episodes of cooling and warming in the absence of proper precooling techniques, results in condensation and accelerated growth of pathogens on delicate petals and other floral parts, particularly when the flowers are packed under conditions that limit air movement. *B. cinerea*, a relatively weak pathogen, is the major pathogen of these products, and a range of chemicals have been used for postharvest protection. The push for organic or sustainable production, and the loss of established chemicals has led to an effort to identify alternative strategies for controlling disease. As noted above, high $CO_2$ levels provide effective control for species whose leaves (or petals) are not damaged by the gas. Studies with $SO_2$ gave the same result (Hammer et al. 1990)—good control of the pathogen, but damage to the host. Recently, Macnish et al. (2010d) reported the efficacy of a simple dip in a solution of $NaHClO_4$, which performed as well as the commercial fungicides under commercial conditions. Other strategies, including the use of $ClO_2$ (Macnish et al. 2008) and ozone generators have been tested, but with inconsistent results (M. Reid, C.-Z. Jiang, and A. Macnish, unpublished). Methyl jasmonate (MJ), a natural plant growth regulator, has been tested for postharvest control of *B. cinerea* in cut flowers of a range of rose cultivars (Meir et al. 1998). Pulse applications of 200–400 mM MJ following either natural or artificial infection seemed to provide systemic protection. MJ applications significantly reduced lesion size and appearance of the infection apparently due to inhibition of *B. cinerea* spore germination and germ-tube elongation. Effective concentrations of MJ caused no loss of flower quality or longevity.

## I. Growth and Tropic Responses

As developing organs, elongation of many cut flowers occurs in response to environmental cues, particularly gravity, and there has been considerable research effort devoted to understanding the mechanisms for these responses, and to devise strategies to prevent them. Researchers agreed that the primary driver for gravitropic responses is the redistribution of auxin in response to its polar transport, and differential growth in response to that redistribution (McClure and Guilfoyle 1989; Vanneste and Friml 2009). The rate-limiting step in changed auxin distribution is the activity of the auxin efflux carriers (called PIN, based on a mutant phenotype of the gene) (Vanneste and Friml 2009). Some research has suggested a role for ethylene and/or calcium in the response (Philosoph-Hadas et al. 1996; Friedman et al. 1998). These researchers reported that the gravitropic response of

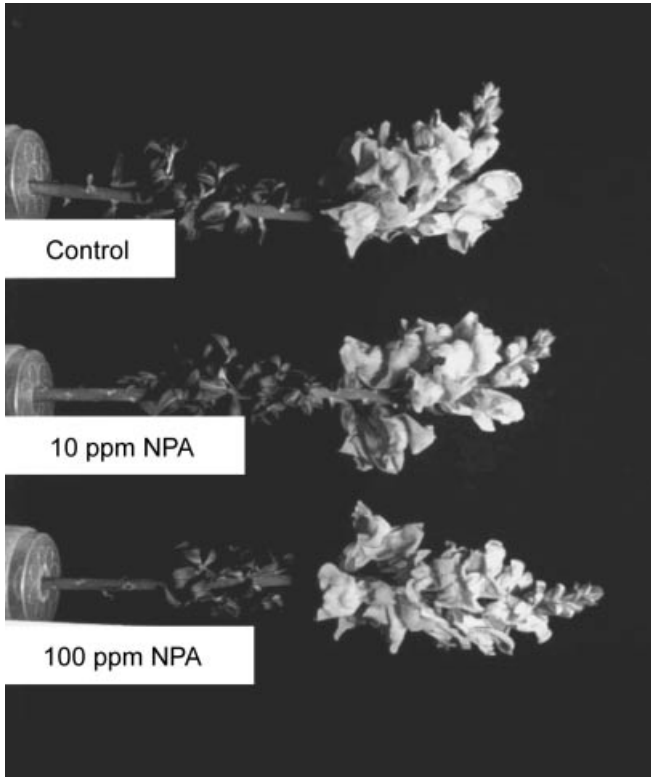


**Fig. 1.11.** Effect of naphthylphthalamic acid (NPA) on geotropic curvature in snapdragons. Flowers were placed in different concentrations of NPA and then held horizontal for 24 h. (M. Reid, unpublished).

*Antirrhinum majus* could be avoided by a pretreatment with silver thiosulfate. Others have not been able to reproduce these results (Woltering et al. 2005; Çelikel et al. 2010). The importance of auxin redistribution in the gravitropic response is well demonstrated by the impressive effects of pretreatment with naphthyl phthalamic acid, an auxin transport inhibitor (Teas et al. 1959); it seems unfortunate that this very effective material has not been developed as a commercial pretreatment for flowers such as antirrhinum, gladiolus, Bells of Ireland, and khiphofia, that have pronounced gravitropic responses (Fig. 1.11).

Unwanted stem elongation can be a problem even for flowers that are held vertical to prevent gravitropic responses. In some (ethylene insensitive) flowers, this problem can be overcome by the treatment

with ethylene or ethephon. In tulip, the negative effects of ethylene can be overcome by simultaneous treatment with GA (to overcome inhibition of opening and stimulation of leaf yellowing), BA (to prevent ethylene-stimulated tepal abscission), and $Ca^{++}$ (to prevent BA-induced stem browning) (van Doorn et al. 2011), and this has become a standard treatment for flowers transported from the Netherlands to the United States in refrigerated marine containers. Clearly a genetic approach that would select for tulips with minimal scape elongation after floral maturation would be a preferable long-term strategy.

## J. Carbohydrate Supply

The high respiration of flowers, and the energy required for flower growth, bud opening, and floral display requires substantial energy reserves in harvested cut flowers. The potential role of carbohydrates in control of petal senescence is discussed in a recent review by van Doorn and Woltering (2004). The fact that the primary component in floral "preservatives" (sometimes termed "fresh flower foods") is a simple sugar—fructose, glucose, or sometimes sucrose—reflects the profound effects of added carbohydrates on flower development, opening, and display life. Responses to sugar in the vase solution include improved floral opening (Doi and Reid 1995), improved pigmentation and size of the opening flowers (Cho et al. 2001), improved water relations (perhaps reflecting an osmotic benefit from the accumulated sugars) (Acock and Nichols 1979), and even reduced sensitivity to ethylene (Nichols 1973). On spike-type flowers, such as gladiolus, senescing flowers appear to supply carbohydrate to those still developing. Removal of senescing florets on gladiolus spices significantly reduced opening and size of florets further up the spike (Serek et al. 1994a). Perhaps the most striking effects of carbohydrate stress in harvested cut flowers is the blackening of leaves of cut flower proteas (Reid et al. 1989). These bird-pollinated flowers produce copious nectar; in the postharvest environment there is insufficient photosynthate to meet the demands of the flower, resulting in necrotic death of the leaves. Girdling the stem just below the flower (Newman et al. 1989), holding the flowers in high light conditions (Bieleski et al. 1992), or providing supplementary carbohydrate (Newman et al. 1989) prevents the blackening symptoms. This study highlighted the importance of the leaves in supplying carbohydrate to the flower. It appears that sugar in the flower preservative is transported in the xylem to the leaves, where it enters the symplast and is transported to the flowers via the phloem (Halevy and Mayak 1979).

Recent research into the effects of added carbohydrates on the life of cut flowers has focused on the potential benefits of trehalose, which has been reported to mitigate the damaging effects of ionizing radiation and to extend the life of gladiolus flowers (Otsubo and Iwaya-Inoue 2000). In a study of the mechanism of the trehalose effect, Yamada et al. (2003) found that trehalose, but not sucrose, delayed symptoms of senescence, and associated programmed cell-death events, including nuclear fragmentation. These data suggest that trehalose is exerting a protective effect, perhaps on membranes (Crowe et al. 1984), rather than supplying the needed carbohydrate.

One of the remarkable technologies that have been successful in improving the opening and vase life of cut flowers is the provision of additional carbohydrate in high concentration or "pulse" pretreatments (Halevy and Mayak 1979). In addition to the well-known effects in gladiolus (Mayak et al. 1973), this treatment has been successful in improving the opening of *Strelitzia* (Halevy et al. 1978), *Eustoma* (lisianthus, or Texas Gentian) (Halevy and Kofranek 1984; Cho et al. 2001), and *Polianthes tuberose* (Naidu and Reid 1989; Waithaka et al. 2001). In lisianthus, the pretreatment greatly improves the color of the newly opened blooms, and in tuberose, it ensures satisfactory bud opening which normally is inhibited by even brief periods of cool storage.

## IV. BIOLOGY OF FLOWER SENESCENCE

Flower senescence has been an attractive model for studies of senescence in plants (Rogers 2006); apart from its commercial importance, it offers a range of advantages for the researcher, including a short, and often tightly controlled, time span. In addition, the onset of senescence is often readily visible (sometimes as a color change (Macnish et al. 2010a), coordinated within a single large organ comprising relatively uniform cells, and may be manipulated by simple triggers (pollination, ethylene, photoperiod). The overall picture of floral senescence that has emerged from recent studies is one of a controlled disassembly of the cells of the corolla, probably by a mechanism homologous with apoptosis [also termed programmed cell death (PCD), vacuolar PCD or necrotic PCD (van Doorn and Wouter 2011)], and transport of the resulting nutrients to other parts of the inflorescence or beyond. In agreement with this picture, increased hydrolytic activity is a common feature of floral senescence; ribonuclease and glucosidase activities increase in senescing corollas of the ephemeral morning glory (Matile and

Winkenbach 1971), acid phosphatase, ribonuclease, and ATPase activities are elevated in senescing petals of carnation (Hobson and Nichols 1977) and cellulase, polygalacturonase and β-galactosidase activities are greater in senescing petals of daylily (Panavas et al. 1998a).

In the past two decades, much of the research addressing the biology of flower senescence has focused on the use of molecular tools, particularly analysis of the transcriptome, to determine the characteristic and key events of flower senescence. A number of studies have also sought to evaluate the functional importance of those changes in the senescence process.

### A. Ultrastructural Changes

The delicacy of petal cells and their rapid collapse during senescence is a challenge to studies of ultrastructural changes during senescence. van Doorn et al. (2003) used *Iris* as a model for examining ultrastructural and molecular changes during opening and senescence, and found dramatic changes in ultrastructure that were clearly related to eventual senescence well before any of the normal hallmarks of senescence (petal inrolling, wilting) had occurred. In particular, they noted that the plasmodesmata of mesophyll cells closed about 2 days before flower opening, while in the epidermis they closed concomitant with opening. Since the onset of visible senescence in the epidermal cells occurred about 2 days later than in mesophyll cells, it seems possible that plasmodesmatal closure may be a very early event in the senescence program. DNA fragmentation or laddering has been detected during petal senescence in a range of species, including pea, petunia, freesia, alstroemeria, gypsophila, sandersonia, and gladiolus (Orz'aez and Granell 1997; Xu and Hanson 2000; Yamada et al. 2001, 2003; Wagstaff et al. 2003; Hoeberichts et al. 2005). In gypsophila, sandersonia, iris, and alstroemeria, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) staining, light microscopy, and transmission electron microscopy showed that nuclear degradation was already under way before the flowers were fully open. Although the epidermal cells remained intact, the mesophyll cells degenerated completely before visible senescence (Bailly et al. 2001; O'Donoghue et al. 2002; van der Kop et al. 2003; Wagstaff et al. 2003; Hoeberichts et al. 2005).

Other ultrastructural events also support the hypothesis that floral senescence is an apoptotic event (Eason and Bucknell 1999; van Doorn and Woltering 2008). Such evidence includes the presence of invaginations in the tonoplast, and the presence of numerous vesicles in the vacuole (Matile and Winkenbach 1971; Phillips and Kende 1980; Smith

et al. 1992), which is suggested to be the main site of membrane and organelle degradation. In addition, increased numbers of small vacuoles and an increase in vacuolar size have been observed in petal cells of *Ipomoea* (Matile and Winkenbach 1971), carnation (Smith et al. 1992), *Hemerocallis* (Stead and van Doorn 1994), and *Iris* (van Doorn et al. 2003). As senescence proceeds, cytoplasmic contents are lost; in Iris (van Doorn et al. 2003) and carnation (Smith et al. 1992) the endoplasmic reticulum and attached ribosomes disappear early in senescence, followed by a reduction in the numbers of Golgi bodies, mitochondria, and other organelles. Although the nucleus remains until late in senescence its ultrastructure changes, with blebbing similar to that seen in apoptosis in animal cells (Serafini-Fracassini et al. 2002) and clumping of chromatin, increased fluorescence (indicative of DNA condensation) and sometimes a decrease in diameter (Yamada et al. 2006). Ultrastructural events during late senescence include nuclear fragmentation (Yamada et al. 2006), loss of remaining organelles, increase in vacuolar size, and eventually collapse of the tonoplast (van Doorn and Woltering 2004).

## B. Changes in the Transcriptome

**1. Gene Expression Analysis.** Researchers have worked with a range of model flowers to compile an impressive catalog of genes whose abundance changes during floral opening and senescence. Woodson and his colleagues (Lawton et al. 1990) investigated changes in transcripts during the onset of ethylene-regulated senescence in carnations and demonstrated changes, among others, in ethylene biosynthetic genes. Differential screening of cDNA libraries identified a number of genes that were strongly up-regulated during tepal wilting in the ethylene-independent daylily (Valpuesta et al. 1995). Of particular interest was the early and massive up-regulation of a cysteine protease, which might be associated with the protein degradation demonstrated by Lay-Yee et al. (1992) in this system.

The detailed information that can be obtained from molecular studies is exemplified by the study by Hunter et al. (2002), investigating changes in the transcriptome of daffodil flowers by using subtractive hybridization—a technique that increased the sensitivity of the differential screen. The 94 unique sequences isolated from incipiently senescent perianth tissue of daffodils selected for further analysis encoded proteins of diverse functions: from enzymes involved in protein, lipid, and nucleic acid breakdown to those involved in wall

modifications, cellular signaling, and transport processes. Similar results were obtained from a study of the ephemeral flowers of *Mirabilis jalapa* (Xu et al. 2007a), and of morning glory (*Ipomoea*) (Yamada et al. 2007). A major collaborative effort identified more than 5,000 unique ESTs encoding genes associated with petal senescence in petunia (Clark et al. 2009). The putative proteins encoded by the genes identified in these studies include many identified in earlier studies, and are obvious candidates for a role in the processes of petal senescence and resource remobilization, including DNA-binding proteins that may be involved in senescence regulation, cysteine proteases, and other enzymes associated with protein turnover, nucleases, and cell-wall associated proteins.

The advent of affordable microarray technology has provided an even more powerful tool to examine changes in the transcriptome. Studies in Iris (van Doorn et al. 2003), in *Alstroemeria* (Breeze et al. 2004), and in wallflower (Price et al. 2008) examined changes in the abundance of transcripts of as many as a thousand genes. The results of these more sensitive analyses mirrored what had already been shown the previous differential screening studies, but did not greatly expand the list of genes that show a clear association with senescence. We have recently developed the Solanaceae microarray (Roche NimbleGen, Madison, Wisconsin, USA). This array included a total of 93,688 expressed sequence tags (EST) from four species (46,024 genes from *Solanum lycopersicum*, 25,119 genes from *Solanum tuberosum*, 13,954 genes from *Capsicum annuum* and 8,591 genes from *Petunia hybrida*). We have used this array to examine changes in the petunia transcriptome during the onset of floral senescence (C.-Z. Jiang, T. Kasuga, D. Kluepfel, and M. Reid, unpublished results). The larger number of transcripts in this microarray has allowed the identification of many more sequences that are up- and down-regulated during the initiation of floral senescence.

**2. Functional Analysis.** Identifying which of the numerous genes that are associated with floral senescence play a role in regulating the process requires functional analysis using transgenic approaches. Since transformation and regeneration is a challenge for most floricultural species, and is, besides, relatively slow and very costly, we have tested two alternative strategies—Virus-Induced Gene Silencing (VIGS), and transient expression analysis. In VIGS, plants are infected with a virus containing a fragment of a target host gene, and the phenotype of the infected plant can provide an indication of the function of target gene. We used a purple petunia plant as our host organism, and used a

fragment of chalcone synthase (CHS) as an indicator of silencing. Where CHS was silenced, the normally purple flowers would be white (Chen et al. 2004). Concatenating one or more host gene fragments into the viral genome allows us to test the effect of silencing those genes, and the color change due to silencing CHS allows us to select the tissues to test for changed phenotype. Using this strategy we have tested a range of candidate genes for their effect on floral senescence. A number of transcription factors identified as being associated with corolla senescence have been tested; silencing a MADS-1 homolog appeared to delay senescence (C.-Z. Jiang and M. Reid, unpublished results), while silencing a NAC homolog accelerated the process (Donnelly et al. 2010).

In transient expression assay, tissues are infected with *Agrobacterium* transformed with a target gene, and the transient phenotype induced following infection can indicate the function of the gene. Petals seem an ideal system for applying transient expression analysis, and we have used this strategy to test senescence-related promoters. For example, by infecting petals with *Agrobacterium* containing a construct comprising a GUS reporter driven by the promoter for a putative Ubiquite E3 ligase (*MjXB3*), we were able to demonstrate that the promoter was strongly and specifically up-regulated in senescing carnation petals (Xu et al. 2007b) (Fig. 1.12). This technique shows promise for examining the effects of candidate genes in the senescence process, by antisense or overexpression in petals at the appropriate stage.

### C. Changes in the Proteome

Although the changes in the transcriptome that have been described suggest the proteins and enzymes that might be key to the regulation of senescence, interpretations based on transcript abundance are subject to the criticism that posttranscriptional, translational, and post-translational modifications might alter the abundance or activity of the proteins that transcripts encode. Few studies have yet attempted to identify specific protein changes that might be associated with the induction of senescence. Lay-Yee et al. (1992), for example, used an *in-vitro* translation technique using rabbit reticulocytes that demonstrated the synthesis of specific polypeptides during the early phases of senescence in daylily flowers. Bai et al. (2010) recently applied powerful proteomic techniques to attempt to define key changes in the proteome of senescing petunia flowers. Two-dimensional gel electrophoresis and mass spectrometry of isolated polypeptide spots was used to identify those that changed in a fashion that might suggest a role in senescence. Unfortunately samples were made at 24, 48, and 72 h after

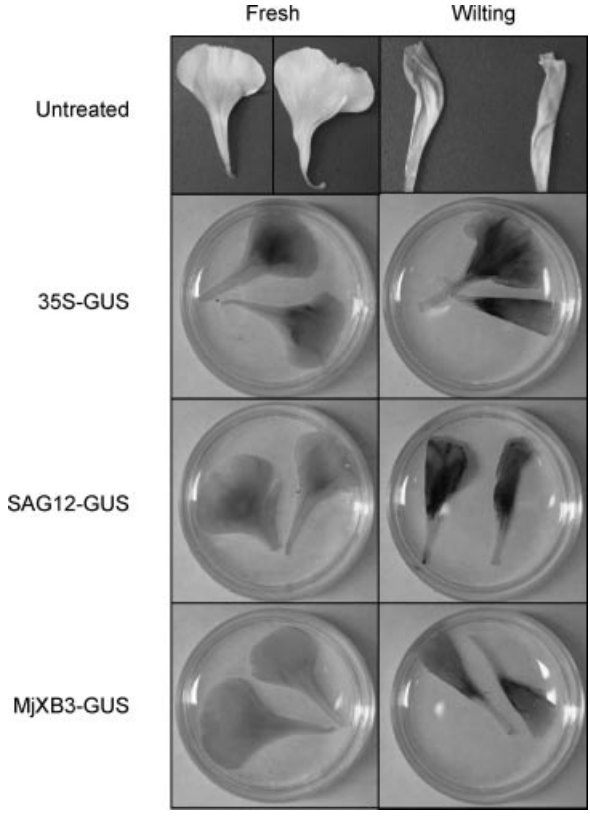


**Fig. 1.12.** Transient expression assay for analysis of senescence-associated promoters. Petals were vacuum infiltrated with *Agrobacterium* transformed with different promoter/GUS constructs. After 48 h, the petals were cleared with alcohol and then stained to visualize GUS activity. (Xu et al. 2007a,b).

pollination; even the earliest of these time points is known to be long after the key triggering events of pollination-induced senescence (Pech et al. 1987). In addition, proteins were applied to the gel on the basis of total protein content, potentially obscuring important changes in the well-known background of general protein degradation (Lay-Yee et al. 1992). The study identified a small number of polypeptides that appeared to be associated with senescence and were identified by mass spectrographic analysis. They were largely catabolic proteins probably involved in later stages of cell disassembly, including a vacuolar invertase, actin depolymerizing factor, senescence-specific proteins, abscisic stress ripening protein, lipoxygenase, and several

xylosidase. The authors' assertion that their data provide evidence for a disconnect between transcript abundance and translated protein products needs confirmation on the basis of a comparison using stable house-keeping proteins (actin, ubiquitin) rather than total protein content.

### D. Senescence Regulation

Floral longevity is tremendously variable; ephemeral flowers may be open for only a few hours, while some flowers may remain open and receptive for many months. Even so, flowers have a very short life compared to most other plant organs, and their senescence is often precisely controlled by environmental or physiological cues. Precisely controlled senescence is likely to have an evolutionary advantage. Not only does it remove a flower from the competition for pollinators once it is pollinated (or no longer is receptive), but it also eliminates an energy sink (Ashman and Schoen 1994), and provides resources to other flowers in the inflorescence (Serek et al. 1994a). The signals for floral senescence are still incompletely understood. In many flowers, an increase in ethylene production, often triggered by pollination (Pech et al. 1987; Stead 1992; van Doorn and Stead 1994; Hunter et al. 2002) clearly initiates the senescence cascade. In many species, however, ethylene appears to play no part in initiating senescence (Woltering and van Doorn 1988), and despite a number of studies on model systems, including daylilies (Panavas et al. 1998a), four o-clocks (Xu et al. 2007a), iris (van Doorn et al. 2003), and daffodil (Hunter et al. 2002), the signals initiating their senescence have not yet been identified.

Ultrastructural studies suggest that autophagy is the major mechanism for large-scale degradation of macromolecules (van Doorn and Woltering 2005, 2010). Such studies also suggest that petal cell death involves rupture of the vacuolar membrane, and subsequent complete degradation of the plasma rather than gradual increase in cell leakiness resulting from progressive degradation of the plasma membrane. The precise control and rapidity of floral wilting, as well as ultrastructural and biochemical observations, have led to the view that floral senescence is a process that mirrors apoptosis in animal cells. van Doorn and his colleagues (van Doorn and Woltering 2005, 2010) have reviewed research that supports this hypothesis, including increased activity of hydrolytic enzymes, DNA laddering, and the appearance of apoptotic bodies. Unlike animal systems, however, a role for caspase-like enzymes or metacaspases has not yet been established in petal senescence, and there has been no clear demonstration of a role for proteins

released by organelles such as the mitochondrion (van Doorn and Woltering 2008). The fact that VIGS-induced silencing of expression of genes encoding prohibitin accelerates floral senescence (Chen et al. 2005) suggests a possible role for the mitochondrion. Prohibitin is essential to mitochondrial assembly and maintenance of mitochondrial function in eukaryotes, so reduction in its synthesis could be argued to lead to impaired mitochondrial function and early release of mitochondrial proteins that initiate the senescence cascade (McClung et al. 1992, 1995).

## V. TRANSGENIC STRATEGIES FOR EXTENDING FLORAL LIFE

Although floriculture crops have been a target for transgenic manipulation, the primary focus of commercial activities has been changing flower color, especially to produce "blue" roses. The fact that the products of these efforts are now commercially available indicates the potential for using transgenic approaches to modify other (and arguably more important) features of floral crops. Floral crops offer several advantages for commercialization of transgenic approaches. The high value of floricultural crops, the diversity of taxa to which the same transgenic approaches can be applied, and the relatively short life cycle of these crops all argue for the value of a transgenic approach to plant improvement as opposed to the time-consuming approaches of conventional breeding. Since ornamentals are nonfood crops, registration of transgenic plants is much less cumbersome and expensive than for food crops, and consumer acceptance has already demonstrated by the transgenic "Moondust" carnations and "Applause" blue roses. Indeed, it seems that ornamentals can be an excellent pilot program for demonstrating the value and safety of transgenic breeding in horticultural crops.

### A. Extending the Life of Ethylene-Sensitive Flowers

Despite the obvious advantages of longer lived floricultural crops, the opportunity to use transgenic approaches to extend flower longevity has so far been only demonstrated in the laboratory, and only with ethylene-sensitive flowers. ACC synthase (ACS) and ACC oxidase (ACO) catalyze the last two reactions in the biosynthesis of ethylene (Wang et al. 2002). Both enzymes are encoded by small gene families whose members are differentially expressed in response to different stimuli (Wang et al. 2002). The induction of ACS and ACO transcripts and the activity of

both enzymes are correlated with ethylene production in senescing and ethylene-treated petunias and carnations (Tang et al. 1994; ten Have and Woltering 1997). Transgenic carnations expressing an antisense fragment of ACO have a longer shelf life than untransformed flowers (Savin et al. 1995). Flowers of transgenic Petunia plants transformed with high homology antisense gene fragments for broccoli ACS and broccoli ACO display delayed senescence (Huang et al. 2007). Likewise, VIGS of ACO in petunia resulted in extended flower life (Chen et al. 2004). These findings have limited commercial value, since inhibiting ethylene biosynthesis does not prevent perception of the exogenous ethylene that is a common contaminant in supermarkets and homes.

The perception of ethylene begins when the hormone binds to membrane-bound receptors, such as ETR1 (Bleecker and Kende 2000). In the absence of ethylene, the kinase activity of ETR1 activates CTR1 which, in turn, results in the suppression of transcription of ethylene response genes (Bleecker and Kende 2000). When ETR1 binds ethylene and is inactivated, the suppression of these genes is released, leading to a variety of ethylene responses including floral senescence and abscission. A mutant receptor, *etr1-1*, identified from *Arabidopsis* was shown to be unable to bind ethylene (Schaller and Bleecker 1995). Plants harboring this mutation have a greatly decreased sensitivity to ethylene since the ethylene response continues to be suppressed even in the presence of the hormone (Schaller and Bleecker 1995). When the *Arabidopsis etr1-1* is constitutively expressed in other species, such as petunia, a decrease in ethylene sensitivity is also observed. Since ethylene signaling is involved in many developmental processes, plants constitutively expressing *etr1-1* show a variety of defects (Wilkinson et al. 1997). Transgenic petunias expressing an antisense fragment of petunia EIN2, a gene that encodes a positive regulator of ethylene action, show similar ethylene insensitivity and delayed flower senescence (Shibuya et al. 2004).

If the mutant receptor *etr1-1* is expressed under the control of a specific promoter a more targeted effect can be achieved (Serek et al. 2006). For example, recent studies have used the FBP1 promoter to drive expression of *etr1-1* in flowers only, thereby producing longer lived flowers without impacting other developmental events in kalanchoe (Sanikhani et al. 2008) and campanula (Sriskandarajah et al. 2007). Another approach to inhibiting ethylene perception that is particularly suited to cut flower crops is to overexpress *etr1-1* using an inducible promoter (Wang et al. 2010). In plants stably transformed with a construct comprising the GVG-inducible system driving overexpression of *etr1-1* we observed a substantial delay of senescence in flowers fed the inducing chemical

(dexamethasone) via the vase solution (Fig. 1.13). Despite the successful demonstration of several strategies for preventing ethylene perception in ethylene-sensitive flowers, this technology has not been carried to the

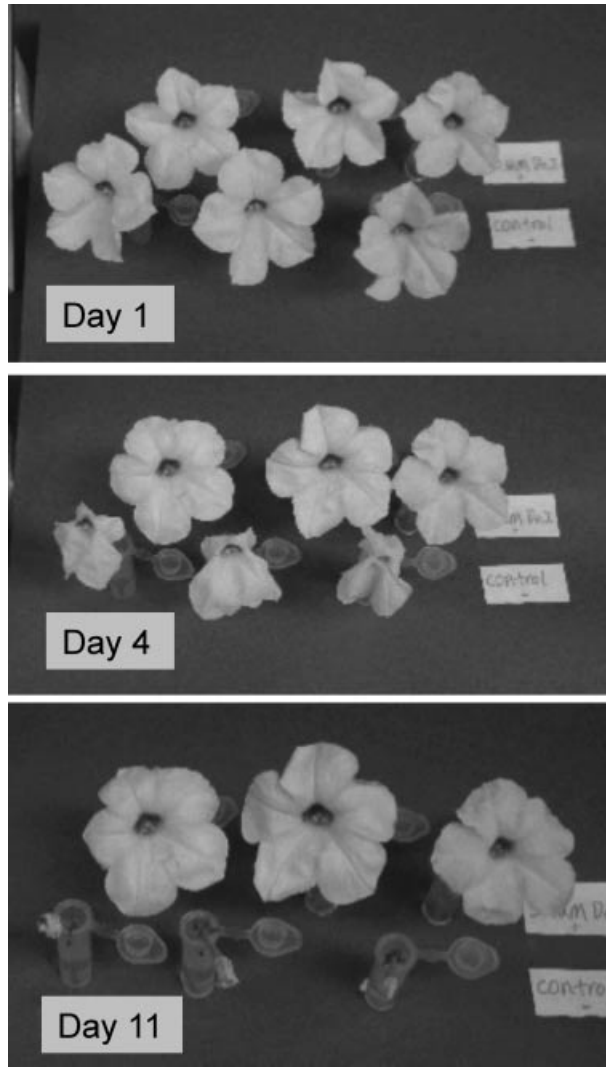


**Fig. 1.13.** Induced production of *etr1-1* extends the life of petunia flowers. Flowers from petunias transformed with the GVG/*etr1-1* transgene were placed in water (lower flowers) or in 30 μM dexamethasone, and held in ethylene-free air at 22°C. (G. Stier, H. Wang, M. Reid, and C.-Z. Jiang, unpublished).

marketplace. In evaluating the reasons for this, Chandler (2007) pointed to a number of issues, including the costs of transformation and registration, the problem of marketing a transgenic crop at a necessary premium, and the availability of alternative technologies to achieve the same phenotype. Several of these issues also apply to the flower-color transgenics, and have not prevented their successful marketing, but certainly the present availability of chemical approaches to delaying ethylene-insensitive senescence provides a viable alternative strategy that may delay introduction of transgenic plants with this phenotype.

### B. Extending the Life of Ethylene-Insensitive Flowers

Floricultural crops with ethylene-insensitive flowers seem a very appropriate target for deploying transgenic strategies to extend postharvest life. Although there is still no clear demonstration of the initiation signal for senescence in these flowers, we do know that their senescence can be delayed substantially by treating the flowers with cycloheximide, an inhibitor of protein synthesis (Jones et al. 1994; Pak and van Doorn 2005) (Fig. 1.14). The use of this metabolic poison is not commercially feasible, but these results suggest a molecular strategy for extending floral life by using a molecular approach to inhibiting protein synthesis. We hypothesized that targeted expression of an antisense sequence to a protein from the ribosome should have the same effect as

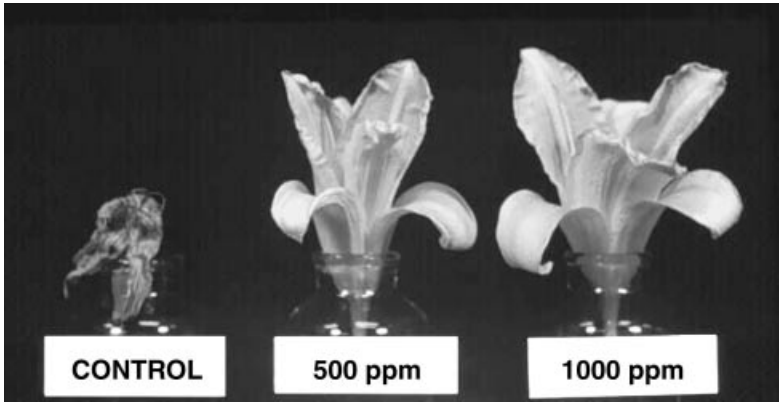


**Fig. 1.14.** Effect of cycloheximide on senescence of daylily (*Hemerocallis*) flowers. Flowers were treated at midnight on the day of opening and then held at 20°C. Control flowers senesced after 1 day. The photograph was taken after 6 days. (Photograph, M. Reid)

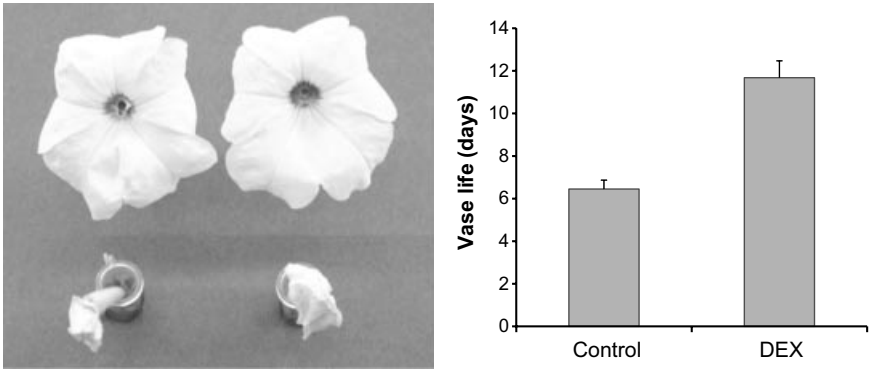


**Fig. 1.15.** Effect of induced silencing protein synthesis on the life of petunia flowers. Flowers from petunias transformed with the GVG/*antisense RPL2* transgene were placed in water (lower flowers) or in 30 μM dexamethasone, and held in ethylene-free air at 22°C. The photograph was taken after 6 days. Data are the means ± SD of flowers from four independently transformed lines. (G. Stier, M. Reid, and C.-Z. Jiang, unpublished).

cycloheximide. Accordingly, we transformed petunia plants with the GVG system driving an antisense construct for RPL2, one of the ribosomal subunits. The plants grew normally, but when flowers were exposed to dexamethasone their life was considerably extended (Fig. 1.15).

Most recently, we have capitalized on studies suggesting the importance of the 26S proteasome in the control of flower senescence. Pak and van Doorn (2005) showed that inhibition of the 26S proteasome (with MG123) would extend the life of iris—an ethylene-insensitive flower. Xu et al. (2007b) had demonstrated a very strong up-regulation, during senescence, of an E3 ubiquitin ligase that we hypothesized to be a component of the proteasome system. They also demonstrated that VIGS silencing of this gene resulted in flowers with extended longevity. We decided to test the possibility that targeted inhibition of the proteasome would extend floral longevity. In initial studies, VIGS was used to silence selected components of the proteasome. Because of the central role of the proteasome, we expected silencing to have drastic effects on plant growth and development and this proved to be the case. Silenced portions of the infected plants grew very slowly, resulting in severe malformation of leaves. The greatest effect of silencing was seen with PBB2, the beta subunit of the 26S proteasome (Stier et al. 2010). This protein is an endopeptidase in the 20S core of the proteasome and is thought to play a key role in targeted protein degradation via the

ubiquitin pathway (Sullivan et al. 2003; Smalle and Vierstra 2004), and has been suggested to have caspase-like activity (Woltering 2010).

Petunia plants were transformed with the glucocorticoid inducer system driving an antisense construct of PBB2. Transformed plants grew normally, and their flowers showed normal longevity when placed in water. However, when the flowers were treated with low concentrations of the dexamethasone inducer, their longevity was greatly increased (Stier et al. 2010) (Fig. 1.16).

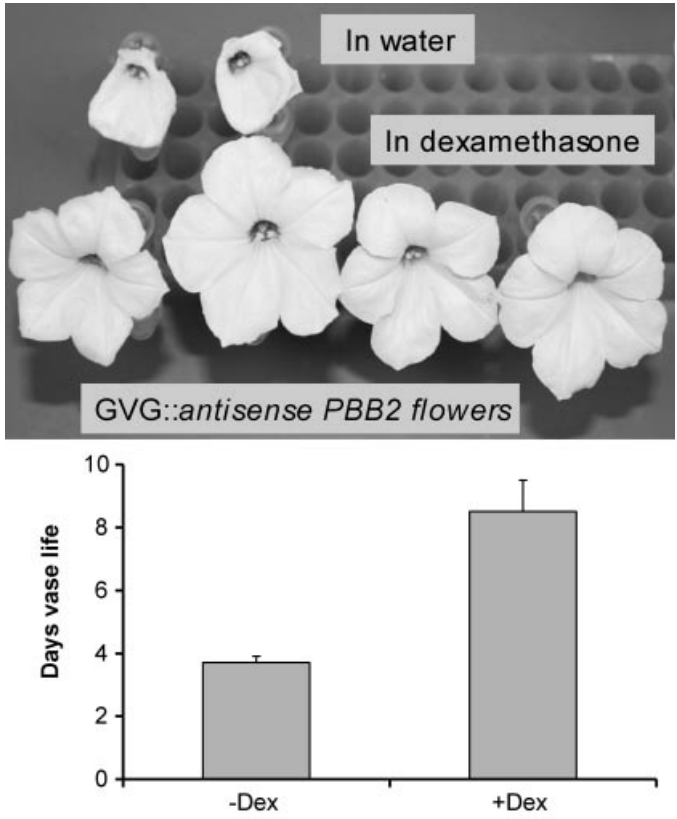


**Fig. 1.16.** Effect of induced silencing of the proteasome on life of petunia flowers. Flowers from petunias transformed with the GVG/*antisense PBB2* transgene were placed in water (upper flowers) or in 30 μM dexamethasone, and held in ethylene free air at 22°C. The photograph was taken after 4 days. Data are the means ± SD of six flowers. (G. Stier, M. Reid, and C.-Z. Jiang, unpublished).

### C. Other Transgenic Targets

The success of transgenic approaches to extending flower life are an indication of future prospects for using such approaches to improve the postharvest performance of all floricultural crops, including cut flowers and potted plants. For example, it has been demonstrated that *Arabidopsis*, tobacco, tomato, and turfgrass plants overexpressing the gene encoding 9-*cis*-epoxycarotenoid dioxygenase (NCED), a rate-limiting enzyme in the biosynthesis of ABA, show decreased transpiration and enhanced drought tolerance (Iuchi et al. 2001; Qin and Zeevaart 2002; Aswath et al. 2005; Wan and Li 2006; Thompson et al. 2007; Zhang et al. 2008). Most of these studies used constitutive promoters to up-regulate gene expression, with attendant and negative pleiotropic effects on plant growth and development. By using an inducible expression system, or a drought-responsive promoter, it will be possible to reduce the negative effects of limited water supply on the postharvest life of ornamentals.

Similarly, the beneficial effects of CKs in improving flower opening and delaying leaf senescence that have long been reported in ornamentals can be obtained by transgenic modification of CK biosynthesis. Gan and Amasino (1995) demonstrated that leaf senescence could be delayed in transgenic plants expressing isopentenyltransferase (IPT), an enzyme that catalyzes the rate-limiting step in CK synthesis. Chang et al. (2003) demonstrated that overexpression of IPT under the control of the promoter from a senescence-associated gene (SAG12) in petunia resulted in a 6–10 day delay in floral senescence, relative to wild-type (WT) flowers. Flowers from IPT plants were less sensitive to exogenous ethylene and required longer treatment times to induce endogenous ethylene production, corolla senescence, and up-regulation of a senescence-related cysteine protease.

The IPT transgene might also be deployed to reduce the negative effects of postharvest water stress in potted plants. Rivero et al. (2007) demonstrated, in tobacco, that suppression of drought-induced leaf senescence in transgenic plants where IPT is driven by a drought-stress promoter resulted in outstanding drought tolerance of the transgenic plants, as well as minimal yield loss when the plants were watered with only 30% of the amount of water used under control conditions.

## VI. FUTURE PROSPECTS

Horticultural and physiological research over the past 30 years has given us a good understanding of the factors that affect the life of cut

flowers, potted plants, and other ornamentals. These findings will be the key to future strategies for improving the postharvest life of cut flowers and potted plants. In addition to improved application of tools such as proper temperature management, existing ornamental taxa will be improved by new and emerging chemical treatments, including water-soluble ethylene inhibitors that may replace the gaseous 1-MCP treatment, and registration and use of growth regulators such as TDZ and NPA for reducing leaf yellowing, improving flower opening and vase life, and preventing gravitropic bending. Chemicals for controlling vase-solution microbes and inhibiting postharvest diseases will be more environmentally friendly. In the longer term, the industry will be improved by a focus on breeding ornamentals with better postharvest characteristics, using the huge genetic variability in the wild populations of most ornamentals. This may be accomplished using conventional breeding, supplemented by modern breeding technologies. Genomics-assisted breeding, already successfully deployed in cereal crops, uses a range of strategies and tools, including marker-assisted breeding, targeted mutagenesis using zinc finger nucleases and oligonucleotides, and identification of point mutations using tilling technology (Lusser et al. 2011). By improving the efficiency and rapidity of predicting phenotypes from genotypes, these techniques will expedite the breeding of improved ornamental cultivars with enhanced postharvest characteristics.

The remarkable effects of transgenic manipulation of genes involved in petal senescence point to the potential for such strategies not only to dramatically improve the postharvest performance of commercial floricultural crops, but also to expand the palette of ornamentals in the trade. Many beautiful ephemeral flowers are seldom seen in the vase because of their short display life, but application of the transgenic techniques described above could enable the rapid commercialization of spectacular flowers such as *Tigridia* (to select just one from a host of possible examples). Similarly, transgenic manipulations may be used to improve the quality and display life of potted plants, by reducing their water loss, preventing leaf yellowing, and extending flower life. Although transgenic ornamentals are presently limited to blue carnations and roses, the rapid acceptance of transgenic technologies in agronomic crops in the United States suggests that when the political, regulatory, and social environment is ready, transgenic ornamentals will quickly play an important role in the ornamental market place.

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