FLOWER PETAL CELL WALLS: CHANGES ASSOCIATED WITH FLOWER OPENING AND SENESCENCE*

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(Received for publication 2 November 2005; revision 8 March 2006)

ABSTRACT

Flowers are prized as objects of great beauty and diversity, and are commercially valuable (~US$4.5 billion in international trade yearly) and highly perishable. Biologically, flower petals have an important role in the lifecycle of plants, as they protect immature reproductive structures, then provide the attraction and accessibility needed for pollination to occur. Flower petal cell wall construction, maintenance, and breakdown are important factors in the life of flowering plants as well as being related to the visual quality of commercial cut flowers. The petal is generally a thin structure, with a mesophyll-type cell layer between an ordered upper and lower epidermis. Petal cell walls are constructed in such a way as to be able to accommodate large and quite fast increases in cellular turgor during petal expansion and flower opening. There is some variety in the way the flower lifecycle may end once pollination has occurred, with petal wilting, shattering, abscission, almost complete autolysis of petal tissue, and the development of a papery shell all occurring in different species. The senescence of flower petals can be accompanied by increased activity of cell wall hydrolases, similar to that found during fruit ripening, and degradation of pectic and hemicellulosic polysaccharides. For some flowers, however, wall dissolution is restricted to depolymerisation of hemicelluloses and loss of neutral sugars, particularly galactose and arabinose. The few species in which the metabolism of cell wall polysaccharides in flower petals has been studied in depth include carnation, sandersonia, and daylily.

Keywords: flower; cell wall; opening; senescence; petal.

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* Based on a paper presented at 1st Joint New Zealand – German Symposium on Plant Cell Walls, 23–24 June 2005, Rotorua, New Zealand

INTRODUCTION

This report covers the composition and changes that occur in petal cell walls during the lifespan of a flower, as well as the cell wall-related gene expression and activities of cell wall-targeted enzymes that accompany these events. Publications on this topic are quite limited for ornamental species. Consequently, this review focuses on the few species that have been studied in detail at the cell wall level: carnation (*Dianthus caryophyllus* L.*)*, sandersonia *(Sandersonia aurantiaca* (Hook.)*),* and daylily (*Hemerocallis* spp.). Included where relevant is information from other commercial cut-flower species, e.g., *Iris hollandia* and *Alstromeria,* and model systems such as *Arabidopsis thaliana* and *Petunia hybrida*, which have been the subject of general flower molecular and gene expression studies. These studies have provided additional knowledge of enzymes associated with cell wall composition and degradation and are of great value in gathering a picture of the cell wall changes associated with opening and senescence in flower petals. The physiology of flower opening and senescence and the anatomical changes to petals during flower development are described in the first two sections of this paper in order to provide a context for the later description of petal cell wall metabolism.

PHYSIOLOGY OF FLOWER OPENING AND SENESCENCE

A complete flower contains petals, sepals, gynoecium, and androecium. The flower is the means by which the species continues to be propagated, and each organ of the inflorescence plays a specific but interacting role in this. Before flowers open, the petals protect the immature reproductive organs. When flower maturity is reached, petals begin to expand and the flower opens. Pollen can then be released and pollination can occur. The colour and arrangement of petals in open flowers also provide visual attraction for pollinators. There is enormous variation among flowering plants in the manner and timing of the flower opening phase. Plant hormones (ethylene, gibberellin, jasmonic acid) have been reported as involved in signalling associated with the change from a bud to an open flower (reviewed by van Doorn & van Meeteren 2003), but consistent roles for inducing or mediating other cues are not clear. Influential mechanisms in flower opening include changes to carbohydrate metabolism (particularly breakdown of storage carbohydrates to soluble, osmotically active sugars), water relations affecting cell turgor, and cell wall metabolism (van Doorn & van Meeteren 2003). Flower opening is physically achieved by petal cell expansion, with uneven expansion of the upper and lower epidermal layers of the petal contributing to the development of the open flower shape (e.g., Asiatic lily — Bieleski *et al.* 2000), or by unfolding pre-formed massed petal tissue although this also appears to require selective cell expansion (e.g., morning glory — Phillips & Kende 1980). While the primary function of any cell wall is to protect the cell and to provide structural stability for the plant, the cell

walls in petals must be rapidly modifiable, so that the pressure of increasing turgor within cells of the petal fabric can be accommodated, thereby allowing opening to proceed.

Senescence of flower petals often appears as wilt, petal abscission, or shattering, or browning and drying. Factors associated with the induction of petal senescence have been reviewed by Rubinstein (2000) and Zhou *et al*. (2005), who both noted the involvement of plant hormones and the increasing molecular evidence supporting the role of programmed cell death in petal senescence, determined by specific gene expression changes and signalling events. In many species (e.g., carnation, petunia, orchid) the onset of flower senescence is associated with increased ethylene evolution, often as a consequence of pollination (reviewed by O'Neill 1997), or with increased ethylene sensitivity. Pollination results in increased ethylene evolution (reviewed by O'Neill 1997). In mature ethylene-sensitive flowers, increased ethylene has the effect of triggering gene expression and new protein synthesis associated with cell and tissue death. On the other hand, senescence of a significant number of flower species (including sandersonia, daylily) cannot be linked to ethylene production or sensitivity. Senescence-initiating mechanisms in these flowers are not fully understood, although abscisic acid is thought to have an central role in the progression of daylily senescence (Rubinstein 2000). For all flowers, external cues such as drought, temperature stress, or the loss of available carbohydrate nutrient (particularly for cut flowers — van Doorn 2004) can influence either the onset or the progression of senescence. Once induced, early physiological hallmarks of senescence include loss of cell membrane integrity, changes in turgor and ion leakage. These changes also involve specific alterations in gene expression and new protein synthesis (Celikel & van Doorn 1995; Eason & de Vré 1995; van Doorn *et al.* 1995; Panavas & Rubinstein 1998). The cell wall is also a target of these changes (discussed in following sections).

The variations in timeframes of opening, maturity, and senescence of carnation (ethylene-sensitive), sandersonia, and daylily (both ethylene-insensitive) flowers are summarised in Fig. 1. This illustrates the diversity of rates of change in the external features of flower development from opening onwards. Clearly, though, there are some similarities in the physiological mechanisms employed to achieve these changes.

PETAL ANATOMY

In general, the basic arrangement of cells in petal tissue is very similar to that in a leaf, with an upper and a lower epidermis (containing stomata), and a wider central layer of parenchyma cells with embedded vascular tissues. In leaves, this middle (mesophyll) layer is differentiated into palisade and spongy parenchyma, but no such differentiation is seen in the parenchyma of petals. Petal parenchyma cells are

very loosely arranged, with large air spaces apparent. As the flower develops and ages, the cell arrangement becomes even further disorganised, air spaces increase, and the cells may lose their regular shape and may even collapse. While the mesophyll layer becomes disordered, the epidermis and vascular tissue appear to remain intact but later undergo changes such as shrinkage and collapse of epidermal cells and dissolution of elements of the vascular tissue cell walls. Features of the progress of change to petal internal structure are species-specific, however, and examples of some commercially important ornamental species — carnation sandersonia, daylily, iris, and alstromeria — are outlined below.

Carnation flowers emerge from sepals into a full form, with petals opening out to lie at 90° to the stem. Flower senescence commences with ethylene evolution after pollination (Borochov & Woodson 1989), and the flower petals subsequently in-roll, wilt, and dry while still attached to the stem. In ultrastructural studies on senescing carnation, Smith *et al*. (1992) also observed cell wall swelling of mesophyll cells, and noted that epidermal cells were shrunken and collapsed in wilted, post-climacteric flowers.

Sandersonia is a liliaceous monocotyledon with a bell-shaped corolla formed from fused petals and sepals. The progression of bud development, flower opening, and senescence in the sandersonia flower proceeds in a manner that provides reliable visual indicators for flower selection. Ethylene is not believed to be involved in co-ordinating the senescence progress (Eason & de Vré 1995). Visual symptoms of the onset of senescence in sandersonia flowers begin with fading of flower colour and wilting of the tissue. When fully senescent, the flowers form a papery shell that remains attached to the plant. In petal tissue of sandersonia, the parenchyma cells progress from being fairly ordered in the tissues of a floral bud to quite disordered in wilted and senescing tissues, with intercellular spaces becoming increasingly evident (O'Donoghue *et al.* 2002).

In the rapidly opening and senescing daylily, light microscopy reveals striking alterations in the arrangement of cells across the width of the petal, with cell size, shape, and adherence to other cells all changing within the 48 hours that it takes for petals to open and senesce (Panavas *et al.* 1998).

Van Doorn *et al*. (2003) observed that the interior cells of the large flag tepals of iris started to become isolated from their neighbouring cells as these tepals expanded in the developing flower. The emergence and condition of the flag tepals largely determines the commercial maturity and extent of vaselife for iris. Transmission and scanning electron micrographs both show extensive swelling of cell walls, beginning 2 days after flag tepal emergence. Cells eventually collapse and many disappear in wilted flowers, leading the authors to suggest that the walls had completely degraded.

In alstroemeria, the walls of epidermal cells become ridged when flowers are fully open, but there is no indication of wall swelling associated with petal senescence in epidermal or mesophyll cells (Wagstaff *et al*. 2003). In mature alstromeria petals (4 days after opening) there is evidence of wall breakage in mesophyll cells near the petal margins, and the walls of neighbouring cells are compressed together as cellular contents are lost.

The anatomical changes in flower petals during opening and senescence suggest that there are some shared features on a general scale, but little similarity in the detail: cell walls may swell and/or break down, and the internal mesophyll cells may become separated from each other, or walls may become compressed. There is a certain amount of looseness in the arrangement of cells in the mesophyll layer even in bud tissue, which may provide the means for subsequent petal expansion, but the loss of order is extreme as petals senesce, with cell collapse and wall fragmentation. Walls of cells in the parenchyma and epidermis appear to degenerate by different timetables but if/how this is regulated is not yet clear, and cell wall studies on petals have not yet ventured into separating these layers for further analysis. The rate at which anatomical changes associated with opening and senescence are made adds yet another perspective to the influence that cell wall compositional changes and altered enzyme activities may have on this process.

PETAL CELL WALL COMPOSITION

Flower petal cell walls, like all primary cell walls in plants, are composed mainly of insoluble and potentially soluble carbohydrate, with small amounts of structural glycoprotein and water. In the primary wall, cellulose microfibrils are embedded in a hydrated matrix of acidic polysaccharides (homogalacturonan, and the branched rhamnogalacturonan I and rhamnogalacturonan II, collectively termed "pectin") and hemicelluloses (neutral, non-cellulosic glycans, e.g., xyloglucan) as well as the structural glycoproteins and enzymes. The sub-structure of the primary wall is more complex, however, and a number of models exist to describe the relationships between the cellulose microfibrils, polysaccharides that surround the microfibrils and form tethering linkages, and the interweaving matrix. These models have been summarised and discussed by Cosgrove (2000), and Brummell (2006) provides a current description of the biochemistry of wall polysaccharides.

There appears to be species-specific variation in the relative amounts of wall polysaccharides present in opening and mature flowers, and there is also variation in the patterns of modification at flower opening and floral senescence. The cell walls of petals of carnation and sandersonia have been studied in the greatest detail, although earlier work by Wiemken-Gehrig *et al.* (1974) reported increases in ethanol-insoluble polysaccharides during expansion and significant losses of hemicellulose and cellulose in senescing morning glory (*Ipomoea tricolor*) petals.

The main features of cell wall composition, polysaccharide size distribution, and cell wall enzyme activity associated with the opening-maturity phase and onset of senescence for carnation and sandersonia are outlined in Table 1. Cell wall enzyme activities at comparative stages for daylily are also included in Table 1. These changes are discussed in more detail below. It is clear from comparisons of these three flowers, however, that there are a few shared features in cell wall change associated with flower opening or senescence — increases in wall deposition during flower opening (including galactose incorporation), some depolymerisation of hemicellulose, and mobilisation of galactose during senescence. It is possible that petal cell wall alterations during development reflect the rate of flower development (Fig. 1) and the adjustments that are made to the cell wall are likely to reflect the role that the petals have in enabling pollination and further seed development and release from the flower.

Carnation petals have been comprehensively analysed for cell wall compositional changes associated with their development using cut flowers at the partial petal emergence stage, fully mature open flowers, and flowers that are progressively wilting and senesced (de Vetten & Huber 1990; de Vetten *et al.* 1991). Compositional changes are summarised in Table 1. These authors found that the full opening of carnation petals was accompanied by increases in cell wall material (cellulose, uronic acid, and neutral sugars) on a fresh flower basis. Following maturity, there was a decrease in cell wall material per flower, with a notable loss of neutral sugars, particularly galactose and arabinose. The percentage of total pectin soluble in chelator increased throughout development and senescence, although there were no significant changes in size of solubilised pectin molecules. The most distinct change in polysaccharide size was a loss of high molecular-weight hemicelluloses, particularly as flower petals progressed from mature to wilted. Of the large-sized hemicelluloses present, neutral sugar analysis indicated xyloglucans, along with small amounts of arabinan and arabinoxylan, were targeted for depolymerisation (de Vetten *et al.* 1991). A low molecular-weight glucomannan fraction, apparently resistant to enzyme breakdown, was also found in hemicellulose extracts from carnation (de Vetten *et al.* 1991). Xyloglucan depolymerising enzyme activity, as well as β-glucosidase and β-galactosidase activities, were detected in protein extracts from senescing flowers, and activity levels were consistent with the changes found in the hemicellulose fraction of the cell wall. The carnation gene *SR12*, putatively encoding a β-galactosidase, has been isolated (Raghothama *et al.* 1991) and its expression demonstrated to be regulated by ethylene (Lawton *et al.* 1990), suggesting the SR12 enzyme is associated with senescence-related events in the carnation petal. The changes in cell wall composition and polymer size were likened by de Vetten & Huber (1990) to those seen in a number of ripening fruit species. Whereas fruit cell wall breakdown is linked to loss of firmness, the authors

TABLE 1–Summary of changes to cell wall polysaccharides (per flower basis) and cell wall enzyme activities, for the opening–mature phase, and the mature–senescent phase of flower development for cut carnation (de Vetten & Huber 1990; de Vetten *et al.* 1991), on-plant sandersonia (O'Donoghue *et al*. 2002), and cut daylily (Panavas *et al*. 1998). Symbols* and abbreviations† outlined in footnote.

	Opening - Mature	Mature - Senescing
Carnation		
Quantity	cellulose ↑ total pectin ↑ chelator-soluble pectin ↑ carbonate-soluble pectin ↑ galactose ↑ arabinose ↑	cellulose ↓ *slight* total pectin ↓ *slight* chelator-soluble pectin ↑ carbonate-soluble pectin ↓ galactose ↓ arabinose ↓
Size distribution	large-sized HC ↓ XG ↓ chelator-soluble pectin ∅ carbonate-soluble pectin ∅	large-sized HC ↓ XG ↓ chelator -soluble pectin ∅ carbonate-soluble pectin ∅
Enzyme activity	PG O	PG O XG-specific glucanase *detected* glucosidase *detected* galactosidase *detected*
Sandersonia		
Quantity	cellulose ↑ total pectin ↑ chelator-soluble pectin ∅ carbonate-soluble pectin ∅ galactose ↑	cellulose ↑ total pectin ↑ chelator-soluble pectin ↓ carbonate-soluble pectin ↓ galactose ↓
Size distribution	HC/XG ↑ chelator-soluble pectin ∅ carbonate-soluble pectin ∅ *but loss of neutral sugar branches*	HC/XG ↓ *slight* chelator-soluble pectin ∅ carbonate-soluble pectin ∅
Enzyme activity	XET ↑ cellulase O PG O PME ↑ *low* galactosidase ∅	XET ↓ cellulase O PG O PME ↓ *low* galactosidase ↑
Daylily		
Enzyme activity	cellulase ↑ PG ∅ PME ↓ galactosidase ∅	cellulase ↓ PG ↑ PME ↓ galactosidase ↑

* *Symbols*: ↑ - element is increasing in quantity or activity during the phase, ↓ - element is decreasing in quantity, size, or activity during the phase; ∅ - factor assayed and unchanged; O - factor assayed but absent.

† *Abbreviations*: HC - hemicellulose; PG - polygalacturonase; PME - pectinmethylesterase; XG - xyloglucan; XET - xyloglucan endotransglycosylase

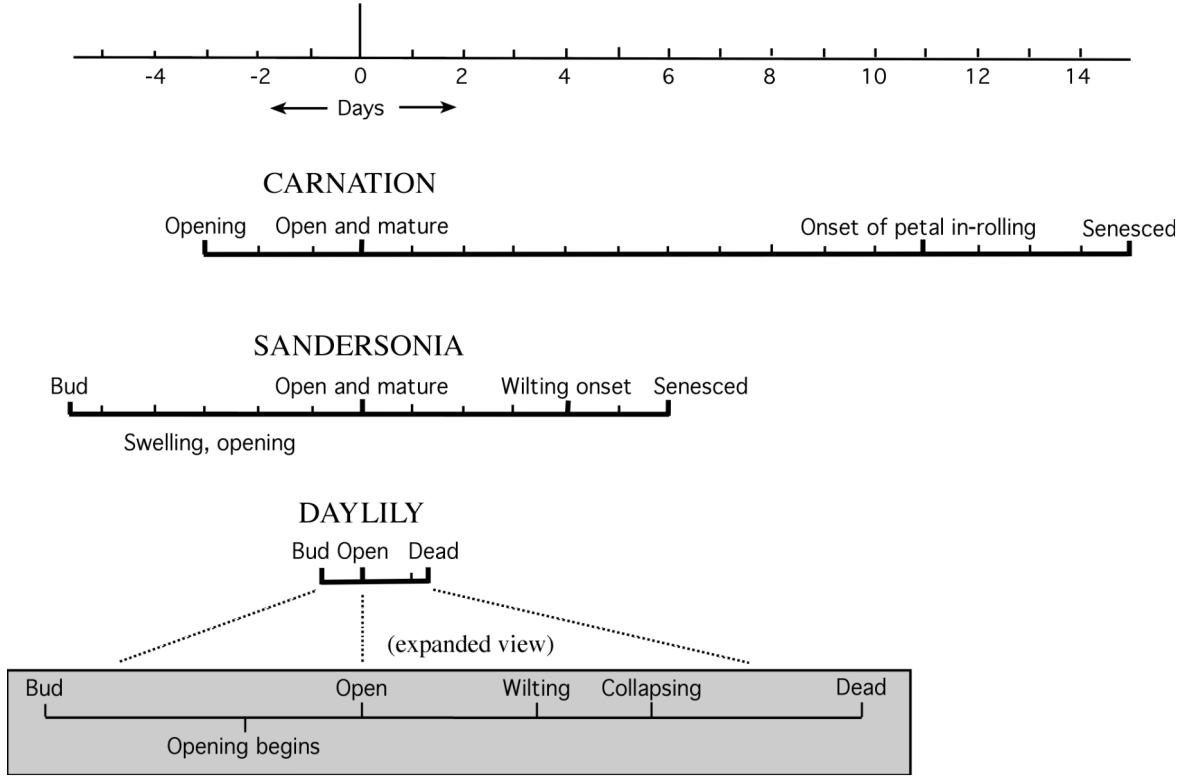

FIG. 1–Comparative timing of opening and senescing events in cut carnation (de Vetten & Huber 1990), on-plant sandersonia (Eason & Webster 1995), and cut daylily (Bieleski & Reid 1992) flowers, standardised to the timepoint of open flowers for each species. The standardised flower lifespans presented are 19 days for carnation, 11.5 days for sandersonia, and 2 days for daylily; the daylily flower lifespan is presented as a standardised and as an expanded view.

did not make a connection between wall degradation and changes to petal texture in carnation, but argued that the impact of these cell wall changes could have other "rheological consequences".

A summary of cell wall changes in petal tissues of sandersonia flowers developing and senescing attached to the parent plant is provided in Table 1. As with carnation, the amount of cell wall material in sandersonia increased as these bell-shaped flowers swelled into a fully mature form (O'Donoghue *et al*. 2002). Approximately 50% of the non-cellulosic neutral sugar associated with cell walls of mature flowers was galactose, primarily in polysaccharides that were not solubilised in chelator, sodium carbonate, or 6 M NaOH. Total galactose in this highly insoluble cell wall fraction increased on a per flower basis as flowers became fully mature, but then declined at the onset of wilting. β-Galactosidase activity was low in expanding tepals but increased as flowers matured and wilted, coinciding with the major loss of galactose from the highly-insoluble cell wall fraction containing polysaccharides associated with cellulose. Three genes putatively encoding β-galactosidase (*SaGAL1*, *SaGAL2,* and *SaGAL3*) have been isolated from sandersonia petal tissue (O'Donoghue *et al*. 2005) and expression of all three begins in petals at the onset of flower wilt, whether flowers are attached to or detached from the parent plant. Total amounts of pectin and cellulose per flower continued to increase throughout sandersonia flower development, even in the cell walls of fully senescent sandersonia flowers. Amounts of chelator-soluble and sodium carbonate-soluble pectin decreased per flower after maturity. Like carnation, changes in the average molecular size of pectin were limited in sandersonia during flower opening and senescence, although in opening flowers there was a distinct loss of neutral sugar-containing side branches from large-sized carbonate-soluble pectins. Polygalacturonase and endo-β-1,4-glucanase (assayed by carboxymethylcellulose hydrolysis) activities were absent in cell wall protein extracts at all developmental stages, and pectinmethylesterase activity was barely detectable, although it did increase as flowers opened and decreased thereafter. Hemicellulose polymers (including xyloglucan) increased in molecular weight from flower expansion up to the point at which wilting occurred. As wilting flowers progressed towards full senescence, there was a slight down-shift in the size of xyloglucan polymers, although this change was not comparable to the extensive degradation of hemicelluloses from carnation. Xyloglucan endotransglycosylase, which cleaves and then forms new linkages between xyloglucan molecules, increased in activity in opening and mature flowers, but declined sharply as flowers wilted. The activity of this enzyme may be responsible for the increase in xyloglucan polymer size prior to sandersonia wilting.

Analyses of wall composition have not been published for daylily flowers, but inferences about wall changes in relation to the rapid opening and senescing of

these petals can be drawn from the study of changes in cell wall hydrolase activity (Panavas *et al*. 1998), outlined in Table 1. In daylily petals, cellulase and pectinmethylesterase activities were greatest as buds were opening, falling sharply after full flower size was reached (Table 1). This suggests that changes to polysaccharides with β-1,4-glucan backbones and changes to esterification levels of homogalacturonans accompanied the flower opening process. Unlike carnation or sandersonia, high and increasing levels of polygalacturonase activity were found in daylily after expansion was completed, providing indirect evidence that pectins were hydrolysed in association with the breakdown of daylily petal structure during senescence. Removal of galactose from cell wall polysaccharides is also likely since β-galactosidase activity increased as petals reached an advanced state of disintegration.

While there are no accompanying analyses of cell wall composition, there is increasing evidence from enzyme and gene expression studies of other flower species to imply that cell wall modifications are a regular feature of flower opening and floral senescence. Petal-specific expansins have been identified in both the rapidly opening and senescing four o'clocks (*Mirabilis jalapa* — Gookin *et al*. 2003) and in petunia (Zenoni *et al*. 2004). The presence of expansin mRNA has also been identified in young pea petals (Michael 1996) and in tomato flowers (Brummell, Harpster, & Dunsmuir 1999). Expansins are non-hydrolytic, non-enzymic proteins that are believed to disrupt the hydrogen bonding that links cellulose microfibrils with matrix polysaccharides. As such, their presence is central to the expansion of cell walls in response to increased turgor pressure within the cell (Cosgrove 2000). There is also evidence that expansins are involved directly and indirectly in cell wall disassembly during fruit tissue senescence (Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir 1999) and in leaf abscission (Belfield *et al*. 2005). The expression patterns of expansin genes identified in four o'clocks indicated that some members of the gene family were expressed at all times during the lifespan of the flower, while other expansin genes were expressed in association with bud development, and then again at petal senescence (Gookin *et al*. 2003). Zenoni *et al*. (2004) showed that down regulation of a petunia floral-specific expansin gene resulted in smaller flowers and a reduction in the crystalline cellulose component of the cell wall, and suggested that this particular expansin was associated with the correct deposition of cellulose during flower growth. Whether this change in expansion-related expansin affected later flower senescence was not reported. Gookin *et al*. (2003) suggested the role of expansins during petal senescence could be to destroy the integrity of the wall by indiscriminate binding. In senescing fruit tissues expansins are proposed to have at least two effects: a direct effect to loosen noncovalent linkages between polymers at the hemicellulose-microfibril interface, and an indirect effect in which relaxation of the wall caused by expansin binding

may increase access of wall hydrolases to tightly bound polysaccharides (Brummell, Harpster, Civello, Palys, Bennett, & Dunsmuir 1999). There may be parallels in senescing flower petals.

The arabidopsis gene-sequencing project has provided information on floral genes that may be usefully applied to research on larger flower systems in the future. In arabidopsis, 67 genes encode polygalacturonase and a family of 111 genes encode pectinmethylesterase (Imoto *et al*. 2005). At least four polygalacturonase genes (Torki *et al*. 1999), and at least one pectinmethylesterase gene (Micheli *et al*. 1998) have been shown to be strongly expressed in arabidopsis flowers, while the microarray experiments of Imoto *et al*. (2005) indicated that five of the polygalacturonase genes and 14 of the pectinmethylesterase genes were differentially expressed in floral bud clusters. In all these examples whole flowers were used for analysis and so it is not clear whether the polygalacturonase and pectinmethylesterase genes were expressed in the pollen or the petal tissues (or both).

At least two α-L-arabinofuranosidase genes have been identified in arabidopsis flowers, and studies of localisation with promoter:reporter gene constructs have shown *AtASD1* and *AtASD2* expression to be spatially and temporally distinct in flower petals — *AtASD1* expressed in vascular tissues of mature and senescing petals, *AtASD2* expressed throughout the petal in fully mature flowers (Fulton & Cobbett 2003). Both genes are also expressed in other tissues in arabidopsis, notably flower abscission zones. Arabinose, along with galactose, usually appears as side chains (linear or branched) attached to the uronic acid backbone of rhamnogalacturonan I. Arabinans are thought to be associated with cell adhesion although their role is still considered "enigmatic" (Oomen *et al*. 2002). This aspect has not yet been studied in flower petals, where cell separation in the mesophyll layer is obvious even in very young flowers.

Recently-developed genomics techniques used to identify holistic gene expression patterns associated with particular developmental events in plants, have been used by several groups to build a molecular knowledge base associated with the lifespan of flower petals. In both iris (van Doorn *et al*. 2003) and alstromeria (Breeze *et al*. 2004), microarray technology identified a significant proportion of cell wall-related genes with changes in expression during flower development and senescence. Although research on individual genes identified by these approaches is yet to come, the distinct changes in groups of genes related to cell wall enzymes, particularly as petals senesce, indicate that wall formation, expansion, and degradation during the lifespan of flower petals involves changes in expression of a large number of genes, and that changes to the wall are indeed genetically programmed and highly controlled.

SIGNALS RELATED TO CELL WALL CHANGES DURING OPENING AND SENESCENCE

As mentioned earlier, flower opening and the onset of petal senescence are regulated by various endogenous and environmental cues. The molecular connections between these initiating events and the down-stream effects at the cell wall level are not clear as yet. This is due partly to the labyrinth of messages that can influence opening and senescence, each with interdependencies and often species-specific responses. Signal transduction research is a relatively new field, and very little work has been done directly on factors affecting the cell wall. However, Lally *et al.* (2001) identified a gene encoding a cell wall associated kinase (*WAK4*) from arabidopsis that is associated with cell elongation. Antisense suppression of *WAK4* in arabidopsis resulted in smaller, unopened flowers compared to wild-type. Studies of leaves in these plants showed that loss of *WAK4* expression consequently resulted in reduced expansin gene expression. It seems likely that correct petal cell expansion and consequent flower opening are reliant on adequate expansin presence influenced by expression of WAK4 protein. The factors that influence WAK4 levels are, as yet, unknown. Other inferences can be made regarding cell wall-related gene expression, e.g., expression of *SR12*, a galactosidase-encoding gene in carnation, which is transcriptionally regulated by ethylene treatment (Lawton *et al.* 1990). Supplemental sugar treatment in vase solutions slightly delays expression of the sandersonia galactosidase genes *SaGAL1* and *SaGAL3* (O'Donoghue *et al.* 2005) suggesting some degree of sugar regulation or responsiveness. Much more work is needed to underline the molecular basis of these connections.

FUTURE DIRECTIONS

In the few examples in which flower petal cell walls have been analysed the petal tissue has been sampled whole, without consideration for the different cell types within. It is, of course, very difficult to separate cell types, particularly in model ornamental crops in which the petal tissues consist of perhaps no more than 20 cell layers. However, there is evidence that control of the extension of stem tissues may lie with the epidermal cells (Catalá *et al*. 1997), and it may be profitable to separate the walls of these cells from those of the mesophyll during petal expansion, as well as during petal senescence, when cells in the mesophyll are becoming disordered and collapsing and the epidermal cells are still reasonably intact. New technologies that enable micro-dissection and biochemical analysis will greatly assist in the full understanding of the specialised roles that cells and their walls may have in the full lifespan of flower tissue, as will the application of localisation techniques with the increasing range of polymer- and site-specific antibodies available, and *in situ* hybridisation using labelled RNA probes for cell-specific gene expression studies.

ACKNOWLEDGEMENTS

Thanks to Dave Brummell for helpful comments on the manuscript.

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