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IN HYBRID GERANIUM

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By

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ABSTRACT

STUDIES ON PETAL ABSCISSION
IN HYBRID GERANIUM

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Studies were conducted to investigate morphological, histological and physiological characteristics of the petal abscission process in the hybrid geranium, and to determine a practical chemical control for the problem. Comparison of petal attachment width and fresh weight to attachment width ratio between easy-to-shatter ('Sprinter Scarlet' and 'Sprinter White') and difficult-to-shatter ('Penny Irene' and 'Marathon') geranium cultivars showed that the morphology of petal attachment does not account for cultivar differences in petal shattering. Petal insertion of the easy-to-shatter cultivars was characterized by a structurally weak abscission zone, formed by small parenchyma cells. Petal separation in 'Sprinter Scarlet' always occurred within the abscission or transition zone. Movement of calcium out of the abscission zone did not appear to precede petal separation in 'Sprinter Scarlet'.

Several compounds were sprayed on floret explants and on intact plants of 'Sprinter Scarlet' geraniums. Petal abscission on explants was delayed or inhibited by the ethylene inhibitors aminoethoxyvinylglycine (AVG) at 100 and 200 ppm, silver nitrate (SN) at 50 and 100 ppm and silver thiosulfate (STS) at 25 and 50 ppm in SN, and by a mixture of gibberellins A4 and A7 (GA4/7) at 20 ppm. A slight delay was observed with a mix of N-(phenyl-methyl)-1H-purine-6 amine and GA4/7(promalin), N6-benzyladenine(N6-BA) and 8-hydroxyquinoline sulfate(8-HQS). Cycloheximide(CHI) accelerated, but the auxins 2(3-chlorophenoxy)propionic acid(3-CPPA) and naphthalene acetic acid(NAA) did not affect the abscis-

sion process. Ethylene production by explants was inhibited by AVG, but it was either unaffected or slightly enhanced by SN. Exogenous ethylene accelerated petal abscission; concentrations as low as 0.1 ppm overcame the inhibitory effects of AVG or SN. Hypobaric ventilation (150 torr) inhibited petal abscission, and its effects were overcome by treatment with 0.1 ppm ethylene. CO_2 enrichment (15%) did not delay petal abscission.

When chemicals were applied to intact plants STS(50 ppm in SN) was the best treatment for practical control of petal abscission. 18 days after treatment petal abscission was less than 10% in inflorescences of plants treated with STS, versus 75% in control plants. SN(100 ppm) and AVG(200 ppm) delayed petal abscission but caused severe phytotoxicity when sprayed on open flowers. GA4/7(20 ppm) also inhibited petal abscission, but caused excessive peduncle elongation. The other chemicals were ineffective. High temperature($30^{o}C$) for more than 8 days completely erased the effect of SN in inhibiting petal abscission. Drench treatment with SN and STS were not effective in delaying petal abscission in intact plants.

To Thereza, Dilza, and in memory of Palmira, who provided me the initial support to "...climb to the shoulders of the giant and look in the right direction".

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Guidance Committee:

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TABLE OF CONTENTS

TABLE OF CONTENTS - continued

LIST OF TABLES

LIST OF TABLES - continued

Table Page

Section III

LIST OF FIGURES

INTRODUCTION

Two types of geraniums are grown in the bedding plant industry: those that are seed-propagated and those that are cutting-propagated. Both belong to the botanical species Pelargonium x hortorum Bailey. The cutting-propagated geranium is listed as the fifth best-selling bedding plant, while the seed-propagated is ranked seventh in a 1980 survey among bedding plant growers(192). The seed-propagated hybrid geranium was favored by many growers because of its disease-free characteristics, wide selection of flower colors, and flower predictability. However, problems like long time to flower, poor germination, and excessive petal shattering have caused growers to return to the cutting-propagated cultivars. Because the hybrid geranium is still competitive in cost of production and consumer appeal, intense research has been conducted in an attempt to solve many of its problems(21,22,23,74,128).

No practical or economical solutions have been found for flower shattering or petal abscission. The non-abscising or difficult-to-abscise cultivars are of either the double or semi-double flower type and usually do not have nectaries(194). The recently introduced hybrid 'Marathon' is a double flower type, lacks nectaries, and accordingly is shatter resistant. Cultivar differences in shattering within hybrid geraniums have also been reported(23). Exogenous ethylene(23,194), and pollination(194) hasten petal abscission. Exposure to low temperature (1 to 5^{o}C) prior to mechanical shaking delayed shattering, while naphthalene acetic acid ($5x10^{-4}$M) treatment or CO_2 (5%) enriched atmosphere did not affect the abscission process(23). Silver nitrate sprays (50 or 100 ppm) delayed petal abscission but caused some phytotoxicity(82). Sprays with gibberellic acid prolonged inflorescence viability in the

cutting propagated 'Spartan White' geranium(116).

The research reported in this dissertation had the following objectives:(a) to investigate the morphological, histological, and physiological characteristics of the petal abscission process in the hybrid geranium;(b) to determine the major differences between the difficult-to-shatter cutting propagated or double flower type and the seed propagated hybrid geranium;(c) to find a practical, economical and non-phytotoxic chemical control for petal abscission.

LITERATURE REVIEW

Specific literature in geranium petal abscission, and abscission of flower parts in general, is very limited. However, numerous anatomical, histochemical, and physiological studies have been made on abscission of leaves and fruits using both explants or intact plants.

This review will summarize information on abscission of flowers or flower parts, leaves and fruits. The subject was divided into the following: (1) anatomical aspects; (2) histochemical changes; (3) chemical regulation by hormones; (4) chemical regulation by other compounds; (5) other physiological and environmental factors.

Whenever they appear in the text, the terms below will have the following meaning:

Abscission - The process of shedding or separation of an auxiliary organ from the axis of a plant, to which the organ is attached. Unless otherwise specified, this review will discuss the natural, or physiologically induced, abscission.

Abscission zone - The region at the base of the abscising organ in which the changes leading to abscission occur.

Separation or abscission layer - The layers of cells in the abscission zone directly involved in separation.

Protective layer - The proximal tissue that remains on the main axis after separation.

Proximal, distal - Towards the main axis, and away from the main axis of a plant, respectively.

Adaxial, abaxial - Upper surface, and lower surface of an organ, respectively, in relation to its insertion in the main axis of a plant.

1) Anatomical aspects

Most of the research on the anatomy and physiology of abscission has been based on abscission of leaves in either explants or intact plant systems. Basic anatomical and physiological features observed in leaf abscission have been associated with the abscission process of perianth parts(84). However, the high variability of abscission processes in different species, and in some cases in the same plant(171,196), do not permit broad generalizations. Tison(in 197) and Lee(107) grouped 105 and 45 species of woody dicotyledons, respectively, according to anatomical features associated with leaf fall.

Description of abscission zone. The most characteristic anatomical change in abscission is the development of the abscission zone. In most leaves, floral parts, and fruits the formation of the abscission zone occurs during ontogeny(76) but may be induced by several factors (101,198). According to Esau(75), the abscission zone comprises two distinct types of cells, the separation layer and the protective layer. The abscission zone is localized at the region of the attachment of the auxiliary organ to the main axis, and it is often distinguishable from the adjacent tissue by the characteristics of the cortical parenchyma cells within it(198). The leaf abscission zone is very conspicuous in some herbaceous species, and can easily be determined by external observation(107,197). The abscission zone of flowers(101,207) and perianth segments(84) may also be conspicuous. A well defined groove frequently occurs at the insertion of some abscising organs(84,100,122,195). However, Kendall(101) observed that these grooves do not necessarily have any relation to abscission. He explained that constrictions are formed because in the development of the abscising organ, certain cells

increase in size less rapidly than neighboring cells on either the proximal or distal side. In the abscission zone of tomato and tobacco flower pedicel the groove has branches which follow along the middle lamella(100). This groove may not be essential for abscission, but it certainly increases the structural weakness of the abscission zone. The surface grooves observed in flower pedicels do not necessarily coincide with the abscission zone(75). Several additional evidences of structural weakness of the abscission zone are present in the literature(13,34,107, 174,177). In leaves the abscission zone is often distinguishable from adjacent petiolar tissue by the cells of the leaf's cortical parenchyma, which are typically thin-walled, densely protoplasmic, closely packed, and uniformly smaller than other cortical cells of the petiole(47,49, 197). These characteristics have also been observed in pedicel abscission of several species of Nicotiana(101). Additional fragility of the abscission zone is caused by a swelling of the cell walls prior to separation(39,77,107,158,207). In the vascular tissue the lignified cells may be represented by tracheary elements only(75). In Phaseolus the separation region is abruptly set off by a lack of sclerification in the cells of the pith and by the characteristic short, broad configuration of the tracheary elements(195). Short tracheary elements are also observed in leaf abscission of woody plants(77,174). The concentration of the vascular tissue in the center, rather than near the periphery, is another structurally weak characteristic often observed in abscission(76). However, peripheral distribution has also been described(in 75). Webster(195) emphasizes that even though the special anatomical features of cells in the abscission zone of Phaseolus can be interpreted as leading to structural weakness, the zone is primarily

a region of abrupt structural transition from the pulvinus to the lower part of the rachis(42). The abscission zone between the pedicel and the fruit of the sour cherry, has an abrupt structural transition region with non-sclerified tissue(182). The presence of a well-defined abscission zone in abscising floral parts is not essential for abscission(75). Pedicels of Datura flowers show no visible difference between the separation cells and other cells of adjacent tissue(101). Moreover, cells in the abscission zone of perianth segments of Magnolia grandiflora are indistinguishable from adjacent cells of the receptacle, even though the zone acts as a separate physiological unit(84). Baird and Webster(26) suggested that anatomical differentiation of an abscission zone is not a pre requisite for leaf separation, but is frequently a must in fruit abscission.

In light of the high variability of abscission patterns, it is prudent to define abscission zone, in generic terms, as a region at the base of the abscising organ, in which the morphological and physiological changes associated with abscission occur(29). However, the occurrence of certain anatomical features which characterize a structurally weak region at the base of the auxiliary organ cannot be neglected in considering the physiological basis of abscission. There are cases in which the region of the attachment is initially as strong as adjacent tissues, but it weakens prior to separation(66,132,191).

Structural changes. Separation of auxiliary organs from the main axis of a plant may or may not involve the formation of an abscission zone, as discussed previously. Despite the great variation in developmental aspects of the abscission process, the abscission zone of a leaf(197) or floral part(75) generally involves two discrete series of structural

changes which can be summarized as separation and protection.

According to Esau(75), separation may occur through a well defined plane or separation layer, or it may happen due to "...peculiarities of the histologic structure of the part of the petiole where the abscission zone is located". This latter case is typical of the already discussed features that cause weakness of the abscission zone. Gossypium (39) and Coleus(202) are classical examples of separation occuring through a precisely delimited abscission or separation layer. In Phaseolus separation happens irregularly through the cortex, vascular tissue, and pith(195). The separation layer, when formed, always appears in a zone of specially differentiated cells and generally in the distal portion(49). The separation layer consists of at least two superimposed rows of cells in which chemical changes in the cell walls take place (75). Separation can initiate in any tissue of the separation layer(198). In leaf abscission of woody dicotyledons, separation starts from the periphery of the petiole and progresses toward the interior(75). In imature apple pedicels separation can begin independently in the pith and in the cortex(126). In abscission of Coleus leaves(135) separation begins in the abaxial side of the petiole extending through the epidermal and cortical tissue, until the leaf is supported only by the adaxial part of the cortex and the xylem elements. In Phaseolus(196) separation may start internally through the pith cells and proceed outward. If the water status of the pulvinus is low, separation begins in the epidermal cells and progresses across the abscission layer(42). Turgor pressure of cortical cells in the pulvinus of citrus leaves facilitates abscission(117).

Separation of cells in the abscission zone may occur in three

ways: dissolution of middle lamella(107); dissolution of middle lamella and primary wall(in 197); mechanical breakage involving non-living cells of the vascular tissue(195). The latter process is often observed in conjunction with one or both of the aforenamed processes. Flower abscission has been reported to follow these general patterns(101,207).

An important, though not obligatory, feature in cell separation is the onset of cell division prior to abscission. Meristematic activity of cells is generally the first conspicuous structural change in the abscission zone(197). Cell division may occur in the pith, cortex, epidermis, and living cells of the vascular tissue(198).Exceptions are not unusual in the literature. Gawady and Avery(81) treated poinsettia, cotton and pepper with ethylene chlorohydrin, observed leaf abscission without the onset of cell division, and concluded that cell division is not needed for separation. Pratt and Goeschl(154) argued that ethylene chlorohydrin is very phytotoxic and any effects on leaf drop could be attributed to the injury it causes. Bednarz(29) clearly demonstrated that cell division and separation are two distinct processes in abscission of the lower pulvinus of unifoliate bean. He treated debladed plants with 5 ppm ethylene and observed separation without previous cell division. Cell division that preceded separation in control plants, or followed separation in ethylene-treated plants, was considered to be related to the formation of the protective layer. Moreover, separation always occurred distally to the zone were cell division was evident and involved only a few of the newly formed cell walls of the protective layer. Baird and Webster(26) reported that meristematic activity is not a conspicuous phenomenon in abscission zone of mature fruits; in this case cell division is also related to the formation of the pro-

tective layer. Immature fruits often develop separation-related cell division at the juncture of the pedicel and the spur(89,90,122,126). In natural abscission of Impatiens leaves, separation occurs without prior mitosis(81); similar observation have been made on woody dicotyledons (107). Separation of floral parts is frequently associated with meristematic activity in the separation layer(84,90,122,126,207). However, no cell division prior to separation was observed in flower abscission of Solanaceae(101) and Phaseolus(199).

Enlargement of cells in the abscission zone prior to and after separation is another common feature of the abscission process(75,198). Growth of cells in the distal tissue of the abscission zone may prevent the progress of abscission(99). However, cell enlargement is frequently associated with separation. Leopold(109) postulated that the differential enlargement in the distal and proximal sides of the abscission zone creates shear forces across the cell walls which culminate with the leaf being forced off the plant by the expansion of cells in the proximal side. This differential enlargement has been observed in leaf abscission of several species(39,81,129,174). Wright and Osborne(203) reported that in leaf abscission of Phaseolus, cell separation is preceded by the enlargement of a single row of cells on the proximal side of the separation layer. Cell enlargement is normally not observed before abscission of floral parts(101,122,126,199). Cell expansion within an abscission zone may constitute a mechanical factor in tissue breakage (197), but has also been considered as a mechanism of surface protection after separation(174).

After separation, the major feature of cicatrization of the exposed tissue is the deposition of substances that protect the new

surface from injuries and water loss(75). In the proximal side of the abscission zone a number of changes take place in several rows of cells, resulting in the formation of a resistant protective tissue(198). These cells constitute the protective layer, which is considered to be an integral part of the abscission zone(75). The protective tissue undergoes several alterations in cell inclusions, cell wall composition, and meristematic activity(198). Secondary cell division, which produces the protective layer, may occur before or after separation(81). During the formation of this layer the vascular tissue may be occluded by tyloses (198). Periderm is frequently produced beneath the protective layer, being incorporated in it after separation(198). All these characteristics of protective layer formation have been described for leaf abscission of several woody plants(107). Similar cytological developments were observed by Griesel(84) during abscission of perianth segments of Magnolia grandiflora, but he did not consider cell division as a requisite for the formation of the protective layer. He also observed similar characteristics of protective layer formation in normally abscised and mechanically detached perianth parts. Scott et al.(174) observed that the cells exposed after separation have a strong enlargement potential, and that suberization or healing of the exposed area is centered in those cells which play an important role in protection.

2) Histochemical changes

The first studies of chemical changes in cell walls involved in organ detachment were performed by Lee(107), Facey(77), and Sampson (171) on abscission of leaves. Despite the improvement of histochemical and analytical techniques, later works(40,132,158) are in almost perfect agreement with Lee's and Facey's basic conclusions. Three types of lyses

are normally associated with organ separation in several species: middle lamella only; middle lamella and primary wall; and entire cell(14). Lee (107) observed that prior to separation there was a swelling of the middle lamella, a change in size of the primary cell walls, and a gradual lamellar deterioration, culminating in separation of intact cells. Facey(77) studied the chemical transformations involved in middle lamella dissolution, and concluded that insoluble pectates, mainly calcium pectate, are reduced to pectic acid, and the pectic acid is methylated to form pectin. Facey(77) disagreed with Sampson's observation that cellulose was converted to pectose(171). Refinements in the techniques employed by Lee and Facey have been used by comtemporary workers and are discussed elsewhere(26,198). Dissolution of middle lamella and/or cell walls has also been observed in abscission of floral parts(101,126, 207). Kendall(101) hypothesized that"...the agency active in the hydrolyses of the cell membranes is probably an enzyme." Modern terminology refers to the enzymes in pectin metabolism as pectin methyl esterase (PME), which catalyses the hydrolysis of ester bonds(143), and polygalacturonase(PG), which catalyses the hydrolysis of polygalacturonic acid(37). Morré(132), working with leaf abscission of Phaseolus, reported a possible involvement of polygalacturonase synthesis in the solubilization of pectin fractions. Rasmussen(158) noted that PG activity in the petiole of bean decreased to undetectable level, but remained constant in the abscission zone from deblading until separation. Morré(132) reported that pectinase activity preceded separation, but concluded that pectin dissolution alone was insufficient to insure actual separation. Final separation occurred only when the thick, cellulosic walls of the vascular elements were severed. Cells of the abscission zone

reportedly synthesize cellulase, which degrades cellulosic walls(91, 112). These enzymes also seem to be involved in the rupture of non-lignified vascular elements(144). Stosser et al.(182,183) showed that the fruit abscission zone of the sour cherry contained less total polysaccharides than adjacent tissues, and that separation was accompanied by a partial breakdown of non-cellulosic polysaccharides and cellulose. Protein synthesis in the abscission zone(3,8,9,183,195) is a requirement for synthesis of wall degrading enzymes(3). The significance of increasing cellulase activity in the abscission zone of leaves is uncertain. Although water-insoluble pectin decreases during the formation of the separation layer in Phaseolus, hemicelluloses and celluloses are unchanged or even increased; treatment of explants with pectinase preparations simulated natural separation, while cellulase preparations had no effect(132). However, Horton and Osborne(91) reported that the cell separation protein synthesized as a result of ethylene treatment was cellulase. Abeles et al.(7) found that cellulase was localized in the separation layer and that its synthesis required a 3 hour induction period after the addition of ethylene. They also reported that cellulase synthesis was inhibited by indoleacetic acid(IAA) and other substances which inhibited abscission. They concluded that tissue at the separation zone can age passively by an autonomous decline in juvenility hormones, or actively via increasing ethylene levels which reduces auxin content. Once the tissue has aged ethylene can initiate cellulase synthesis in cells of the separation layer(7). Craker and Abeles(60) found that abscisic acid (ABA) increased the synthesis of cellulase once initiation of protein synthesis was under way. They concluded that the action of ABA on abscission of isolated explants was

two-fold: first, ABA accelerated ethylene production; second, it increased cellulase activity. Later Abeles et al.(7) concluded that ABA facilitates the synthesis of cellulase during abscission.

3) Chemical regulation by hormones

The involvement of hormones in abscission was first reported by Laibach in 1933(cited in 15). Since then extensive research has been conducted to determine the role of each of the known plant hormones in the abscission process.

The following is a chronological sequence of the most important hypothesis that have been proposed to explain the physiological control of abscission:

a) Auxin-ethylene balance; by Hall, 1952(85): Auxin inhibits and ethylene promotes abscission; however the relative balance between the two compounds controls abscission.

b) Auxin-ethylene; by Barlow, 1952(28): Cells that are able to separate will do so, unless growth substances are continually supplied to them. An activator of cell separation is produced in the general metabolism of the cell, and may be in the nature of ethylene.

c) Auxin-gradient; by Addicott et al., 1955(18): Abscission occurs after a fall in the normal ratio of distal to proximal auxin. A reverse of this gradient causes rapid acceleration of abscission.

d) Auxin concentration; by Gaur and Leopold, 1955(80): High concentration of auxin inhibits abscission, while low concentration promotes abscission.

e) Auxin-auxin balance; by Jacobs, 1955(97): Leaf abscission is inhibited by auxin moving from the leaf blade into the petiole. When this flow decreases to a certain level, the auxin coming from the younger

neighbor leaves promote abscission.

f) Auxin-senescence factor; by Osborne, 1955(142): Abscission seems to be controlled by endogenous auxin interacting with some abscission-promoting substance produced as the leaves mature and undergo senescence.

g) Membrane integrity; by Sacher, 1957(169): Auxin retards abscission by maintaining the integrity of cell membranes. Auxin level drops during senescence resulting in a loss of membrane integrity, thus facilitating separation.

h) Methionine-auxin; by Yager and Muir, 1958(205): Methionine plays a role in abscission by promoting methylation of the carboxyl groups of adjacent pectin molecules causing the splitting of carbon bridges, thus leading to abscission. Auxin retards abscission; high concentrations of IAA completely overcome the accelerating effect of methionine.

i) Auxin-gibberellin-abscission accelerating hormone; by Carns et al., 1961(51): These three substances interact in a common mechanism that regulates abscission.

j) Two stage theory; by Rubinstein and Leopold, 1963(166): Leaf abscission is divided into two chronological stages. In the first, auxin inhibits; in the second, auxin accelerates abscission.

k) Endogenous abscission accelerating substances; by Addicott et al.,1964 (see 15): Abscisic acid(abscisin II, dormin) is a natural abscission accelerator.

l) Localized cellular senescence; by Leopold, 1967(109): Abscision regulation comprises two stages. In the first, a metabolic difference develops on the two sides of the future separation layer. This is followed by the second stage which includes mobilization of materials

out of the distal tissue, a repression of synthetic activity in the distal cells, and finally the degradation of cell wall components. Inhibitory effects of auxin occur mainly in the first stage; promotive effects of ethylene or auxin occur in the second stage.

m) Auxin-ethylene; by Abeles, 1968(3): Ethylene is the hormone responsible for the synthesis of hydrolytic enzymes in the abscission zone. Cells in the abscission zone remains insensitive to ethylene as long as a supply of juvenility factors or aging retardants(auxin and cytokinin) are available from adjacent distal cells.

n) Calcium maintenance of stage I; by Poovaiah and Rasmussen, 1973(150): Calcium maintains membrane integrity in cells of the abscission zone, thus delaying the onset of senescence and the responsiveness of the abscission layer to ethylene and therefore maintaining the plant in the stage I(166) condition.

Each of the known plant hormones have been associated with abscission at least once. In all hypothesis auxin is either directly or indirectly involved in the regulation. Auxin may either inhibit or promote abscission. Both inhibitory and stimulative responses, depending on plant species tested, hormone concentration, localization of treatment, or other factors, have been reported for all of the known plant hormones except ethylene. Ethylene is always associated with promotion of the abscission process.

The following review will discuss the major results obtained with research on each of the plant hormones and their interrelations in the control of abscission.

Auxin. Auxin is the hormone consistently involved in hypothesis concerning the control of abscission(15,106,135,202). Early reports atribute

to auxin only an inhibitory role in the abscission process(28,87). However, in 1955, Addicott and co-workers(18) observed that auxin delays abscission only when applied distally to the abscission zone, proximal application accelerating the process. Similar observations were made by Jacobs(97). The ratio of the concentrations of free-extractable auxin on the proximal versus distal sides of abscission zones in bean(178) and cotton(50) decreased as separation approached. Auxin concentration in leaf blades also decreased during abscission(185).

Both retardation and acceleration were also observed by Leopold and co-workers(36,53,80,165) but depending on the concentration applied; a high concentration of naphthalene acetic acid(NAA) inhibited and a low concentration promoted abscission of leaf explants in several species. They also observed that early application, regardless of concentration, inhibited and late application promoted abscission of explants(166). A similar critical period was observed for the inhibitory effect of IAA in abscission of apple leaves(27). This was the basis for the two-stage hypothesis(167), in which auxin is assumed to exert its effects mainly through the inhibition of the passage out of stage I. Addition of auxin during this stage can retard abscission; after stage I is completed auxin has no inhibitory effect but rather promotes abscission.

The two-stage hypothesis could not explain either earlier(18) or later (118) data which favored the "auxin-gradient" hypothesis. Rasmussen and Bukovac(159) showed that similar amounts of ^{14}C-labelled NAA accumulated on the distal side of the abscission zone, regardless of the concentration applied. Louie and Addicott(119) applied several IAA concentrations to the distal and proximal sides of cotton explants

simultaneously; they observed that when applied distally higher concentrations accelerated abscission, otherwise the process was retarded. Jacobs(98) and Jacobs et al.(99) proposed that auxin affects abscission indirectly, promoting the growth of the petiole, and presented evidence to substantiate this hypothesis. Chaterjee and Leopold(53) found that all auxin-like substances tested could retard abscission when applied during stage I, but only those auxins capable of promoting growth could promote abscission when applied during stage II. Wright(204) suggested that the auxins that control fruit growth may be different from the ones that control abscission. As leaf abscission appears to depend on auxin production in the leaf blade, the abscission of fruits seems to depend on the supply of auxin from the seeds(120). Luckwill(120) suggested that periodicity of apple fruit abscission results from the fact that hormone production in fruit is not as continuous as in leaves.

According to Leopold and Kriedemann(111) when the auxin level of a fruit decreases, it will abscise. Leopold(110) suggested that the natural progress of leaf abscission through stage I into stage II is associated with the decline in endogenous auxin levels. This decrease can be caused either by an increase in IAA oxidase activity or an interference with auxin transport(44). Enzymes localized in the abscission zone of sour and sweet cherry fruits(153) do not appear to be related to the auxin effect in abscission(26). Craker et al.(59) presented data to support the hypothesis that auxin-controlled abscission is not dependent upon translocation of auxin, but is probably mediated by immobilization of auxin through the formation of auxin conjugates, such as indolacetylaspartate, indolacetylamide, and ethylindolacetate. Chang and Jacobs(52) reported that ABA decreased the free IAA content and

increased IAA aspartate. Addicott(15) suggested that the involvement of auxin in retardation of auxin may be related to the maintenance of ongoing physiological and biochemical functions and the mobilization of nutrients. The promotive effect of auxin on abscission seems to be via promotion of ethylene synthesis(2).

Spraying with NAA and other auxins delayed petal abscission in some species of flowering woody ornamentals(201) and Linum lewisii (16). Ethylene. Two of the early hypothesis regarding hormonal control of abscission involve ethylene as an abscission accelerator(28,85). References to the effect of ethylene on abscission are also found in earlier works(63,101). Addicott(15) points out that ethylene is not always required for abscission to develop(41) although it is intimated involved with abscission in several species.

Application of exogenous ethylene is frequently associated with a fast induction of abscission(42). Abeles(2) observed that all accelerants of abscission caused an increase in ethylene evolution, although in some cases little correlation was found between the amounts of ethylene released and the rates of abscission induced.

Most of the recent information about the role of hormones in abscission of plant parts comes from investigations with explants. Immediately after excision, the rate of ethylene released from explants is relatively high(168). This fact always constitutes a problem in monitoring ethylene production by explants(145). Jackson and Osborne(95) reported that within 12 hours after excision, the ethylene released by bean explants dropped to a very low level and remained low until abscission at 82 h. Just after abscission there was a rapid rise in ethylene released from the tissues distal to the abscission zone; how-

ever, a lower ratio of ethylene production was observed from the petiole. The peak of ethylene production by explants post-excision is apparently due to a build-up of wound ethylene(see in 44). Addicott(15) suggested that a long time may be needed for the completion of physiological changes which prepare the explants for abscission. When the accumulation of ethylene was prevented by putting mercuric perchlorate in containers with the explants, abscission did not take place; after removal of the scrubber and accumulation of ethylene for 12 hours, the leaves abscised(15). Experiments with excised pulvinus and petiole segments from leaves in the pre-abscission stage and just after abscission showed that the amount of ethylene released from the pulvinus was greater than that released from the petioles(95); both explants evolved more ethylene as abscission approached with a rapid decline after abscission. Ethylene production may occur when a particular stage of senescence is reached and this may initiate the biochemical sequences responsible for abscission(95).

Abeles et al.(10) and Jackson and Osborne(94,95) reported that bean explants differ in sensitivity to ethylene, depending on the stage of abscission. During stage I the explants are relatively insensitive to applied ethylene; however, if ethylene is allowed to accumulate around the explants, the first stage is shorter than if ethylene is removed(95). During stage II the abscission responses to ethylene become very evident; applied ethylene during this period induces abscission(5). If ethylene is withdrawn, abscission is similar to the controls(67).

Ethylene increased incorporation of ^{32}P into RNA, with further enhancement of protein synthesis(8). Ability of ethylene to increase

RNA synthesis depends on an aging process(55). If aging is blocked by IAA or cytokinins, ethylene has almost no effect on RNA synthesis(3). Ethylene-mediated enhancement of RNA synthesis occurs only in the abscission zone and not in tissues proximal or distal to it(10).

Horton and Osborne(91) refuted the claim that there is a stage in the abscission process in intact plants which is insensitive to ethylene. Burg(44) reported results to support this interpretation, suggesting that ethylene accelerates the aging process(Stage I) which precedes abscission. Evidence for the hypothesis stage I cells are insensitive to ethylene while stage II cells are sensitive was presented by Abeles and Holm(8), who showed that ethylene-mediated increases in protein synthesis occurred in stage II explants but not in stage I explants. Contradictory results have also been reported(175), but these appear to be due to the techniques used in each case(3).

Abeles et al.(7) reported that ethylene treatment during stage I increased the effectiveness of ethylene given during stage II in reducing break strength, but the first treatment was not effective by itself. However, ethylene effects may not be entirely restricted to stage II. Burg(44) showed that even very young abscission zones (stage I) can respond to ethylene if a high concentration is applied. Bukovac (personal communication, 1978) attributes this response to the extremely high, non-physiological level used. Other authors report that exogenous ethylene applied in stage I inhibits polar transport of auxin, increases IAA oxidase activity, and decreases the level of diffusible auxin(87,130,147,190). The major effects due to ethylene application during stage II are enhancement of pectinase activity(132), increase in cellulase activity(4,91), and decline in break strength(60).

Ethylene increases the permeability of the tonoplast in cells of Tradescantia petals during flower fading(184). Vacuolar compartmentation of hydrolytic enzymes has been described by Matile(cited in 184); ethylene could cause the leakage of hydrolases, thus allowing eventual autolysis of the cell. Some authors consider that ethylene triggers the physiological processes involved in senescence(see in 170); others believe that senescence is initiated by metabolic processes preceding ethylene synthesis, and that ethylene regulates the rate of terminal deteriorative changes(102,104,184). Osborne(145) suggests that the initial stimulus for abscission is a hormonal imbalance due to environmental changes and endogenous competition; this hormonal imbalance would mediate localized senescence of cells in the abscission zone, leading in turn to an increase in ethylene synthesis which would be the signal for abscission. Only cells in the abscission zone are sensitive to ethylene, which promotes the synthesis of hydrolytic enzymes or enzymes involved in growth of cells in the proximal tissue (145). A gradual increase in ethylene sensitivity has been observed in senescence processes other than abscission(45,46,102,184).

Abscisic acid. Although many references document the leaf abscission accelerating effects of applied ABA on explants(2,19,39,60,73,96,144, 180) and intact plants(58,71,73,169), as well as high levels of endogenous ABA in abscising organs(69) it is now believed that ABA has little or no direct effect on leaf abscission(127). The rise in ABA level during abscission of explants or old leaves in intact plants, could be the result of wilting, which is now known to be accompanied by a sharp increase in ABA(127). Milborrow(127) points out that applied ABA is only effective in inducing abscission when abnormally high

concentrations are used.ABA also enhanced petal fall in Linum lewisii(16).

The idea that ABA acts as an abscission regulator was introduced when Ohkuma et al.(140) demonstrated the presence of ABA in rapidly abscising cotton bolls. Bornman(38) reported that the activity of ABA in promoting abscission was equal to or slightly greater than that of ethylene. Craker and Abeles(60) proposed that the abscission-promoting effect of ABA is indirect via an increase in ethylene production; they also reported that ethylene did not affect the rate of aging. If an increase in ethylene production was the sole effect of ABA on abscission, then a saturating level of ethylene should mask any effect of ABA. However, Abeles et al.(7) reported that the combination of ABA plus saturating levels of ethylene was more effective than ethylene alone in promoting abscission. According to the results of Craker and Abeles (60) the abscission promoting effects of ABA occur during stage II, and ABA enhances the synthesis of hydrolytic enzymes involved in the abscission process, more than does a saturating concentration of ethylene. ABA also reportedly inhibits the synthesis of enzymes involved in other processes(144).

While accelerating abscission, ABA also enhances senescence in attached and detached leaves, leaf discs, and the distal portions of explants(24,73,180). ABA may also accelerate senescence changes in tissues distal to the abscission zone, including enhancement of ethylene evolution(15). Senescing tissues are low in auxin, although they produce large amounts of ethylene, therefore auxin induced ethylene synthesis(2) does not appear to be involved in senescence related processes(146). Based on this premise Osborne et al.(146) suggested that a non-volatile substance present in aqueous diffusates from senescent

leaves of deciduous, evergreen and herbaceous plants(142) is the ethylene stimulator. This substance, called senescence factor(SF), both accelerated abscission and stimulated ethylene production in explants (146). For some time it was thought that SF and ABA could be the same compound. Dorffling et al.(70) isolated ABA, xanthoxin and a third substance with acidic characteristics from senescent petioles of Coleus, Phaseolus, and Acer, and from pedicels of apple fruits. None were identical with Osborne's SF because they did not stimulate ethylene synthesis. Dorffling et al.(70) also reported that ABA accelerated abscission, but not via ethylene synthesis.

ABA also stimulates fruit abscission(72,123,211). However, it also induces parthenocarpy and inhibits abscission in Rosa(93).

Cytokinins and gibberellins. Cytokinins produced in roots appear to play a major role in retarding senescence of leaves(56,162,179,200). Osborne and Moss(148) observed that cytokinin applied directly to the abscission zone of bean explants retarded leaf senescence and abscission. However, when applied away from the abscission zone, either on the proximal or distal side, abscission was accelerated. Cytokinins are known to act as "mobilizers" of nutrients(61,179), and this may be their role in abscission.

Direct evidences of abscission mediated by gibberellins are not common in the literature. Applied GA stimulated fruit development and reduced abscission of young fruits(62,157,193). However, this seems to be an indirect effect due to promotion of development of the young fruit and possibly the synthesis of auxin which could be affecting directly the abscission process(78,105,172).

GA treatment of explants accelerates leaf abscission at high

concentrations(35,50,54), but retards it at low concentrations(121). Muir and Valdovinos(133) correlated GA-induced abscission with an increase of diffusible auxin in the stem. They concluded that GA acts as a modifier of the auxin gradient at the abscission zone.

Cytokinins and gibberellins are thought to play an indirect role in abscission, perhaps by modifying the endogenous level of auxin or ethylene, or by affecting the mechanism of cell sensitivity to ethylene(145).

Summary of hormonal regulation. Abscission is a process which seems to be directly dependent on the action of enzymes which affect cell division and cell wall plasticity. A hormone which affects the synthesis of these enzymes is said to act directly in the abscission process. There are some evidences that ethylene promotes the synthesis of cellulase, which in many cases is directly responsible for the loosening of cell wall in the separation layer. An indirect action of ethylene is considered to be its ability to induce the synthesis of IAA-oxidase, thus causing the degradation of auxin. Inhibition of auxin transport and synthesis may be other indirect roles of ethylene in abscission.

The direct effect of auxin is not very well stablished. Some evidence exists that auxin and other substances inhibit cellulase synthesis, thus retarding or preventing abscission. IAA delays the loss of calcium ions, thus delaying abscission. An indirect role of auxin is considered to be its ability to prevent ethylene action during stage I. The promotive effect of auxin during stage II is also considered to be indirect through the induction of ethylene synthesis.

ABA seems to promote abscission indirectly via induction of

ethylene synthesis. A more direct effect of ABA is its ability to increase cellulase activity, and also to promote cellulase synthesis during abscission. Another indirect effect of ABA is its ability to decrease the level of free IAA and to increase the concentration of IAA conjugates.

Gibberellins and cytokinins affect abscission indirectly, possibly via modification of tissue sensitivity to ethylene, or by altering endogenous levels of ethylene and auxin.

4) Chemical regulation by other compounds

Compounds that inhibit ethylene synthesis or action are of major importance in controlling abscission. Silver nitrate has long been used to increase vase life of cut flowers(1); silver is effective as a bactericide(125,163) and it is a potent inhibitor of ethylene action in various plants(32). Petal abscission of some geranium cultivars was substantially delayed by silver nitrate treatment(82). Following the discovery of a pathway for ethylene synthesis via methionine, S-adenosyl methionine(SAM), and amino cyclopropyl carboxylic acid(ACC), the rhizobitoxine analog aminoethoxy vinyl glycine(AVG) was identified as a potent inhibitor of *in vivo* synthesis of ethylene(114). AVG acts via competitive inhibition of ACC synthase, a key enzyme in ethylene biosynthesis(208,209).

A proteinaceous inhibitor of ethylene production reportedly inhibited leaf abscission of *Coleus* and *Phaseolus* explants, but had no effect on flower abscission of potted *Begonia* plants(187).

Localized application of calcium chloride on abscission zones of bean leaves delayed abscission(150). Poovaiah and Leopold(149) reported that calcium inhibited ethylene-induced abscission. Pectin

dissolution involves the polymerization and removal of divalent ions (38,132,158). Calcium frequently appears as oxalate or free ions in the abscission zone, suggesting a weakening of the pectin binding between cell walls(151,160,174,182), since pectin acts as the cementing substance between cells and the pectin molecules are linked together by divalent ions such as calcium(176). Walls of the sour cherry abscission zone also lose calcium and magnesium during separation(182). The affinity of cell walls in the abscission layer for calcium binding decreases with the layer development(43). Calcium and magnesium link the molecular chains of pectic acid to hemicellulose and cellulose(155,186). According to Bukovac(43), changes in the calcium and magnesium distribution and calcium binding affinity during layer development in the abscission zone of cherry fruits may be related to degradation of the cell walls in the separation layer.

Cycloheximide is an inhibitor of protein synthesis and may either inhibit(9,152) or promote abscission(3,9,57). Abscission promotion by cycloheximide is via ethylene production and is only observed if the site of application is removed from the site of action(9).

5) Other physiological and environmental factors

Senescence and aging. Senescence of cells distal to the abscission zone is more important for the abscission process than senescence of the abscising organ itself(10,145). The tissues involved in abscission do not die until after separation(3). Senescing cells in the proximal side of the bean leaf abscission zone produce relative large amounts of ethylene(95). Auxin accelerates the synthesis of ethylene in senescing leaf blades(88), and may also delay senescence of tissues distal to the abscission zone by preventing the cells in the proximal side to respond

to ethylene(88). However, this is only observed before the process of abscission is initiated; once it is in progress auxin can no longer retard abscission(27,96).

Old leaves are more sensitive to ethylene than young leaves (131,145) and therefore senesce and abscise first. Auxin level is lower in older than in younger leaves(178,202). Young leaves accumulate cytokinins more readily than older leaves(65). Aging and senescence are not the same, so the statement that auxin and cytokinin delay aging actually means that they slow the physiological processes that lead to the onset of senescence(3).

Respiration, oxygen, and carbon dioxide. Respiration is essential for abscission(14,48). Abscission zones exhibit a climacteric rise in respiration similar to the climacteric of ripening fruits(48,108). However, no such rise occurs during petal abscission in geranium flowers(23).

Zhang and Lu(210) suggested that uncoupling of respiration and oxidative phosphorylation might be one of the effects of the growth inhibiting substances in inducing the abscission of cotton bolls.

The presence of oxygen is absolutely essential for abscission (6,51,206). Lowering the atmospheric oxygen level to 5% inhibited abscission in Coleus explants (164); raising it above 20% accelerated abscission(51). Carns et al.(51) suggested that oxygen was needed to supply energy for the metabolic processes during abscission. Modern models of ethylene biosynthesis indicate that oxygen is needed for the ultimate conversion of methionine(113), or of the intermediate ACC(11, 12), to ethylene.

Although some early references show an abscission accelerating effect of CO_2(see in 17), this gas generally retards abscission in ex-

plants(6). In his revieww, Rasmussen(158) indicated that CO_2 stimulated abscission in non-photosynthesizing tissue and retarded it in photosynthesizing tissue, implying that CO_2 acts through carbohydrate synthesis. Today we know that CO_2 is an antagonist of ethylene, and this property probably is responsible for its action in retarding abscission(8,17,31, 33,46,113).

Hypobaric conditions. Low pressure treatment increases storability of fruits and flowers by facilitating diffusion of endogenous ethylene out of the tissue(68). Morgan and Durham(131) reported that hypobaric conditions delayed leaflet abscission of excised Melia azedarach L. leaves. Termination of hypobaric treatment allowed a normal progression of abscission as well as normal ethylene evolution rates.

Pollination. Pollination induces flower senescence in several species (20,47,83,138), corolla abscission in Digitalis flowers(181), and petal abscission in geranium(194). Stead and Moore(181) determined that the abscission stimulus due to pollination moves down the pedicel at $4\ mm\ h^{-1}$.

Sugars. The effect of added sugars on organ abscission depends on the sugar reserves within the plant or explant(36,42). When carbohydrate level is high additional sugars help to maintain the integrity of the cell wall polysacharides; when the level is low, additional sugars appear to be utilized to provide energy for abscission(15). Sucrose stimulated leaf abscission of bean explants(188), and an increase in amylase activity has been associated with sucrose-induced abscission (103).

Temperature. The temperature response curve for abscission of explants exhibit a maximum near 30^{o} for beans and near 35^{o} for cotton(17).

Petal abscission on plants of several species was accelerated by brief exposure of flowers to temperatures of 33^{0} to 40^{0}(cited in 17). Temperatures just above freezing delay abscission in cotton (86). In cold sensitive plants, mild freezes cause metabolic changes that hastens abscission; severe freezes kill the tissues before abscission can occur(17). Leaves of several deciduous trees exhibit an arrested separation which may last the whole winter(39,92,124).

Nichols(137) reported that a peak of ethylene production occurred in carnation flowers 5 to 8 days after excision at temperatures higher than 7.2^{0}; at lower temperatures ethylene production was not detectable. However, if ethylene synthesis is initiated at temperatures higher than 7.2^{0} it continues at reduced rates at 1.6^{0}or 4.4^{0}. The effect of temperature on ethylene production is consistent with a system which is enzyme activated(45,115).

Light. Long days, or light interruption of long nights, delay abscission(79,134,141,173), apparently because of accumulation of photosynthates(36), or an increase in the level of endogenous auxin and gibberellin(25,139,156). In explants obtained from young bean leaves both red(R) and far-red(FR) light inhibited abscission; however, in explants taken from well developed leaves R retarded while FR stimulated abscission(188).

Water. Drought or flooding can initiate abscission(161); however, moderate amounts of water must be available to the abscission zone for abscission to occur(51).

Wind. Under natural conditions final mechanical separation of an auxiliary organ is frequently facilitated by wind action(17). Shaking by artificial means or by wind action causes petal abscission in

several flowering plants(16,see 17,64). The shaking action of the wind could increase wound ethylene production, thus stimulating abscission (see 136,189).

Conclusions

The detachment of an auxiliary organ from the main axis of a plant generally occurs in an anatomically well-defined abscission zone localized at the base of the organ. Epidermal indentation may delineate the region of the attachment. The abscission zone is structurally weak and is often characterized by meristematic acitivity which leads to the formation of a separation layer, protective layer, or both. Cell enlargement is frequently associated with separation but may also be involved in the prevention of abscission, or protection of the exposed surface after separation.

Three types of cell lyses may occur during organ separation: middle lamella only; middle lamella and cell wall; entire cell. Pectinases and cellulases are the hydrolytic enzymes that appear to be involved in the degradation of cell wall components in the abscission layer. Divalent ions, mainly Ca^{++} and Mg^{++}, play an important structural role in the abscission zone by linking the pectin in the middle lamella to the other cell wall components.

A general hormonal imbalance seems to be the initial stimulus for abscission, However, ethylene appears to be the ultimate signal for the onset of abscission. Ethylene action is primarily via promotion of synthesis of hydrolytic enzymes and perhaps via increasing membrane permeability thus reducing enzyme compartmentation. Auxin and possibly all hormones act as modifiers of tissue sensitivity to ethylene, or as promoters of ethylene synthesis.

Localized senescence in cells of the abscission zone is a requisite for the triggering of metabolic activities leading to abscission. A respiratory climacteric pattern seems to occur during development of abscission, at least in some cases. Oxygen defficiency limits ethylene synthesis and therefore abscission. CO_2 acts as competitive inhibitor of ethylene and may inhibit abscission if present in high concentration. Low temperature inhibits the abscission process, possibly by affecting the enzymatic systems involved in ethylene synthesis and action.

Although several different compounds affect the abscission process, the practical control of abscission may be achieved by treatment with chemicals that inhibit either ethylene action or synthesis.

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Section I

Morphology, Histology and Calcium Localization in the Petal Abscission Zone of the Hybrid Geranium

Morphology, Histology and Calcium Localization in the Petal Abscission Zone of the Hybrid Geranium

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Abstract. Comparison of petal attachment width and fresh weight to attachment width ratio between easy-to-shatter ('Sprinter Scarlet' and 'Sprinter White') and difficult-to-shatter ('Penny Irene' and 'Marathon') geranium cultivars showed that the morphology of petal attachment does not account for cultivar differences in petal shattering. However, petal attachment of the easy-to-shatter cultivars was characterized by a structurally weak abscission zone, formed by small parenchyma cells. Petal separation in 'Sprinter Scarlet' always occurred within the abscission or transition zone. Cell division and enlargement of cells proximal to the abscission zone were observed but were not essential for the onset of separation of 'Sprinter Scarlet' petals. Movement of calcium out of the abscission zone did not appear to precede petal separation in 'Sprinter Scarlet'.

The seed propagated hybrid geranium is an important item in the bedding plant industry(3,4,18). However, petal abscission is much more of a problem with seedlings than with cutting propagated cultivars(3). Morphological features such as semi-double or double flower type have been associated with the shattering-resistant cultivars(18). Abscission of auxiliary plant organs is frequently associated with the development of an abscission zone at the base of the abscising organ(7). An abscis-

sion zone can be defined as a region of structural weakness in which changes associated with cell separation occur(5,6,22). Mobilization of calcium out of the abscission zone may be a major controlling factor in abscission(17).

The objective of this investigation was to compare morphological and histological features of petal attachment in shattering and non-shattering cultivars. Histological aspects during abscission development and final separation, as well as the pattern of calcium distribution in the abscission zone and related tissues of 'Sprinter Scarlet', were also investigated.

Materials and Methods

General. Two easy-to-shatter ('Sprinter Scarlet' and 'Sprinter White') and two difficult-to-shatter ('Penny Irene' and 'Marathon') geranium cultivars(1,19) were used. Stock plants were grown with a commercial peat-lite mix in 15.2 cm clay pots, in a greenhouse at 15^{o}C night and 21^{o}day. Plants were watered when necessary with a water soluble fertilizer at 200 ppm N of 20N-8.7P-16.7K.

Morphology. To study morphological aspects of petal attachment, five florets from each of four plants of 'Sprinter Scarlet', 'Penny Irene' and 'Marathon' were collected. The petals were carefully removed, weighed on an analytical balance, and the width of attachment was determined using a stereomicroscope fitted with an ocular micrometer. Width of attachment and ratio of petal fresh weight to width of attachment (FW/WA) were used to compare cultivars. Depending on size and position on receptacle, petals were classified as having wide attachment (>1.15 mm), narrow attachment (<1.14 mm) or as petaloids.

Histology. For anatomical comparison, ten 5 mm floret explants

containing petal and receptacle tissue were excised from inflorescences of each of the four cultivars at two different stages: 2 days before and 2 days after flower opening. Flower opening was defined as the stage when the pistil and stamens were first seen. Similar explants were excised from 'Sprinter Scarlet' plants from 2 days before until 6 days after flower opening to study the histological development of abscission. Immediately after excision the specimens were fixed in FAA(50% ethyl alcohol, 10% formalin, 5% glacial acetic acid and 35% water), aspirated, dehydrated using the ethanol-TBA series, infiltrated with paraffin and sectioned (10 μm) on a rotary microtome. Sections were afixed to glass slides with Weaver's solution and passed through a staining series with safranin and fast-green before being mounted with Lipshawn^{R} mounting media(12). Sections were observed with a light microscope and longitudinal or cross sections were photographed.

Localization of calcium. The distribution of calcium in the profile of longitudinal sections of 'Sprinter Scarlet' was determined using an electron microprobe X-ray analyser (Applied Research Laboratories Model EMX-SM). Specimens were collected from 3 days before until 6 days after flower opening. The samples were prepared as described for the histological study, except that sections were 20 μm thick, and they were affixed to quartz slides using a gelatin adhesive(11). The paraffin was removed with xylene and the specimens were air dried for at least 24 h. Sections were coated with carbon prior to examination. The operating conditions were 20 kV accelerating potential and 0.02 μA sample current(17). A 100 μm band along the region of petal insertion was analysed on each side of the vascular tissue, using a scan speed of 192 μm min^{-1}. Elemental analysis was conducted for calcium and

magnesium, and the relative amount of each element in counts per second was recorded. Magnesium was not detected. To evaluate the distribution of calcium in tissues in relation to the region of petal attachment during the abscission process, seven zones were identified: one at the insertion, three towards the petal and three towards the receptacle. Each zone had the same length as the layers of small cells characteristic of the abscission zone (about 192 μm). The percentage of calcium relative to the whole profile was determined for each zone, and its variation over time was plotted.

Results

Morphology. No difference in width of attachment between cultivars was observed for petals of wide attachment or average width (Table 1); however, petals of narrow attachment in 'Sprinter Scarlet' were significantly narrower than in 'Penny Irene', those of 'Marathon' being intermediate. Petaloid attachment in the difficult-to-shatter 'Penny Irene' and 'Marathon' was generally much narrower than the narrow petals in the easy-to-shatter 'Sprinter Scarlet' (Table 1). The FW/WA ratio was slightly larger in 'Penny Irene' than in 'Sprinter Scarlet' for all petal types, indicating that the former is subjected to a greater natural breaking force than the latter (Table 2).

Histology. In longitudinal sections the easy-to-shatter cultivars had a well distinguished transition zone at the base of the petal, between the petal and the receptacle tissue, which was absent from or less evident in the difficult-to-shatter cultivars (Fig.1 and Fig. 2 a,c). This transition zone was characterized by several layers of small parenchyma cells which may define a region of structural weakness (Fig. 1). In all cultivars a single vascular bundle appeared

in the center of the region of attachment (Fig. 1) being ramified toward the petal or receptacle tissue. The ratio of vascular tissue width to width of petal attachment appears to be the same for all cultivars(Fig. 1 and 2). In 'Sprinter Scarlet' sampled 2 days before flower opening (Fig. 1a),and in 'Marathon' (Fig. 1g) and 'Penny Irene' (Fig. 1h) sampled 2 days after flower opening, the vascular tissue appears to be slightly dislocated from the media section; this was common in all cultivars. Sclerified cells often associated with vascular tissues were not evident at the region of attachment in cross sections of either 'Penny Irene' or 'Sprinter Scarlet' (Fig. 2b and d). In 'Sprinter Scarlet' cells proximal to the transition zone tended to enlarge during abscission (Fig. 3a to f). However, cell enlargement was not necessarily associated with separation, which occurred anywhere within the transition zone (Fig. 4a to c). The transition zone of the petal attachment was recognized to be the abscission zone or abscission region as defined in the literature(7). As described for various organs in other species(8,20) the vascular tissue constituted the major structural resistance against petal separation (Fig. 4b and c), which finally occurred by mechanical means(see in 2). Tissue separation often began in the adaxial side of the petal insertion (Fig. 5a to c), generally from the periphery towards the center (Fig 4a). However, a few cases of initial central separation were observed, including partial rupture of the vascular tissue (Fig. 5b and c). The small cells characteristic of the abscission zone of the easy-to-shatter cultivars remained on the exposed surface after separation and constituted the protective layer (Fig. 4b) as described by Esau(7). Modified parenchyma cells formed callus tissue which occluded the vascular tissue after

separation (Fig. 4d). An abscission layer, as defined by a series of active dividing cells involved in final separation, was apparent in the abscission zone (Fig. 6) but did not appear to be essential for separation.

Localization of calcium. Marked variation was obtained for calcium localization within and among specimens. In general a high level of calcium occurred on the proximal side of the abscission zone (receptacle) before flower opening and tended to decrease afterward (Fig. 7a). However, calcium content was stable during flower development in proximal tissue closer to the abscission zone (Fig. 7b and c). At the abscission zone, calcium appeared to accumulate until 1 day after flower opening, decreased steadily for the next 2 days and then increased again (Fig. 7d). Calcium content of petal tissue immediately distal to the abscission zone rose during flower development (Fig. 7e); however, the level in more distal tissue was consistently low throughout flower development (Fig. 7f and g).

Discussion

Since no differences in width of petal attachment or FW/WA ratio were observed between the easy-to-shatter and the difficult-to-shatter cultivars, petal morphology cannot be accounted for cultivar differences in petal abscission. However, the histological differences appear to be important. Cells in the transition zone between petal and receptacle tissue in 'Sprinter Scarlet' and 'Sprinter White' appear to be the site of anatomical and/or physiological changes associated with abscission, for separation always occurs within this zone. Similar characteristics have been described in abscission zones of leaves(5,6,21), and in some flowers(13) but not all(7). Both cell division(9,10,15,16,

22) and cell enlargement(14) are associated with abscission of leaves and flower parts. Even though both were observed in the abscission zone of 'Sprinter Scarlet' petals, neither seems to be essential for the onset of separation. In 'Sprinter Scarlet' the vascular tissue remains intact after separation of the parenchyma cells in the abscission zone, as is the case in leaf and fruit abscission(20). Final separation appears to involve all three processes described in the literature: middle lamella dissolution, middle lamella and primary wall dissolution, and mechanical breakage involving non-living cells of the vascular tissue(20).

Only small changes in calcium distribution at the point of petal insertion were observed during abscission, thus mobilization of calcium out of the abscission zone may not be a pre-requisite for separation(17); however, the high variability prevented a definite conclusion.

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Table 1. Width of petal attachment (mm) in the receptacles of easy-to-shatter ('Sprinter Scarlet') and difficult-to-shatter ('Penny Irene' and 'Marathon') geranium cultivars.

Cultivar	Width of attachment(mm) Petal type			
	Wide attachment[z]	Narrow attachment[y]	Avg. width[z]	Petaloid[x]
Sprinter Scarlet	1.28	0.88 a	1.04	--
Penny Irene	1.28	0.98 b	1.11	0.37
Marathon	1.25	0.95 ab	1.08	0.65

[z]Means not significantly different by F test (5%).

[y]Mean separation by Duncan's Multiple Range test (5%).

[x]Means significantly different by F test (5%).

Table 2. Ratio of petal fresh weight (mg) to width (mm) of attachment (FW/WA) in easy-to-shatter('Sprinter Scarlet') and difficult-to-shatter('Penny Irene') geranium cultivars.

Cultivar	FW/WA Petal type			
	Wide attachment	Narrow attachment	Avg. width	Petaloid
Sprinter Scarlet	22.42	42.95	32.78	--
Penny Irene	26.09	47.86	37.68	35.88
Significance of F	5%	5%	5%	--

Fig. 1. Longitudinal sections of the region of petal attachment in four geranium cultivars: 'Sprinter Scarlet'(a and c), 'Sprinter White'(b and f), 'Marathon'(c and g), and 'Penny Irene'(d and h); 2 days before(a to d) and 2 days after(e to h) flower opening. (P) petal tissue), (R) receptacle tissue.

P
R
a
b
c
d
e
f
g
h
100 µm

Fig. 2. Longitudinal(a and c) and cross(b and d) sections of 'Penny Irene'(a and b) and 'Sprinter Scarlet'(c and d) petal attachment, 2 days after flower opening. (P) petal tissue, (R) receptacle tissue.

P
R
a
R
P
b
P
R
c
R
P
d
100μm

Fig. 3. Longitudinal sections showing sequential development of the petal abscission zone in 'Sprinter Scarlet': (a) 2 days before flower opening, (b) 1 day before flower opening, (c) day of flower opening, (d) 2 days after flower opening, (e) 4 days after flower opening, (f) 6 days after flower opening. (P) petal tissue, (R) receptacle tissue.

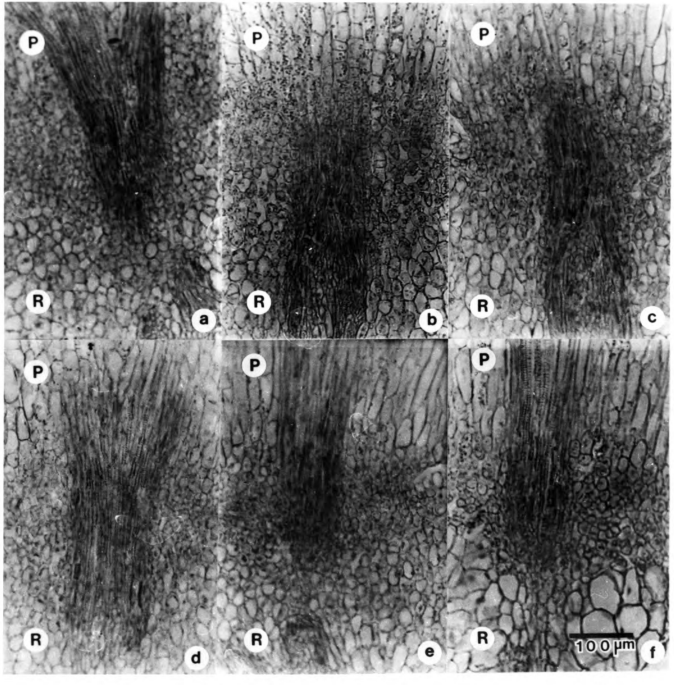

P
R
a
P
R
b
P
R
c
P
R
d
P
R
e
P
R
100 µm
f

Fig 4. Patterns of separation in the petal abscission zone of 'Sprinter Scarlet' geranium: (a) separation starting on the periphery of the petal attachment, at the morphological groove; (b) advanced stage of tissue separation, showing the vascular tissue still intact; (c) higher magnification of (b) showing detail of cell separation; (d) formation of callus tissue obstructing the vascular bundle after separation. (P) petal tissue, (R) receptacle tissue, (S) cell separation, (A) abscission zone, (pl) protective layer, (c) cells of the callus tissue.

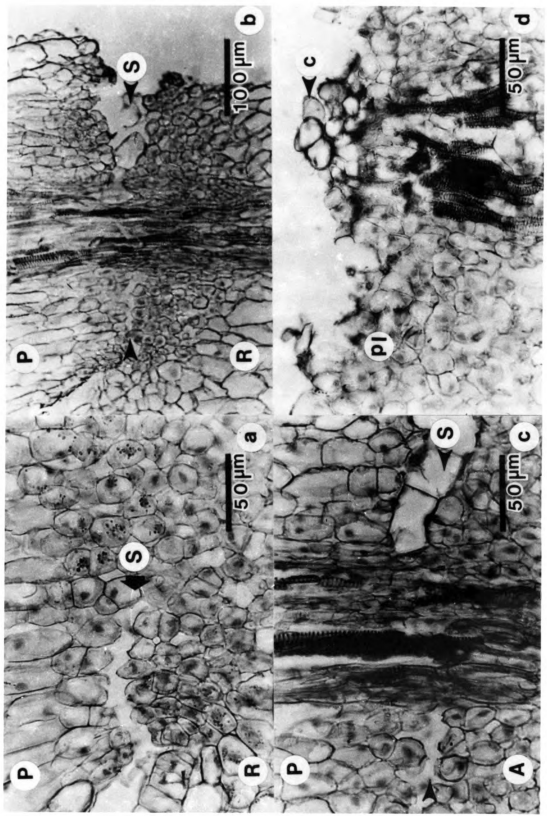

P
S
50 µm a
R
P
S
100 µm b
R
Pl
c
50 µm d
P
S
50 µm c
A

Fig. 5. Sequential longitudinal sections of 'Sprinter Scarlet' petal attachment showing the tissue separation beginning on the adaxial side(a) and progressing towards the center(b and c). (P) petal tissue, (A) abscission zone, (S) tissue separation.

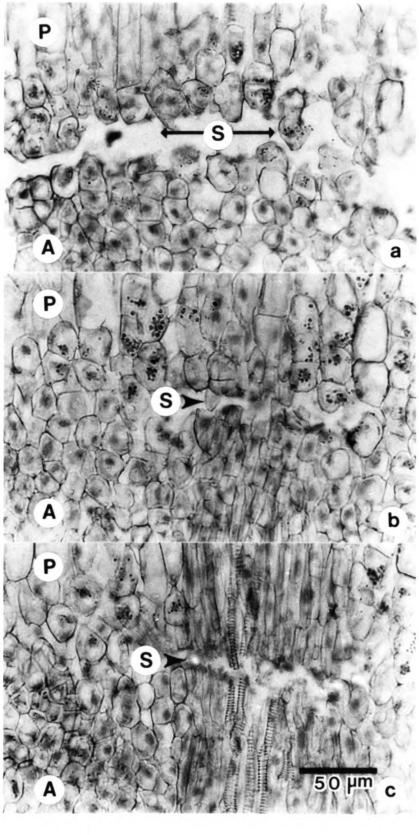

P
S
A
a
P
S
A
b
P
S
A
50 µm
c

Fig. 6. Longitudinal section of petal attachment of 'Sprinter Scarlet' showing cell dissolution and apparent cell division on the distal side of the abscission zone (cd). (P) petal tissue, (A) abscission zone.

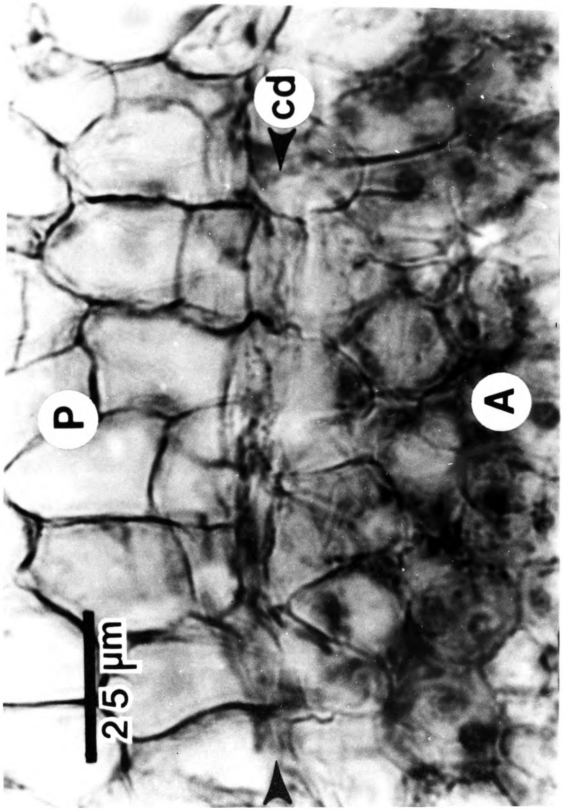
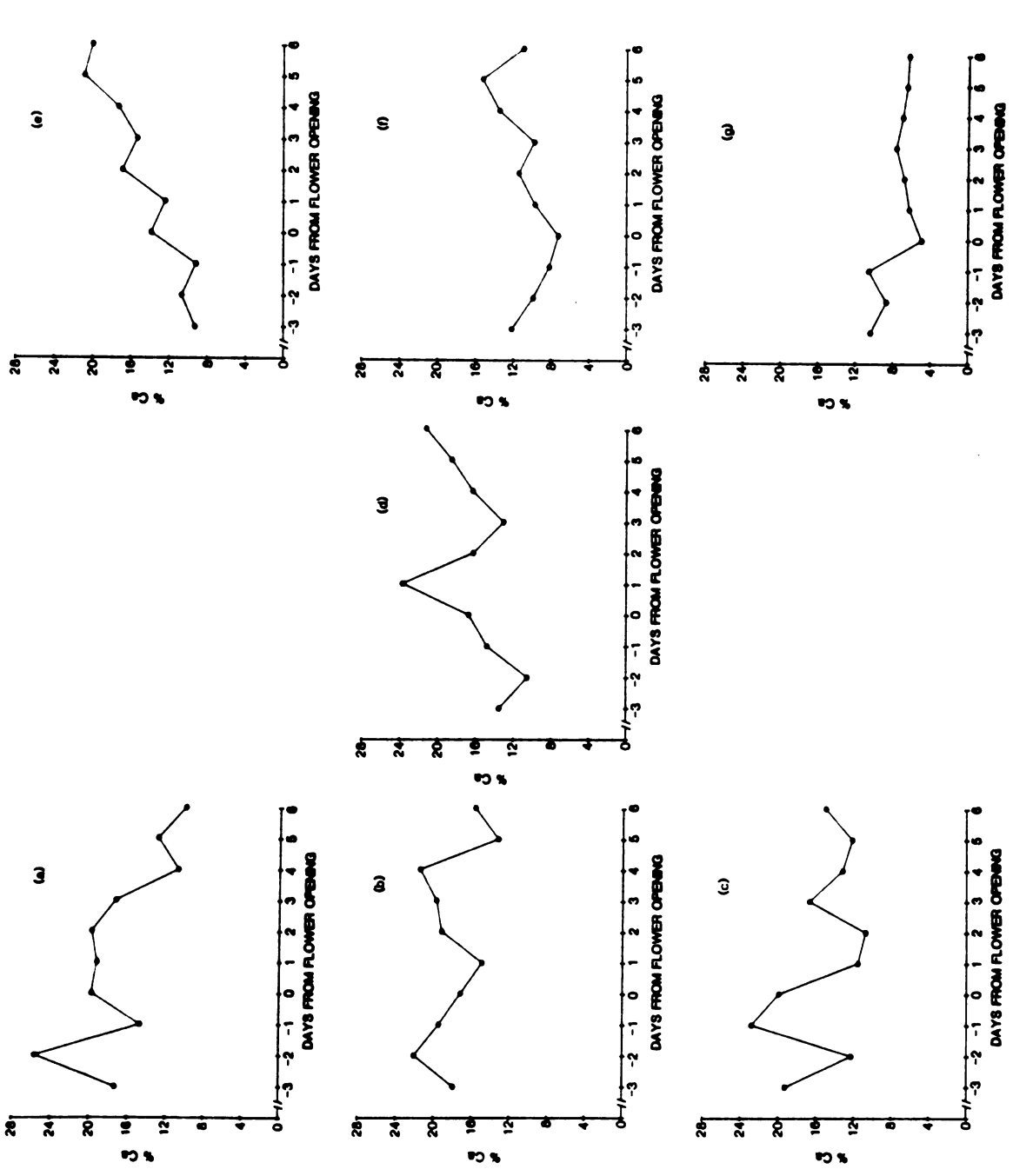

cd
P
A
25 µm

Fig. 7. Changes in calcium distribution accross the region of petal attachment in 'Sprinter Scarlet' geranium, during abscission. Each graph represents the change in calcium content in a 192 μm longitudinal section of the petal insertion zone, from 3 days before until 6 days after flower opening.
(a to c) third, second, and first sections proximal to the abscission zone; (d) abscission zone; (e to g) first, second, and third section distal to the abscission zone.


(a)
(b)
(c)
(d)
(e)
(f)
(g)
DAYS FROM FLOWER OPENING
♂ #

Section II

Characterization of the Role of Ethylene in Petal Abscission of Hybrid Geranium Using Floret Explants

Characterization of the Role of Ethylene in Petal Abscission of Hybrid Geranium Using Floret Explants

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Abstract. Petal abscission on floret explants was delayed or inhibited by the ethylene inhibitors aminoethoxyvinylglycine (AVG) at 100 and 200 ppm, silver nitrate(SN) at 50 and 100 ppm and silver thiosulfate(STS) at 25 and 50 ppm in SN, and by a mixture of gibberellins A4 and A7(GA4/7) at 20 ppm. A slight delay was observed with a mix of N-(phenyl-methyl)-1H-purin-6 amine and gibberellins A4 and A7(promalin),N6-benzyladenine(N6-BA) and 8-hydroxyquinoline sulfate(8-HQS). Cycloheximide(CHI) accelerated, but the auxins 2(3-chlorophenoxy) propionic acid (3-CPPA) and naphthalene acetic acid(NAA) did not affect the abscission process. Ethylene production by explants was inhibited by AVG, but it was either unaffected or slightly enhanced by SN. Exogenous ethylene accelerated petal abscission; concentrations as low as 0.1 ppm overcame the inhibitory effects of AVG or SN. Hypobaric ventilation(150 torr) inhibited petal abscission, and its effects were overcome by treatment with 0.1 ppm ethylene. CO_2 enrichment(15%) did not delay abscission.

Petal abscission in hybrid geraniums during transit and handling is a well recognized problem in the marketing of this crop(5).

Several endogenous and exogenous factors have been associated with flower shattering including genetic and morphological characteristics(6,34) and accumulation of exogenous ethylene(6,15). However, morphological differences in geranium petal attachment were not related to cultivar differences in readiness toshatter(26). Refrigeration before shippment and during transit, prevention of ethylene accumulation, cultivar selection(6), and silver nitrate treatment prior to shipping(15) all reduce petal abscission. Most investigators assume that ethylene plays a major role in the process of petal abscission, but few data are available to support this assumption. Ethylene may play both passive and active roles in the control of abscission of plant organs(19,20,31,33).

This research was undertaken to clarify the role of ethylene in the petal abscission process, and to find a chemical means of control.

Materials and Methods

General. Excised florets of the easy-to-shatter hybrid geranium 'Sprinter Scarlet' were used in all experiments; in one experiment the difficult-to-shatter 'Penny Irene' and 'Marathon' were also utilized. The petals were not subjected to any breaking force other than the petal weight itself. Because the calyx provided a natural support for the petals, even after separation, standard explants were prepared by carefully removing the sepals with forceps just after floret excision, without damaging the region of petal attachment to the receptacle. Florets were excised within 12 hours of flower opening, unless otherwise specified. Flower opening was defined as the stage when the pistil and stamens were first evident. Immediately after excision, the explants were placed in 10 ml petri dishes with the pedicels immersed

in deionized water and sustained by glass beads and marbles. Before treatment the explants were exposed to ambient air for 4 hours to allow dissipation of wound ethylene(10). In preliminary experiments wound ethylene production peaked approximately 2 hours after excision, then decreased for the next two hours. Chemical treatments were applied by spraying the explants with 0.5 ml of the test solution, using a 1 cc syringe. Unless otherwise specified, 10 explants were used in each of 3 replications and abscission data were expressed as time to 50% cumulative petal fall (T50A). Chemicals reported to be effective in the control of abscission or senescence of plant organs were selected(3,7, 15, 23,28,29,35,37). The compounds used fell into 3 classes: a) ethylene inhibitors: aminoethoxyvinylglycine(AVG), silver nitrate(SN, $AgNO_3$), silver thiosulfate(STS, $Ag(S_2O_3)_2^{3-}$); b) hormones or hormone-like compounds: 1-naphthalene acetic acid(NAA), 2(3-chlorophenoxy)propionic acid(3-CPPA), N-(phenylmethyl)-1H-purine-6-amine + gibberellins A4/A7 (promalin), N6-benzyladenine(N6-BA), gibberellins A4/A7(GA 4/7) ; (c) miscellaneous: calcium chloride($CaCl_2$), 8-hydroxyquinoline sulfate (8-HQS), cycloheximide(CHI). Solutions of compounds other than STS in groups (a) and (c) were prepared by dissolving the chemical in deionized water at room temperature, then diluting this stock solution to the desired concentrations. STS anionic complex was prepared as described by Reid et al.(30), except that a molar ratio between silver and thiosulfate of 1:3.13 was used; a stock solution with 1.5 mM Ag^+ or 250 ppm in $AgNO_3$ was prepared. Compoundsin group (b) were dissolved in warm water (50^oC) or 25% ethanol. Controls were sprayed with deionized water or with appropriated diluted solutions of ethanol. In preliminary experiments the ethanol controls had no effect on petal abscission. All

explants were excised from greenhouse stock plants grown at 15°C night and 21° day temperature, in 15.2 cm clay pots containing a commercial peat-lite mix. Plants were watered as needed with a water soluble fertilizer at 200 ppm in N of 20N-8.7P-16.7K.

Application of chemicals. Explants of 'Sprinter Scarlet' geraniums were sprayed with each of the test solutions at the following concentrations: AVG(0,100,200,400 ppm); STS(0,25,50,100 ppm in $AgNO_3$); SN, promalin, 3-CPPA(0,50,100,200 ppm); GA 4/7 , NAA(0,5,10,20 ppm); N6-BA(0,15,30,45 ppm); 8-HQS(0,200,400,600 ppm); $CaCl_2$(0, $6.8x10^{-3}$, $6.8x10^{-2}$, $6.8x10^{-1}$ M); CHI(0,10^{-5},10^{-4},10^{-3}M). Inhibitors of ethylene synthesis(AVG) and action(SN) were also combined in a 3 by 3 factorial experiment, each chemical being used at zero, sub-optimal and optimal concentrations, in order to evaluate their interactive effect on petal abscission. One replicate consisted of five explants placed in a 10 ml petri dish containing deionized water. Three replications were arranged in a completely randomized design inside a growth chamber(Sherer-Gillette, Marshall, MI) under a 12 h light 12 h dark regime of 280 μE m^{-2} s^{-1} from cool-white fluorescent tubes at a constant temperature of 21 ± 1°C. The explants were checked for petal abscission every 12 h after treatment and water was added to the petri dishes as needed. Results are expressed as time to 50% abscission (T50A). Because no break strength test was used, data for treatments that entirely prevented abscission were expressed as time to 50% petal fading (T50F). Petal fading was indicated by loss of turgidity and petal rolling.

Ethylene production. Explants of 'Sprinter Scarlet' were sprayed with SN, AVG or deionized water, then placed in 500 ml air-tight glass jars fitted with serum caps. In one experiment the jars were kept

closed during the experimental period, except for one 5 minute aeration period every 12 hours just after the removal of aliquots. In another experiment a flow-through system was used,as described by Saltveit(32), with one complete gas exchange every 3.2 hours and a flow rate of approximately 2.6 ml of ethylene-free air per minute. The treatments were applied in a completely randomized design with 3 replications. Every 12 hours two air samples of 1cc each were taken from each vial with a syringe. Ethylene content was measured with a gas chromatograph employing a flame ionization detector and a column of activated alumina, using N_2 as carrier gas. Data was expressed as ppb g^{-1} for the static system, and as nl $h^{-1}g^{-1}$ for the flow through system.

Effects of exogenous ethylene. Explants sprayed with AVG, SN and STS were placed in 10 l desiccators and exposed to exogenous ethylene at concentrations of 0, 0.1, 1.0 and 10.0 ppm either for periods of 12, 24, 36, 48 h or continuously throughout the experimental length. The ethylene control (0 ppm) was achieved by placing a petri dish with Purafill[1] pellets on the bottom of the desiccator; this prevented ethylene accumulation without interfering with synthesis or action of endogenous ethylene. Treatment combinations were arranged as a split-plot design, with ethylene as main treatment and chemicals as sub-plots. T 50 for abscission or petal fading was determined for each treatment combination.

Effects of hypobaric conditions. To observe the effects of lowering the endogenous level of ethylene on petal abscission, explants of 'Sprinter Scarlet' were sprayed with SN, AVG or deionized water, then

[1]Potassium permanganate impregnated pellets; distributed by H. E. Burroughs & Assoc., Inc., Chamblee,GA.

exposed to hypobaric conditions. Hypobaric conditions were attained by evacuating 10 l desiccators to aproximately 2 torr. Pure oxygen was flushed through the system until the total pressure inside the desiccators reached 760 torr. The desiccators were then reevacuated to 150 torr in order to maintain the normal partial pressure of O_2 . In one treatment,at each pressure, ethylene (0.1ppm) was injected into the desiccators. Control desiccators were evacuated to 2 torr and flushed with ethylene-free air until normal pressure was achieved. A petri dish with 10 M KOH and a beaker of distilled water were placed in each desiccator to prevent CO_2 accumulation and to maintain humidity, respectively. Treatments were arranged in a split-plot design with pressure as main treatment and chemical treatments as sub-plot. T50 to abscission or petal fading was determined.

Effects of carbon dioxide. Explants of 'Sprinter Scarlet', 'Penny Irene' and 'Marathon' were excised at three different stages of flower development(A = 2 days before flower opening; B = day of opening; C = 2 days after flower opening), and placed in 10 l desiccators wherethey were exposed to0 or 1.0 ppm ethylene in either air or 15% CO_2. Observations were recorded 8 to 120 hours after beginning treatment. The data are expressed as percent petal abscission over time instead of as T50 because in most instances the difficult-to-shatter cultivars did not drop their petals throughout the experimental period. Treatments were arranged in a split-plot, with ethylene and CO_2 as main-plot and cultivar and flower stage as sub-plots.

Results

Compoundsthat inhibit ethylene synthesis or action were the most efficient in controlling petal abscission (Table 1). Since each com-

pound was tested alone comparisons among them are not valid because of possible differences in experimental conditions. All three ethylene inhibitors were effective at the lowest concentration tested, but maximum response occurred at 200 ppm AVG, 100 ppm SN, and 50 ppm STS in $AgNO_3$ (Table 1). The dose/response curves for AVG and SN are best explained by a cubic model (Fig. 1a,1b), and for STS by a quadratic model (Fig. 1c). Dark spots on petals were observed on explants treated with AVG or SN, but STS treatments caused no phytotoxicity. Although $CaCl_2$ delayed petal abscission, this was probably the result of the petal dehydration observed after 60 h, rather than of activity of the salt itself (Table 1). GA 4/7 was also effective in delaying petal abscission at 10 and 20 ppm. The dose/response curve for GA treatment follows a linear model (Fig. 1d). Promalin, N6-BA and 8-HQS inhibited petal abscission only slightly (Table 1). CHI accelerated petal abscission significantly, and NAA had a slight but non-significant promotive effect. AVG and SN when applied together had neither additive nor synergistic effect (Table 2).

Ethylene evolution was inhibited by AVG in both aeration systems (Table 3). SN promoted ethylene synthesis slightly shortly after treatment, specially in the static system (Table 3). In the static system ethylene production was consistently stimulated by SN, the difference being significant at 5% level for the first 24 h. Although AVG consistently inhibited ethylene production, the effect was not significant until 84 h after treatment when production in the control began to increase dramatically in both systems. Exogenous ethylene caused a sharp acceleration of petal abscission. regardless of treatment duration (Table 4). Response was saturated at 1 ppm. Ethylene

treatment also overcame the inhibitory effects of the anti-ethylene compounds (Table 4). Although the differences between SN and control at 0.1 and 1.0 ppm ethylene are statistically significant, the actual inhibition of abscission is very little in each case (Table 4). The only significant interaction was the two-way chemical x ethylene indicating that the chemical differences were not consistent for all ethylene levels (Table 4).

Both hypobaric conditions and ethylene inhibitors inhibited petal abscission, in addition the interaction pressure x chemical treatment was significant; however, flower fading was delayed slightly longer by the ethylene inhibitors (Table 5). Exposing explants to ethylene negate the effect of hypobaric treatment (Table 5).

CO_2 enrichment did not inhibit petal abscission in 'Sprinter Scarlet'; in fact, CO_2 caused promotion of petal abscission in some situations (Table 6). Petal abscission occurred in 'Sprinter Scarlet' explants 24 hours after being exposed to 1.0 ppm ethylene, even before the opening of the flowers in some cases (Table 6). Flowers of difficult-to-shatter cultivars 'Penny Irene' and 'Marathon' faded when exposed to 1.0 ppm ethylene for 48 hours or longer, but did not abscise while still turgid (Table 6).

Discussion

Petal abscission of 'Sprinter Scarlet' floret explants can be inhibited by spray applications of the ethylene-synthesis inhibitor AVG and by the ethylene-action inhibitors SN and STS. AVG inhibits ethylene production by blocking the conversion of S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid(ACC) via competitive inhibition of ACC synthase(36). Ag^+ salts inhibit ethylene action possibly by

competing with ethylene's metallic binding site(9,11). Gibberellin A4/A7 (GA 4/7) also inhibited petal abscission and can promote (13,14) or inhibit(13,24) leaf abscission depending on species. The effect of gibberellins in the abscission process may be indirect via modification of tissue sensitivity to ethylene(27). Auxin is a major controlling factor in the abscission process, even though inhibitory effects are mainly associated with an early stage of organ development, the so-called "stage I" (21). Perhaps auxin was not effective in delaying petal abscission in the explants because stage I is restricted to a very early phase of flower development; at the time of excision all explants were already well advanced in stage II, in which ethylene promotes abscission(2). The slight acceleration of abscission by auxin may have been an effect of auxin-mediated ethylene synthesis(1). This may also explain the promotive effect of cycloheximide(3).

AVG inhibited ethylene synthesis in geranium explants particularly as the explants aged. Although Armitage et al.(6)in contrast with our results observed a steadly decline in ethylene synthesis after explant excision, ethylene production increases with time in bean explants(16,17,18). Simultaneous application of AVG and SN did not give additive effects; this may indicate that their different mode of action do not interact in the control of a response to ethylene. None of the inhibitors were effective in delaying petal abscission when the treated explants were exposed to exogenous ethylene. Nullification of the AVG effect by exogenous ethylene was expected since AVG inhibits ethylene synthesis rather than action of ethylene(36). However, suppression of the inhibitory effect of silver salts by exogenous ethylene possibly reflected saturation of cell sensitivity to ethylene(4) at which point

ethylene could bind freely to its metal-containing receptor site(11) without interference by Ag^+(8,9).

The results obtained with hypobaric conditions provided additional evidence that Ag^+ and AVG inhibit petal abscission via inhibition of ethylene, and indicated that ethylene must be present for abscission to occur. This experiment also showed that the presentation time for ethylene, at saturating levels, is as low as 5 hours. Failure of 15% CO_2 to inhibited athylene-promoted petal abscission confirmed observations by Armitage et al.(6) who reported similar results with 5% CO_2. Even though CO_2 is often described as a competitive inhibitor of ethylene(7,9,10), its mode of action is not fully understood(9). In addition CO_2 has been reported to enhance ethylene action in some circumstances(22). Because a concentration of 10% CO_2 will normally overcome the effect of 1.0 ppm ethylene(12), it is unlikely that a concentration higher than 15% would be more effective.

Two ethylene-mediated processes appear to be occurring during floret development in geraniums, one being petal abscission, the other petal and flower fading. Petals of cultivars that have an abscission zone at the base(26) will abscise shortly after being exposed to ethylene, as seen with 'Sprinter Scarlet' explants in Table 4. Cells in the abscission zone seem to develop an extreme sensitivity to ethylene much earlier than more distal or proximal tissues of the flower, since petals abscise while still turgid. This sensitivity to ethylene appears to be modified by hormone imbalance, thus the inhibitory effect of gibberellins A4/A7. Inhibiting ethylene synthesis with AVG, or providing additional binding sites with Ag^+, does not seem to affect cell sensitivity to ethylene, but certainly inhibits the abscission process

by reducing either ethylene availability or ethylene binding respectively. Ethylene mediation of overall petal senescence or fading appears to be a consequence of aging of cells in the whole petal. This process can be retarded by ethylene inhibitors until cell sensitivity to ethylene, or ethylene availability, reaches a threshold level at which irreversible tissue deterioration is triggered. This process happens in both shattering and non-shattering cultivars, as seen in Table 6. Both processes, abscission and fading, apparently require some endogenous preparatory conditions before they can be initiated. Preparatory conditions for petal abscission seem to be underway very soon in the flower development, since some closed flowers exposed to ethylene dropped petals even before they were opened (Table 6).

Our data provide conclusive evidence that petal abscission in hybrid geraniums is ethylene-mediated and can be effectively controlled by inhibitors of ethylene synthesis or action. The use of such chemicals for practical control of petal abscission in intact plants is discussed in a subsequent paper(25).

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Table 1. Effect of 11 chemicals on time to 50% abscission (T50A) of petals on floret explants of 'Sprinter Scarlet' geraniums.

	Hours to 50% abscission(T50A)										
	Chemical treatment										
Concentration	AVG	SN	STS	NAA[z]	3-CPPA[z]	N6-BA	GA 4/7	Promalin	$CaCl_2$	8-HQS	CHI
0(control)	61 a	61 a	53 a	67	61	60 a	71 a	55 a	54 a	45 a	68 a
A[y]	90 b	87 b	100 b	68	57	58 a	76 a	57 a	107[w]b	44 a	65 ab
B	143[x]c	142[x]c	116[x]c	56	58	71 b	93 b	70 b	132[w]c	63 b	54 bc
C	150[x]c	155[x]c	115[x]c	54	59	72 b	115 c	70 b	130[w]c	68 b	46 c

[abc]Mean separation within columns by Duncan's Multiple Range test (5%).

[z]F test non-significant (5%).

[y]Concentrations (A=lowest, B=medium, C=highest): AVG(100,200,400 ppm); SN, Promalin, 3-CPPA(50,100,200 ppm); STS(25,50,100 ppm in $AgNO_3$); GA 4/7, NAA(5,10,20 ppm); N6-BA(15,30,45 ppm); 8-HQS(200,400,600 ppm); $CaCl_2$($6.8x10^{-3}$,$6.8x10^{-2}$,0.68 M); CHI(10^{-5},10^{-4},10^{-3} M).

[x]Time to 50% petal fading.

[w]Petals wilted after 60 hours.

Table 2. Interactive effects of inhibitors of ethylene synthesis (AVG) and action (SN) on petal abscission of 'Sprinter Scarlet' floret explants.

SN (ppm)	Hours to 50% abscission(T50A) AVG (ppm) 0	100	200
0	66[z]a	92 b	140[y]c
50	88 b	91 b	146 c
100	144[y]c	143[y]c	142[y]c

[z]Mean separation by Duncan's Multiple Range test (5%).

[y]Time to 50% petal fading (T50F).

Table 3. Effects of spraying with SN(100 ppm) and AVG(200 ppm) on ethylene synthesis by 'Sprinter Scarlet' floret explants in a flow-through vs. a static aeration system.

Aeration system	Chemical treatment	Ethylene production[z] Hours from treatment 0-12	12-24	24-36	36-48	48-60	60-72[y]	72-84	84-96	96-108	108-120
		nl.g^{-1}.h^{-1}									
Flow[x] through	Water	3.70[w]	2.94[w]	3.81[w]	3.96[w]	4.76[w]	6.77[w]	8.34 b	11.76 b	14.26 b	16.36 b
	SN	2.39	3.14	3.32	3.61	4.16	5.85	6.28 ab	7.45 ab	8. 1 ab	8.84 ab
	AVG	2.66	1.97	1.91	2.19	2.07	2.45	2.39 a	2.56 a	2.55 a	2.69 a
		nl.l^{-1}.g^{-1}									
Static[v]	Water	17.07 a	24.74 a	33.29 ab	36.04 ab	42.64 ab	51.32 ab	59.62 b	66.45 b	93.64 b	110.09 b
	SN	27.89 b	40.63 b	49.19 b	57.88 b	65.06 b	76.49 b	81.36 b	80.69 b	98.09 b	118.51 b
	AVG	18.03 a	19.32 a	19.12 a	20.04 a	21.57 a	25.89 a	27.68 a	28.80 a	29.56 a	30.59 a

[z]Mean separation within columns, for each aeration system, by Duncan's Multiple Range test (5%).

[y]More than 50% petal abscission, in both aeration systems in the water treatment.

[x]One air change every 3.2 hours.

[w]F test non-significant (5%).

[v]Opened for 5 min every 12 hours.

Table 4. Effects of exogenous ethylene on floret explants treated with AVG(200 ppm), SN(100 ppm) or STS(25 ppm in $AgNO_3$).

Chemical treatment	Hours to 50% abscission(T50A)[z,y,x] Ethylene concentration(ppm) 0	0.1	1.0	10.0
Water	65.25 a v	24.07 a u	7.33 a t	7.27 t
SN	135.87[w]c v	33.96 b u	11.80 b t	7.19 t
AVG	141.94[w]c v	28.57 ab u	7.69 a t	7.05 t
STS	94.89 b v	26.82 a u	7.36 a t	7.08 t

[z]Letters following numbers (abc) indicate mean separation within columns, by Duncan's Multiple Range test (5%). Absence of letters indicates that F test was not significant (5%).

[y]Letters below numbers (tuv) indicate mean separation within rows by Duncan's Multiple Range test (5%).

[x]Means were averaged for different durations of ethylene exposure (12, 24,36,48 hours or continuously), because the F value for duration was not significant (5%).

[w]Hours to 50% petal fading (T50F).

Table 5. Effect of hypobaric conditions on 'Sprinter Scarlet' floret explants treated with either AVG(200 ppm) or SN(100 ppm), or exposed to ethylene(0.1 ppm).

Chemical treatment	T50A(hours)[z,y] Total pressure(torr) 150	760
Dry control	128.62[x]a w	63.99 a v
H_2O control	132.52[x]a w	60.53 a v
SN	152.00[x]b w	146,39[x]b w
AVG	164.26[x]b w	156.09[x]b w
Ethylene (0.1 ppm)	5.76 w	5.37 w

[z]Letters following numbers (ab) indicate mean separation within columns by Duncan's Multiple Range test (5%).

[y]Letters following numbers (wv) indicate mean separation within rows by Duncan's Multiple Range test (5%).

[x]Time to 50% flower fading (T50F).

Table 6. The effect of CO_2 enrichment, with and without exogenous ethylene, on petal abscission of 'Sprinter Scarlet'(SS), 'Penny Irene'(PI), and 'Marathon'(Ma) geraniums at three different stages of flower development.

Ethylene (ppm)	CO_2(%)	Flower[z] stage	% petal abscission Hours from treatment: 8 SS	8 PI	8 Ma	24 SS	24 PI	24 Ma	48 SS	48 PI	48 Ma	72 SS	72 PI	72 Ma	96 SS	96 PI	96 Ma	120 SS	120 PI	120 Ma
0	0.033 (ambient)	A	--	--	--	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		B	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	0
		C	0	0	0	0	0	0	0	0	0	0	0	0	50	12[y]	0	100	20[y]	0
	15.00	A	--	--	--	0	0	0	0	0	0	30	0	0	50	0	0	100	15[y]	0
		B	0	0	0	0	0	0	10	0	0	20	0	0	100	0	0	100	50[y]	0
		C	80	0	0	80	0	0	80	0	0	80	0	0	100	18[y]	0	100	70[y]	0
1.0	0.033	A	--	--	--	60	0	--	60	0	--	70	0[x]	0[x]	100	0[x]	0[x]	100	0[x]	0[x]
		B	10	0	0	70	0	0	70	0[x]	0[x]	80	0[x]	0[x]	100	0[x]	0[x]	100	0[x]	0[x]
		C	60	0	0	60	0	0	60	0[x]	0[x]	60	0[x]	0[x]	100	0[x]	0[x]	100	0[x]	0[x]
	15.00	A	--	--	--	100[w]	0	0	100[w]	0	0	100	0[x]	0[x]	100	0[x]	0[x]	100	0[x]	0[x]
		B	50	0	0	50	0	0	60	0[x]	0	60	0[x]	0[x]	100	0[x]	0[x]	100	0[x]	0[x]
		C	40	0	0	60	0	0	60	0[x]	0[x]	70	0[x]	0[x]	100	0[x]	0[x]	100	0[x]	0[x]

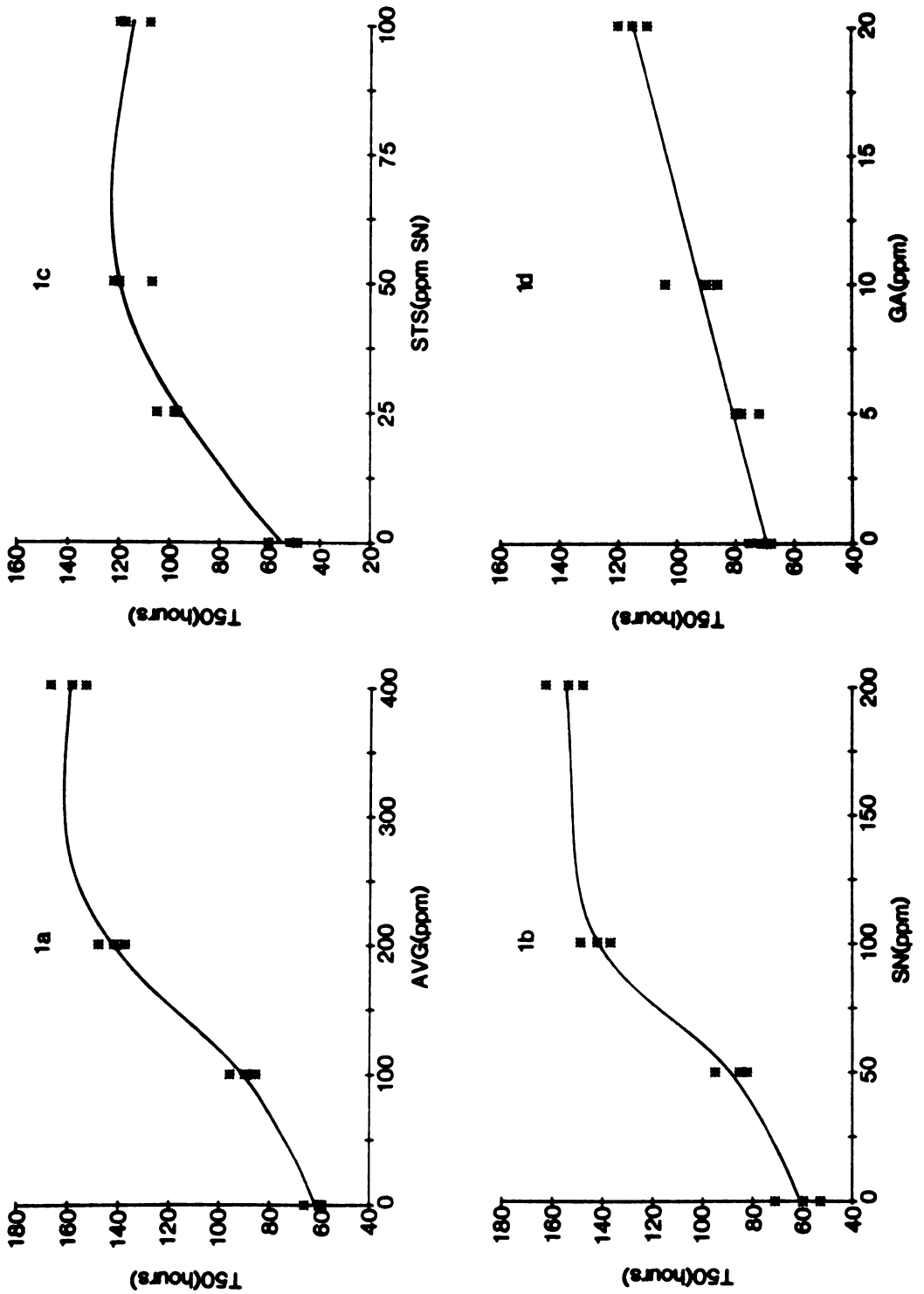
[z]A = 2 days before flower opening;B = day of flower opening; C = 2 days after flower opening.

[y]Petals dropped after losing turgidity.

[x]Flower fading.

[w]Petals dropped before flower opening.

Fig. 1. Dose/response curves for chemicals effective in delaying petal abscission of 'Sprinter Scarlet' floret explants. Data was expressed as time to 50% petal abscission (T50A) up to 110 hours after treatment, and as time to 50% petal fading (T50F) thereafter. (a) AVG, $r_{(cubic)} = .99^{**}$;(b) SN, $r_{(cubic)} = .98^{**}$; (c) STS, $r_{(quadratic)} = .97^{**}$;(d) GA 4/7, $r_{(linear)} = .93^{**}$.


1a
T50(hours)
AVG(ppm)
1b
T50(hours)
SN(ppm)
1c
T50(hours)
STS(ppm SN)
1d
T50(hours)
GA(ppm)

Section III

Chemical Control of Petal Abscission in the Hybrid Geranium *Pelargonium* x *hortorum* Bailey

Chemical Control of Petal Abscission in the Hybrid Geranium *Pelargonium* x *hortorum* Bailey

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Abstract. Of several compounds tested for controlling petal abscission of 'Sprinter Scarlet' geranium plants growing in the greenhouse silver thiosulfate (STS), at 50 ppm in $AgNO_3$ was the most effective. Petal abscission was less than 10% in plants treated with STS,18 days after treatment, versus 75% in controls. Silver nitrate (SN) at 100 ppm and aminoethoxyvinylglycine (AVG) at 200 ppm were also efficient, but caused severe phytotoxic symptoms when sprayed on open flowers. A mixture of gibberellins A4 and A7(GA 4/7) at 20 ppm also inhibited petal abscission, but caused excessive peduncle elongation. 2(3-chlorophenoxy)propionic acid (3-CPPA), naphthalene acetic acid (NAA), N6-benzyladenine (N6-BA), a mixture of N-(phenyl-methyl)-1H-purin-6 amine with gibberellins A4 and A7 (promalin), 8-hydroxyquinoline sulfate (8-HQS), cycloheximide, calcium chloride and calcium nitrate, were not effective in controlling petal abscission. High temperatures (30°C) for more than 8 days completely eliminated the effect of Ag^+ in inhibiting petal abscission. Soil drench treatments with silver nitrate or silver thiosulfate were ineffective.

Seed propagated hybrid geranium is an important item in the bedding plant industry(22); however, severe petal abscission during shipping drastically limits the marketing of this crop(4). Petal abscission is accelerated by exogenous ethylene(5,14) and appears to be associated with high sensitivity to ethylene(14) of specialized cells located at the base of the petals(15). Petal shattering on explants(14) and on intact plants(9) can be reduced or eliminated by inhibitors of ethylene synthesis or action. Exposure to low temperature (1 to 5^{o}C) before or during shipping delays petal abscission(5).

In this investigation several chemicals reported to affect abscission or senescence processes in various species(2,6,9,12,17,19, 23,25) were tested on intact 'Sprinter Scarlet' geranium plants. The concentrations used were based on dose/response experiments using floret explants(14). The effects of solutions of Ag^{+} salts when either sprayed on plants held at several temperatures or applied as soil drench were also investigated.

Materials and Methods

General. 'Sprinter Scarlet' geranium plants were grown from seeds germinated under mist and transplanted to 15.2 cm diameter clay pots containing a commercial peat-lite mix. Plants were grown in greenhouse at 15^{o}C night and 21^{o} day temperature under natural light conditions through out the experimental period, except in experiment V when high pressure sodium illumination (HPS) was used from transplanting until treatment. Plants were watered as needed with a water soluble fertilizer at 200 ppm in N of 20N-8.7P-16.7K. Chemicals were applied with a hand sprayer at a rate of approximately 10 ml per plant. Several stages of floret development were recognized in each experiment.

Stage 0 was used to designate florets that were open when the treatments were applied. Florets that opened thereafter were designated as 1, 2, etc. with reference to the time (days) of treatment prior to flower opening. Flower opening was identified as the stage when the pistil and stamens were first visible. At intervals after flower opening, each pot was affixed to the central plate of a Burrel^{R} side-arm shaker and shaken for 1 minute at moderate speed. After shaking, the numbers of open flowers, flowers with at least one petal shattered, and total number of petals shattered were recorded. Percent of petal abscission was recorded for each flower stage. Time was defined as number of days after treatment for stage 0 and overall petal abscission (T), or after flower opening for other stages. "T" designated the total cumulative petal abscission for all open flowers, at a given time from the beginning of the experiment. Since inflorescences were not excised, it was possible to observe the sequential effect of the treatments on all flower stages. Treatments were arranged in a randomized complete block design with 3 blocks. Plants were blocked according to uniformity of flowers in stage 0. Two plants were used per experimental unit. Square root data transformation was done before statistical analysis as the Bartlet's test for homogeneity of variances was significant when data were not transformed.

Experiments I, II and III. Twelve compound were sprayed on 'Sprinter Scarlet' geraniums. Insufficient plants were available to test all compounds at once; therefore 3 experiments were performed as follows:

Experiment I: SN at 100 ppm; AVG at 200 ppm; STS at 25 ppm in $AgNO_3$; and $CaCl_2$ at $6.8x10^{-2}M$.

Experiment II: GA 4/7 at 20 ppm; promalin at 100 ppm; 3-CPPA at 100 ppm; and NAA at 10 ppm.

Experiment III: N6-BA at 30 ppm; CHI at 10^{-4}M; 8-HQS at 400 ppm; and $Ca(NO_3)_2$ at 6.8×10^{-2}M.

Plants used as controls in each experiment were sprayed with deionized water or diluted solutions of ethanol(14). Percent of petal abscission over time and flower quality were recorded.

Experiment IV. To determine the efficiency of chemical control of abscission under adverse environmental conditions, plants treated with SN at 0 and 100 ppm were placed inside large cardboard boxes, which were closed and held inside growth chambers at 15, 20, 25 or 30°C for various time periods. At the end of each period plants were shaken and percent abscission was determined.

Experiment V. Since silver salts were the most promising treatment for the practical control of petal abscission, another experiment was conducted using SN, STS and the water control, applied as a spray or soil drench(200 ml per plant). The concentration of STS was increased to 50 ppm in $AgNO_3$. In order to evaluate the long term effect of the treatments, data was taken over a longer period than in the previous experiments.

Results

Experiment I. Stage 0 flowers reached at least 50% petal abscission 6 days after flower opening in all treatments except AVG and SN (Table 1). STS-treated plants showed less petal abscission than control or $CaCl_2$-treated plants, but SN and AVG were more effective (Table 1). Effects on flowers treated in stages 1, 3 and 4 were similar except that STS did not reduce petal abscission on flowers that had

been open for more than 8 days (Table 1). Overall petal abscission (stage T) was effectively inhibited by AVG, SN and STS for 2 to 10 days after flower opening (Table 1). $CaCl_2$ appeared to be effective during the initial days after flower opening but did not differ from the control treatment after 4 days, except for stage 4 after 6 days (Table 1). Flower quality parameters were not affected by any of the compounds tested, except that phytotoxic symptoms were observed in stage 0 flowers of plants treated with AVG, SN and $CaCl_2$ (Table 2).

Experiment II. GA4/7 inhibited petal abscission when sprayed on flowers at any stage and was effective in the control of overall petal abscission (Table 3). However, GA-treated plants showed excessive peduncle elongation in the second inflorescence, and excessive pedicel elongation on flowers of the first inflorescence (Table 4). Promalin also inhibited abscission when applied on flowers in stages 3 and 4 (Table 3), but caused severe phytotoxicity on flowers and leaves (Table 4). Neither 3-CPPA nor NAA affected petal abscission or flower quality significantly (Tables 3 and 4).

Experiment III. None of the compounds tested in this experiment delayed petal abscission significantly (Table 5). However, abscission was slightly inhibited by cycloheximide during the first 6 days after flower opening (Table 5). N6-BA, 8-HQS, and $Ca(NO_3)_2$ appeared to promote abscission in stage 0 flowers after 6 days, but no other effects were significant (Table 5). Flower characteristics were also not affected by chemicals tested in this experiment, except that CHI and $Ca(NO_3)_2$ caused phytotoxic symptoms on petals and leaves (Table 6).

Experiment IV. SN was effective in delaying petal abscission in flowers of all stages and at all temperatures tested, except when

held for 12 days at 30°C (Table 7). Overall petal abscission increased with temperature, but SN treatment was much more effective than low temperature alone (Table 7).

Experiment V. STS sprayed at 50 ppm in $AgNO_3$, was as effective as 100 ppm SN in the control of petal abscission (Table 8). However, drench treatments were rarely effective. Both SN and STS treated plants abscised less than 10% of their petals from flowers at stages 4 and 6 during at least 22 days following treatment (Table 8). Stage 0 flowers treated with SN and STS showed a faster petal abscission than younger flowers (Table 8). Only at 20 days after flower opening did petals of stage 2 flowers begin to abscise; inhibition of petal abscission was more effective when flowers were treated prior to opening (Table 8). SN induced toxic symptoms on petals of stage 0 flowers, but STS did not.

Discussion

Inhibition of petal abscission in explants of hybrid geraniums by AVG, silver nitrate and silver thiosulfate has been reported(14). AVG inhibits ethylene synthesis(24) and Ag^+ inhibits ethylene action (6). SN(50 or 100 ppm) delayed petal abscission in some geranium cultivars when sprayed on intact plants(9), but caused severe phytotoxic symptoms when applied to open flowers (Table 1). In experiment I STS was less effective than SN in controlling petal abscission (Table 1) because of the low concentration (25 ppm) of $AgNO_3$ used in the STS complex. When STS was applied at 50 ppm in $AgNO_3$ it was as effective as SN alone at 100 ppm (Table 8) and was not phytotoxic. Absorption, translocation, and therefore availability of cations is greatly increased upon chelation of the metal in an anionic complex(8,9,13), thus STS can be as effective as SN (2) at a lower Ag^+ concentration. Calcium treatment

delays abscission(19) for calcium acts as the cementing substance linking pectin polymers within the middle lamella(18). However, treatment with Ca^{2+} salts was not effective in delaying petal abscission in intact plants (Tables 1 and 5). The inhibitory effects of gibberellin on abscission may result from alteration of the hormonal balance which affects cell sensitivity to ethylene(16). GA 4/7 caused a delay in petal abscission in both explants(14) and intact plants(Table 3), but also induced excessive peduncle elongation in intact plants(Table 4). Stimulation of peduncle elongation by GA treatment in intact geranium plants(12) is undesirable because the weight of the inflorescence bends the long, thin peduncle. The failure of auxin to inhibit petal abscission when applied at several stages of flower development (Table 3) does not negate its role in the abscission process (e.g. 3), for it could be acting during flower organogenesis. The two stage hypothesis proposed for leaf abscission(20) may also be valid for petal abscission; however, at flower opening the petal seem to already be in stage II(14). An extremely high NAA concentration (100 ppm) caused a strong epinastic response on florets and entire inflorescences (data not shown), which has been associated with the auxin-promoted ethylene synthesis(1,11). As little as 0.1 ppm exogenous ethylene accelerates petal abscission in geranium explants(14). High temperature ($30^{o}C$) overcame the effect of SN in inhibiting petal abscission (Table 7), presumably because of accumulation of endogenous ethylene(11) and high metabolic activity(7). Control plants generally showed less petal abscission at 15^{o} than at higher temperatures, as low temperatures delay abscission(5).

AVG, SN, STS and GA 4/7 were effective in controlling or inhib-

iting petal abscission. However, GA 4/7 caused undesirable peduncle elongation, and AVG and SN caused severe phytotoxicity on petals of flowers that were open at the time of treatment. Therefore STS appears to be the best compound to control petal abscission in hybrid geraniums. Cool temperatures (1 to 5^{o}C) during transport effectively inhibit petal abscission(5); however, this is more expensive and less practical than a single spray treatment with STS.

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Table 1. Effects of SN(100 ppm), AVG(200 ppm), STS(25 ppm in $AgNO_3$), and $CaCl_2$($6.8x10^{-2}$M) on petal abscission of 'Sprinter Scarlet' geraniums.

Flower stage at treatment[y]	Chemical treatment	% petal abscission[z] Days from flower opening 2	4	6	8	10
0	control	46 a	97 a	100 a	100 a	100 a
	SN	14 b	21 c	30 cd	31 bc	32 c
	AVG	8 bc	8 c	15 d	19 c	26 c
	STS	38 ab	45 bc	57 bc	58 b	64 b
	$CaCl_2$	3 c	68 ab	90 ab	98 a	100 a
1	control	20 a	65 a	90 a	100 a	100 a
	SN	1 b	8 b	10 c	11 b	20 b
	AVG	2 b	14 b	23 bc	34 b	45 b
	STS	3 b	23 b	58 ab	75 a	98 a
	$CaCl_2$	1 b	34 ab	80 a	96 a	100 a
3	control	8 a	31	97 a	100 a	100 a
	SN	0 b	0	1 b	9 b	10 c
	AVG	0 b	9	13 b	33 b	38 b
	STS	0 b	5	25 b	76 a	97 a
	$CaCl_2$	0 b	16	87 a	100 a	100 a
4	control	0	60 a	99 a	100 a	100 a
	SN	0	0 b	2 c	5 c	8 b
	AVG	0	5 b	12 c	19 bc	23 b
	STS	0	0 b	12 c	42 b	88 a
	$CaCl_2$	0	2 b	66 b	86 a	100 a
T	control	29 a	39 a	33 a	38 a	52 a
	SN	8 bc	9 b	7 b	6 c	8 b
	AVG	6 bc	6 b	9 b	6 c	16 b
	STS	15 b	14 b	15 b	20 b	25 b
	$CaCl_2$	1 c	14 b	21 ab	29 ab	45 a

[z]Mean separation within column, for each stage, by Duncan's Multiple Range test (5%); if differences were not significant, letters were not used.

[y]Days relative to flower opening: 0 = flower opening; 1, 3 or 4 = 1, 3 or 4 days prior to flower opening. "T" represents total or overall petal abscission at a given time.

Table 2. Effects of SN, AVG, STS and $CaCl_2$ on flower quality of 'Sprinter Scarlet' geraniums. Data recorded 10 days after treatment.

Chemical treatment	Phytotoxic symptoms[z]	Floret size[y] (cm)	Pedicel length[y] (cm)	1st peduncle length[y](cm)	2nd peduncle length[y](cm)	Floret stage in 2nd inflorescence[y,x]
control	no	3.8	2.8	12.4	12.9	5.0
SN	yes	3.9	2.9	12.7	13.7	4.6
AVG	yes	4.0	2.8	14.0	13.6	5.0
STS	no	4.0	2.7	12.5	12.2	6.0
$CaCl_2$	yes	4.0	2.8	13.1	12.7	4.0

[z](SN) white spots with black center on petals of stage 0 florets; (AVG) gray spots with white center on petals of stage 0 florets;($CaCl_2$) bright whitish spot on petals.

[y]Differences among means were not significant by F test(5%).

[x]Flower stages: (1) green bud in closed inflorescence, (2) green bud, (3) pink bud, (4) beginning of red coloration, (5) red bud, (6) pedicel upright, petals starting to open, (7) open flower.

Table 3. Effects of GA 4/7 (20 ppm), promalin (100 ppm), 3-CPPA(100 ppm), and NAA (10 ppm) on petal abscission of 'Sprinter Scarlet' geraniums.

Flower stage at treatment[y]	Chemical treatment	% petal abscission[z] Days from flower opening 2	4	6	8	10
0	control	10	51	70 a	85 a	100 a
	GA 4/7	13	20	23 b	33 b	36 b
	Promalin	50	78	91 a	100 a	100 a
	3-CPPA	30	46	61 a	80 a	100 a
	NAA	46	70	73 a	83 a	100 a
1	control	0	0	44 a	96 a	100 a
	GA 4/7	0	0	3 b	30 b	40 b
	Promalin	6	10	56 a	80 a	83 ab
	3-CPPA	0	0	25 ab	87 a	100 a
	NAA	0	0	36 a	83 a	100 a
3	control	0	8	48 a	88 a	95 a
	GA 4/7	0	0	0 b	2 b	18 b
	Promalin	2	6	8 b	10 b	15 b
	3-CPPA	0	5	48 a	79 a	96 a
	NAA	0	16	54 a	81 a	100 a
4	control	0	16	70 a	90 a	93 a
	GA 4/7	0	0	0 b	0 b	14 b
	Promalin	7	11	13 b	16 b	28 b
	3-CPPA	0	11	50 a	73 a	95 a
	NAA	1	17	58 a	100 a	100 a
T	control	1	13	12 b	24 a	46 a
	GA 4/7	3	2	5 b	5 b	8 c
	Promalin	22	19	26 a	25 a	26 b
	3-CPPA	18	7	9 b	16 ab	38 ab
	NAA	12	13	13 b	22 a	46 a

[z]Mean separation within column, for each stage, by Duncan's Multiple Range test (5%); if differences were not significant, letters were not used.

[y]Days relative to flower opening: 0 = flower opening; 1, 3 or 4 = 1, 3 or 4 days prior to flower opening. "T" represents total or overall petal abscission at a given time.

Table 4. Effects of GA 4/7, promalin, 3-CPPA, and NAA on flower quality of 'Sprinter Scarlet' geraniums.Data recorded 10 days after treatment.

Chemical treatment	Phytotoxic[z] symptoms	Floret[y] size (cm)	Pedicel[y] length (cm)	1st[y] peduncle length(cm)	2nd[y] peduncle length(cm)	Floret stage[y,x] in 2nd inflorescence
control	no	3.9 b	2.6 b	13.4	12.0 b	6.7
GA 4/7	yes	4.3 a	3.3 a	13.1	17.0 a	5.0
Promalin	yes	3.7 b	2.8 b	12.6	12.4 b	2.4
3-CPPA	no	3.9 b	2.7 b	12.5	11.5 b	5.0
NAA	no	3.9 b	2.5 b	12.8	10.5 b	3.0

[z](GA 4/7) faded flower color; (promalin) brown spots on flowers and leaves.

[y]Mean separation within columns by Duncan's Multiple Range test (5%); if differences were not significant, letters were not used.

[x]Flower stages: (1) green bud in closed inflorescence, (2) green bud, (3) pink bud, (4) beginning of red coloration, (5) red bud, (6) pedicel upright, petals starting to open, (7) open flower.

Table 5. Effects of N6-BA (30 ppm), CHI (10^{-4}M), 8-HQS (400 ppm), and $Ca(NO_3)_2$ ($6.8x10^{-2}$M) on petal abscission of 'Sprinter Scarlet' geraniums.

Flower[y] stage at treatment	Chemical treatment	% petal abscission[z] Days from flower opening 2	4	6	8	10
0	control	0	3	33 b	96	96
	N6-BA	3	10	70 a	90	100
	CHI	0	0	20 b	73	100
	8-HQS	0	11	66 a	86	100
	$Ca(NO_3)_2$	0	0	73 a	83	100
1	control	0	10	60 a	93	100
	N6-BA	0	20	65 a	100	100
	CHI	0	0	39 b	98	100
	8-HQS	0	23	60 a	90	100
	$Ca(NO_3)_2$	0	0	63 a	80	100
3	control	0	17	66	95	100
	N6-BA	0	26	68	98	100
	CHI	0	5	39	97	100
	8-HQS	0	3	56	100	100
	$Ca(NO_3)_2$	0	20	49	99	100
4	control	10	20	74	88	100
	N6-BA	0	6	38	80	100
	CHI	0	4	41	88	100
	8-HQS	0	6	38	90	100
	$Ca(NO_3)_2$	0	6	32	83	100
T	control	0	0	12 a	23	42
	N6-BA	1	2	16 a	30	41
	CHI	0	0	2 b	25	42
	8-HQS	0	2	17 a	26	41
	$Ca(N)_3)_2$	0	0	11 a	25	45

[z]Mean separation within columns, for each flower stage, by Duncan's Multiple Range test (5%); if differences are not significant, letters were not used.

[y]Days relative to flower opening: 0 = flower opening; 1, 3 or 4 = 1, 3 or 4 days prior to flower opening. "T" represents total or overall petal abscission at a given time.

Table 6. Effects of N6-BA, CHI, 8-HQS, and $Ca(NO_3)_2$ on flower quality of 'Sprinter Scarlet' geraniums. Data recorded 10 days after treatment.

Chemical treatment	Phytotoxic[z] symptoms	Floret[y] size (cm)	Floret[y] length (cm)	1st[y] peduncle length(cm)	2nd[y] peduncle length(cm)	Floret stage[y,x] in 2nd inflorescence
control	no	3.8	2.8	12.6	11.3	3.6
N6-BA	no	3.8	2.5	13.5	11.7	3.6
CHI	yes	3.7	2.5	13.4	10.0	3.0
8-HQS	no	3.7	2.3	13.3	11.3	3.0
$Ca(NO_3)_2$	yes	3.8	2.5	13.4	10.6	2.9

[z](CHI) gray spots on petals; ($Ca(NO_3)_2$) bright white spots on petals and brown spots on leaves.

[y]Differences among means are not significant by F test.

[x]Flower stages: (1) green bud in closed inflorescence, (2) green bud, (3) pink bud, (4) beginning of red coloration, (5) red bud, (6) pedicel upright, petals starting to open, (7) open flower.

Table 7. Effects of temperature on SN inhibition of petal abscission in 'Sprinter Scarlet' geraniums.

Flower[z] stage at treatment	Temp. (°C)	% petal abscission, Days from flower opening: 2			4			8			12		
		H_2O	SN	F[y]	H_2O	SN	F[y]	H_2O	SN	F[y]	H_2O	SN	F[y]
0	15	0	0	NS	0	3	NS	93	13	**	100	13	**
	20	3	0	NS	53	0	**	100	10	**	100	10	**
	25	0	0	NS	44	0	**	100	0	**	100	26	**
	30	0	0	NS	66	0	**	100	13	**	100	91	NS
1	15	0	0	NS	26	0	*	100	0	**	100	0	**
	20	0	0	NS	26	0	*	86	0	**	100	0	**
	25	0	0	NS	50	0	**	73	0	**	100	16	**
	30	0	0	NS	100	0	**	100	33	*	100	84	NS
2	15	0	0	NS	0	0	NS	40	0	**	86	0	**
	20	0	0	NS	11	0	NS	66	0	**	100	0	**
	25	0	0	NS	29	0	*	100	0	**	100	0	**
	30	20	0	*	100	0	**	100	0	**	100	73	**
T	15	0	0	NS	9	4	NS	22	2	*	28	1	*
	20	2	0	NS	11	0	*	45	2	**	53	2	**
	25	0	0	NS	11	0	*	50	0	**	61	4	**
	30	0	0	NS	21	0	*	59	2	**	75	48	NS

[z]Days relative to flower opening: 0 = flower opening; 1 or 2 = 1 or 2 days prior to flower opening. "T" represents total or overall petal abscission at a given time.

[y]NS = not significant; * = significant at 5% level; ** significant at 1% level.

Table 8. Long term effects of SN (100 ppm) and STS (50 ppm in $AgNO_3$), applied as spray or soil drench, on petal abscission of 'Sprinter Scarlet' geraniums.

Flower stage	Application method	Chemical treatment	% petal abscission, Days from flower opening: 2	4	8	14	18	27
0		control	31 a	100 a	100 a	100 a	100 a	100
	spray	SN[z]	8 b	20 b	23 b	28 c	77 b	100
		STS	5 b	8 b	20 b	50 b	93 a	100
	drench	SN	33 a	98 a	100 a	100 a	100 a	100
		STS	41 a	85 a	100 a	100 a	100 a	100
2		control	10 a	42 a	100 a	100 a	100 a	100
	spray	SN	0 b	0 b	0 b	0 b	32 b	y
		STS	0 b	0 b	0 b	0 b	30 b	y
	drench	SN	0 b	52 a	100 a	100 a	100 a	100
		STS	0 b	41 a	100 a	100 a	100 a	100
4		control	0	38 a	100 a	100 a	100 a	100
	spray	SN	0	0 c	0 c	0 b	9 b	y
		STS	0	0 c	0 c	0 b	8 b	y
	drench	SN	0	39 a	93 a	100 a	100 a	100
		STS	0	18 b	76 b	100 a	100 a	100
6		control	0	48 a	100 a	100 a	100 a	100
	spray	SN	0	0 c	0 b	0 b	4 b	y
		STS	0	0 c	0 b	2 b	4 b	y
	drench	SN	0	15 b	87 a	93 a	100 a	100
		STS	0	13 b	94 a	100 a	100 a	100
T		control	13 a	32 a	44 a	59 a	75 a	96 a
	spray	SN	4 b	5 b	4 b	2 b	7 b	51 b
		STS	2 b	2 b	3 b	3 b	9 b	39 b
	drench	SN	16 a	46 a	41 a	61 a	65 a	88 a
		STS	19 a	26 a	32 a	48 a	71 a	91 a

[abc]Mean separation within columns, for each flower stage, by Duncan's Multiple Range test (5%); if differences are not significant, letters were not used.

[z]Dark spots on petal.

[y]Flower fading.