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Role of Cytokinins in Carnation Flower Senescence[1]

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ABSTRACT

Stem and leaf tissues of carnation (*Dianthus caryophyllus*) plants appear to contain a natural antisenescence factor since removal of most of these tissues from cut carnation flowers hastened their senescence. However, kinetin (5–10 μg/ml) significantly delayed senescence of flowers with stem and leaf tissues removed. In addition, the life span of cut flowers with intact (30-cm) stems was increased with kinetin treatment. Peak ethylene production by presenescent flowers was reduced 55% or more with kinetin treatment and was delayed by 1 day. Kinetin-treated flowers were less responsive to applied ethylene (100 μl/l for 3 hours) than untreated flowers. Possible natural roles of cytokinins in carnation flower senescence are discussed.

Cytokinins are known to defer leaf senescence (18) and improve the keeping qualities of cut carnation (6, 11) and rose flowers (12). MacLean and Dedolph (11) reported a decrease in the respiration rate of cytokinin-treated flowers and proposed that cytokinins increase flower longevity as a result of this reduction in respiration. However, Heide and Oydvin (6) found only small and inconsistent effects of cytokinins on the respiration rate of cut carnation flowers. They concluded that processes other than respiration mediate the cytokinin retardation of senescence. Mayak and Halevy (14) have shown that kinetin increases net water uptake of expanding rose petals and delays wilting of petals especially when flowers are subjected to heat (28 C) and low relative humidity (40–50%). Kinetin had no effect on protein content of rose petals under these stressful conditions, but kinetin retarded the increase in RNase activity normally seen in rose flowers at the onset of senescence. Kinetin is proposed to increase rose flower longevity by improving water balance and delaying senescence processes.

Ethylene gas has been proposed as the natural regulator of flower senescence (1, 16). Older flowers spontaneously release a large burst of ethylene which predicts the onset of senescence. Exposure of many kinds of flowers to exogenous ethylene leads to premature senescence (2, 5). Such exposure to ethylene or ethylene analogs triggers the flower to produce a burst of ethylene (1). This "autocatalytic" synthesis of ethylene is thought to be a normal part of flower senescence. Dilley *et al.* (3) have shown that if cut carnation flowers (and other cut flowers) are prevented from responding to their endogenous ethylene production by hypobaric storage, senescence can be delayed for more than 3 months. Although reductions in levels of O_2 and other volatiles may be important in the long term delay of senescence, these hypobaric studies suggest that ethylene gas is primarily responsible for the onset of senescence in cut carnation flowers.

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The results described here indicate that ethylene-induced senescence in carnation flowers may be controlled by cytokinins.

MATERIALS AND METHODS

Carnation flowers (*Dianthus caryophyllus* cv. Peterson Red) were cut at full bloom and held at 4 C for about 4 hr before treatment. Stems were cut to uniform lengths under water and flowers were held under continuous fluorescent room lighting at 21 C. The stems of the flowers were placed in jars or vials which contained deionized water or 5% (w/w) sucrose and 300 μg/ml 8-hydroxyquinoline citrate (HQC, Merck & Co., Hawthorne, N.J.). Various concentrations of kinetin were added to this mixture. Flowers were rated on the following scale: 3 = fresh (no desiccated petals), 2 = intermediate, and 1 = senescent (nearly all petals desiccated). Data are presented as averages of at least three replicate experiments each with internal controls.

Ethylene production by flowers was measured by placing six flowers with 3-cm stems in a 9-liter vacuum desiccator whose volume was reduced to 6.5 liters with inert materials. The atmosphere was sampled after 3 hr and ethylene was determined using a flame ionization gas chromatograph (Perkin-Elmer model 800) with a (90 × 0.5 cm) Porapak S (100–120 mesh, Waters Associates, Milford, Mass.) column. The desiccators remained open during the 21-hr interval between daily readings.

RESULTS

Removal of the major portion of the stem (with leaves) of cut carnation flowers hastened the onset of senescence. Flowers with 3-cm stems (leafless) had an average life span of 13 ± 2 days compared to an average life span of 17 ± 2 days for 30-cm stemmed control flowers. Removal of only the leaves from flowers with 30-cm stems reduced the life span of the flowers but to a lesser extent. However, 10 μg/ml kinetin significantly delayed the onset of senescence of both short and long stemmed flowers. The life span of 3-cm stemmed flowers was increased to 20 ± 2 days and leafless, 30-cm stemmed flowers were restored to a similar level. Equivalent kinetin treatment of flowers with intact 30-cm stems increased their average life span to 22 ± 1 day. It is important to note that little difference was seen in the average life span of long and short stemmed flowers treated with kinetin (20 ± 2 *versus* 22 ± 1 days). This kinetin treatment had no visible effects on the flowers other than increased longevity.

Kinetin was effective in extending the life span of carnation flowers at concentrations between 1 and 15 μg/ml (Fig. 1). Concentrations of 0.1 μg/ml or less had no significant effect; 5 to 10 μg/ml was optimal in nearly all experiments; and concentrations of 15 μg/ml or greater were supraoptimal. Heide and Oydvin (6) did not expose their cut carnation flowers to 6-benzylamino-purine continuously, but immersed the stems in various concentrations for varying periods of time. They observed optimal effects by immersion for 2 min at 225 μg/ml, 50 min at 22.5 μg/ml, or 12 hr at 2.25 μg/ml. Twelve-hr exposure to 225 μg/ml was found to be detrimental. Mayak and Halevy (14), using cut rose flowers under temperature and water stress,

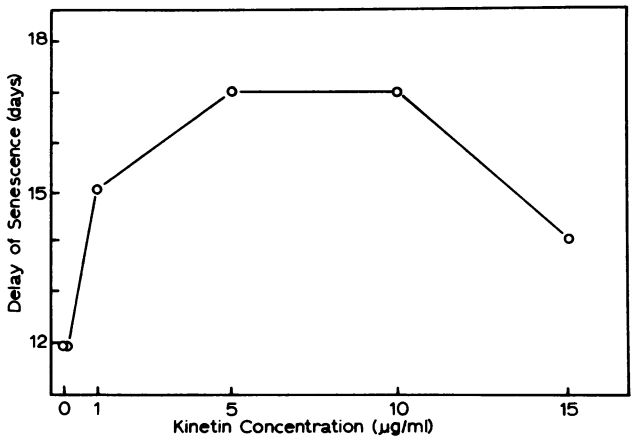

FIG. 1. Effects of various concentrations of kinetin on the delay of senescence of carnation flowers (3-cm stems). The day listed is the first the flowers averaged intermediate quality (as described in the text).

found that continuous exposure to 60 μg/ml kinetin was more effective than none or 20 μg/ml. This indicated that cut carnation flowers have very different cytokinin requirements than cut rose flowers.

Optimal concentrations of kinetin decreased the peak rate of ethylene production by the flowers and delayed the onset of that peak (Fig. 2A). Kinetin at 5 μg/ml reduced peak ethylene production by 55% and delayed the peak by 1 day. The only significant ethylene production by flowers treated with 10 μg/ml kinetin was 5 nl/3 hr on day 8. Supraoptimal kinetin concentrations (15 μg/ml or greater) induced ethylene production in excess of control flowers. Kinetin at 0.1 μg/ml had no significant effect on ethylene production. The part of effect of kinetin on ethylene production may be mediated through an alteration of auxin metabolism (9).

Control flowers began a period of rapid decline in flower quality on the day of peak ethylene production (Fig. 2B). Kinetin-treated flowers showed only a slight reduction in flower quality following the day of peak ethylene production.

The responsiveness of cut carnation flowers to applied ethylene was considerably reduced with kinetin treatment (Fig. 3). Control flowers began to decline 2 days after a 3-hr exposure to 100 μl/l ethylene in a sealed 9-liter desiccator. However, 5 μg/ml kinetin retarded this decline for 8 additional days.

DISCUSSION

Kinetin may be extending the life span of cut carnation flowers because it is replacing the natural cytokinins which are normally supplied to the flower from the parent plant. The fact that kinetin, a synthetic cytokinin, increases the life span of cut flowers, especially those with short stems (3 cm), indicates that the natural antisenescence factor in carnation flowers may be endogenous cytokinins. This is further supported by the fact that increased stem length provides no significant additive effect with kinetin-treated flowers. The stem and leaf tissues probably serve only as a reservoir since cytokinins are believed to be synthesized in the roots of plants (20).

The increased longevity of cytokinin-treated flowers might be the result of many different physiological effects of the hormone on the flower tissues. They may operate by maintaining membrane permeabilities (7), water balance (14), and/or protein and nucleic acid metabolism (17). The presence of optimal concentrations of cytokinins may also increase longevity of flowers by reducing their ethylene production and responsiveness. Research with rose flowers suggests that cytokinins play a role in natural senescence. Rose flowers show a peak in natural cytokinin levels as the flower opens, but decline as the flower ages on the parent plant (15). Also, higher natural cytokinin levels were found in more long lived rose varieties (12).

When flowers are detached from the parent plant, the natural cytokinin source (the roots) would be lost and internal levels would decline (10). This decline in endogenous cytokinin levels could serve as a trigger for senescence initiated by increased ethylene production and responsiveness by the cut flowers. Intact flowers might also use this trigger since their endogenous cytokinin levels decline with age (15). In nature, pollination often serves as the trigger for increased ethylene production (1), and as a result of this ethylene exposure, carnation flowers begin the process of senescence 2 to 4 days after pollination (16).

Using a different tissue system, Kende and Hanson (8) reported that BA delayed ethylene production by isolated rib segments of *Ipomoea tricolor* (morning-glory) flowers and the rolling up response of these rib segments. Thus, the morning-glory flowers show the same kind of delay of ethylene production as do carnation flowers in response to cytokinin treatment. The reduced responsiveness to exogenous ethylene seen with kinetin-treated carnations was not seen in the leaf rolling up response of their rib segments with BA treatment.

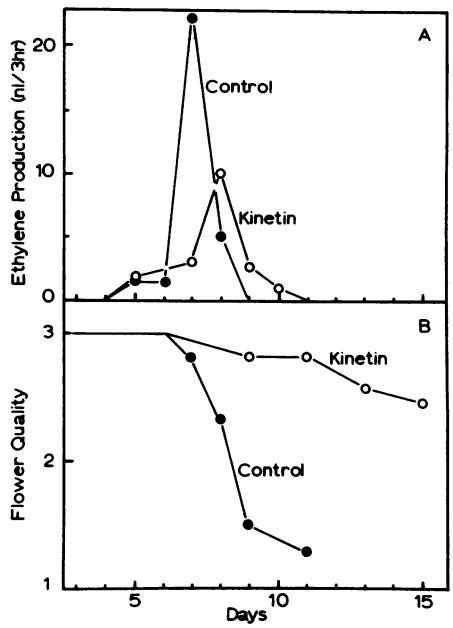

FIG. 2. Effect of kinetin (5 μg/ml) on ethylene production (per six flowers) and flower quality.

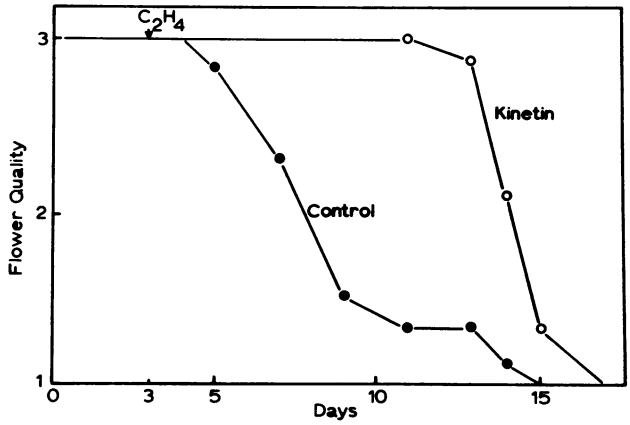

FIG. 3. Effects of kinetin (5 μg/ml) on ethylene sensitivity of cut carnation flowers (3-cm stems). Flowers were exposed to ethylene (100 μl/l) for 3 hr on day 3.

Kende and Hanson (8) found that attempts to prevent the rib segments from responding to endogenous ethylene delayed, but did not prevent, the eventual rolling up response. They concluded that ethylene does not initiate rolling up (senescence), but merely acts to accelerate it. Kende and Hanson admit that trace amounts of ethylene still produced by the segments could be responsible in the initiation of the rolling up response. The hypobaric studies by Dilley *et al.* (3) suggest that ethylene is the initiating factor in cut carnation flower senescence.

The natural role of other plant hormones in flower senescence is less clear. Abscisic acid which can induce senescence in other plant organs (4, 19) also induces senescence in rose petals (13) and in carnation flowers in my laboratory. Abscisic acid must play a secondary role in natural senescence since it increases only after peak ethylene synthesis (13), and hypobaric removal of ethylene (and other volatiles, [3]) delays the onset of senescence for months. Addition of auxin alone or in conjunction with kinetin in my laboratory had no significant effect on carnation flower longevity. Although GA_3 alone was ineffective, an apparent synergistic interaction between GA_3 and kinetin was sometimes observed. Thus, several plant hormones may play important roles in the natural regulation of flower senescence.

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LITERATURE CITED

1. Burg SP, MJ Dijkman 1967 Ethylene and auxin participation in pollen induced fading of Vanda orchid blossoms. Plant Physiol 42: 1648–1650
2. Crocker W, LI Knight 1908 Effect of illuminating gas and ethylene upon flowering carnations. Bot Gaz 46: 259–276
3. Dilley DR, WJ Carpenter, SP Burg 1975 Principles and application of hypobaric storage of cut flowers. Acta Hort 41: 249–268
4. El-Antalby HM, PF Wareing, J Hillman 1967 Some physiological responses to D,L-abscisin (dormin). Planta 73: 47–90
5. Fischer LW 1950 Ethylene gas a problem in cut flower storage. NY State Flower Growers Inc Bull Vol 61
6. Heide OM, J Oydvin 1969 Effects of 6-benzylamino-purine on the keeping quality and respiration of glasshouse carnations. Hort Res 9: 26–36
7. Kende H, B Baumgartner 1974 Regulation of aging in flowers of *Ipomoea tricolor* by ethylene. Planta 116: 279–289
8. Kende H, AD Hanson 1976 Relationship between ethylene evolution and senescence in morning-glory flower tissue. Plant Physiol 57: 523–527
9. Lau OL, SF Yang 1973 Mechanism of a synergistic effect of kinetin on auxin-induced ethylene production: suppression of auxin conjugation. Plant Physiol 51: 1011–1014
10. Livne A, Y Vaadia 1972 Water deficits and hormone relations. *In* TT Kozlowski, ed, Water Deficits and Plant Growth Vol 3. Academic Press, New York pp 255–276
11. MacLean DC, RR Dedolph 1962 Effects of N^6-benzylaminopurine on postharvest respiration of *Chrysanthemum morifolium* and *Dianthus caryophyllus*. Bot Gaz 124: 20–21
12. Mayak S, AH Halevy 1970 Cytokinin activity in rose petals and its relation to senescence. Plant Physiol 46: 497–499
13. Mayak S, AH Halevy 1972 Interrelationships of ethylene and abscisic acid in the control of rose petal senescence. Plant Physiol 50: 341–346
14. Mayak S, AH Halevy 1974 The action of kinetin in improving the water balance and delaying senescence processes of cut rose flowers. Plant Physiol 32: 330–336
15. Mayak S, AH Halevy, M Katz 1972 Correlative changes in phytohormones in relation to senescence processes in rose petals. Physiol Plant 27: 1–4
16. Nichols R 1966 Ethylene production during senescence of flowers. J Hort Sci 41: 279–290
17. Osborne D 1962 Effects of kinetin on protein and nucleic acid metabolism in *Xanthium* leaves during senescence. Plant Physiol 37: 595–602
18. Richmond A, A Lang 1957 Effect of kinetin on protein content and survival of detached *Xanthium* leaves. Science 125: 650–651
19. Sankhala N, D Sankhala 1968 Abscisin II-kinetin interaction in leaf senescence. Experientia 24: 294–295
20. Weiss C, Y Vaadia 1965 Kinetin activity in root apices of sunflower plants. Life Sci 4: 1323–1326