Role of Cytokininins in Carnation Flower Senescence

WILLIAM EISINGER
Department of Biology, University of Santa Clara, Santa Clara, California 95053

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.

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 1.0 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 Odvind (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,
found that continuous exposure to 60 \( \mu g/ml \) kinetin was more effective than none or 20 \( \mu g/ml \). 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 \( \mu g/ml \) reduced peak ethylene production by 55% and delayed the peak by 1 day. The only significant ethylene production by flowers treated with 10 \( \mu g/ml \) kinetin was 5 nl/3 hr on day 8. Supraoptimal kinetin concentrations (15 \( \mu g/ml \) or greater) induced ethylene production in excess of control flowers. Kinetin at 0.1 \( \mu g/ml \) 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 \( \mu l/l \) ethylene in a sealed 9-liter desiccator. However, 5 \( \mu g/ml \) 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. In tact 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.
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 GA3 alone was ineffective, an apparent synergistic interaction between GA3 and kinetin was sometimes observed. Thus, several plant hormones may play important roles in the natural regulation of flower senescence.

Acknowledgment—The technical assistance of D. Iaconis, T. Huston, N. Huber, and L. Loftus is gratefully acknowledged. Many of the flowers used in these experiments were gifts of Silver Terrace Nursery of South San Francisco, Calif. I am grateful to S. P. Burg, University of Miami, for his critical reading of the manuscript.

LITERATURE CITED


5. Fischer LW 1950 Ethylene gas a problem in cut flower storage. NY State Flower Growers Inc Bull Vol 61


