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Effects of Methyl Jasmonate on Anthocyanin Accumulation, Ethylene Production, and CO_2 Evolution in Uncooled and Cooled Tulip Bulbs

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Abstract. Effects of methyl jasmonate (JA-Me) on anthocyanin accumulation, ethylene production, and CO_2 evolution in uncooled and cooled tulips (*Tulipa gesneriana* L. cvs. Apeldoorn and Gudoshnik) were studied. JA-Me stimulated anthocyanin accumulation in stems and leaves from uncooled and cooled bulbs of both cultivars. The highest level of anthocyanin accumulation was observed in leaves from cooled bulbs treated with 200 μL/liter JA-Me. In sprouting bulbs treated with 100 μL/liter and higher concentrations of JA-Me, the ethylene production began to increase at 3 days after treatment, being extremely greater in uncooled bulbs than in cooled ones. JA-Me also stimulated CO_2 evolution in both cultivars, depending on its concentrations. CO_2 evolution in sprouting bulbs was not affected by cooling treatment. These results suggest that anthocyanin accumulation by JA-Me in tulip leaves is not related to ethylene production stimulated by JA-Me.

Key Words. Anthocyanin—Bulbs—CO_2—Ethylene—Methyl jasmonate—Tulip

Induction of a wide spectrum of secondary metabolites by jasmonates, mainly by methyl jasmonate (JA-Me), was documented not only in many cell suspension cultures (Beerhues and Berger 1995, Dittrich et al. 1992, Gundlach et al. 1992, Mizukami et al. 1993, Nojiri et al. 1996, Urbanek et al. 1996), but also in differentiated plants (Aerts et al. 1994, Baldwin et al. 1994, Bodnaryk 1994, Doughty et al. 1995). JA-Me has already been reported to stimulate anthocyanin accumulation in hypocotyls of light-grown seedlings of soybean (Franceschi and Grimes 1991), in leaves of seedlings of a wild-type of *Arabidopsis* (Feys et al. 1994), and in detached corollas of *Petunia* (Tamari et al. 1995). Moreover, JA-Me caused a rapid and intense senescence of leaves and induced gum formation in bulbs, stems, and the basal part of the leaf in tulips (Saniewski 1989, Saniewski and Puchalski 1988, Saniewski et al. 1997). Saniewski and Węgrzynowicz-Lesiak (1994) also showed that JA-Me stimulated ethylene evolution and 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity during gum induction in stems of tulips. However, the relationships between anthocyanin accumulation and ethylene production in tulips are not clear yet. In this paper we report that JA-Me substantially promoted anthocyanin accumulation, ethylene production, and CO_2 evolution in uncooled and cooled tulips of cvs. Gudoshnik and Apeldoorn. The interactions of JA-Me and ethylene on anthocyanin accumulation in tulips are also discussed.

Materials and Methods

Experiment A

Tulip bulbs cv. Gudoshnik were dry cooled at 5°C and then planted individually in pots and cultivated at 17–20°C under natural light conditions from January to February. When the first (basal) internode was fully elongated, the middle part of it was treated with 0.5% JA-Me (w/w) or 1.0% (w/w) in lanolin paste (50–80 mg) as a ring, about 5 mm in width, around the stem. Untreated plants and plants treated with lanolin paste only were used as a control. Ten plants/treatment were used. The content of anthocyanin was determined in 2-cm-long segments of the stem above and below the place of treatment at 6 days after treatment.

Experiment B

Tulip bulbs of a circumference of 11–12 cm of cvs. Gudoshnik and Apeldoorn were stored at 18–20°C until October 15 after lifting. One group of the bulbs was kept at 17°C (uncooled bulbs), and another

Abbreviations: JA-Me, methyl jasmonate; ACC, 1-aminocyclopropane-1-carboxylic acid.

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Fig. 1. JA-Me induced anthocyanin accumulation in tulip stem cv. Gudoshnik; *left*, control; *right*, 0.5% JA-Me.

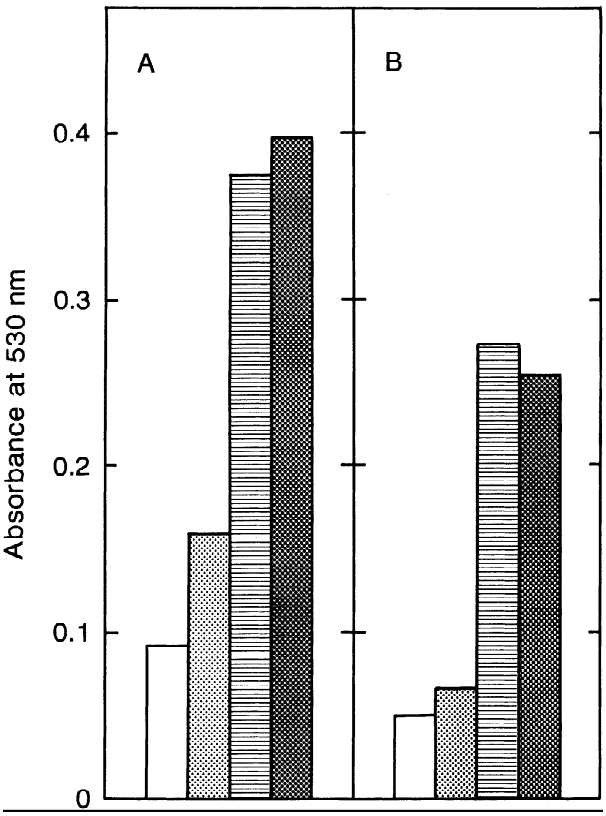

Fig. 2. Effect of JA-Me on anthocyanin accumulation in tulip stem cv. Gudoshnik. *A* (*left*), below the treated area; *B* (*right*), above the treated area. □, control; ▨, control (lanolin paste); ▤, 0.5% JA-Me; ▩, 1.0% JA-Me.

Fig. 3. JA-Me-induced anthocyanin accumulation in leaves of uncooled (*upper row*) and cooled (*lower row*) of tulip bulbs cv. Apeldoorn. From *left* to *right*, control (water), JA-Me at 10, 50, 100, and 200 μL/liter.

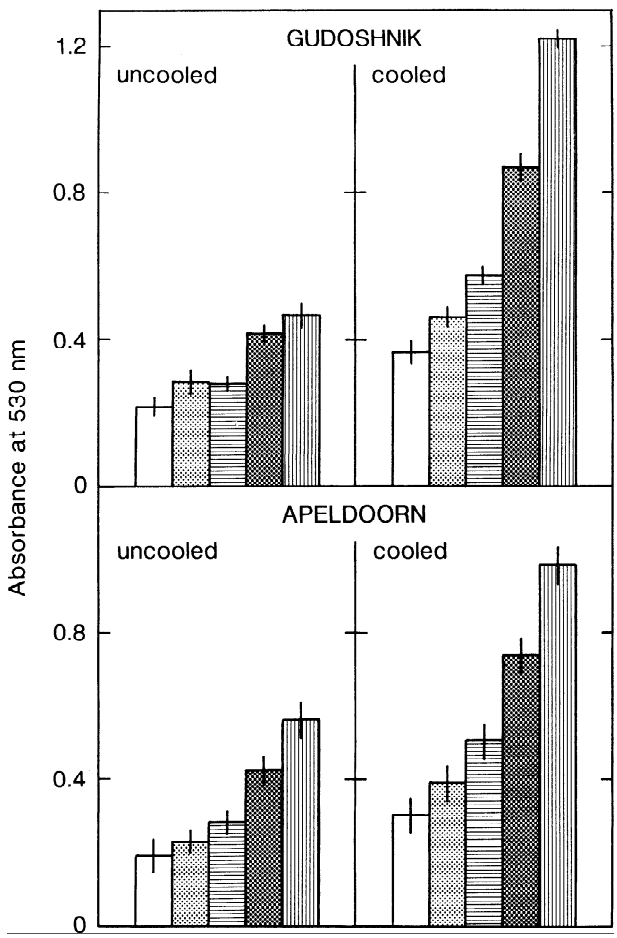

Fig. 4. Effect of JA-Me on anthocyanin level in tulip leaves. □, control; ▨, JA-Me at 10 μL/liter; ▤, JA-Me at 50 μL/liter; ▩, JA-ME at 100 μL/liter; ▥, JA-Me at 200 μL/liter.

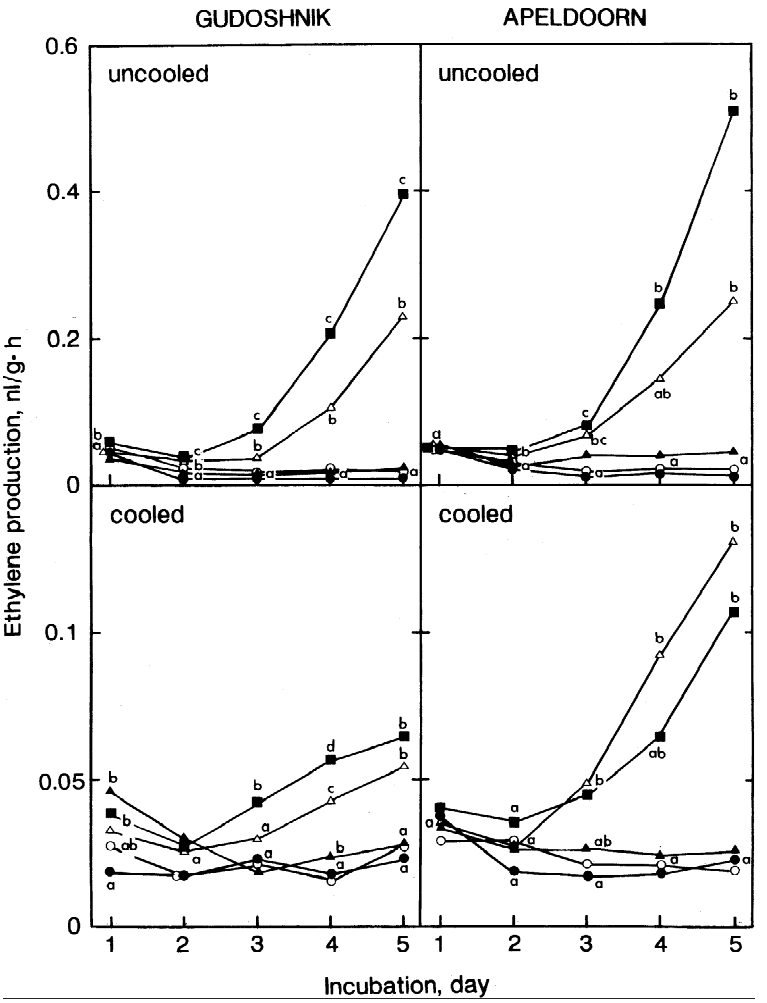

Fig. 5. Effect of JA-Me on ethylene production in tulip bulbs. Means followed by the same letter are not significantly different (5%) according to the Duncan *t*-test. Values are calculated separately for each cultivar, for uncooled and cooled bulbs, and for each day. ●, control; ○, JA-Me at 10 μL/liter; ▲, JA-Me at 50 μL/liter; △, JA-Me at 100 μL/liter; ■, JA-Me at 200 μL/liter.

group was transferred to 5°C for dry cooling (cooled bulbs). On January 22 of the next year all of the roots in uncooled and cooled bulbs were removed, and then bulbs were placed in 5-liter jars with 150 mL of water (control) and 10, 50, 100 or 200 μL/liter concentrations of JA-Me. Each treatment had four replications of seven bulbs/jar. Jars were sealed tightly, and ethylene and CO_2 evolutions were measured daily for 5 days after treatment. Leaves were harvested at 6 days after treatment for the determination of anthocyanin content. Jars were ventilated for 2 h every day after gas sampling. Lyophilized leaves were macerated, and anthocyanins were extracted at 2°C in 1% HCl in methanol for 24 h. The content of anthocyanin was determined spectrophotometrically at 530 nm. Ethylene was determined using gas chromatography, and CO_2 was analyzed using infrared ADC gas analyzer (Miszczak et al. 1995).

The Duncan *t*-test was used to estimate the difference between means at a 5% level of significance.

Results and Discussion

JA-Me stimulated anthocyanin accumulation in the stem both above and below the place of the treatment (Figs. 1 and 2) and in leaves from the bulbs (Figs. 3 and 4). The highest level of anthocyanin accumulation was observed in leaves from cooled bulbs treated with 200 μL/liter JA-Me. High concentrations of JA-Me (100 and 200 μL/liter) increased ethylene production in sprouting bulbs of both cultivars, although lower concentrations of it (10 and 50 μL/liter) did not. The stimulating effect of JA-Me on ethylene production was much greater in uncooled bulbs than cooled ones (Fig. 5). The stimulatory effects of JA-Me on anthocyanin formation have already been found in other plants as well. JA-Me induced anthocyanin accumulation in a wild-type of *Arabidopsis* (Feys et al. 1994) and in hypocotyls of light-grown seedlings of soybean after 5 days, but anthocyanin accumulation was inhibited by about 50% in etiolated ones (Franceschi and Grimes 1991). In this case, the changes in anthocyanin accumulation in soybean hypocotyl after treatment with JA-Me were not accompanied by an increase of ethylene production, similar to the results in this experiment. According to the results in this study together with the fact that JA-Me could induce chalcone synthase and dihydroflavonol-4-reductase gene expression in detached co-

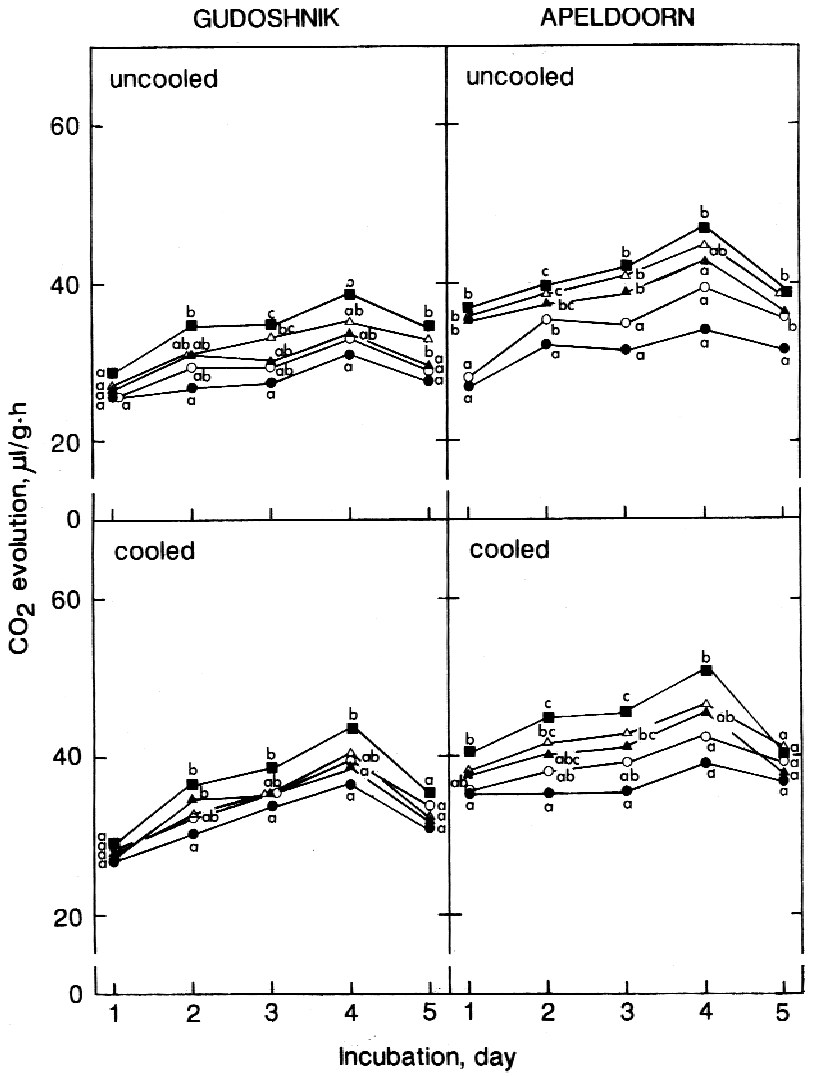

Fig. 6. Effect of JA-Me on CO_2 evolution in tulip bulbs. Means followed by the same letter are not significantly different (5%) according to the Duncan *t*-test. Values are calculated separately for each cultivar, for uncooled and cooled bulbs, and for each day. ●, control; ○, JA-Me at 10 µL/liter; ▲, JA-Me at 50 µL/liter; △, JA-Me at 100 µL/liter; ■, JA-Me at 200 µL/liter.

rollas of *Petunia* (Tamari et al. 1995), anthocyanin accumulation in tulip leaves might depend on the gene expressions of enzymes related to its biosynthesis.

It has been shown previously that JA-Me increases CO_2 production, independent of the stimulation or inhibition of ethylene biosynthesis in Jonagold apples (Miszczak et al. 1995) and the respiration of barley leaves during the promotion of senescence (Popova et al. 1988, Satler and Thimann 1981). Saniewski and Węgrzynowicz-Lesiak (1995) showed that spraying of intact *Kalanchoe blossfeldiana* with JA-Me induced leaf abscission for 2–3 days and evidently increased CO_2 evolution. The stimulation of CO_2 production induced by JA-Me in sprouting bulbs of tulips was also observed in this study and was found to depend on the concentration of JA-Me. The effect of JA-Me on CO_2 evolution was similar in both cultivars and independent of storage conditions (Fig. 6).

These results suggest that the stimulation of anthocyanin accumulation induced by JA-Me in tulip leaves is unrelated to ethylene production. Lower concentrations of JA-Me did not influence the ethylene production, but accelerated anthocyanin formation and anthocyanin accumulation were greater for cooled bulbs, ethylene production was much greater for uncooled ones.

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