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EFFECT OF METHYL JASMONATE AND ETHYLENE ON LEAF GROWTH, ANTHOCYANIN ACCUMULATION AND CO_2 EVOLUTION IN TULIP BULBS

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A B S T R A C T

The effect of JA-Me at a concentration of 100 μl l^{-1} and ethylene applied in the gaseous form at a concentration of 10 μl l^{-1} on leaf growth, anthocyanin accumulation and CO_2 evolution in uncooled and cooled, derooted bulbs of 'Apeldoorn' and 'Oxford' tulips, was investigated. JA-Me partially reversed the inhibitory effect of ethylene on the increase in leaf length in uncooled bulbs, and to a smaller degree in those cooled. JA-Me applied alone did not affect leaf growth. Ethylene evidently inhibited anthocyanin accumulation induced by JA-Me applied simultaneously. CO_2 evolution was similar after treatments with JA-Me, ethylene and JA-Me + ethylene. Simultaneous application of JA-Me with ethylene induced gum formation more than these compounds applied individually. Thus, interaction between JA-Me and ethylene is synergistic or antagonistic in the regulation of some physiological processes in tulips.

Key words: methyl jasmonate, ethylene, anthocyanin, tulip, bulb, CO_2

INTRODUCTION

It has been previously shown that methyl jasmonate (JA-Me) stimulates ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity and CO_2 evolution in tulip bulbs, and anthocyanin accumulation in stems and leaves from uncooled and cooled bulbs of 'Apeldoorn' and 'Gudoshnik' (Saniewski et al., 1998a). JA-Me greatly increased ethylene

production from simultaneously applied ACC, through stimulation of ACC oxidase activity, in comparison to ACC applied alone. ACC evidently inhibited anthocyanin accumulation induced by JA-Me applied simultaneously. CO_2 evolution was similar after treatments with JA-Me and ACC. Simultaneous application of JA-Me + ACC increased CO_2 evolution in uncooled derooted bulbs in comparison to JA-Me and ACC applied alone (Saniewski et al., 2003). These results suggest that anthocyanin accumulation by JA-Me in tulip leaves is not related to ethylene production stimulated by JA-Me. However, the synergistic effect between JA-Me and ethylene (after treatment with ethephon or ACC) on gum formation in tulips was observed (Saniewski et al., 1998b; 2003).

The objective of this study was to determine the effect of methyl jasmonate and ethylene (applied in the gaseous form) and their simultaneous treatment on some physiological processes in tulip bulbs: leaf growth, anthocyanin accumulation and CO_2 evolution.

MATERIAL AND METHODS

Tulip bulbs (10-12 cm in circumference) of 'Apeldoorn' and 'Oxford' were stored at 18-20°C until October 15 after lifting. Then one group of the bulbs was kept at 17°C (uncooled) and the other group was transferred to 5°C for dry cooling (cooled). On January 20 (uncooled bulbs), January 13 and March 3 (cooled bulbs) the entire tunic and all roots were removed and the following treatments were applied at room temperature:

1) control, distilled water,
2) JA-Me at a concentration of 100 $\mu l\ l^{-1}$,
3) distilled water + gaseous ethylene at a concentration of 10 $\mu l\ l^{-1}$,
4) JA-Me + gaseous ethylene in above concentrations.

Bulbs were placed in 5-liter jars with 150 ml of water or JA-Me. Each treatment had four replications of 7 bulbs per jar. Jars were sealed tightly and in treatments 3) and 4) the appropriate amount of ethylene was added in order to obtain 10 $\mu l\ l^{-1}$ of ethylene in jar atmosphere. CO_2 evolution was measured daily for 4 days after treatment and was analysed using infrared ADC analyser (Miszczak et al., 1995). Jars were ventilated for 2 h every day after gas sampling, sealed and again ethylene was added. Four days after treatment the length of sprouting leaves was measured and outermost leaves were harvested for the determination of anthocyanin content. 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.

The analysis of variance and Duncan t-test were used to estimate the difference between means at P=0.05.

RESULTS AND DISCUSSION

Ethylene at a concentration of 10 μl l^{-1} in atmosphere surrounding bulbs inhibited the growth of leaves in derooted, uncooled (Fig. 1A) and cooled (Fig. 2A and 3A) tulip bulbs. JA-Me at a concentration of 100 μl l^{-1} partially reversed the inhibitory effect of ethylene on the increase in leaf length in uncooled bulbs, and to a smaller degree in those cooled. JA-Me applied alone at this concentration did not affect leaf growth. Similar results were observed when ACC at a concentration of 1 mmol was used

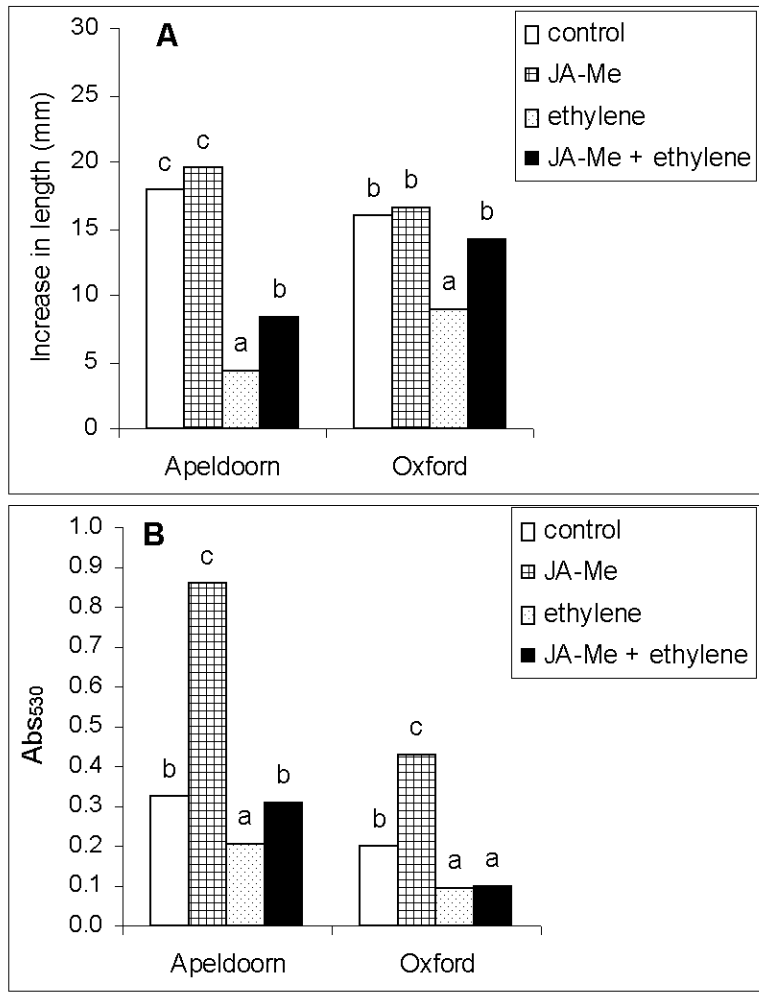

Figure 1. Effect of JA-Me at 100 μl l^{-1} and ethylene at 10 μl l^{-1} on increase in leaf length (A) and anthocyanin level in leaves (B) measured 4 days after treatment on January 20 of uncooled, derooted tulip bulbs; columns for each cultivar with a letter in common are not significantly different at P=0.05

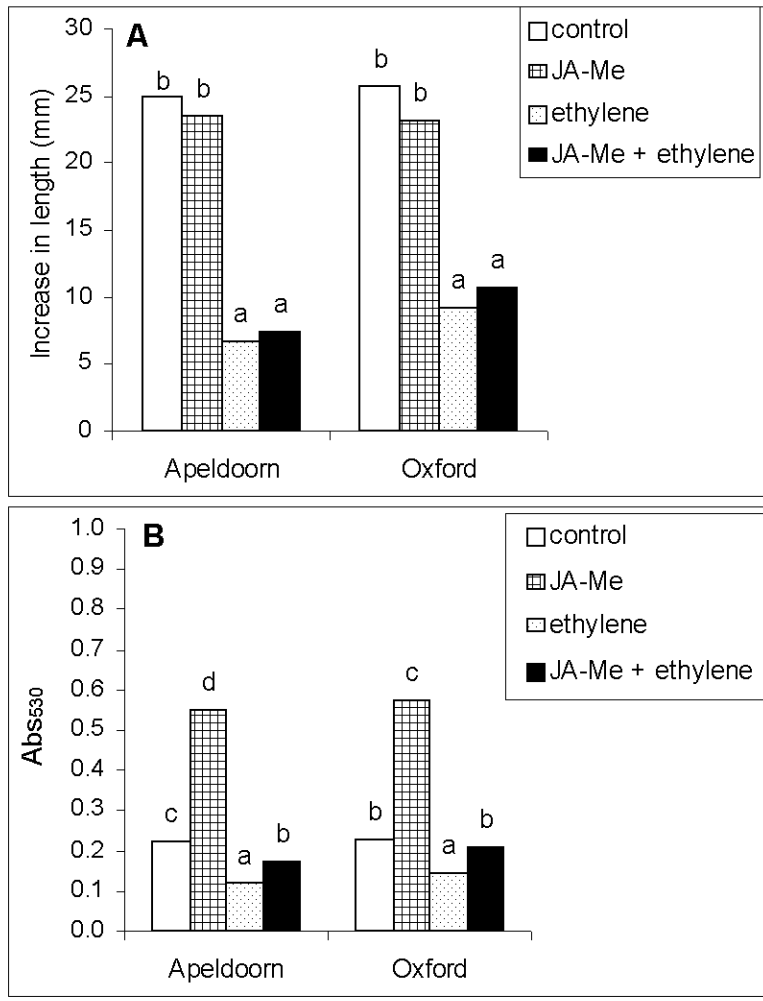

Figure 2. Effect of JA-Me at 100 µl l^{-1} and ethylene at 10 µl l^{-1} on increase in leaf length (A) and anthocyanin level in leaves (B) measured 4 days after treatment on January 13 of cooled, derooted tulip bulbs; columns for each cultivar with a letter in common are not significantly different at P=0.05

as a source of ethylene (Saniewski et al., 2003). It is well known that ethylene or ethephon inhibits tulip stem growth and the silver thiosulphate (STS) treatment, the inhibitor of ethylene action in plants, completely reverses the inhibition of tulip stem elongation caused by treatment with ethylene or ethephon. Tulip stem growth induced by IAA, after the removal of the flower bud and all leaves, was also inhibited by ethephon applied on different internodes in lanolin paste and STS

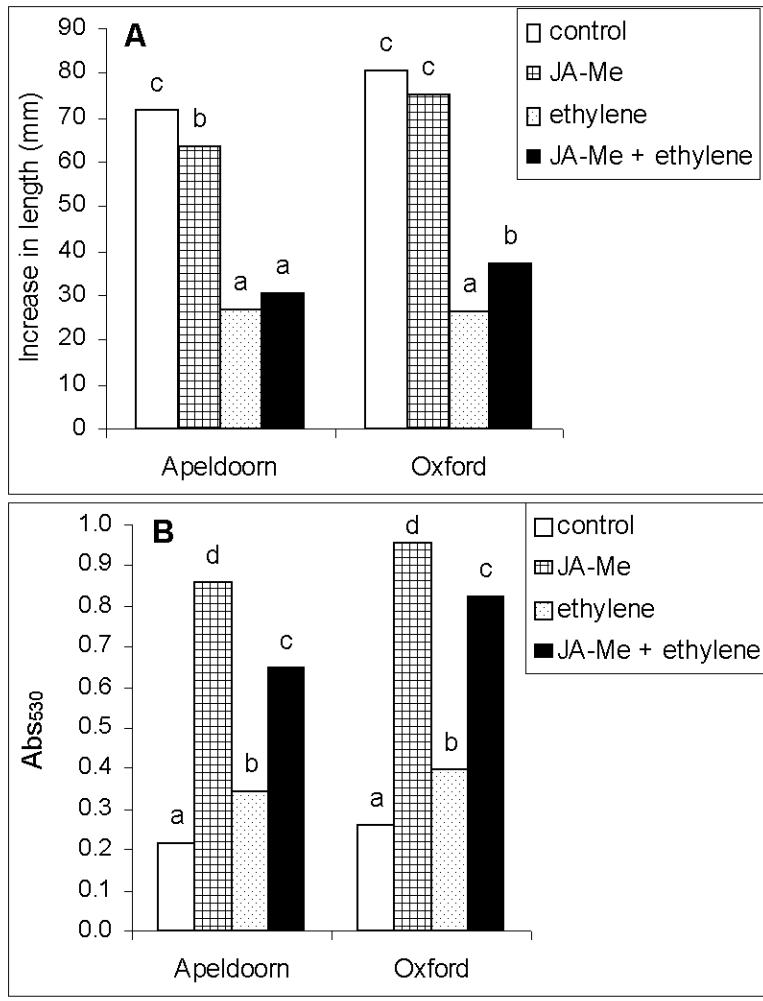

Figure 3. Effect of JA-Me at 100 μl l^{-1} and ethylene at 10 μl l^{-1} on increase in leaf length (A) and anthocyanin level in leaves (B) measured 4 days after treatment on March 3 of cooled, derooted tulip bulbs; columns for each cultivar with a letter in common are not significantly different at P=0.05

completely reversed the inhibitory effect of ethephon (Saniewski and Kawa, 1988).

JA-Me at a concentration of 100 μl l^{-1} greatly stimulated anthocyanin accumulation in the leaves of un-cooled and cooled, derooted tulip bulbs (Fig. 1B, 2B and 3B), as previously documented (Saniewski et al., 1998a; 2003). Ethylene applied alone at a concentration of 10 μl l^{-1} substantially inhibited anthocyanin accumulation in leaves in comparison to the control and evidently inhibited

Table 1. Effect of JA-Me at 100 µl l^{-1} and ethylene at 10 µl l^{-1} on CO_2 evolution [µl g^{-1} h^{-1}] in uncooled, derooted tulip bulbs

Treatment	'Apeldoorn'				'Oxford'			
	days after treatment on January 20							
	1	2	3	4	1	2	3	4
Control	31.1 a*	35.2 a	37.2 a	35.5 a	58.9 b	62.4 ab	61.5 ab	59.9 b
JA-Me	38.5 a	39.9 a	45.9 c	44.8 b	49.3 a	61.1 a	65.6 b	59.8 b
Ethylene	30.8 a	39.0 a	38.3 ab	36.4 a	47.1 a	57.2 a	41.9 a	43.8 a
JA-Me + ethylene	37.6 a	48.0 b	43.7 bc	46.0 b	61.9 b	67.2 b	61.8 ab	64.9 b

*In columns means followed by the same letter are not significantly different at P=0.05 according to Duncan's t-test

Table 2. Effect of JA-Me at 100 µl l^{-1} and ethylene at 10 µl l^{-1} on CO_2 evolution [µl g^{-1} h^{-1}] in cooled, derooted tulip bulbs

Treatment	'Apeldoorn'				'Oxford'			
	days after treatment on March 3							
	1	2	3	4	1	2	3	4
Control	35.9 a*	40.2 a	40.5 b	41.9 b	37.6 b	41.8 a	42.0 b	42.9 a
JA-Me	37.2 ab	42.1 a	41.8 b	42.9 b	34.9 a	40.8 a	38.5 a	43.5 a
Ethylene	37.8 ab	42.1 a	35.7 a	38.3 a	39.2 b	43.5 ab	42.9 b	40.2 a
JA-Me + ethylene	40.6 b	45.2 a	44.3 b	41.9 b	40.1 b	45.3 b	44.6 b	40.1 a

*Explanations see Table 1

anthocyanin accumulation induced by JA-Me when applied simultaneously (Fig. 1B, 2B and 3B). Anthocyanin accumulation in response to JA-Me was also observed in other species: in hypocotyls of light-grown seedlings of soybean (Franceschi and Grimes, 1991), in leaves of seedlings of wild-type *Arabidopsis* (Feys et al., 1994), in detached corollas of *Petunia* (Tamari et al., 1995), and in shoots of peach (Saniewski et al., 1998b). Ethephon substantially inhibited anthocyanin accumulation induced by JA-Me in peach shoots.

It is known that ethylene enhances the respiration of tulip bulbs (Kanneworff and van der Plas, 1994; Wild et al., 2002a). Our experiments showed that JA-Me, ethylene and JA-Me + ethylene treatments at used concentrations did not affect respiration of uncooled and cooled tulip bulbs (Tab. 1 and 2). In the previous study an increased CO_2 evolution occurred in uncooled derooted bulbs after simultaneous application of JA-Me + ACC in compa-rison to ACC and JA-Me applied alone (Saniewski et al., 2003).

It is well known that in tulip bulb infection by *Fusarium oxysporum* f. sp. *tulipae* and the application of ethylene or ethylene-releasing compound ethe-

phon induces gum formation. The effect of ethylene on gummosis depends on its concentration and tulip cultivar (Wild et al., 2002a). Wild et al. (2002b) showed that bulb pretreatment with 1-MCP (1-methylcyclopropene), which blocks the ethylene receptor binding-site (Sisler and Serek, 1997), prevented the ethylene-induced gummosis. JA-Me applied in lanolin paste also induced gummosis in different organs of tulips (Saniewski and Puchalski, 1988; Saniewski 1989; Saniewski and Węgrzynowicz-Lesiak, 1994; Saniewski et al., 2000).

In the present study we observed that the simultaneous application of JA-Me with gaseous ethylene induced gum formation in bulb scales. JA-Me and ethylene applied alone did not have such an effect. Also in the case of joint application of JA-Me + ACC gum formation in tulip bulbs was observed, and no gum when these compounds were applied alone (Saniewski et al., 2003). It seems that both, JA-Me and ethylene are important factors for gum formation in tulip bulbs. In used treatments, ethylene makes tulip tissue sensitive to JA-Me action or JA-Me makes tissue sensitive to ethylene action.

Thus, in the case of tulips, in our previous and present studies we have documented a stimulatory effect of JA-Me on ethylene production through the stimulation of ACC oxidase activity, and that JA-Me simultaneously with ethylene synergistically induced gum formation. On the other hand, ethylene and ACC, ethylene precursor, inhibited anthocyanin accumulation induced by JA-Me in tulip leaves. However JA-Me partially reversed the inhibitory effects of ethylene and ACC on tulip leaf growth.

It is known from literature that crosstalk between ethylene and jasmonates can be either synergistic or antagonistic (Saniewski et al., 1999; 2002; Kessler and Baldwin, 2002). The synergistic effect between JA-Me and ethylene has been reported in various phenomena such as gum formation in peach shoots (Saniewski et al., 1998b), gene expression of pathogen-related proteins in tobacco seedlings (Xu et al., 1994), breakdown of cell integrity and cell membrane in sunflower cotyledons (Emery and Reid, 1996), and expression of proteinase inhibitor in wounded tissues (O'Donnell et al., 1996). In contrast, there are some reports that ethylene substantially suppresses physiological processes induced by jasmonates. It was documented that ethylene suppresses jasmonate-induced gene expression in nicotine biosynthesis (Shoji et al., 2000). Antagonistic interactions between jasmonates and ethylene are also reported to regulate the antifeedant plant lectin GS-II in locally wounded leaves of *Griffonia simplicifolia* (Zhu-Salzman et al., 1998).

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WPŁYW JASMONIANU METYLU I ETYLENU NA WZROST LIŚCI, AKUMULACJĘ ANTOCYJANÓW I WYDZIELANIE CO_2 W CEBULACH TULIPANA

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S T R E S Z C Z E N I E

Celem badań było określenie wpływu jasmonianu metylu i etylenu podawanego w formie gazowej oraz ich współdziałania na niektóre procesy fizjologiczne zachodzące w niechłodzonych i chłodzonych, pozbawionych korzeni cebulach tulipanów 'Apeldoorn' i 'Oxford', tj. wzrost liści, akumulację antocyjanów i wydzielanie CO_2. Etylen hamował wydłużanie się liści w cebulach niechłodzonych i chłodzonych. JA-Me częściowo odwracał hamujący wpływ etylenu na wydłużanie się liści w cebulach niechłodzonych, w mniejszym stopniu w cebulach chłodzonych. Traktowanie cebul samym JA-Me nie wpływało na wzrost liści. JA-Me silnie stymulował, a etylen hamował akumulację antocyjanów w liściach z cebul niechłodzonych i chłodzonych. Stymulujący wpływ JA-Me na produkcję antocyjanów był hamowany przy równoczesnym potraktowaniu cebul etylenem. Traktowanie cebul JA-Me lub etylenem oraz ich łączne podanie nie miało większego wpływu na ilość CO_2 wydzielanego przez cebule. Przy równoczesnym traktowaniu cebul JA-Me i etylenem obserwowano tworzenie się gum w łuskach. Współdziałanie jasmonianu metylu i etylenu może mieć charakter synergistyczny lub antagonistyczny w regulacji niektórych procesów fizjologicznych w tulipanach.

Słowa kluczowe: jasmonian metylu, etylen, antocyjany, tulipan, cebule, CO_2