Interaction of 1-Methylcyclopropene and Thidiazuron on Cut Stock Flowers Vase Life

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Abstract: Leaf yellowing or petal senescence is the main postharvest disorder for many cut flowers. Plant hormones such as cytokinins are able to inhibit leaf yellowing in some cut flowers and potted plants. In our experiments, we applied thidiazuron aiming to delay leaf yellowing and 1-methylciclopropene (1-MCP) for inhibiting flower senescence of cut stock flowers during vase life. Cut flowers were pulse treated with water (control), 5 μM TDZ, 500 nL L^{-1} 1-MCP or combinations 5 μM TDZ+500 nL L^{-1} 1-MCP, 5 μM TDZ+500 nL L^{-1} 1-MCP+10 μL L^{-1} ethylene or 10 μL L^{-1} ethylene. All treatments were applied for 24 h (pulse), except for the 1-MCP that was applied for 6 h. The effect of treatments was evaluated by chlorophyll determination, petal fall, leaf yellowing appearance, vase life and ethylene production.

Pulse treatment with TDZ was able to delay leaf yellowing in light during whole experimental period (30 days). During the first days after TDZ treatment, the chlorophyll *b* biosynthesis was strongly induced, after 5 days were the initial values, 3-fold higher while the total chlorophyll did not change. The TDZ stimulated ethylene production such as 1-MCP as soon as after 24 h. The ethylene production was found in cut flowers treated with ethylene, reaching 9 nl g^{-1} h^{-1} in leaves and 6.5 nl g^{-1} h^{-1} in detached flowers. The 1-MCP dramatically reduced the efficiency of TDZ even if the vase life of cut flowers was extended compared with controls.

Keywords: Chlorophyll, ethylene, 1-MCP, postharvest, senescence, TDZ.

INTRODUCTION

Quality losses of cut flowers during the postharvest stage can be due to petal wilting, abscission, colour changes, leaf yellowing or weight loss. Some cut flowers are sensitive to leaf yellowing which occurs before petal wilting and lose their commercial value. The physiological disorders responsible for chlorophyll loss are mainly driven by hormonal and environmental factors [1-3]. Ethylene and abscisic acid are triggers of flower senescence or leaf yellowing [4]. Several cut flowers show chlorophyll loss before petal wilting such as *Alstroemeria* spp., *Lilium*, *Matthiola incana*, *Pelargonium*, *Euphorbia pulcherrima*. One of the main factors involved in the induction of chlorophyll degradation is the lack of cytokinins. This plant hormone is preferentially biosynthesized in roots [5]. Therefore, after harvest cut flowers do not receive cytokinins and leaf yellowing takes place. The postharvest treatments with benzyladenine applied as spray or in vase water, usually prevent or reduce the leaf yellowing appearance. Among the environmental issues, dark greatly activates leaf yellowing process. Cut flowers may be more or less sensitive to leaf yellowing depending from the species and cultivars [6]. The presence of ethylene in the cold rooms or supermarket enhances the leaf yellowing, but also accelerates flower senescence [7]. However, commercial formulations are available for preventing leaf yellowing of cut flowers [8]. Usually, they contain cytokinins (6-benzyladenine) or gibberellic acids (GA_3, GA_4 and GA_{4+7}). Exogenous applications of cytokinins can greatly delay leaf discoloration [9]. Exogenous applications of GA_3 with concentrations that ranged between 10^{-4} and 10^{-5} M were able to prevent leaf yellowing of cut alstroemeria flowers [9, 10]. In this species it has been found that leaf yellowing can be greatly delayed either by a pulse treatment for 24 h with 5 μM thidiazuron (TDZ). The TDZ both inhibits chlorophyll degradation and stimulates new chlorophyll biosynthesis [11]. The aim of this work was to investigate the effect of TDZ in combination with 1-MCP, an efficient inhibitor of ethylene action, on leaf yellowing and flower senescence of cut stock flowers. The synergism between TDZ and other plant growth regulators may be useful for preventing leaf yellowing of cut flowers dark stored or transported for a short or long period of time.

MATERIALS AND METHODOLOGY

Plant Material

Cut stock (*Matthiola incana* L.) flowers were directly bought from a cooperative of flower growers (Florexport, Viareggio Italy). Cut flowers were selected to avoid malformations or damage related with harvesting and transport handling. Cut flowers were cut to 60 cm and the basal leaves were removed. Each stem had 10-15 leaves in total. The whole cut flowers, with leaves and inflorescences, were used in the experiments. The experiments were repeated three times using cut flowers grown from January to March.

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Chemical Treatments

Cut flowers were pulse treated for 24 h (expect 1-MCP) in postharvest laboratory under conditions described above. After the pulse treatment the vase solutions were replaced with distilled water. The 1-MCP treatment instead was applied only for 6 h in airtight container then was taken out and left with others pulse treatments.

Flower stems were cut to 60 cm and placed in distilled water (control) or vase solution containing 5 μM thidiazuron (TDZ, Duchefa), 500 nL L^{-1} 1-methylcilcopropene (1-MCP, AgroFresh) or combination of 500 ppb 1-MCP plus 5 μM TDZ, 500 ppb 1-MCP plus ethylene, 500 ppb 1-MCP plus 5 μM TDZ plus 10 μL L^{-1} ethylene, finally 10 μL L^{-1} ethylene.

Cut flowers were placed in a postharvest room maintained at 20 °C, 60% relative humidity and 15 μmol m^{-2} s^{-1} light intensity for 12 h day^{-1} by cool-white fluorescent lamps.

Chlorophyll Determination

Total chlorophyll was determined from 10 mm diameter discs (3 discs for each sample) sampled from middle leaves of the stem. Leaf pigments were extracted using 99% methanol and samples were kept in darkness at 4 °C for 24 h. The absorbance readings were taken at 665.2 and 652.4 nm. Total chlorophyll content was calculated as described by Lichtenthaler [12].

Ethylene Production

Ethylene production was measured enclosing flowers detached from the spikes or leaf disks in airtight containers (30 ml). Two ml gas samples were taken from the headspace of the containers with a hypodermic syringe after 1 h incubation at room temperature. The ethylene concentration in the sample was measured by gas chromatograph (HP5890, Hewlett-Packard, Menlo Park, CA) using a flame ionisation detector (FID), a stainless steel column (150 x 0,4 cm ø packed with Hysep T), column and detector temperatures of 70 and 350 °C, respectively, and nitrogen carrier gas at a flow rate of 30 ml min^{-1}. Quantification was performed against an external standard and results were expressed on a fresh weight basis (pl h^{-1} g^{-1} F.W.).

Leaf Yellowing, Flower Senescence and Vase Life

The effect of chemical treatments was evaluated by daily monitoring leaf yellowing and petal abscission appearance. The useful vase life was considered as the minor value between the leaf yellowing and flower senescence.

Statistical Analysis

The data are reported in figures and table as means with standard errors. Each treatment was composed of six replicate stems. The data were subjected to one-way or two-ways analysis of variance and the differences among treatments were analysed by Bonferroni's multiple comparison test. Experiments were repeated at least three times.

RESULTS

Leaf Yellowing, Flower Life and Vase Life

The vase life of cut stock flowers was delayed by pulse treatments with 5 μM TDZ up to 10 days. Leaf yellowing in 5 μM TDZ treatment was inhibited during the whole experimental period (30 days). Flower senescence instead was also improved but was extended for only 3.8 days compared to controls (Table **1**). In order to improve the overall vase life of cut stock flowers, the TDZ was also combined with 1-MCP. This ethylene action inhibitor alone increased the vase life of cut stock flowers compared with the control but slightly improve the leaves quality (Table **1**). The 1-MCP treatment combined with TDZ reduced the beneficial effect of TDZ on leaf yellowing, even if the treatment significantly delayed the leaf yellowing compared with control (Table **1**).

Treatment with exogenous ethylene resulted in 100 % flower abscission and leaf yellowing in few days However, 1-MCP or 1-MCP+TDZ treatments completely prevented the negative ethylene effect on cut flowers (Table **1**).

Table 1. Leaf Yellowing, Flower Senescence (Days of Apparearence) and Vase Life (Minus Value between Leaf and Flower Senescence). Values are the Means with Standard Errors (*n*=6).

Treatments	Leaf yellowing (days)	Flower senescence (days)	Vase life (days)
Control	6.2 ± 0.31d	6.3 ± 0.44b	6.2 ± 0.31b
5 μM TDZ	30 ± 0.00a	10 ± 0.84a	10 ± 0.84a
500 ppb 1-MCP	8.7 ± 0.21c	8.2 ± 0.17ab	8.2 ± 0.17ab
500 ppb 1-MCP+5 μM TDZ	16.2 ± 0.60b	9.8 ± 0.65a	9.8 ± 0.65a
500 ppb 1-MCP+10 μl L^{-1} Ethylene	7.5 ± 0.22d	8.0 ± 0.26ab	7.5 ± 0.22ab
500ppb 1-MCP+5μM TDZ+10 μl L^{-1} Ethylene	17.33 ± 0.56b	9.5 ± 0.72a	9.5 ± 0.72a
10 μl L^{-1} Ethylene	4.3 ± 0.9 d	3.4 ± 0.6 c	3.4 ± 0.6 c

Data were subjected to one-way analysis of variance and differences among treatments were analysed by Bonferroni post-test. Different letters denote significant differences at $p<0.05$ for each column.

Chlorophyll Content

Leaves of cut flowers pulse treated with TDZ did not show total chlorophyll reduction (Fig. **1C**). Other chemical treatments showed lower total chlorophyll content.

The two chlorophyll pigments *a* and *b* had different behaviour in TDZ treated plants compared with other treatments. The chlorophyll a (Fig **1A**) after 3 and 5 days slightly declined, and increased again after 10 days. Opposite trend was observed for chl *b* (Fig. **1B**) that sharply increased after 3 and 5 days and slightly declined after 10 days. The 1-MCP alone or in combination with ethylene delayed the chlorophyll reduction if compared with control. On the contrary after 10 days of vase life, if 1-MCP was used in combination with TDZ, both compounds lost the ability to prevent chlorophyll losses and there were not significant differences compared with control (Fig. **1C**).

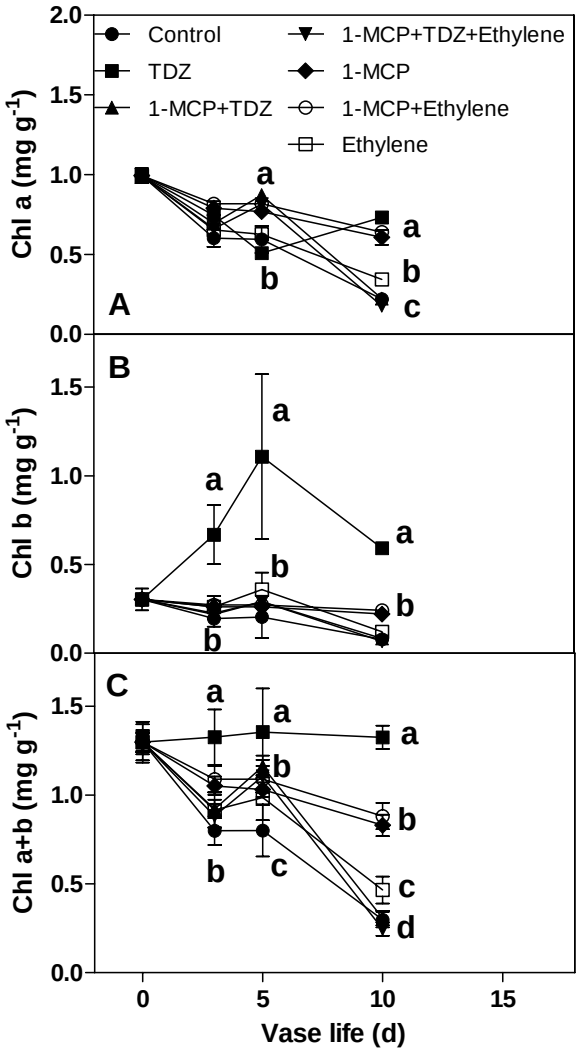

Fig. (1). Chlorophyll a (**A**), b (**B**)and a+b (**C**) concentration (mg g^{-1} F.W.) of cut stock leaves harvested from cut flowers pulse treated, distilled water (Control), 5 μM TDZ , 500 ppb 1-MCP, 10 μl L^{-1} C_2H_4, 500 ppb 1-MCP plus 10 μl L^{-1} C_2H_4 or 5 μM TDZ, 500 ppb 1-MCP plus 5 μM TDZ plus 10 μl L^{-1} C_2H_4. Values are the means with the relative standard errors of six leaves randomly harvested. Data were subjected to two-way analysis of variance and differences among treatments were analysed by Bonferroni post-test. Different letters denote significant differences at least $p<0.05$.

Ethylene Production

Ethylene production was only measured in treatments that improved the vase life or in treatments that influenced the ethylene biosynthesis and action.

Ethylene biosynthesis was stimulated by all treatments and after 24 h the highest production was found in leaves treated with 1-MCP+TDZ+ethylene. After five days the ethylene production was not statistically different among treatments. After ten days the higher ethylene production was found from leaves of control and 1-MCP+TDZ+ethylene treated plants (Fig. **2A**).

Ethylene production measured from open flowers detached from the inflorescence (Fig. **2B**) was enhanced only after 24h by 1-MCP+TDZ+ethylene treatment.

DISCUSSION

Stock flowers are very sensitive to leaf yellowing that usually occurs within 4-10 days after harvest [13]. Application of TDZ in vase water for 24 h completely inhibited chlorophyll loss as reported in previous works [14] of cut flowers or potted plants [12, 13, 15, 16]. The treatment with TDZ prevented the chlorophyll degradation or even in-

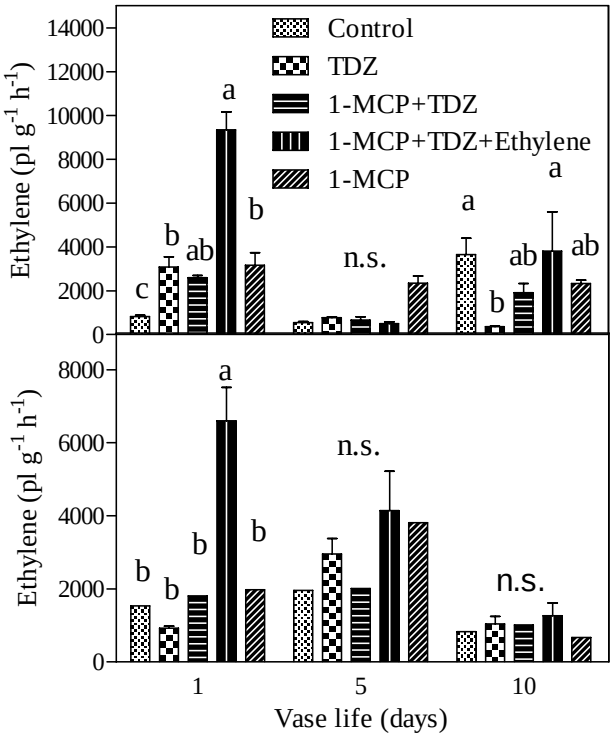

Fig. (2). Ethylene production from leaves of cut stock harvested from cut flowers pulse treated for 24 h, distilled water (Control), 5 μM TDZ, 500 ppb 1-MCP, 500 ppb 1-MCP plus 5 μM TDZ, 500 ppb 1-MCP plus 5 μM TDZ plus 10 μl L^{-1} C_2H_4. Values are the means with the relative standard errors of six leaves randomly harvested. Data were subjected to two-way analysis of variance and differences among treatments were analysed by Bonferroni post-test. Different letters denote significant differences at least $p<0.05$.

creased the leaf total chlorophyll content as observed in treated cut tulips or cut chrysanthemum [11, 3]. Our results confirm the ability of TDZ to stimulate new chlorophyll biosynthesis.

Moreover, TDZ increased the ethylene production in cut stock flowers and these findings were in according with previously observations [17]. The ethylene produced by TDZ has been used for promoting leaf abscission in cotton plants [18]. Experiments performed on cotton demonstrated that TDZ induced abscission of young and mature leaves. The role of TDZ-induced ethylene during leaf abscission was extensively characterized using ethylene inhibitors such as silver thiosulfate that completely inhibited leaf abscission in intact cotton leaves [19]. On the basis of these results the lack of negative effect of TDZ in cut stock should be explained considering the tissues sensitivity to ethylene. This hypothesis might be justified considering the behaviour of transgenic plants that overproduce cytokinins. Transgenic petunia containing the construct SAG12:IPT that overexpressed cytokinins showed that the higher level of cytokinins reduced the sensitivity of flowers to exogenous ethylene [20].

The ethylene action inhibitors, instead, such as silver thiosulfate or 1-MCP were able to prevent flower senescence if flowers were exposed to 1 μL L^{-1} of ethylene [21].

In our experiments, 1-MCP+TDZ +ethylene dramatically stimulated ethylene production, but did not accelerate the senescence. Ethylene induced autocatalytic ethylene, but the leaves and flowers were protected by 1-MCP. The amount of ethylene produced was organ dependent, in fact the leaf response was much higher, 10 fold compared with control flowers, while flower response was just doubled in treated cut flowers compared with controls. The absence of ethylene deleterious effect may be again explained considering the low ethylene sensitivity of leaves and flowers. Moreover, the climacteric ethylene peak in these plants was delayed and flowers had longer life. However, further investigations should be carried out at molecular level by the isolation of genes encoding for ethylene receptors and studying if the TDZ affect the organ sensitivity.

The organ differential response was also observed in cut flowers treated with 1-MCP alone or in combination with TDZ. The increase of ethylene production was also observed in 1-MCP treated flowers. These findings were in according with previous observations in citrus, where the ethylene evolution was directly correlated with the concentration of 1-MCP used [22]. In this case the lack of ethylene effect may be attributed to the 1-MCP treatment that blocked the ethylene receptors. The leaf yellowing was delayed 2.5 days compared with control by 1-MCP treatment. This effect on chlorophyll retain was already found in leafy veegtables. In fact, 1-MCP treatments avoided chlorophyll loss in rocket [23] and spinach leaves during storage [24]. The 1-MCP and TDZ used together lose their ability to counteract the chlorophyll degradation. No literature information are available to explain this result. Therefore, further experiments should be performed for understand this interaction. Moreover, 1-MCP did not efficiently prevent the flower senescence of cut stock flowers, suggesting that this ethylene inhibitor is not very effective on this species. Analogous results were already shown by comparative treatments between 1-MCP and STS in cut stock flowers [8,13].

CONCLUSION

Results demonstrated that in light conditions the leaf yellowing can be avoided using pulse treatments with TDZ and the efficacy of the treatments can be seen at 5 μM. Combination of TDZ with 1-MCP is not advisable because does not give any beneficial effect or reduces the TDZ efficiency.

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CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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