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Postharvest Biology and Technology 25 (2002) 333–338

Postharvest Biology and Technology

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Thidiazuron—a potent inhibitor of leaf senescence in *Alstroemeria*

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Received 7 August 2001; accepted 29 October 2001

Abstract

The time to flower senescence and leaf yellowing in 20 cut flower cultivars of *Alstroemeria* was studied. In deionized water (DI), the time to abscission of the first petal ranged from 9 to 16 days. Time to leaf yellowing ranged from 5 to 18 days. These two processes proceeded independently so that in some cultivars leaf yellowing occurred long before flower senescence, and in others, much later. Thidiazuron (TDZ, *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea), a substituted phenylurea with cytokinin-like activity, markedly extended leaf longevity. TDZ was much more effective than two other substituted phenyl-urea compounds tested, in delaying leaf yellowing. A single 24-h pulse treatment with 10 μM TDZ prevented yellowing of isolated leaves for more than 2 months.

Keywords: Thidiazuron; TDZ; *Alstroemeria*; Senescence; Leaf yellowing

1. Introduction

Over the last 20 years, hybrid *Alstroemeria* cultivars have become major commercial cut flowers, because of their long vase life, elegant flowers, and range of patterns and colors. Unlike many other cut flowers, the first sign of senescence in *Alstroemeria* commonly is yellowing of the leaves, which may occur within a few days, and proceeds very rapidly. Previous researchers have studied leaf senescence both in detached and attached *Alstroemeria* leaves (Jordi et al., 1995). They demonstrated that the onset of yellowing is associated with chlorophyll breakdown, that treatment with gibberellins and cytokinins delayed senescence, and that treatment with auxins and polyamines had no effect. Treatments with cytokinins reduced chlorophyll degradation only at concentrations higher than 10^{-5} M. GA_4 was very effective in delaying chlorophyll loss and was active at concentrations as low as 10^{-8} M. A GA_4 pretreatment is now a standard prophylaxis for preventing leaf yellowing in *Alstroemeria*, although the biological basis of this beneficial effect

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0925-5214/02/$ - see front matter
PII: S0925-5214(01)00195-8

is still unclear (van Doorn et al., 1992). Thidiazuron (TDZ, *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea) is a substituted phenylurea compound that is registered for use as a herbicide and defoliant. It has high cytokinin-like activity that probably is the basis of its herbicidal and defoliation properties. Researchers discovered that it was a good substitute for N^6-benzylaminopurine (BA), zeatin, and other cytokinins that are commonly used in plant tissue culture, because of its high activity (Genkov and Iordanka, 1995).

As part of a study using *Alstroemeria* as a model system for exploring the molecular basis of leaf senescence, we examined the role of cultivar, environment, and vase solution components on leaf and flower senescence. We report here experiments testing the hypothesis that there might be significant variation in the rate of senescence among present cultivars that could serve as a basis for future breeding programs to increase longevity. We also examined the possible use of TDZ, as a substitute for the expensive growth regulator treatments presently employed to prevent leaf yellowing in *Alstroemeria* and other crops.

2. Materials and methods

2.1. Plant material

Cut *Alstroemeria* flowers were obtained from a commercial grower (Mellano, San Diego, CA) at commercial maturity (oldest buds about to open). They were transported dry to the laboratory within 24 h. Stems were cut to 60 cm and placed in solutions in a vase life room at 20 °C, 60% RH and either in a photosynthetically active quantum flux of 15 μM m^{-2} s^{-1} (12 h per day) or in complete darkness, for postharvest evaluation.

2.2. Standard treatments

Cut flowers were placed in solutions containing 100 μM gibberellic acid (GA_3), 100 μM 6-benzylaminopurine (BA, Sigma), a standard floral preservative ('Crystal Clear', Floralife, Evanston, IL) containing 1.0% sugar, or 1 μM thidiazuron (TDZ). Flowers were exposed to ethylene gas by sealing them for 24 h in a tank ventilated with a flowing stream (30 l h^{-1}) of 1 μl l^{-1} ethylene.

2.3. Pulse treatments

Flowers were 'pulse' treated with a range of concentrations of TDZ (from 0.01 to 50 μM), other substituted phenyl-ureas (4-CPPU at 10–100 μM and 1,3-DPU at 10–100 μM), BA (10–100 μM), or GA_3 (10–100 μM) by being placed in the solution for 24 h in the vase life room under the conditions described above. After pulse treatment all flowers were placed in deionised water (DI) and postharvest performance was compared with that of control flowers held continuously in DI.

2.4. Chlorophyll determination

The chlorophyll content of fully expanded leaves harvested at intervals from cut flower stems was determined by extraction with methanol and measurement of the absorption at 665.2 and 652.4 nm. Total chlorophyll content was calculated as described by Lichtenthaler (1987).

2.5. Vase life parameters

Days to initiation of visible leaf yellowing and days to abscission of the first petal were recorded for each replicate stem.

2.6. Statistical analysis

The experimental design was randomized with six replications per treatment. Total chlorophyll content was measured from three replicate leaves harvested from each stem.

The data were subjected to one-way analysis of variance and the differences among treatments were analyzed by Tukey's test ($P < 0.05$). Experiments were repeated at least three times.

3. Results

3.1. Variation among cultivars

There was a wide variation in the rate of leaf yellowing and petal fall among the different cultivars (Fig. 1). In several ('Cuba', 'Saba', 'Petra', 'Tamara'), leaf yellowing was visible within 5 days. In contrast, no leaf yellowing was seen in 'Rio', until the flowers had been in the vase for 18 days. A similar (though less striking) range was seen in the time to first petal fall, which ranged from 10 days ('Cuba', 'Tamara', 'Petra' and 'Rio') to 17 days ('Tiara' and 'Jubilee'). No relationship was found between the time to leaf yellowing and petal fall amongst the cultivars tested. For subsequent experiments we used cv. Diamond, whose leaf yellowing was uniform and rapid.

3.2. Effect of chemicals

Leaves on 'Diamond' flower stems placed in the dark in DI, held in a standard floral preservative containing 1% sugar, or treated for 1 day with 1 μl l^{-1} ethylene, yellowed more rapidly than DI controls in the light (Fig. 2). Leaf yellowing was substantially delayed if the flowers were placed in GA_3, and was delayed for more than 70 days if the flowers were placed in a solution containing 1 μM TDZ (Fig. 2). TDZ had no apparent effect on petal abscission (Fig. 2).

3.3. Changes in chlorophyll content

During the first 10 days of the experiment, the total chlorophyll content remained high in leaves treated with TDZ and GA_3 (Fig. 3), but fell in the leaves of flowers held in water or given the other treatments such as darkness, ethylene or a floral preservative containing sucrose.

3.4. Effect of pulse treatments

A 24 h pulse treatment with TDZ at 20 °C and concentrations below 1 μM was ineffective at retarding leaf yellowing (data not shown). However, TDZ pulse treatments were very effective at concentrations of 10 μM or greater, which prevented leaf yellowing for more than 60 days (data not shown). During the first 3 weeks, chlorophyll content of the treated leaves was as high as, or even higher than their initial content, while the

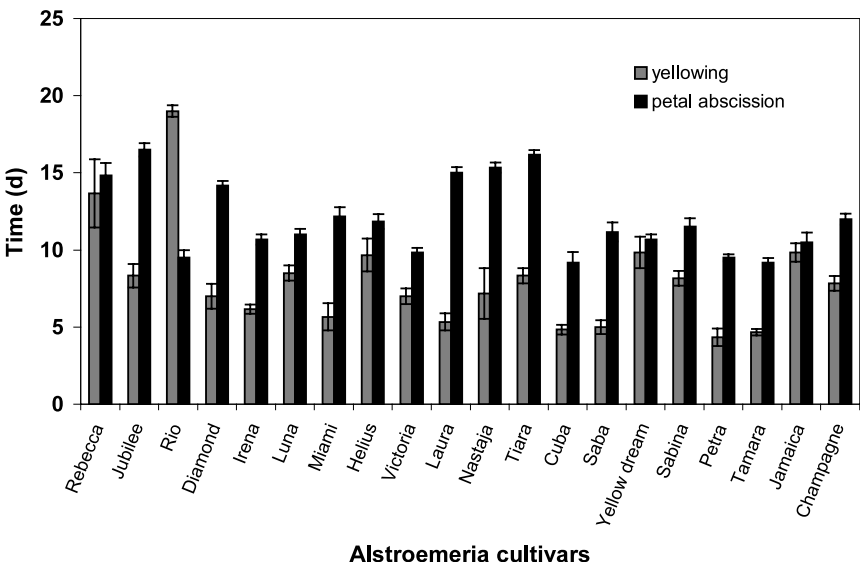

Fig. 1. Days to leaf yellowing and petal abscission in 20 *Alstroemeria* cultivars. Freshly-harvested flowers were shipped by air, and were placed in DI within 1 day of harvest. The flowers were held under standard conditions (20 °C, 60% R.H., and 12 h day illumination from cool-white fluorescent lights (15 μmol m^{-2} s^{-1} PAR)). Means ± S.E. for six replicate flowers per cultivar.

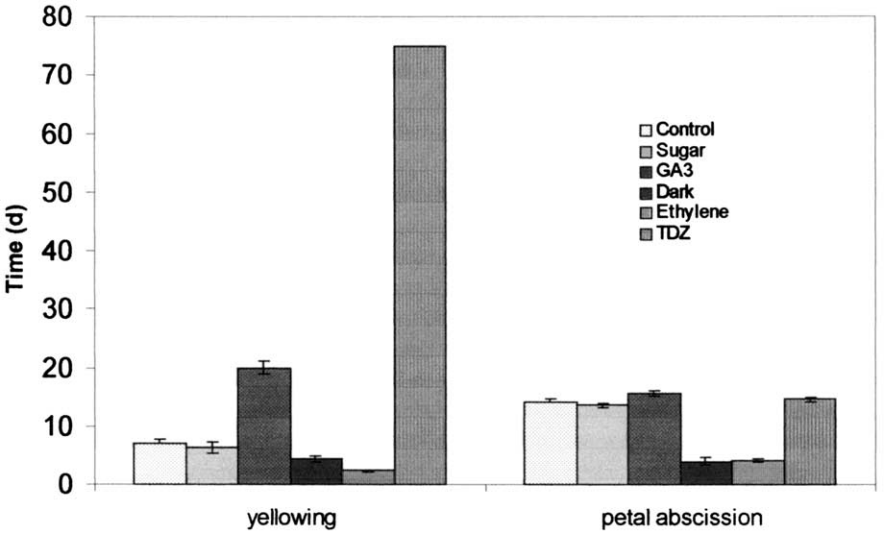

Fig. 2. Effect of darkness and chemicals treatments on leaf yellowing and petal abscission in *Alstroemeria* 'Diamond'. Freshly-harvested flowers were shipped by air, and were placed in vase solutions containing different chemicals within 1 day of harvest. Ethylene treatment was for the first 24 h in a tank ventilated with air containing 1 $\mu l\, l^{-1}$ ethylene. The flowers were evaluated under standard conditions (20 °C, 60% R.H., and 12 h day illumination from cool-white fluorescent lights (15 $\mu mol\, m^{-2}\, s^{-1}$ PAR)). Means $\pm$ S.E. for six replicate flowers per treatment. Leaves on TDZ-treated flowers were all still green at the end of the experiment, so no S.E. was calculated.

content of the controls fell steadily after 6 days in the vase (Fig. 4).

The striking effect of TDZ in preventing leaf yellowing stood out from all the other chemicals tested as pulse treatments (Fig. 5). Of the other chemicals, GA_3 was the most effective, particularly at 100 μM; all the other materials had modest but significant preventative effects on leaf yellowing at the concentrations tested. None of the pulse treatments had a significant effect on petal wilting.

4. Discussion

Leaf yellowing is a serious postharvest problem with cut *Alstroemeria* flowers. In over half the cultivars that we tested the leaves were already yellowing before the start of floral senescence (Fig. 1). Our data demonstrate the remarkable variation among modern cultivars in time to leaf yellowing under indoor conditions, suggesting that future breeding programs could greatly improve the postharvest performance of these flowers by selecting for flower *and* leaf longevity. Although variation in leaf yellowing of *Alstroemeria* can also be the result of variable cultural conditions, we obtained our flowers from a single major producer. Moreover, repeated experiments gave reproducible results.

It is well established that the onset of leaf yellowing can be delayed in many plants by treating their leaves with plant hormones such as cytokinins (Richmond and Lang, 1957) and gibberellins (Han, 1997; Ichimura and Goto, 2000). In *Alstroemeria*, gibberellic acid was previously shown to be more effective than the adenine-based cytokinins at preventing yellowing of the leaves of the cut stems (Hicklenton, 1991; van Doorn et al., 1992; Jordi et al., 1995).

Given its difference in structure to the adenine-based endogenous cytokinins, it is intriguing that TDZ, a substituted phenylurea, is so effective at inducing cytokinin-like responses. TDZ was first shown by Arndt et al. (1976) to cause defoliation of cotton, most probably through increasing ethylene production and/or stomatal closure (Elstner et al., 1983; Suttle, 1984, 1985). On a concentra-

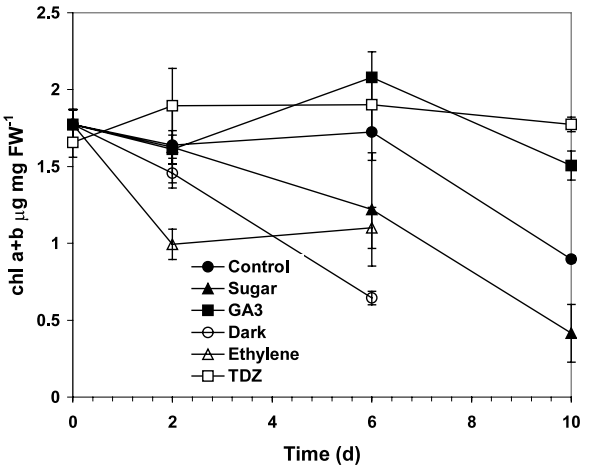

Fig. 3. Effect of darkness and chemicals on total chlorophyll content of *Alstroemeria* 'Diamond' during vase life evaluation. Leaves were harvested at intervals from replicate stems of the flowers in Fig. 2, and their total chlorophyll content was measured using standard procedures. Means ± S.E. for six replicate leaves per time point.

tion basis, TDZ was a 100-fold more active than the synthetic adenine-based BAP in growth of carnation explants (Genkov et al., 1997) and

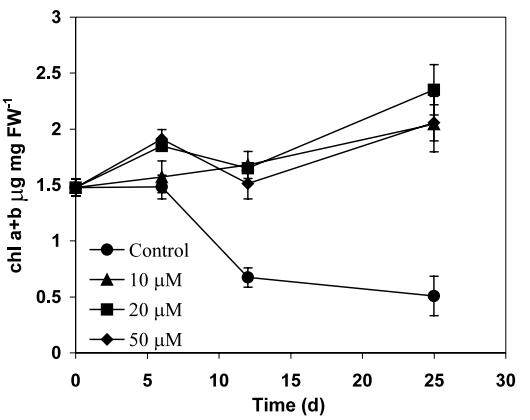

Fig. 4. Effect of pulse treatments with TDZ on changes in chlorophyll content of leaves of *Alstroemeria* 'Diamond'. Freshly-harvested flowers were shipped by air, and were 'pulsed' by being placed in different concentrations of TDZ for 24 h. The flowers were then placed in DI and evaluated under standard conditions (20 °C, 60% R.H., and 12 h day illumination from cool-white fluorescent lights (15 μmol m^{-2} s^{-1} PAR)). The total chlorophyll content of harvested leaves was determined at intervals by standard procedures. Means ± S.E. for six replicate leaves per time point.

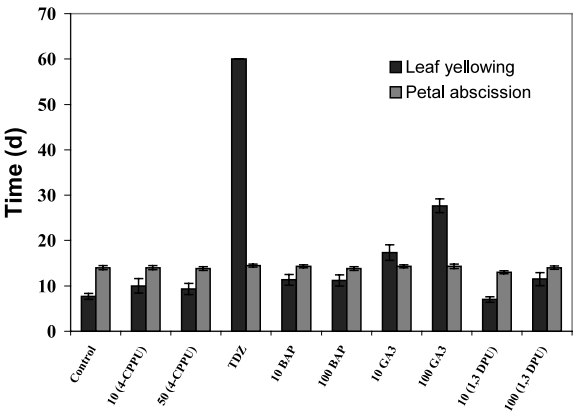

Fig. 5. Effect of pulse treatments with different chemicals on the postharvest life of *Alstroemeria* 'Diamond'. Freshly-harvested flowers were shipped by air, and were 'pulsed' by being placed in DI or different concentrations of a range of growth regulating chemicals for 24 h under standard conditions (20 °C, 60% R.H., and 12 h day illumination from cool-white fluorescent lights (15 μmol m^{-2} s^{-1} PAR)). The flowers were then placed in DI and evaluated in the same conditions. Means ± S.E. for six replicate flowers per treatment.

10 000 fold more active than BAP and kinetin in stimulating soybean callus growth (Thomas and Katterman, 1986).

The reason that exogenously applied thidiazuron is so much more effective than the other cytokinins in causing cytokinin responses, including preventing leaf yellowing in *Alstroemeria* is not known. Mok and Mok (1985) showed, using [^{14}C]-thidiazuron, that it was not substantially broken down in *Phaseolus lunatus* callus tissue over a 33 day time period and concluded that thidiazuron itself, and not its catabolites was stimulating the physiological responses. It seems probable that TDZ is very stable in *Alstroemeria* leaves.

It is still unclear whether TDZ acts to invoke cytokinin responses by interacting directly with the cytokinin receptors in the leaves (Fox, 1992; Christianson and Hornbuckle, 1999), or indirectly through either stimulating conversion of cytokinin nucleotides to their biologically more active ribonucleosides (Capelle et al., 1983) or inducing the accumulation of endogenous adenine-based cytokinins (Thomas and Katterman, 1986; Vankova et al., 1991) which could be due to

inhibition of cytokinin oxidase (Chatfield and Armstrong, 1986; Hare and Van Staden, 1994). The effectiveness of TDZ may result from a combination of these mechanisms.

No previous studies have mentioned the effect of TDZ on preventing leaf senescence, although Chernyad'ev (1994) demonstrated that spray application of TDZ and BAP increased the photosynthetic rate and activities of key photosynthetic enzymes in sugar beet, pea, meadow fescue and red fescue. We have found, in preliminary experiments, that TDZ also retards leaf yellowing in a range of other cut flowers (lilies, stock, tulip, and iris) and potted flowering plants (poinsettia and miniature roses). Since it is already registered in the US as an agricultural chemical (for defoliation of cotton under the trade name 'Dropp') its extraordinary efficacy at low concentrations makes it a potential commercial treatment for delaying leaf senescence in many species.

Acknowledgements

We thank Mellano Wholesale Florists for their generous donation of the flowers used in this study.

References

Arndt, F., Rusch, R., Stilfried, H.V., 1976. SN 49537, a new cotton defoliant. Plant Physiol. 57, 99.

Capelle, S.C., Mok, D.W.S., Kirchner, S.C., Mok, M.C., 1983. Effects of thidiazuron on cytokinin autonomy and the metabolism of N^6-(Δ^2-isopentenyl)[8-^{14}C]adenosine in callus tissues of *Phaseolus lunatus* L. Plant Physiol. 73, 796–802.

Chatfield, J.M., Armstrong, D.J., 1986. Regulation of cytokinin oxidase activity in callus tissues of *Phaseolus vulgaris* cultivar Great Northern. Plant Physiol. 80, 493–499.

Chernyad'ev, I.I., 1994. Effect of 6-benzylaminopurine and thidiazuron on photosynthesis in crop plants. Photosynthetica 30, 287–292.

Christianson, M.L., Hornbuckle, J.S., 1999. Phenylurea cytokinins assayed for induction of shoot buds in the moss *Funaria hygrometrica*. Am. J. Bot. 86, 1645–1648.

Elstner, E.F., Keller, G., Paradies, I., 1983. Contrasting effects of the cotton defoliant thidiazuron and aminoethoxyvinylglycine, an inhibitor of ethylene formationon stomatal aperture and on ethylene formation in bean (*Phaseolus vulgaris*) leaves. Ber. Dtsch. Bot. Ges. 96, 459–467.

Fox, J.E., 1992. Molecular modeling of cytokinins and the CBF-1 receptor. In: Kaminek, M., Mok, D.W.S., Zazimalova, E. (Eds.), Physiology and Biochemistry of Cytokinins in Plants. SPB Academic Publishing, The Hague, The Netherlands, pp. 127–132.

Genkov, T., Iordanka, I., 1995. Effect of cytokinin-active phenylurea derivatives on shoot multiplication perioxidase and superoxide dismutase activities of in vitro cultured carnation. Bulg. J. Plant Physiol. 21, 73–83.

Genkov, T., Tsoneva, P., Ivanova, I., 1997. Effect of cytokinins on photosynthetic pigments and chlorophyllase activity in in vitro cultures of axillary buds of *Dianthus caryophyllus* L. J. Plant Growth Regul. 16, 169–172.

Han, S.S., 1997. Preventing postproduction leaf yellowing in Easter lily. J. Am. Soc. Hortic. Sci. 122, 869–872.

Hare, P.D., Van Staden, J., 1994. Inhibitory effect of thidiazuron on the activity of cytokinin oxidase isolated from soybean callus. Plant Cell Physiol. 35, 1121–1125.

Hicklenton, P.R., 1991. GA3 and benzylaminopurine delay leaf yellowing in cut *Alstroemeria* stems. HortScience 26, 1198–1199.

Ichimura, K., Goto, R., 2000. Effect of gibberellin A3 on leaf yellowing and vase life of cut *Narcissus tazetta* var.chinensis flowers. J. Jap. Soc. Hortic. Sci. 69, 423–427.

Jordi, W., Stoopen, G.M., Kelepouris, K., Van-der-Krieken, W.M., 1995. Gibberellin-induced delay of leaf senescence of Alstroemeria cut flowering stems is not caused by an increase in the endogenous cytokinin content. J. Plant Growth Regul. 14, 121–127.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic membranes. Methods Enzymol. 148, 350–382.

Mok, M.C., Mok, D.W.S., 1985. The metabolism of [14C] thidiazuron in callus tissues of *Phaseolus lunatus*. Physiol.Plant. 65, 427–432.

Richmond, A.E., Lang, A., 1957. Effect of kinetin on protein content and survival of detached Xanthium leaves. Science. 125, 650–651.

Suttle, J.C., 1984. Effect of the defoliant thidiazuron on ethylene evolution from mung bean hypocotyl segments. Plant Physiol.75, 902–907.

Suttle, J.C., 1985. Involvement of ethylene in the action of the cotton defoliant thidiazuron. Plant Physiol. 78, 272–276.

Thomas, J.C., Katterman, F.R., 1986. Cytokinin activity induced by thidiazuron. Plant Physiol. 81, 681–683.

van Doorn, W.G., Hibma, J., de-Wit, J., 1992. Effect of exogenous hormones on leaf yellowing in cut flowering branches of *Alstroemeria pelegrina* L. Plant Growth Regul. 11, 445–448.

Vankova, R., Hsiao, K.C., Bornman, C.H., Gaudinova, A., 1991. Effects of synthetic cytokinins on levels of endogenous cytokinins and respiration patterns of *Beta vulgaris* cells in suspension. J. Plant Growth Regul. 10, 197–200.