Effect of Thidiazuron on Senescence of Flowers in Cut Inflorescences of *Lupinus densiflorus* Benth

N. Sankhla, W.A. Mackay and T.D. Davis
Texas A&M University, TAES-Dallas, 17360 Coit Rd., Dallas, TX 75252-6599
USA

Keywords: ethylene sensitivity, lupines, postharvest, vase life

Abstract

Cut inflorescences of *Lupinus* species have potential as specialty cut flowers, but their sensitivity to ethylene varies widely among species and even in selections within a species. In some species (e.g., *L. havardii*) flower abscission, is particularly sensitive to the presence of ethylene. In contrast, we observed that in cut inflorescences of *L. densiflorus*, even in the presence of a relatively high concentration of 2-chloroethylphosphonic acid (CEPA), flowers did not exhibit any abscission, although flower senescence was hastened considerably. This investigation was initiated to study the effect of thidiazuron (TDZ), alone or in combination with sucrose, silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP), on flower senescence of 'dark yellow' and 'light yellow' flowered lines of *L. densiflorus*. Incorporation of TDZ in the holding solution delayed the onset of flower senescence in both lines of *L. densiflorus*. The senescence of flowers was also delayed by the addition of sucrose or by pretreatment with STS or 1-MCP. Sucrose and TDZ, in combination, proved even more effective in delaying senescence of flowers. Both sucrose and TDZ, like STS and 1-MCP, also partially counteracted the flower senescence- accelerating effect of CEPA. These results suggest that, in addition to its cytokinin-like activity, TDZ may also have some role in modulating the effects of ethylene in cut inflorescences of *L. densiflorus*.

INTRODUCTION

Long, attractive and brightly colored inflorescences of several *Lupinus* spp. have potential as specialty cut flower crops. However, their postharvest performance depends on sensitivity to ethylene which varies widely among species and even in selections within a species (Mackay et al., 2001). The key components affecting display life are flower abscission and/or flower senescence. In some species (e.g. *L. havardii*), in the presence of ethylene, flower abscission plays a relatively more important role than flower senescence in postharvest longevity. In contrast, in *L. densiflorus*, in which the cut flowers do not abscise during vaselife, flower senescence plays a central role in postharvest performance (Mackay et al., 2001).

It is known that in some flowers (e.g. carnation, petunia) ethylene induces senescence, while exogenous cytokinins suppress senescence of flowers apparently via inhibition of ethylene biosynthesis and action (Rubinstein, 2000). Recently, thidiazuron (N'-phenyl-N'-1,2,3-thiadiazol-5-ylurea, TDZ), a phenylurea derivative, has been characterized as a highly efficacious type of non-purine cytokinin with strong morphogenic potency in a wide range of plant species (Murthy et al., 1998; Mok et al., 2000). Like purine cytokinins, TDZ has also been shown to be a potent inhibitor of leaf senescence and flower abscission (Ferrante et al., 2002; Sankhla et al., 2003).

This study was initiated as a part of our ongoing research aimed at optimizing postharvest protocols for cut inflorescences of *Lupinus* spp. and evaluates the role of TDZ, alone and in combination with sucrose, on senescence of *L. densiflorus*. Experiments were also conducted to study the effect of ethylene inhibitors (STS and 1-MCP) on flower senescence.

MATERIALS AND METHODS

Plant Material

Plants of *L. densiflorus* Benth and *L. havardii* Wats. were grown in a non-shaded greenhouse. Two lines of *L. densiflorus*, one producing 'light' yellow and the other 'dark' yellow flowers and a blue flowered line of *L. havardii* was used for experiments. Freshly cut inflorescences were transported to the laboratory for postharvest evaluation.

Growth Regulator Treatment

Cut inflorescences were placed in solutions containing TDZ (45µM), sucrose (60µM) and CEPA (10-1000µM). Deionized water was used to prepare the fresh solutions. All vases also contained 100 $mg.l^{-1}$ 8-hydroxyquinoline sulphate (HQS). For some experiments, cut inflorescences were pretreated with STS (10 mg/l) or 1-MCP (50 mg EthylBloc®) for a period of 4 hours.

The vases containing inflorescences were then randomly placed on benches in the laboratory at 22-25ºC under illumination from a light bank of florescent lamps (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) for 12 hrs a day. The number of abscised and senescent flowers was recorded daily, and the vase life evaluated at the termination of the experiment.

Statistical Analysis

The experimental design was completely randomized with four replications per treatment. Each experiment was repeated at least twice. Standard error was calculated using the Microsoft Excel statistical analysis tool pack.

RESULTS

In *L. densiflorus*, flowers are produced in a whorl of 6-7 per node, while in *L. havardii* only one flower is produced per node. The cut inflorescences of the two species differ with respect to abscission of flowers in the vase: *L. densiflorus* does not exhibit any flower abscission; while in *L. havardii* the flowers do abscise during vase life (Mackay et al., 2001). The dramatic difference between the two species with respect to flower abscission and flower senescence in response to the presence of CEPA is shown in Table 1. Within 96 hours, almost 100% abscission of flowers was clearly observed in *L. havardii*, while in *L. densiflorus* not a single flower abscised. Even at relatively very high concentrations of CEPA (500-1000µM), the cut inflorescences of *L. densiflorus* did not exhibit any flower abscission (data not presented). Instead, increasing concentrations of CEPA (10-100µM) brought about a strong promotion in flower senescence. Therefore, further experiments were focused to study the effect of various treatments on only flower senescence in *L. densiflorus*.

Effect of TDZ and Sucrose

Incorporation of TDZ in the holding solution considerably delayed the onset of flower senescence in both the dark yellow and light yellow lines of *L. densiflorus*. Since the response of both the lines to various treatments was almost identical, only the results obtained using the dark yellow line are presented herewith. In the presence of TDZ, the cut inflorescences remained quite fresh and healthy for at least 8-10 days. As with TDZ, the presence of sucrose in the vase solution also delayed flower senescence (Table 2).

Effect of STS and 1-MCP

Like TDZ and sucrose, both the ethylene action inhibitors (STS and 1-MCP) were also found to be effective in delaying flower senescence in *L. densiflorus*. Both STS as well as 1-MCP also counteracted the effect of CEPA (50 µM) on induction of flower senescence (Table 2).

Effect of Various Chemicals in Combination

Sucrose and TDZ, in combination, proved even more effective in delaying flower

senescence. When administered in the presence of CEPA, a combination of TDZ and sucrose, also proved much more effective in counteracting the effect of the latter chemical on flower senescence than either compound alone (Table 2).

DISCUSSION

Petal senescence is a key factor affecting vase life and quality of cut flowers. Currently, petal senescence, like leaf senescence is considered a part of programmed cell death (PCD), although little is known about the mechanisms triggering cell death in petals (Rubinstein, 2000; Yamada et al., 2003). At least two pathways, ethylene sensitive and ethylene insensitive, exist that trigger the process of petal senescence (Woltering and van Doorn, 1988). In ethylene sensitive flowers, ethylene inhibitors such as STS and 1-MCP, prevent flower abscission/senescence and extend vase life (Ichimura and Hiraya, 1999; Sankhla et al., 2001). Our results suggest that ethylene is a distinct trigger of petal senescence in ethylene-sensitive flowers such as *L. densiflorus*.

In flowers of many plants, petal senescence is often accompanied by a decline in the endogenous cytokinin levels (Borochov and Woodson, 1989). It has been suggested that this could be partially due to promotion of cytokinin inactivation by ethylene via O-glycosylation and degradation (Taverner et al., 1999), which may facilitate senescence. On the other hand, cytokinins delay senescence of petals either by preventing ethylene synthesis or by decreasing the sensitivity to ethylene (Rubinstein, 2000).

The mode of action of TDZ is not completely known (Murthy et al., 1998; Mok et al., 2000). However, in a variety of systems TDZ was much more effective than the purine type cytokinins in influencing typical cytokinin responses, including induction of somatic embryogenesis and organogenesis and prevention of leaf yellowing and senescence (Murthy et al., 1998; Mok et al., 2000). Recently, evidence has also been provided suggesting that the TDZ molecule remains quite stable and intact in both a free and conjugated form within the plant tissues (Murch and Saxena, 2001). The effectiveness of TDZ may result from a combination of the above mechanisms of its action. Previously, we observed that TDZ prevented flower abscission in phlox and, like the purine cytokinins, delayed the senescence of leaves (Sankhla et al., 2003). Current results indicate that TDZ is also able to delay senescence of petals in *L. densiflorus* flowers.

In addition to TDZ, as with STS and 1-MCP, even sucrose was found to be quite effective in delaying the senescence of petals in *L. densiflorus*. Both sucrose and TDZ, alone and in combination, also partially counteracted the flower senescence accelerating effect of CEPA as well as ACC (our unpublished results). Sucrose is known to prolong vase life in several cut flowers (Mayak and Dilley, 1976; Ichimura and Hiraya, 1999). Besides promoting phosphorylation of hexose, glycosylation of anthocyanins, and pH of the petal cell sap (Ichimura and Hiraya, 1999; Oren-Shamir et al., 2001), sucrose also suppresses ethylene production and reduces ethylene sensitivity (Mayak and Dilley, 1976; Ichimura and Hiraya, 1999). It is likely that the increased effectiveness of TDZ and sucrose in delaying senescence of flowers in *L. densiflorus* is partially due to their effect on ethylene production/sensitivity.

Literature Cited

Borochov, A. and Woodson, W.R. 1989. Physiology and biochemistry of flower petal senescence. Hort. Rev. 11:15-43.

Ferrante, A., Hunter, D.A., Hackett, W.P and Reid, M.S. 2002. Thidiazuron – a potent inhibitor of leaf senescence in *Alstromeria*. Postharvest Biol & Tech. 25:333-338.

Ichimura, K. and Hiraya, T. 1999. Effect of silver thiosulphate (STS) in combination with sucrose on the vase life of cut sweet pea flowers. J. Japan. Soc. Hort. Sci. 68:23-27.

Mackay, W.A., Davis, T.D. and Sankhla, N. 2001. Effect of ethephon and silver thiosulphate on postharvest characteristics of inflorescences of several lupine species. Acta Hort. 543:69-73.

Mayak, S. and Dilley, D.R. 1976. Effect of sucrose on response of cut carnation to kinetin, ethylene, and abscisic acid. J. Amer. Soc. Hort. Sci. 101:583-585.

Mok, M.C., Martin, R.C. and Mok, D.W.S. 2000. Cytokinins: Biosynthesis, metabolism and perception. In Vitro Cell. Dev. Biol. Plants 36:102-107.
Murch, S.J. and Saxena, P.K. 2001. Molecular fate of thidiazuron and its effects on auxin transport in hypocotyls tissue of *Pelargonium* × *hortorum* Bailey. Plant Growth Regul. 35:269-275.
Murthy, B.N.S., Murch, S.J. and Saxena, P.K. 1998. Thidiazuron: A potent regulator of in vitro plant morphogenesis. In Vitro Cell. Dev. Biol. Plant 34:267-275.
Oren-Shamir, M., Dela, G., Ovadia, R., Nissim-Levi, A., Philosoph-Hadas and Meir, S. 2001. Differentiation between petal blueing and senescence of cut 'Mercedes' rose flowers. J. Hort. Sci. & Biotech. 76:195-200.
Rubinstein, B. 2000. Regulation of cell death in flower petals. Plant Mol. Biol. 44:303-318.
Sankhla, N., Mackay, W.A. and Davis, T.D. 2001. Extension of vaselife and prevention of ethylene-induced flower shattering in *Lupinus havardii* by 1-methylcyclopropene. Acta Hort. 543:75-78.
Sankhla, N., Mackay, W.A. and Davis, T.D. 2003. Reduction of flower abscission and leaf senescence in cut phlox inflorescences by thidiazuron. Acta-Hort. 628 (Vol 2): 837-841
Taverner, E., Letham, D.S. Wang, J., Cornish, E. and Willcocks, D.A. 1999. Influence of ethylene on cytokinin metabolism in relation to *Petunia* corolla senescence. Phytochemistry 51:341-347.
Woltering, E.J. and van Doorn, W.G. 1988. Role of ethylene in senescence of petals - Morphological and taxonomical relationships. J. Exp. Bot. 39:1605-1616.
Yamada, T., Takatsu, Y., Manabe, T., Kasumi, M. and Marubashi, W. 2003. Suppressive effect of trehalose on apoptic cell death leading to petal senescence in ethylene-insensitive flowers of gladiolus. Plant Sci. 164:213-221.

Tables

Table 1. Effect of CEPA on flower abscission (FA) and flower senescence (FS) after 96 hours.

Treatment (µM)	*L. densiflorus*		*L. havardii*	
	FA%	FS%	FA%	FS%
Control	-	30±2.8[a]	16±2.0	36±2.8
$CEPA_{10}$	-	66±4.0	38±4.0	42±3.2
$CEPA_{50}$	-	85±3.0	72±3.0	6±1.2
$CEPA_{100}$	-	96±2.0	94±2.0	-

[a]Standard Error (±SE)

Table 2. Effect of various treatments on flower senescence in *L. densiflorus*.

Treatment	Flower senescence % after days	
	4	6
Control	30±2.8[a]	55±3.0
TDZ	0	10±2.0
Sucrose	0	15±2.5
STS	15±3.0	25±3.0
1-MCP	12±2.5	22±2.0
CEPA	85±3.0	96±2.0
CEPA+TDZ	25±4.0	35±5.0
CEPA+Sucrose	20±1.5	25±3.0
CEPA+STS	28±2.8	35±3.0
CEPA+1-MCP	30±3.0	38±4.0
Sucrose+TDZ	0	0
CEPA+Sucrose+TDZ	10±1.8	15±2.0

[a]Standard Error (±SE)