Senescence Symptoms after Emasculation Vary among *Cymbidium* Cultivars

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Abstract. Response of cut *Cymbidium* flowers to emasculation was investigated. All *Cymbidium* cultivars that we examined produced ethylene during senescence. There were marked cultivar differences in the changes of fresh weight, ethylene production, and lip coloration after emasculation. In the case of 'Venus', flower emasculation did not alter fresh weight, ethylene production, or lip coloration. There were differences in ACC oxidase *in vivo* activity among the three cultivars before and after emasculation. There was a correlation between the initial ACC oxidase activity and time to lip coloration in the emasculated flowers, but not in control flowers. It was suggested that the initial ACC oxidase activity of the column may be related to flower response to emasculation rather than vase life during senescence.

In *Cymbidium* flowers, the first visible sign of senescence is a red coloration in the floral lip as a result of anthocyanin accumulation and concomitant ethylene production. Both emasculation and pollination dramatically hasten lip coloration and wilting of the perianth (Arditti et al., 1973). *Cymbidium* flowers have a protruding gynostemium (column), and the anther cap may be detached accidentally during harvesting and packaging procedures. Depending on the cultivar and handling procedure, some 10% to 20% of the flowers may lose their caps during distribution in The Netherlands (Woltering and Harkema, 1994) and 10.3% in Korea (Suh, 1996). Some postharvest treatments may ameliorate the apparently harmful effect of emasculation. However, cultivar selection might eliminate the need for

chemical treatments (van Doorn and Woltering, 1991). For this reason we investigated the senescence symptoms of some *Cymbidium* cultivars following emasculation as a screening method to determine their potential postharvest performance.

Flower spikes were obtained from a commercial grower and transported in water to our laboratory. All experiments were performed with individual flowers cut from the middle region of the inflorescence. After excision, the flowers were placed in water under controlled environmental conditions of $20 \pm 2^\circ\text{C}$, relative humidity $40 \pm 10\%$, and a 24-h photoperiod using cool-white fluorescent lamps with a photosynthetically active radiation of $4.5 \pm 1.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at flower level. In all cultivars, a comparison was made between intact flowers and those with their anther cap and pollinia removed (emasculated).

The development of visible senescence symptoms was recorded daily as described by Woltering (1990). Lip coloration was classified on a scale from 0 (original color) to 4 (dark red coloration). The average number of days to lip coloration (color score ≥ 3) was calculated.

After a 1-h incubation of individual flowers in a 155-mL vial, ethylene content of the vial was measured with a gas chromatograph (Hitachi 163, Tokyo) equipped with a flame ionization detector and an activated alumina column held at 80°C . For the determination of *in vivo* 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity (Woltering, 1990), columns were excised from flowers of eight cultivars and placed in 15-mL vials with their cut bases in 2 mL of 10 mM ACC (Sigma, St.

Fig. 1. Effects of emasculation on changes in fresh mass, ethylene production per flower, and lip coloration of *Cymbidium* cultivars. Open symbols = emasculated; closed symbols = control. Color score of lip: 0—original color; 1—pink tint; 2—pink; 3—red; 4—dark red.

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Table 1. Effect of emasculation on ACC oxidase activity *in vivo* in columns of three *Cymbidium* cultivars.

Cultivar	Treatment	ACC oxidase activity per column (nL C ₂ H ₄ ·h ⁻¹)						
		Time after emasculation (d)						
		0	2	6	9	11	13	18
Elise	Control	3.7 ± 0.8 ^z	6.7 ± 2.0	12.0 ± 2.4	36.6 ± 4.7	33.0 ± 5.8	---	---
	Emasculated	---	18.6 ± 4.0	38.0 ± 4.5	30.7 ± 3.5	13.0 ± 3.1	---	---
Anmitsu Hime	Control	1.6 ± 0.6	1.8 ± 0.7	3.0 ± 1.4	2.4 ± 1.3	3.1 ± 1.1	5.6 ± 0.0	31.1 ± 3.8
	Emasculated	---	2.0 ± 0.0	2.6 ± 0.9	2.6 ± 1.1	9.9 ± 2.3	28.9 ± 6.4	12.3 ± 4.5
Venus	Control	0.5 ± 0.3	1.0 ± 0.3	0.8 ± 0.0	3.4 ± 1.4	24.5 ± 4.4	21.0 ± 1.8	---
	Emasculated	---	0.8 ± 0.2	0.5 ± 0.0	2.5 ± 1.1	27.3 ± 5.3	18.9 ± 2.3	---

^zMean ± standard error (n = 5).

^yNot determined.

Fig. 2. Relationship between initial ACC oxidase activity per column and days to lip coloration (color score 3) in cut flowers of eight *Cymbidium* cultivars. All regressions are significant at $P \leq 0.05$. 1—'Princess Masako'; 2—'Elise'; 3—'Marie Laurencia'; 4—'Anmitsu Hime'; 5—'Venus'; 6—'Husky Honey'; 7—'Jungfrau'; 8—'Jane Seresute'.

Louis). After 2 h of gentle rotation at 20 °C, the vials were flushed with fresh air, sealed with serum vial septa, and incubated for 1 h, after which the headspace was sampled for ethylene content.

Results and Discussion

There were marked differences among cultivars in response to emasculation (Fig. 1). Emasculation of 'Venus' flowers did not alter fresh mass, ethylene production, or lip coloration, the lip never reaching a color score of more than 3 (red coloration) in these flowers whether emasculated or not.

The three cultivars differed in *in vivo* ACC oxidase activity both before and after emasculation (Table 1). When more cultivars were compared, initial ACC oxidase activity was negatively correlated with time to lip coloration, r^2 being greater for emasculated flowers than for control flowers (Fig. 2). This suggests that the initial ACC oxidase activity of the

column may be related to the flower's responsiveness to emasculation, but not to its vase life during natural senescence. Nadeau et al. (1993) suggested that differences in ACC oxidase gene expression prior to pollination might be the basis for the longevity of certain flowers, since expression in the style might ultimately lead to enough "leaky" ethylene synthesis to stimulate full-scale autocatalytic production.

During flower senescence the production and rate of ethylene vary greatly among flowers of different taxa (Woltering and van Doorn, 1988) as well as among cultivars within the same species (Mayak and Tirosh, 1993; Wu et al., 1991). This fact indicates the potential for the development of genetic lines that produce little or no ethylene and therefore have longer vase life. All of the 33 *Cymbidium* cultivars we examined produced some ethylene during senescence (data not shown). However, both ethylene production and response to emasculation varied significantly among them. Some

were less sensitive (e.g., 'Anmitsu Hime' and 'Princess Masako'), while others were insensitive to emasculation (e.g., 'Venus' and 'Love'). This trait will be useful to breeders in improving the vase life of the flower.

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