Putrescine Increases Effective Pollination Period in Roses

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Summary. Flowers of two cultivars of *Rosa hybrida* were treated or not with putrescine before being pollinated from 2 to 8 days after anther emasculation. On both cultivars the 10^{-3} M putrescine treatment extended the effective pollination period, as shown by the best hip formation rates and mean number of seeds per hip. On one cultivar, the 10^{-5} M putrescine treatment increased fertilization efficiency (more hips obtained). The effect of putrescine was proportionally more important on the cultivar characterized by the highest stigmatic exudate pH. Putrescine also influenced in vitro pollen germination by increasing the length of emitted pollen tubes (10^{-3} and 10^{-5} M putrescine) and the quantity of germinated pollen grains (10^{-5} M putrescine).

Putrescine applications have been shown to increase fruit set in apple (Costa and Bagni, 1983), olive (Rugini and Mencuccini, 1985), and pear (Crisosto et al., 1988) trees. In pear, Crisosto et al. (1988) also demonstrated that putrescine is liable to extend the effective pollination period by enhancing ovule longevity. We attempted to determine if one or both of these effects could be observed in roses. A cultivar characterized by a "high" pH stigmatic exudate and a cultivar characterized by a "low" pH stigmatic exudate (such as classified by Gudín and Aréne, 1991) were used, as external pH has been shown to interact with cellular polyamine content (Galston, 1983), especially putrescine

content (Young and Galston, 1983). The influence of putrescine on in vitro pollen germination was also investigated.

On 18 June 1989, 900 flowers each of *R. hybrida* '255-75-A43' and *R. hybrida* '364-73-A' grown in a greenhouse at Selection Meilland in Antibes were cut at the commercial cropping stage (Gudín et al., 1991). Their petals and stamens were removed (Gudín et al., 1990, 1991). The pH of the stigmatic exudates of both cultivars was measured with Dosatest paper strips as described by Gudín and Aréne (1991). One hundred flowers of each cultivar were treated with a pH 5.6 aqueous solution of 10^{-5} M putrescine, 400 flowers with a pH 5.6 aqueous solution of 10^{-3} M putrescine, and 400 flowers with pH 5.6 distilled water by soaking the styles and stigmas with a paint brush (Raphael no. 8).

Two days later, 100 flowers of each treatment were pollinated with pollen of *R. hybrida* '86-73-UO' (Gudín et al., 1990). One hundred 10^{-3} M putrescine-treated and 100 water-treated flowers were pollinated similarly at 48-h intervals for 4 days. Four months after pollination the hips were collected and counted, and the number of seeds per hip was determined.

On each pollination day some pollen was removed from a frozen lot stored at -30°C from May 1989 (Gudín et al., 1990). It was allowed to thaw for 4 h in the laboratory at 20°C before use. A sample of the pollen (also kept 4 h in the laboratory before use) was used for in vitro germination tests (Gudín et al., 1991). The sample was deposited on an agarized medium described previously (Gudín et al., 1991), with the addition of three drops of pH 5.6 (same pH as the medium) HCl-acidified distilled water, 10^{-5} M putrescine or 10^{-3} M putrescine aqueous solutions. Twelve hours later the mean number of pollen grains germinated per 50 grains and the average length of emitted pollen tubes were determined (Gudín et al., 1991).

Measurements of stigmatic exudates showed that '255-75-A43' was characterized by a pH of 9, while '364-73-A' had a pH of 5.

Table 1 shows that, for both crosses, the 10^{-3} M putrescine treatment was beneficial to hip and seed production when pollinations were performed between the 4th and 8th days following treatment. Only the

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Table 1. Effects of style and stigma treatments with aqueous solutions of putrescine at time of flower castration on subsequent hip and seed formation resulting from pollinations performed 2, 4, 6, or 8 days after flower castration among two crosses between *Rosa hybrida* cultivars.

		Pollination time (no. days following flower castration)								
		2		4		6		8		
		Style and stigma treatment								
Cross	Pollination result criteria	10 ⁻⁵ M Put.	10 ⁻³ M Put.	0 Put. (H ₂ O)	10 ⁻³ M Put.	0 Put. (H ₂ O)	10 ⁻³ M Put.	0 Put. (H ₂ O)	10 ⁻³ M Put.	0 Put. (H ₂ O)
255-75-A43 x 86-73-UO	Hip formation									
	rate (%)	92 a ^c	88 ab	80 b	70 c	50 d	44 d	16 f	30 c	0 g
	Mean no. seeds/hip (± SE)	10 ± 6 a	9 ± 5.75 a	8.15 ± 5.95 a	5 ± 3 b	2.6 ± 2 c	2.3 ± 2.4 c	1 ± 0.4 d	1.3 ± 0.9 cd	---
364-73-A x 86-73-UO	Hip formation									
	rate (%)	98 a	96 a	97 a	84 b	48 c	25 d	10 d	9 d	0 e
	Mean no. seeds/hip (±SE)	24 ± 9.7 a	22.1 ± 10.5 a	20.2 ± 9.4 a	20.4 ± 10.1 a	13 ± 6 b	5 ± 5 c	1.7 ± 1.1 d	2.4 ± 1.2 cd	---

Among each cross, means (±SE) and rates followed by at least one identical letter are not significantly different from each other at $P = 0.05$ (the χ^2 and Student's t tests were used for difference analysis of rates and means, respectively); 100 pollinated flowers per cross and treatment.

10⁻⁵ M putrescine treatment on '255-75-A43' had a significant effect on flowers pollinated 2 days after flower preparation, while it had no significant influence on the other cultivar.

The putrescine treatment was more effective on '255-75-A43' than on '364-73-A', as pollinations performed on '255-75-A43' on the 8th day yielded hip formation rate and mean number of seeds per hip of 34% and 14%, respectively, of those obtained with flowers pollinated after 48 h, while these same ratios represented only 9% and 10% in the second cultivar.

Putrescine at 10⁻⁵ M influenced in vitro pollen germination by increasing germinated quantities and average length of emitted tubes (Table 2). At 10⁻³ M, it only significantly increased the length of emitter tubes.

Hip and seed production have previously been shown to be a good index of fertilization success in roses (Gudin and Arene, 1991; Gudin et al., 1991). Our results are, therefore, most likely due to this polyamine influence on the fertilization process.

Fruit set can be increased by a putrescine treatment on roses polli-

nated at a stage corresponding to anthesis, depending on cultivar ('255-75-A43') and putrescine concentration (10⁻⁵ M). This concentration was also reported as the most efficient among a wide range tested (10⁻⁶ to 10⁻³ M) on fruit set of apple (Costa and Bagni, 1983).

Although the demonstration by Crisosto et al. (1988) of putrescine's increasing of ovule longevity is sufficient to explain the extension of the pollination period, the influence of putrescine on pollen germination demonstrated here might also have an effect. The observed effect of putrescine on promotion of pollen-tube elongation supports the assumption of the role of polyamines in cellular division (Flores et al., 1985; Heby, 1981), elongation (Galston, 1983), and differentiation (Montague et al., 1978; Nielsen, 1990).

It is interesting that the putrescine treatments were more effective on the cultivar with the higher stigmatic exudate pH. As external low pH has been shown to indicate high cellular polyamine contents (Flores et al., 1985; Galston, 1983; Young and Galston,

1983), the cultivar characterized by a high stigmatic exudate pH might be hypothesized to be poor in stigmatic and stylar tissue polyamine content compared with the cultivar with a low stigmatic exudate pH. This possible "lack" of cellular polyamine content then could be at least partially compensated for by an external putrescine application. The pollination efficiency results we obtained seem to support this assumption. This hypothesis must be verified by the analysis of endogenous polyamine levels, since rose cultivars with low stigmatic exudate pH recently have been shown to be fertilized more easily than cultivars with high stigmatic exudate pH (Gudin and Arene, 1991). Cellular polyamine content of female sexual tissues is known to increase dramatically following pollination (Galston, 1983). Our results clearly indicate that putrescine influences the efficiency of this step toward fertilization in rose.

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Table 2. Influence of an addition of three drops of aqueous solutions of different putrescine concentrations to agarized medium on mean quantities of in vitro-germinated pollen grains (±SE) and average length of emitted tubes (±SE) of *Rosa hybrida* '86-73-UO'.

	10 ⁻⁵ M Put.	10 ⁻⁵ M Put.	10 ⁻⁵ M Put. (H ₂ O)
Mean no. germinated pollen grains out of 50	20 ± 3 a ^c	18 ± 3 ab	15 ± 3.4 b
Mean length of emitted pollen tubes (µm)	480 ± 64 a	380 ± 66 b	300 ± 48 c

For each parameter, means followed by at least one identical letter are not significantly different from each other at $P = 0.05$ (Student's t test). The means given for the pollen germinated quantity parameter are the result of 10 counts. The means given for the pollen tube length parameter result from the observation of 25 germinated pollen grains taken at random.

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