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Pollen Viability in *Rosa*

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Abstract. **The viability of fresh pollen from 6 *Rosa* species (*Rosa spinosissima L., R. fedtschenkoana* Regel, *R. pendulina* L., *R. arkansana* Porter, *R. carolina* L., *R. virginiana* Miller), one botanical variety (*R. spinosissima altaica* Rehd.) and 3 cultivars ('Betty Bland', 'George Will', 'Prairie Princess') were evaluated. Correlation between pollen staining and germination was positive and significant, but absolute pollen viability was found to be a better indicator of viability than staining. *Rosa spinosissima* and *R. fedtshenkoana* were considered to be the best pollen parents from the genotypes sampled.**

Modern garden rose cultivars are tetraploid, complex interspecific hybrids which have arisen from about 10 rose species (5). New germplasm is needed to increase hardiness, however, as garden roses in most parts of Canada suffer injury or death each winter. Wide crosses using wild species are sometimes difficult because of cross imcompatibility and either inviability or sterility of the microgametophytes.

Although various stains (4, 6, 10, 11) have been used in studies of rose pollen, staining may not indicate its true viability. Erlanson (3) reported that many apparently morphologically perfect grains were unable to effect fertilization. Visser et al. (13) called stainable pollen grains "normal pollen", but considered them as having only the potential to germinate. The actual amount of viable pollen has been determined *in vitro* by rose pollen germination (2, 13, 15). This study was conducted to evaluate the viability of pollen from 7 rose species and 3 cultivars and hence the potential of these taxa as parents in breeding cold hardy garden roses. In addition, a comparison was made between 2 indicators of pollen viability, staining and *in vitro* germination.

The 7 species (*Rosa spinosissima* L., *R. spinosissima altaica* Rehd., *R. fedtschenkoana* Regel, *R. pendulina* L., *R. arkansana* Porter, *R. carolina* L., *R. virginiana* Miller) are once-flowering hardy shrubs, indigenous to cold areas of the world. The 3 shrub rose cultivars ('Betty Bland', 'George Will', and 'Prairie Princess') have a hardy species as one of their primary or secondary parents (Table 1). Pollen was collected during the spring flowering period in 1978, 1979, and 1980. Unopened flower buds, showing petal color and having reflexed sepals, were collected at 8:00 AM and placed in labeled bags. In the laboratory, the mature unopened anthers were renoved from the flowers and allowed to dry in small 5 g vials under diffuse laboratory light. When the anthers dehisced 2 to 4 days later, pollen as collected and stored in capped vials at 4° to 5°C over crystalline $CaCl_2$.

Stainability of fresh pollen samples was determined by the acetocarmine stain technique (10). One hundred pollen grains were counted per slide, with 6 slides for each of the 3 cultivars and 10 species. From this sample, the percentage of stained pollen and the percentage of aborted pollen grains were determined in each. Germination of fresh pollen was assessed by hanging-drop culture (13). A drop of germination medium, a 15% aqueous sucrose solution containing 40 ppm boric acid, was placed on a coverslip and the pollen dusted onto the drop. The coverslip then was inverted and placed over a concave depression on a slide, using glycerol to seal the coverslip and prevent desiccation. After 4 to 6 hr at 22°C in the light, the 6 slides were scanned for each pollen source. The percentage of pollen viability was determined for each by counting the number of pollen grains germinated per 600 normally shaped pollen grains. All observations of slides were carried out at x100 magnification using a grid mounted in the eyepiece of a Leitz Wetzlar photomicroscope. Following Visser et al. (13, 14), absolute pollen viability, or the effective germination capacity *in vivo*, was calculated for fresh 1980 pollen samples using the formula.:

$$\frac{\%\ \text{normal pollen} \times \%\ \text{pollen viability}}{100}$$

All lightly stained misshapen pollen grains were counted as abnormal. Normal pollen grains were spherical and stained carmine. The proportion of stained pollen grains produced by the same individual over 2 to 3 seasons varied, more so in *R. spinosissima altaica, R. pendulina, R. carolina, R. virginiana,* 'Betty Bland', and 'Prairie Princess' than in *R. spinosissima, R. arkansana* and 'George Will' (Table 2). In addition, there were differences in stained pollen grains from different taxa in the same year.

Pollen germination was considered to occur *in vitro* when a pollen tube was formed that was equal to, or greater than, the diameter of the pollen grain. Normal pollen grains that did not germinate seemed unchanged, even after several hours in the germination medium. The amount of fresh rose pollen that germinated was much less than the percentage stained (Table 3). Although the correlation between germination percentage and staining percentage was significant ($r = +0.5$), the corresponding coefficient of determination ($R^2 = 0.25$) suggests that staining rose pollen with ace-

Table 1. Origin of the 3 cultivated varieties.[z]

Cultivar	Parentage
Betty Bland	*R. blanda* × a Hybrid Perpetual (possibly Captain Hayward)
George Will	(*R. rugosa* × *R. acicularis*) × a garden variety
Prairie Princess	Carrousel × (Morning Stars × Suzanne) Suzanne is an F_2 of *R. laxa* × *R. spinosissima*

[z]Literature cited (9, 12).

Table 2. The stainability of fresh rose pollen of 10 taxa in 3 consecutive years.

Genotype	Normal pollen grains (%)[z] 1978	1979	1980
R. spinosissima	94.0 ab[y]	92.2 ab	87.3 bc
R. spinosissima altaica	68.8 ef	71.5 e	60.8 fg
R. fedtschenkoana	---	---	84.5 cd
R. pendulina	---	84.5 cd	58.7 gh
R. arkansana	53.0 ghi	43.8 i	48.0 hi
R. carolina	95.7 a	93.2 ab	75.7 e
R. virginiana	87.3 bc	93.3 ab	96.3 a
Betty Bland	93.7 ab	77.3 de	84.5 cd
George Will	69.3 ef	72.2 e	70.8 ef
Prairie Princess	---	45.5 i	57.2 gh

[z]Mean of 6 replications, each containing 100 pollen grains.
[y]Means of transformed data are separated by Duncan's multiple range test, 5% level.

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Table 3. Comparison of percentage stainable and germinated pollen; and the calculation of percentage absolute pollen viability for fresh pollen samples in 1980.[z]

Genotype	Stained pollen (%)	Normal pollen germinated (%)	Absolute pollen viability (%)
R. spinosissima	87.3 b[y]	64.7 a	56.6 a
R. spinosissima altaica	60.8 de	3.3 ef	2.0 fg
R. fedtschenkoana	84.5 b	69.2 a	58.5 a
R. pendulina	58.7 e	1.7 f	1.0 g
R. arkansana	48.0 f	10.8 de	5.2 ef
R. carolina	75.7 c	32.8 b	24.8 b
R. virginiana	96.3 a	14.5 cd	14.0 cd
'Betty Bland'	84.5 b	25.8 bc	21.8 bc
'George Will'	70.8 cd	26.0 bc	18.4 bcd
'Prairie Princess'	57.2 ef	20.5 cd	11.7 de

[z]Mean of 6 replications, each containing 100 pollen grains.
[y]Means in a column represent transformed data and are separated by Duncan's multiple range test, 5% level.

tocarmine provides only a rough estimate of viability.

Absolute pollen viability in the genotypes ranged from 1.0% to 58.5% (Table 3). This calculation is improved for estimation of actual pollen viability because many morphologically normal pollen grains lacked the ability to germinate.

Lewis (7) and Macfarlane (8) both noted that the North American tetraploid species overlap in range and that species complexes often arise through hybridization. *Rosa arkansana, R. carolina,* and *R. virginiana* are 3 members of the section Cinnamomeae that do overlap in north-eastern North America. These rose species could be hybrid through introgressive hybridization and the development of hybrid swarms with subsequent reproductive incapacity. With the exception of *R. fedtschenkoana* and *R. spinosissima,* the other taxa had low absolute pollen viabilities. *Rosa fedtschenkoana,* a native of Turkestan had highly stainable pollen and the best absolute viability. *Rosa spinosissima,* which ranges from Europe to western Asia, consistently produced high amounts of stainable pollen and had the 2nd highest absolute viability. These species are reliably hardy in zone 2 (1). On the basis of their hardiness and comparatively good pollen viability, these 2 taxa were selected as the best staminate parents in our hardiness breeding program.

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