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Barriers to Intersubgeneric Crosses between *Muscadinia* and *Euvitis*

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Abstract. **Fluorescence microscopy was used to examine the unilateral intersubgeneric incongruity of muscadine grape (*Muscadinia* Planch.) x bunch grape (*Euvitis* Planch.). Pollen grains of bunch grape hydrated and germinated on the stigmas of muscadine grape. Healthy pollen tubes of the bunch grape also penetrated the stigma and entered into the style without obstacles. However, most bunch grape pollen tubes were arrested in the style near the stigma, and few bunch grape pollen tubes were found at the base of the style. Barriers to the intersubgeneric crosses apparently occurred before fertilization; abortion of pollen tubes in the style was the major cause of failure for the cross of *V. rotundifolia* Michx. x *Euvitis*.**

Wild and cultivated grapevines belong to the genus *Vitis* L. in the family Vitaceae Planch. The genus *Vitis* is divided into two subgenera: *Euvitis* Planch. and *Muscadinia* Planch. (Olien, 1990; Winkler et al., 1974). The former has 38 chromosomes and many berries borne in each cluster, so the general term "bunch grape" is given to the subgenus *Euvitis*. The latter has 40 chromosomes and smaller clusters, with a common name of muscadine grape. More than 60 species have been described in *Euvitis*, and *V. vinifera* L. is the predominant commercial species cultivated worldwide (Alleweldt et al., 1991; Zhang et al., 1990). Only three species (*V. rotundifolia* Michx., *V. munsoniana* Simpson ex Munson, and *V. popenoei* Fennell) have been identified in the subgenus *Muscadinia*. *Vitis rotundifolia,* normally referred to as the muscadine grape, is the only species with commercial value.

Vitis rotundifolia is native to the southeastern United States and is characterized by high disease, insect, and nematode resistance. It has resistance to most fungal diseases and Pierce's disease (PD), caused by the bacterium *Xylella fastidiosa* Wells (Olmo, 1971), a limiting factor in the production of *V. vinifera* grapes in the southeastern United States. Except for a few species native to the southeastern United States, bunch grapes are susceptible to these pests but have positive characteristics, such as a large cluster, edible skin and pulp, and seedlessness, which have not been found in muscadine grapes. Hybridization of these two grapes to obtain bunch grape fruit quality and good disease resistance from *V. rotundifolia* has been a long-term goal for grape breeders.

Intersubgeneric crosses between muscadine and bunch grapes have been performed for more than a century by breeders in several grape breeding programs (Bouquet, 1981; Detjen, 1919; Dunstan, 1962; Olmo, 1971, 1986). *Vitis rotundifolia* will hybridize quite readily with some species of *Euvitis* when used as the male parent, but when used as the female parent, it will hybridize only rarely.

The failure to produce hybrids from the muscadine stigma pollinated with bunch grape pollen in various attempts over a century clearly indicates that a unilateral incompatibility exists between the *Muscadinia* x *Euvitis* crosses. Based on a histochemical study, Patel and Olmo (1955) concluded that the reason for the complete failure of *rotundifolia* x *vinifera* crosses was not due to failure of pollen tube growth but to barriers in the embryo sac. Since interspecific incongruity may vary depending on the species that are crossed, we determined whether the inconcruity described by Patel and Olmo also appears in crosses of *V. rotundifolia* pollinated by pollen of other *Vitis* species. In addition, we used fluorescence microscopy to determine more efficiently the distance pollen tubes penetrate in grape. Fluorescence staining and squashing technique also can trace pollen tube growth from stigmas to ovules.

Materials and Methods

Twenty-four, cross combinations were used for this study (Table 1). Sixteen of them were *V. rotundifolia* x *Euvitis*, including four female muscadine parents ('Fry', 'Higgins', 'Jumbo', 'Summit') and four bunch grape pollen parents ('Thompson Seedless', 'Flame Seedless', 'Orlando Seedless', and 'Blanc du Bois'). Four combinations of *Euvitis* x *V. rotundifolia* and four combinations of *V. rotundifolia* x *V. rotundifolia* were used as control crosses. The muscadine grapes, 'Orlando Seedless', and 'Blanc du Bois' were grown in the experimental vineyard at Florida A&M Univ., while fresh pollen of 'Thompson Seedless' and 'Flame Seedless' was obtained from the Horticultural Crops Research Laboratory, U.S. Dept. of Agriculture, Agricultural Research Service, Fresno, Calif.

Table 1. Pollen tube growth in inter- and intraspecific crosses of muscadine and bunch grapes.

Crosses	Pistils examined[z]	Pistils with pollen tubes: Near stigma No.	Near stigma %	At base of style No.	At base of style %
Vitis rotundifolia x *Euvitis*					
Fry x Flame Seedless	25	17	68.0	0	0
Fry x Thompson Seedless	25	20	80.0	2	8.0
Fry x Orlando Seedless	24	14	58.3	4	16.7
Fry x Blanc du Bois	23	18	78.3	1	4.3
Higgins x Flame Seedless	25	20	80.0	0	0
Higgins x Thompson Seedless	25	14	56.0	0	0
Higgins x Orlando Seedless	25	20	80.0	3	12.0
Higgins x Blanc du Bois	25	15	60.0	2	8.0
Jumbo x Flame Seedless	24	18	75.0	0	0
Jumbo x Thompson Seedless	25	15	60.0	3	12.0
Jumbo x Orlando Seedless	20	11	55.0	0	0
Jumbo x Blanc du Bois	20	11	55.0	1	5.0
Summit x Flame Seedless	25	14	70.0	0	0
Summit x Thompson Seedless	23	21	91.3	1	4.3
Summit x Orlando Seedless	25	17	68.0	0	0
Summit x Blanc du Bois	22	11	50.0	2	9.1
Euvitis x *V. rotundifolia*					
Blanc du Bois x Carlos	23	14	60.9	13	56.5
Blanc du Bois x Noble	22	12	54.5	10	45.5
Orlando Seedless x Carlos	21	13	61.9	11	52.3
Orlando Seedless x Noble	22	9	40.9	9	40.9
V. rotundifolia x *V. rotundifolia*					
Fry x Carlos	25	21	84.0	21	84.0
Fry x Noble	25	18	72.0	16	64.0
Jumbo x Carlos	25	24	96.0	24	96.0
Jumbo x Noble	25	23	92.0	23	92.0

[z]Numbers were combined from all five collection-time treatments.

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'Orlando Seedless' and 'Blanc du Bois' were emasculated 1 day before anthesis, and paper bags were used to cover the flower clusters immediately thereafter. No emasculation was needed for the muscadine cultivars Fry, Higgins, Jumbo, and Summit since they produce pistillate flowers. Instead, paper bags were used to cover the flower clusters several days before pollination. Pollination was performed in the morning between 8:00 AM and 10:00 AM. A glass rod carrying pollen grains was gently touched onto the stigma surface. Five to 10 clusters of flowers, with a total of ≥250 flowers, were pollinated for each cross combination. Five pollinated flowers for each cross were collected 8 h, 1 day, 2 days, 3 days, and 5 days after pollination and were fixed in 1 formalin : 1 acetic acid : 8 alcohol. The fixed pistils were stored in a refrigerator at 4C until microscopic examination. The remaining flowers were left on the vines for further observation.

The pistils were cleared and softened in 1 N NaOH for 2 h at 25C, then moved to a 60C water bath for 45 min, and stained with 0.1% aniline blue in 0.1 N K_3PO_4 for 2 h. The pistils were squashed under a cover slip and were examined under a fluorescent microscope.

Results and Discussion

The aniline blue staining technique worked fairly well for staining grape pollen tubes, as they were distinguished clearly from the surrounding stylar tissue by the presence of a strong fluorescence (Fig. 1). In these intraspecific crosses, pollen tubes appeared healthy and grew all the way to the base of the style. For example, >90% of the examined pistils of 'Jumbo' x 'Carlos' and 'Jumbo' x 'Noble' had pollen tubes at the base of the style (Table 1). The pollen tubes were found at the base of the style as early as 8 h after pollination. By using the fluorescence microscopy staining technique, we could examine pollen tubes on the same day that the pistils were excised from the vine. The simplicity and quickness of the fluorescence microscopy staining technique encouraged us to use it to study pollen tube growth in intersubgeneric crosses.

As with the intraspecific crosses of *V. rotundifolia*, the pollen grains of muscadine grapes germinated on the stigmas of the *Euvitis* 'Orlando Seedless' and 'Blanc du Bois'. Pollen tubes also penetrated the stigma without obstacles. Healthy pollen tubes were found at the base of the *Euvitis* styles from 8 to 24 h after pollination. In the *V. rotundifolia* x *Euvitis* crosses, the *Euvitis* pollen grains also germinated on the muscadine stigmas. The pollen tubes that entered the stigma and penetrated the style were similar to those of *Euvitis* x *V. rotundifolia*. However, unlike the crosses of *Euvitis* x *V. rotundifolia*, most of the bunch grape pollen tubes were arrested in the style near the stigma end of the muscadine grapes (Fig. 2). Only occasionally were one or two pollen tubes of the bunch grapes at the base of the *V. rotundifolia* styles (Fig. 3, Table 1). This phenomenon appeared in all of the *V. rotundifolia* x *Euvitis* crosses in this study.

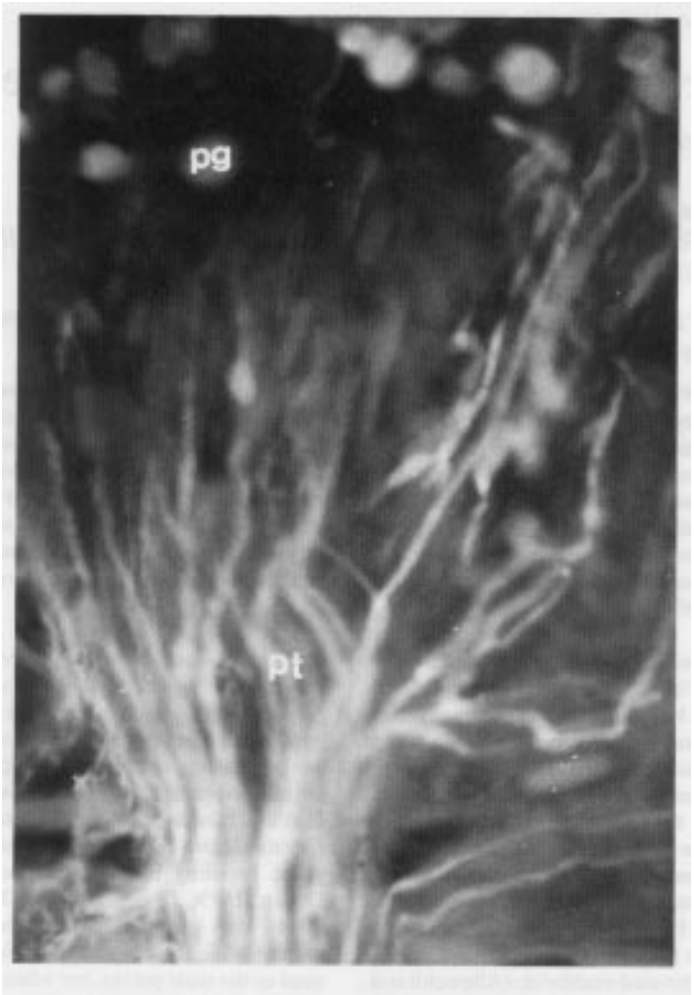

Fig. 1. Fluorescence micrograph of the squashed pistil of 'Fry' muscadine grape 24 h after pollination with pollen of 'Carlos' muscadine grape; pg = pollen grain and pt = pollen tube, ×200.

Fig. 2. Arrest of pollen tubes in the style near the stigma in the intersubgeneric cross of 'Fry' x 'Orlando Seedless' 48 h after pollination; ×100.

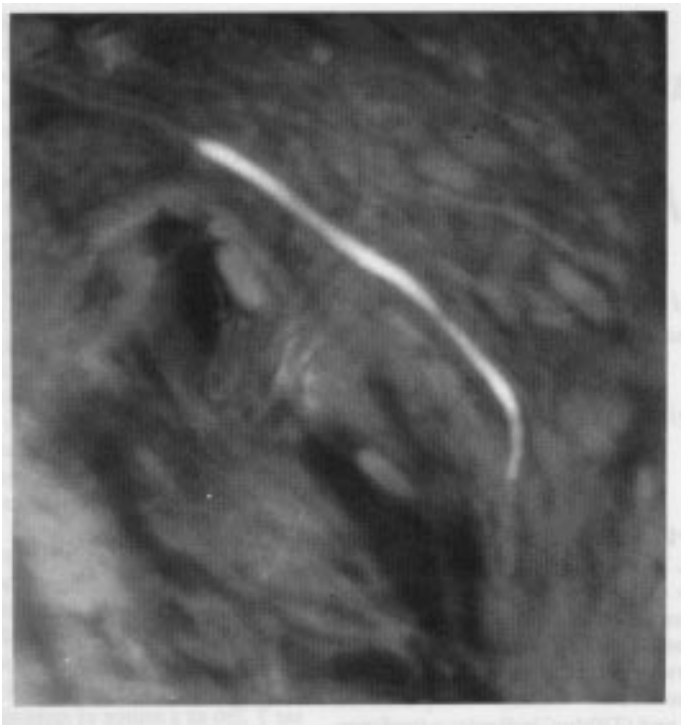

Fig. 3. A single pollen tube aborted at the base of the style in the intersubgeneric cross of 'Fry' x 'Blanc du Bois' 48 h after pollination; ×220.

When 'Flame Seedless' was used as a pollen parent, none of the pollen tubes grew all the way down the style, while in all four crosses of *V. rotundifolia* x 'Blanc du Bois', at least one pollen tube reached the base of the style. However, it is difficult to interpret such a difference into genotype variation since the percentage of styles with pollen tubes reaching to the stylar base was so low. When a comparison was made at the species level (*vinifera* vs. the American hybrids), no significant difference was found whether *V. vinifera* ('Flame Seedless', 'Thompson Seedless') or American hybrid bunch grapes ('Orlando Seedless', 'Blanc du Bois') were used as the pollen parents ($\chi^2 = 3.2$, 1 df).

Among flowers that had been left on the vines, only one hybrid was obtained from a cross of 'Jumbo' x 'Thompson Seedless' among the 16 cross combinations of *V. rotundifolia* x *Euvitis*.

As part of our breeding program in 1993, pollen of the stenospermocarpic grapes used in the intersubgeneric crosses also were put onto stigmas of *Euvitis* grape 'Blanc du Bois' ('Blanc du Bois' x 'Orlando Seedless', 'Blanc du Bois' x 'Flame Seedless', and 'Blanc du Bois' x 'Thompson Seedless'). Hundreds of F_1 hybrids were produced from these crosses (data not shown). This result implies that the pollen grains used in the intersubgeneric crosses were viable and vigorous, and the abnormality of pollen tube growth in the style of *V. rotundifolia* was not caused by defective pollen.

In angiosperms, many events occur between pollen germination and seed production, such as pollen hydration and germination, pollen tube penetration through the stigma surface, pollen tube growth in the style, and fertilization and embryo development (Williams, 1987). Barriers to any of these series of events can lead to the failure of seed formation. The pollen tube abortion in the styles of *V. rotundifolia* appeared to be the major cause of failure in the muscadine x bunch grapes crosses. This conclusion differed from the one of Patel and Olmo (1955)—that the reason for complete failure of crossing *rotundifolia* x *vinifera* was due to the barrier in the embryo sac but not due to the failure of pollen tube growth. A possible reason for this difference may be that they used genotypes that differed from ours. However, the reoccurrence of the pollen tube being aborted in the style for all the muscadine x bunch crosses implies that pollen tube abortion in the style is a common phenomenon in *V. rotundifolia* x *Euvitis*. Since few pollen tubes were at the base of some styles but only one hybrid was developed among the 16 cross combinations from >3000 pollinated flowers left on the vines, barriers also may exist somewhere between the base of the style and the embryo sac. The latter conclusion is similar to the findings of Patel and Olmo (1955), but it implies a second barrier site in addition to the one in the style. Embryo rescue may help to recover hybrids if the failure of seed set results from embryo abortion, which is common in many wide crosses. However, our results show that this is not a possibility since the incompatibility of muscadine x bunch grapes took place before fertilization. Overcoming the prefertilization barriers, therefore, must be the first step in obtaining hybrids when muscadine is used as the female parent. Further research is warranted to see if techniques (such as bud pollination, style pollination, application of gibberellic acid, etc.) can be used to overcome prefertilization barriers. However, the low fruit set in *Euvitis* x *V. rotundifolia* crosses may be due, in part, to postfertilization barriers since no stylar barrier was observed in those crosses. This prediction is in agreement with the report of Goldy et al. (1989), who found that success could be enhanced if hybrid embryos were rescued 6 weeks after pollination.

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