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Artificial pollination in *Vitis coignetiae* Pulliat

by

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Summary: Artificial pollination experiments were conducted on female vines of *Vitis coignetiae* and the effects of pollen variety and pollination methods on pollen tube growth were analyzed. Pollination by dusting pure pollen from Muscat of Alexandria (*V. vinifera*), Cabernet Sauvignon (*V. vinifera*), Campbell Early (*V. vinifera* x *V. labrusca*) and male *V. coignetiae* vines resulted in a large number of pollen tubes penetrating the stigma and an average of more than four tubes per pistil reached the micropyles. Dry pollinations with pollen from Muscat of Alexandria diluted in powdered milk or *Lycopodium* spores at the proportion of 1:2 or 1:10 (pollen : diluent w/w) resulted in pistils with ca. 140 to 250 pollen grains on the stigma. Forty to 60 pollen tubes per pistil penetrated the style and four or more pollen tubes reached micropyles in all treatments. Clusters sprayed with or dipped in a 10 % sucrose solution containing 0.1, 0.5 or 1.0 g pollen/l produced stigmas with an average of 5.2 to 38.5 pollen grains. Pollen germination was poor both for spray and dipping treatments and less than one pollen tube per pistil reached micropyles for any of the pollen concentration used.

Key words: artificial pollination, pollen diluent, pollen tube growth, Vitis coignetiae.

Introduction

Flowers of most commercial grape cultivars are functionally hermaphrodite and produce self-compatible pollen (MULLINS *et al.* 1992). Problems of insufficient fertilization observed in some cultivated varieties of grapes occur due to abnormal development of the ovule (PRATT and EINSET 1961; CARRARO *et al.* 1979). However, several reports on cultivars with low productivity have focused on the inability of pollen to germinate as the main factor affecting berry set (LOMBARDO *et al.* 1976 and 1978; CARGNELLO *et al.* 1980; CARRARO *et al.* 1981). Grape cultivars which require cross- or supplementary pollination are rarely used for commercial production, and no studies seeking an efficient and economically viable method for artificial pollination in grapes have yet been reported. However, several commercial fruit crops such as peach, kiwifruit, pear, date and apple use artificial pollination commercially and equipments for this process have been developed (LEGGE and WILLIAMS 1975; WILLIAMS and LEGGE 1979; CONTE *et al.* 1991; LOGHAVI 1991; FERGUSON and PUSCH 1992). The use of honey-bees to improve pollination in fruit crops is also a common practice. In grapes, however, attraction of honey-bees to flowers is poor and placement of hives within a vineyard seems inefficient in improving yield (SHARPLES *et al.* 1965; BRANTIES 1978).

Vitis coignetiae, a wild species of grape, have been cultivated for wine making in Japan since the last decade (KIMURA *et al.* 1997). This species is dioecious and needs cross-pollination to set berries. The level of natural cross-pollination in vineyards of *V. coignetiae* is low, and growers have been spraying pollen from male vines diluted in a 10 % sucrose solution on clusters in an attempt to improve yield. However, the effectiveness of this practice is unclear. The present study was conducted to select pollen cultivars suitable for pollination of this species as well as to analyze the effects of pollination methods on pollen distribution on the stigma and pollen tube growth in the pistil.

Material and methods

Experiments were conducted in 1996 at Hiruzen, Okayama Prefecture, using 12-year-old female vines of *V. coignetiae*. The vines were own-rooted, 2.0 m x 2.5 m spaced, hedge-trained and cane-pruned. Fertilization efficiency of pollen source variety was tested using pollen obtained from local vineyards. Pollen was collected from three commercial cultivars, i.e. Muscat of Alexandria (*V. vinifera*), Cabernet Sauvignon (*V. vinifera*), Campbell Early (*V. vinifera* x *V. labrusca*) and from male vines of *V. coignetiae*.

Germination of pollen *in vitro*, for all cultivars, was tested on agar blocks prepared with 1 % agar: 20 % sucrose and incubating at 25 °C for 8 h, with 3 replicate blocks. The germination criterion was the growth of a tube longer than two times the pollen grain diameter. Germination of Muscat of Alexandria pollen diluted in a 10 % sucrose solution was also tested under similar conditions.

For *in vivo* pollen tube growth studies, individual florets of clusters on female vines were tagged at bloom and hand-pollinated two days after with pure pollen from the cultivars being tested. There were 4 replicate vines and all vines had two clusters pollinated with each pollen type. From each vine, 30 pistils per treatment were randomly collected 3 d after pollination and fixed in a solution prepared with 50 % ethanol, formalin and glacial acetic acid (FAA - 18:1:1 v/v). Pistils were later embedded in paraffin and sectioned with a microtome. Cross-sections from the style and ovary were stained with aniline blue and the number of pollen tubes present in each part were counted under a fluorescent microscope.

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Effectiveness of dry and liquid artificial pollination methods with different pollen concentrations was tested using Muscat of Alexandria pollen. Dry pollinations were done by dusting mixtures comprised of one part of pollen plus two (1:2 w/w) or ten (1:10 w/w) parts of powdered skimmed milk or *Lycopodium* spores as diluents. For dry pollinations, clusters were enclosed with plastic bags containing the pollen mixture and shaken. For liquid pollinations, clusters were dipped in or sprayed with a 10 % sucrose solution containing 0.1, 0.5 or 1.0 g/l of pollen. A hand-mister was used to generate the spray. Each treatment used 5 clusters distributed on 5 different vines. Florets with delayed development were excluded, and after emasculation clusters were covered with paper bags for 3 d until pollination to allow the pistils reach an adequate maturity.

After pollination, 30 pistils per treatment were randomly collected and the number of pollen grains on their stigmas was determined. Stigmas were singly excised from pistils, placed on micro-slides together with a drop of acetocarmine (Nacalai Tesque Inc., Kyoto) and lacerated using a needle to disperse pollen. The number of pollen grains was counted under a light microscope. Another 30 pistils per treatment were collected and analyzed 3 d after pollination for pollen tube growth. All data were subjected to Duncan's multiple range test.

Results and Discussion

Pollen germination test: Germination of pollen *in vitro* averaged 41.0 % for Muscat of Alexandria, 33.4 % for Cabernet Sauvignon, 27.3 % for Campbell Early and 16.7 % for male *V. coignetiae*. Pollen germination of Muscat of Alexandria was significantly higher than Campbell Early and male *V. coignetiae*. Percent pollen germination of *V. coignetiae* was significantly lower (at $p < 0.01$) than that of the other varieties tested. Pollen germination within cultivars presents some variation according to vine age, vineyard environment and storage conditions, therefore the results obtained did not always coincide with data published on previous works (PARFITT and ALMEHDI 1983; KOMATSU and NAKAGAWA 1989; KIMURA *et al.* 1997). Conditions for the *in vitro* pollen germination test in this study were similar for all pollen cultivars. The optimum condition for pollen germination such as medium composition and incubation temperature may affect pollen germinability and may vary among cultivars (STONE *et al.* 1995) and may have influenced the results. However, this test does provide information about the relative germination capacity of the pollen used.

Efficiency of pollen variety: Pistils pollinated by dusting pure pollen from Muscat of Alexandria or Cabernet Sauvignon had more pollen tubes growing through the style than those pollinated with pollen from Campbell Early and male *V. coignetiae* (Tab. 1). The amount of pollen deposited on the stigmas after pollination was not determined. However, pollen from all cultivars was similarly applied in a way that stigmas were completely covered with pollen. Since the difference of pollen grain size between the cultivars is small (KIMURA *et al.* 1997), pollen load on the stigmas might be also similar. The difference observed in the number of pollen tubes in the upper parts of the pistils may have occurred due to difference in pollen germinability, as indicated by *in vitro* tests. However, in all treatments, the average number of pollen tubes reaching the micropyles exceeded four and was not significantly affected by pollen variety. This suggests that all the pollen cultivars used can provide efficient fertilization if excess amounts of pollen are used.

Dry pollination: Clusters pollinated with pollen of Muscat of Alexandria mixed with powdered milk or *Lycopodium* spores had a large amount of pollen retained on stigmas (Tab. 2). Pollen mixed with milk at a proportion of 1:2 and 1:10 presented no significant differences in the amount of pollen grains deposited on the stigmas, or on pollen tube development. The average number of pollen grains per stigma was 250 and 236 in the high and low concentrations, respectively, and ca. 40 pollen tubes germinated through the upper part of the style. The number of pollen tubes gradually decreased along the style and ovary, with four or more pollen tubes reaching the micropyles for both treatments. Physical properties of pollen and powdered milk, such as granule size, density or electrical properties may be different and may have accounted for the lack of difference in the level of pollination in the two pollen concentrations used (FERGUSON and PUSCH 1992).

When pollen was diluted with *Lycopodium* spores at 1:2 and 1:10, there were significant differences in the level of pollination and pollen tube development. Stigmas showed

Table 1

Average number of pollen tubes growing in pistils of female vines of *Vitis coignetiae* pollinated with pollen from different cultivars

Parts of the pistils	Pollen cultivars			
	Male *Vitis coignetiae*	Campbell Early	Muscat of Alexandria	Cabernet Sauvignon
Style upper	38.2b[1)]	36.6b	87.8a	72.9a
Style base	27.4b	22.9b	59.3a	33.4b
Locule	19.0b	17.3b	29.9a	19.5b
Ovary middle	7.2b	8.6ab	12.1a	9.9ab
Micropyle	4.2a	4.1a	4.5a	4.1a

[1)] Means within lines followed by the same letter are not significantly different at $p \leq 0.05$.

Table 2

The effect of dusting pollinations with pollen diluted in *Lycopodium* spores or powdered milk on deposition of pollen grains on the stigma and on pollen tube development

Treatment[1)]	No. of pollen grains on the stigma	No. of pollen tubes in Style		Ovary		
		Upper	Base	Upper	Middle	Micropyle
Pollen : spores						
1:2	216.0a[2)]	61.6a	37.2a	26.0a	11.5a	5.4a
1:10	139.7b	41.8b	21.2b	17.0b	7.1b	3.9b
Pollen : milk						
1:2	250.3a	39.5b	21.8b	20.9ab	8.1b	4.5ab
1:10	236.6a	39.0b	24.9b	16.0b	7.5b	4.1b

[1)] Values are pollen dilution, expressed as part of pollen per parts of diluent (w/w).
[2)] Means within columns followed by the same letter are not significantly different at $p \leq 0.05$.

an average of 139 and 216 pollen grains in the high and low concentrations, respectively. The average number of pollen tubes in sections from the upper part of the style to the middle of the ovary was higher in pistils pollinated with pollen at lower dilution rate. The final number of tubes reaching the micropyles for pollen dilution at 1:2 and 1:10 were 5.4 and 3.9, respectively. The mixture of pollen and *Lycopodium* spores was more homogeneous than for powdered milk, suggested by the lower pollination rate at the lower pollen concentration.

Pollination results were good for both types of diluent used. However, the use of diluent such as powdered milk, which does not provide an uniform mixture, might present problems for pollination at lower pollen concentration. In this experiment, dry pollinations resulted in pistils with four or more pollen tubes reaching the micropyles. Since pistils of *V. coignetiae* have in average two normal mature ovules at bloom (KIMURA *et al.* 1997), pollen could be used at lower concentrations than tested in this study. However, at very low pollen concentrations, the use of *Lycopodium* spores seems preferable over that of powdered milk.

Liquid pollination: Clusters sprayed with pollen at concentrations of 0.1, 0.5 and 1.0 g/l had pistils with respective averages of 5.2, 6.0 and 11.5 pollen grains, while values for dipped clusters were 5.2, 17.8 and 38.5 pollen grains, respectively (Tab. 3). Both for spraying and dipping treatments, only few pollen tubes germinated into the pistils. Even pistils from clusters dipped in solution containing 1.0 g/l of pollen which showed the highest amount of pollen on the stigma, had less than two pollen tubes growing through the style. This indicates that no more than 5 % of the pollen grains retained on the stigma germinated. Percent berry set was not determined. However, the number of pollen tubes reaching micropyles per pistil was less than one in all treatments, suggesting low levels of ovule fertilization and berry set. We suspected that pollen germinability or stigma receptivity might have decreased rapidly as a result of contact with the solution. This prompted us to analyze the correlation between pollen germinability and the period which pollen remains in contact with the solution used.

Pollen diluted in 10 % sucrose solution and maintained at room-temperature (26 °C) for a few hours had a dramatic decrease in germination (Tab. 4). After 4 h the percent pollen germination was five times lower than the initial value. Under refrigeration (10 °C), the decrease in germination during the same period was ca. 33 %. After 12 h, both for refriger-

Table 3

Effects of liquid pollinations on deposition of pollen grains on the stigma, and on pollen tube development in pistils of female vines of *Vitis coignetiae*

Treatment[1)]	No. of pollen grains on the stigma	No. of pollen tubes in Style		Ovary		
		Upper	Base	Upper	Middle	Micropyle
Spray						
0.1 g/l	5.2d[2)]	0.4c	0.4bc	0.4bc	0.3bc	0.3bc
0.5 g/l	6.0d	1.6a	1.2a	1.0a	1.0a	0.9a
1.0 g/l	11.5c	0.6bc	0.2c	0.2c	0.2c	0.2c
Dipping						
0.1 g/l	5.2d	0.2c	0.2c	0.1c	0.1c	0.1c
0.5 g/l	17.8b	0.8b	0.7abc	0.6ab	0.4ab	0.4ab
1.0 g/l	38.5a	1.9a	1.1ab	0.6ab	0.5ab	0.5ab

[1)] Values are pollen dilution, expressed as grams of pollen per litre of 10 % sucrose solution.
[2)] Means within columns followed by the same letter are not significantly different at $p \leq 0.05$.

ated and non-refrigerated solutions, percent pollen germination decreased to approximately 10 % of the initial values. It was clear that the time which pollen remains immersed in the solution affects pollen germination. In this study, the time taken from the preparation of the solution until pollination was not calculated, and this may have influenced the results. The success of liquid pollination seems to be dependent on how quickly pollination is made after mixing the pollen in the solution and on how much pollen is added to compensate the decrease in germinability.

Table 4

Germinability[1)] of Muscat of Alexandria pollen after being in contact with a 10 % sucrose solution for different periods

Storage condition	Percent germination of pollen after 0 h	4 h	12 h
Room temperature	47.5	9.6	5.3
Refrigeration (10 °C)	--	31.8	4.6
		**	NS

[1)] Germination test on 1 % agar + 20 % sucrose medium.
** significant at $p \leq 0.01$.
NS = not significant.

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