

## Compatibility Among Plum Cultivars Adapted to Southern Brazil<sup>1</sup>

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### Abstract

The best pollinizers for the Japanese plum cultivars 'Amarelinha', 'Burbank', 'Harry Pickstone', 'Ozark Premier', 'Pluma 7', 'Reubennel', 'Rosa Mineira', 'Santa Rosa', 'The First', 'Wade' and for the European prune 'D'Agen' were studied through controlled hybridization in the field and also in the laboratory, using the cultivars that bloom at the same time. Self fertility was also assessed. *In-vivo* pollen tube growth was studied in the laboratory using fluorescent microscopy and common light microscopy with differential dye and results were compared to fruit set in the field.

Under southern Brazil conditions 'Amarelinha', 'Harry Pickstone', 'Methley', 'Reubennel' and 'Rosa Mineira' had a relatively good fruit set when self pollinated. The same was true with the European cultivar 'D'Agen'.

'Burbank' can be pollinated by 'Ozark Premier' and 'Ozark Premier' by 'Burbank'. 'Pluma 7' showed a high degree of incompatibility but 'Reubennel' and 'Amarelinha' are good pollinizers for it. The cultivar 'Reubennel' can be self-pollinated or can be pollinated by 'Amarelinha', 'Pluma 7' or 'Rosa Mineira'. 'Rosa Mineira' can be self-pollinated and 'Amarelinha', 'Methley' and 'Reubennel' are good pollinizers for it also.

Results from the use of the light microscope with lacmoid as a dye did not differ significantly from those obtained with the fluorescent microscope.

### Introduction

Pollination is one of the most crucial aspects in orchard management. The choice of cultivars as pollinizers is very important in planning a new plum orchard (13). Most Japanese type of plums are self-incompatible and some are also cross-incompatible (16). Some authors (3, 5) state that 'Methley', 'Santa Rosa', 'Harry Pickstone', 'Clima' and 'Beauty' are self-compatible.

Several of the European cultivars, such as 'Rainha Claudia', 'Transparent' and 'Giant', are self-incompatible. How-

ever, there are some cultivars, such as 'D'Agen' and 'Tragedy', that are self-fertile only under certain conditions.

Incompatibility in plums is a genetic character (16) but is influenced by the plant's nutritional status (2, 8). In addition environmental conditions may retard pollen tube growth and reduce effective pollination in some genotypes thus resulting in apparent incompatibility (9).

Temperature is one of the factors influencing pollen tube growth (15, 11). Under optimum conditions a pollen tube considered as incompatible with the pistil can grow rapidly and reach the ovary before the death of the style (4). Thus even cultivars reported in literature as self-fertile, or combinations of cultivars which successfully cross pollinate under certain environmental conditions, may behave differently under conditions of southern Brazil.

The objectives of this study were to determine: 1) which of the commercially cultivated plums in southern Brazil are self-fertile; 2) the best pollinizer combinations and 3) the possible mechanisms influencing pollen tube growth. During the first year of the study an attempt was also made to find an alternative method for studying pollen tube development *in-vivo*, because of the expense of fluorescent microscopy equipment.

### Material and Methods

In the spring of 1988 and 1989 flowering branches of the cultivars 'Amarelinha', 'Burbank', 'Ozark Premier',

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'Pluma 7', 'Reubennel', 'Rosa Mineira', 'Santa Rosa', 'The First' and 'Wade' were brought to the laboratory and placed in beakers of water. The cultivars 'Burbank', 'D'Agen', 'Harry Pickstone', 'October Purple' and 'Songold' were observed only in 1989.

When the flowers were at the balloon stage they were emasculated and pollinated with their own pollen or with pollen of other cultivars overlapping in time of bloom. Pollinations were performed at room temperature (15-19°C).

The pollen viability was tested *in vitro* prior to its use (Table 1). The germination medium, was 1 g of agar plus 10 g of sucrose in 100 ml distilled water maintained at 25°C. The counts were made 4 hours after spreading the pollen on the medium.

In 1988, 5 to 6 days after pollination, the pistils were fixed for at least 24 hours in formalin, acetic acid and ethyl

alcohol (1:1:8). They were then rinsed in distilled water and transferred to 8 N NaOH solution for another 24 hours. They were kept refrigerated until observed by two different procedures.

The first procedure, described by Perez and Moore (12), used the fluorescent microscope and blue aniline dye. The second procedure, adapted from Wilson and Brown (17), allows the use of a common light microscope. For slide preparation, pistils were soaked in a 20% solution of sodium hypochloride for 10 minutes, then stained in a water solution of 1% lacmoid for 5 to 10 minutes. Pistils were then rinsed in distilled water to remove the excess stain. Pistils were placed on a slide with two drops of a light solution of lacmoid. A coverslip was pressed lightly to mash the ovary. Pollen tube germination was observed and the samples (pistils) were classified using a modification of the Franken et al. (1) model. The classes used were:

1. Pollen germinated but the pollen tube is still in the stigma.
2. Pollen tube penetrated the style to about 1/2 of its length.
3. Pollen tube penetrated about 2/3 of the style's length.
4. Pollen tube penetrated more than 2/3 the length of the style and near ovary entrance.
5. Pollen tube penetrated the ovary.
6. Pollen tube reached the ovule.

Emasculations and pollinations were also done in the field. For each combination, from 100-200 flowers were pollinated and fruit set was recorded 60 days after pollination.

In the spring of 1989 approximately 20 pistils of each cultivar were self-pollinated and cross pollinated using the combinations done in 1988, fixed, and kept as described above. Five days after pollination they were observed using fluorescent microscopy. The number of pistils in which the pollen tube reached the ovary and the ovule was recorded.

**Table 1. Percent of pollen germination of the cultivars used in the experiments, and average length of the pollen tube *in vitro*, after 4 hours at 25°C.**

Cultivars	% Germination*			Avg. length (micron)
	1987	1988	1989	
Ace	—	7	—	493
Amarelinha	70	43	23	849
Burbank	—	30	23	438
D'Agen	—	—	22	—
Friar	66	—	15	—
Harry Pickstone	47	62	25	376
Methley	66	25	—	1378
Ozark Premier	—	26	25	383
October Purple	—	—	—	—
Pluma 7	—	9	—	—
Reubennel	30	47	30	1103
Rosa Mineira	52	36	48	440
Santa Rita	—	13	42	1009
Santa Rosa	—	22	—	691
Songold	47	57	—	454
The First	—	59	—	480
Wade	—	32	25	997

\*Not tested.



Figure 1. Germination of pollen on the stigma, observed under the fluorescent microscope.

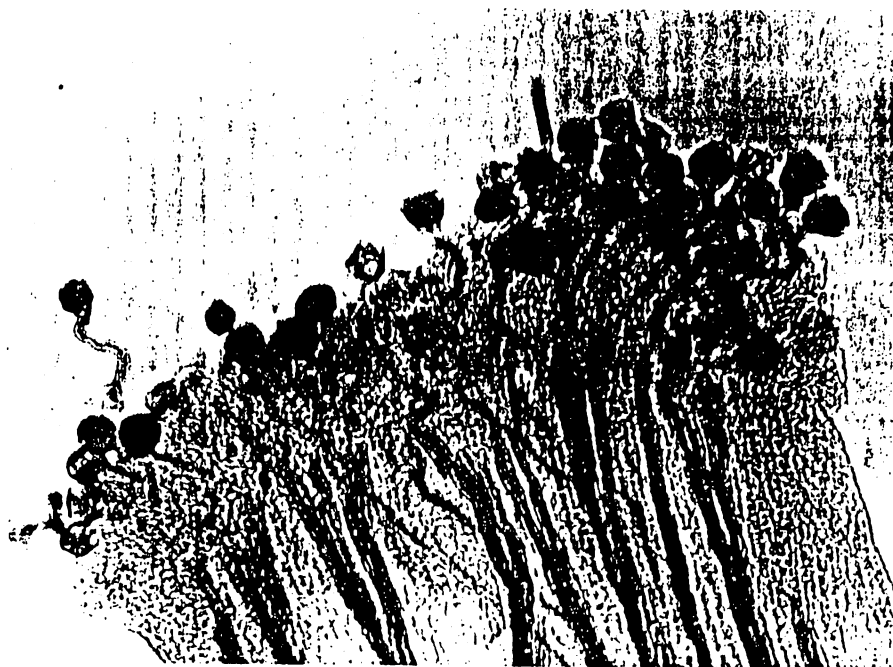


Figure 2. Germination of pollen on the stigma under the common light microscope.

In the field, 100 to 200 flowers of each cultivar were emasculated at balloon stage and immediately pollinated with pollen of cultivars which coincide in blooming time with the commercial cultivars being studied.

### Results and Discussion

There were no statistically significant differences between the procedures for assessing pollen germination and growth, except for two ('Methley' and 'Ozark Premier') of 13 cultivars tested (data not shown). Fluorescent microscopy is an easier process, however, the possibility of using the common light microscope to observe *in-vivo* germination is very important where laboratory facilities with more sophisticated equipment are not available (Table 1). Figures 1 and 2 show pistils observed by the two different processes.

The combinations tested and the most compatible cultivars according to the laboratory results are presented in Table 2. Among the cultivars that were observed in the laboratory, the majority of the combinations showed a good rate of pollen tube development. Exceptions, were 'The First' x 'The First', 'Santa Rosa' x 'The First' and 'Methley' x 'Wade'.

Most cultivars in the field tests had a low fruit set as previously reported by Hurter and Van Tonder (6), Hurter and Vanzyl (8) and Kloppers and Hadlow (10). These authors consider the low fruit set characteristic of Japanese plums.

According to Hurter et al. (7) a 5% fruit set is sufficient for commercial production. Based on this index, we may conclude that an adequate set was obtained (Table 3) with 'Wade' by 'Methley'; 'Methley' by 'The First' or 'Santa Rosa'; 'Songold' by 'Bruce' and by 'Ace'; 'The First' by 'Methley' or 'Santa Rosa'; 'Burbank' by 'Ozark Premier'; and 'Ozark Premier' by 'Burbank'. The cultivars 'Harry Pickstone' and 'Methley' showed a good degree

of self-compatibility with percentages of 5.7% and 5.3% respectively, with the results of 'Methley' comparable to the findings of Hurter and Van Tonder (6).

A comparison of field fruit set results and the number of pistils in which the pollen tube reaches the ovule (144

**Table 2. Pollen tube development following laboratory pollination of plum cultivars, 1988.**

Female	Cultivar	Pollen Growth Stage <sup>z</sup>
	Pollen	
Ace	Ace	2.9 a
	Songold	2.7 b
Amarelinha	Rosa Mineira	3.3 a
	Reubennel	3.2 b
Burbank	Ozark Premier	3.5 a
	Burbank	3.3 b
Methley	The First	3.1 a
	Santa Rosa	3.0 ab
	Wade	2.8 bc
	Methley	2.7 c
Ozark Premier	Ozark Premier	4.2 a
	Burbank	4.1 b
Pluma 7	Amarelinha	3.3 a
	Reubennel	3.0 b
Reubennel	Pluma 7	3.1 a
	Rosa Mineira	3.0 a
Rosa Mineira	Methley	3.5 a
	Amarelinha	3.4 a
Santa Rosa	Santa Rita	3.0 a
	The First	2.9 a
	Santa Rosa	2.8 a
Santa Rita	Santa Rosa	3.3 a
	Santa Rita	3.2 b
Songold	Songold	3.0 a
	Ace	2.9 a
The First	Methley	4.1 a
	Santa Rosa	3.7 b
	The First	3.1 c
Wade	Methley	3.6 a
	Wade	3.1 b

<sup>z</sup>Based on modification of Franken et al. (1) model (see text). Means of 10 pistils. The data were transformed in  $\log(x + 1)$  for statistical analysis. The means followed by the same letters did not differ (Duncan Multiple Range Test,  $P = .05$ ).

hours after pollination) in the laboratory is presented in Table 3. Based on these results, we can observe that the pollen of 'Burbank' reaches its ovule with reasonable facility (8 out of 10 pistils that were observed 144 hours after pollination). However, the fruit set obtained in the field was less than 1%. Since there is no inhibition of the pollen tube growth, possibly two hypotheses could be discussed. First, the low fruit set may be due to the influence of temperature in the field, which varied from a minimum of 7.6° to a maximum of 26.8°C during Burbank's blooming period.

According to Keulemans (9) pollen tubes grew about ten times faster at 12°C than at 4°C. However, in the present case, 'Burbank' pollen used on 'Ozark Premier' in the same orchard and pollinated at the same time as in the case of 'Burbank' gave a fruit set of 13.8%, which is considered adequate for commercial production.

The second hypothesis is that self-incompatibility may occur at the ovule level or after fertilization occurs. Keulemans (9) notes also that some cultivars, such as 'Reine Claude d' Altham', 'Sanctus Hubertus' and 'Monsieur Hatif', can be considered "slow growers," and points out that all varieties did not react the same way to temperature.

In the case of the cultivars 'The First' and 'Santa Rosa', they might be considered "slow growers," in artificial media or under their own styles (Tables 1, 2, 3). However 'The First' grew well on 'Methley's' style, and 'Santa Rosa' did well on 'Santa Rita.' Self-pollination of 'Songold' and 'Ozark Premier' not only caused a slow development of the pollen tube under room temperature (15 to 19°C) but also gave a very poor fruit set under field conditions. Perhaps, self pollen triggers some inhibitory mechanism at the ovule or at post-fertilization, since the same mother plants, under the same conditions, gave a satisfactory fruit set with 'Bruce' and 'Burbank' pollen.

**Table 3. Comparison of the number of ovaries in which the pollen tube reached the ovule after 144 hours post-pollination and fruit set obtained in the field (1988).**

Crosses	No. ovaries <sup>2</sup>	Fruit <sup>3</sup> set (%)
Amarelinha x Rosa Mineira	10	—
x Reubennel	4	—
Burbank x Burbank	8	0.7
x Ozark Premier	9	15.3
Harry Pickstone x Amarelinha	4	xxx
x Harry Pickstone	+	5.7
Methley x The First	7	9.1
x Methley	6	5.3
x Santa Rosa	3	6.9
x Wade	2	2.7
Ozark Premier x Burbank	7	13.8
x Ozark Premier	5	0.7
Pluma 7 x Amarelinha	5	—
x Reubennel	5	—
Reubennel x Pluma 7	4	xxx
x Rosa Mineira	8	xxx
Rosa Mineira x Amarelinha	6	3.0
x Methley	5	xxx
Santa Rita x Santa Rosa	7	xxx
x Santa Rita	9	xxx
Santa Rosa x The First	1	4.6
x Santa Rita	8	3.5
x Santa Rosa	2	2.0
Songold x Ace	3	6.0
x Songold	4	0
x Bruce	+	11.0
The First x The First	0	0
x Santa Rosa	4	26.6
x Methley	8	24.5
Wade x Methley	6	21.8
x Wade	7	2.0

<sup>2</sup>10 ovaries were observed being five by each method of observation.

<sup>3</sup>(xxx) crosses not done in field; (—) frost damage; (+) no observations were done at the laboratory.

**Table 4. Number of observed pistils (out of 10) in which the pollen tube reached the ovary, number that reached the ovule 120 hours after pollination, and fruit set obtained in controlled pollinations (1989).**

Female Cultivar	Pollen	No. of Pistils <sup>x</sup> with pollen tube in ovary	No. of Pistils with pollen tube in ovule	Fruit set (%) in the field
Amarelinha	Amarelinha	2	0	1.4
	Rosa Mineira	10	2	2.0
	Pluma 7	10	7	2.1
	Reubennel	10	3	13.3
D'Agen	President	8	0	10.0
	D'Agen	8	2	19.7
October Purple	Burbank	0	0	1.9
	October Purple	0	0	7.8
	Ozark Premier	0	0	8.4
Ozark Premier	Ozark Premier	1	0	0
	October Purple	7	2	2.9
	Burbank	0	0	7.6
Pluma 7	Pluma 7	0	0	0
	Amarelinha	9	2	9.8
	Rosa Mineira	8	3	1.0
	Reubennel	9	6	11.4
Reubennel	Reubennel	6	0	22.4
	Rosa Mineira	8	3	11.0
	Amarelinha	5	2	25.0
	Pluma 7	•	•	14.1
Rosa Mineira	Reubennel	10	5	14.2
	Amarelinha	7	2	17.5
	Methley	•	•	15.1
	Rosa Mineira	•	•	19.0
Santa Rosa	Santa Rosa	2	0	0.5
	The First	7	2	0
	Wade	9	3	0
	Santa Rita	5	2	1.2
The First	The First	0	0	1.3
	Santa Rosa	10	2	8.7
	Wade	9	3	2.3
Wade	Wade	3	0	3.0
	Harry Pickstone	5	2	1.0
	Santa Rosa	8	2	••
	The First	8	0	0

• — Not tested in the lab.

•• — Not tested in the field.

x — Twenty or more pistils were observed, in each combination, but the results are presented here, transformed to a 10 basis.

In 1989 the cultivar 'Santa Rosa' was again the best pollen donor for 'The First'. In the field, 'Burbank' was the best pollinizer for 'Ozark Premier' as it was in 1988 (Table 4).

All the cultivars tested as pollinizers for 'Reubennel' ('Rosa Mineira,' 'Reubennel,' 'Amarelinha' and 'Pluma 7') produced a good fruit set. The same was true for pollinations of 'Rosa Mineira' by 'Reubennel,' 'Amarelinha,' 'Methley' and 'Rosa Mineira.' The laboratory results were also satisfactory in all cases tested.

For 'Amarelinha,' the cv. 'Reubennel' was the best pollinizer, even though the laboratory results were better for 'Pluma 7'. Perhaps there was some problem after fertilization had occurred in the case of 'Pluma 7'.

Field results were not good for 'Wade.' A large proportion of pistils showed pollen tubes on the ovary and/or ovule when 'Wade' was pollinated with 'Santa Rosa' and 'The First,' but some inhibitory process may have occurred at ovule level or after fertilization since the fruit set in the field was very low. 'Methley' was not tested in 1989, but based on the 1988 results it is a good pollinizer for 'Wade.'

'Pluma 7' showed a high degree of self-incompatibility; 'Reubennel' and 'Amarelinha' are good pollinizers for it. In laboratory tests 'Rosa Mineira' seemed to be a good pollinizer for 'Pluma 7,' however the performance in the field was not satisfactory. 'Reubennel' or 'Amarelinha,' even having a lower percent of pollen germination, were better. The cultivar 'Santa Rosa' had a very low fruit set with all the pollen tested, i.e., its own pollen or pollen of 'The First,' 'Wade' or 'Santa Rita.' The laboratory tests showed that in 7 out of 10 pistils (Table 4) pollinated with 'The First' and in 9 out of 10 pistils pollinated with 'Wade,' the pollen tube had reached the ovary 120 hours after pollination. However, fruit set was not satisfactory in the field experiment. Low fruit set was also obtained

in 1988, even though it was slightly better than in 1989. 'Santa Rosa' has a marginal adaptation to southern Brazil and this might be one factor to consider. According to Sud et al. (14) this cultivar has a very poor fruit set in adverse weather. These authors obtained sets of 2% using 'Burbank' pollen and 3.65%, using 'Santa Rosa' pollen.

Even though pollen tube development in the laboratory was slow, 'October Purple' showed a good fruit set when pollinated with 'Ozark Premier' or with its own pollen.

'Reubennel' and 'Rosa Mineira' showed a high degree of self-fertility, as did the European cultivar 'D'Agen.'

### Conclusions

The cultivars 'Amarelinha,' 'Harry Pickstone,' 'Methley' and 'Reubennel,' and the European cultivar 'D'Agen' have a good degree of self-fertility under the conditions of southern Brazil. 'Burbank,' 'The First,' 'Santa Rosa,' 'Songold' and 'Wade,' do not set good crops of fruit when self-pollinated.

'Pluma 7' can be pollinated by 'Reubennel' or 'Amarelinha'; 'Wade' by 'Methley'; 'Songold' by 'Bruce'; 'The First' by 'Santa Rosa' or 'Methley'; 'Burbank' by 'Ozark Premier'; and 'Ozark Premier' by 'Burbank.' The cultivar 'Reubennel' can set a satisfactory crop by itself but can also be pollinated by 'Amarelinha,' 'Pluma 7,' or 'Rosa Mineira.'

'Rosa Mineira' can be self-pollinated or can be pollinated by either 'Reubennel,' 'Amarelinha' or 'Methley.'

The choice of using the fluorescent microscope to observe *in vivo* pollen tube germination or the common light microscope with lacmoid as a differential dye depends on the availability of the equipment, since the efficiency is about the same.

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Fruit Varieties Journal 46(1):35-36 1992

## 'Chulli'—A Wild Apricot From Himalayan Cold Desert Region

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### Abstract

'Chulli' is a wild apricot found growing in cold desert regions of the Himalayas, adjoining Tibet. This wild fruit possesses some unique traits not found in the cultivated types. The fruits are borne in clusters and not sparsely arranged like the cultivated types. This fruit grows under very harsh climate and on poor soils. The fruits are late ripening, maturing after about three weeks than the commercial cultivars in the same region.

### Origin and Utilization

The trees of 'Chulli' are found growing wild in large numbers, in dry temperate regions of Kinnaur, Chamba and Lahaul and Spiti districts of the Indian state of Himachal Pradesh. There is no report about the existence

of this fruit elsewhere. The existence of wild plants in such a large numbers in this area indicates that 'Chulli' might have been originated in this region.

The fruits of 'Chulli' are eaten fresh and also sun-dried. The dried fruits are used mostly for the preparation of a hard alcoholic liquor, which is very popular with the tribal people of that region. The kernel is mostly devoid of the bitter alkaloid and thus edible. It is used as a substitute for almond in food preparations. The kernel contains 48.6 per cent oil (2), which is used for cooking, in lamps and as a hair oil. It is also reported to cure arthritis and joint pains and is, therefore, used for massage (1).

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