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The results of intergeneric pollination of *Fragaria* x *ananassa* Duch. and *Fragaria virginiana* Duch. by *Potentilla* species

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Abstract

Female varieties of *Fragaria* × *ananassa* (Sonja, Freja, Mieze Schindler, Dir. Wallbaum, Reine des Precoces, Pozdnaya Slodkaya and others) and, tentatively, one female clone, *F. virginiana* "Scheldon", were pollinated with nineteen *Potentilla* species in the course of four years (1977-1980). A total of 99 194 seeds was obtained, and from them, 739 seedlings (0.75%). After the first year of growth, a mean 24% of seedlings survived (178). Vegetatively mature plants were obtained from the above named octoploid maternal forms (Reine des Precoces excepted) with six *Potentilla* species (the diploid *P. rupestris, P. purpureoides, P. geoides, P. glandulosa* and *P. fruticosa* as well as the hexaploid *P. fragiformis*). *F.* × *ananassa* var. Sonja × *P. rupestris* was classified as the best combination in producing surviving plants.

Key words: intergeneric pollination, female clones, seedlings

INTRODUCTION

The first mentions of crossing representatives of the genera *Fragaria* L., *Potentilla* L. and *Duchesnea* Smith. date back to the beginnings of the 20th century. Initially, these are attempts to put the systematics of these genera in order, and next, to see if the hybrids obtained this way could be used in breeding. A full comparison of the results of intergeneric crosses presented chronologically and a botanical description of these genera were given in a review of literature on this subject (Niemirowicz-Szczytt 1980). On the basis of this comparison, differences could be found in the frequency of using representatives of

individual genera for crossing. These differences resulted from the chance to collect certain genera, the coincidence of blooming of both forms being crossed and from the results of earlier works (Mangelsdorf and East 1927, Jones 1955 cit. Ellis 1960/1961, Harland 1957 cit. Asker 1971, Ellis 1958, 1960/1961, 1971, Haskell 1963 cit. Asker 1971, Shangin-Berezovskiy 1963, Senanayake and Bringhurst 1967, Asker 1970, 1971, Barrientos and Bringhurst 1973, 1974, Hughes and Janick 1974). The relatively largest number of seeds and plants was obtained by crossing the octoploid *F.* x *ananassa* with diploid *Potentilla* species. One to two percent of the seeds germinated and many young seedlings did not survive in the early stages of growth. Such a low percentage of germinating seeds indicates the need for a high number of pollinations in order to obtain a high number of seeds and for creating optimal conditions for germination and growth of young plants.

The aim of this study was to obtain seeds and viable plants from crossing representatives of the genus *Fragaria* with *Potentilla* species and to find which of the studied combinations gives the most viable offspring.

MATERIAL AND METHODS

The following female varieties (clones) of *F.* x *ananassa* were chosen as the maternal forms for intergeneric crosses: Freja, Sonja, Mieze Schindler, Reine des Precoces, Dir. Wallbaum, Pozdnaya Slodkaya, Pozdnaya from Kubań, Dr. Seffegast, Papa Lange, and also (during the first two years) the female clone *F. virginiana* (Scheldon) obtained from the Institute for Breeding Horticultural Plants in Wageningen, Holland. The "Scheldon" clone was excluded from the experiments in the third and fourth years because its fruits easily fell off from the stem, its inflorescences were limp and touched the ground under the weight of the fruit and rotted, and also due to the unviability of its seedlings. The varieties: Pozdnaya from Kubań, Dr. Seffegast and Papa Lange played only a small role in this experiment because they were susceptible to frost and the seedlings obtained from crossing them with *Potentilla* had a low viability. Use of female forms enabled large numbers of pollinations to be carried out since emasculation was unnecessary. Differentiation of varieties in respect to their blooming periods allowed the extension of the pollination period, which was of great importance under field conditions.

The strawberry plants were grown in 5-liter jars or in the ground covered with black plastic. Each variety was represented by 10 to 20 plants. Whole inflorescences were isolated with tomofan isolators just

before the first buds bloomed. Part of the flowers in the isolators were left unpollinated, as a control (in an unpollinated flower the receptacle does not increase in size).

Species of *Potentilla*, whose blooming periods agreed with those of the strawberry varieties used in this study, were chosen as the paternal forms. Nineteen species obtained from botanical gardens from all over the world were used. A list of the species used in the crosses, along with their ploidy according to "Khromosomnye chisla cvetkovykh rastyeniy" (1969) and "Index to chromosome numbers 1967—1971" (1973) and determined by this author, is given in Table 1. *Potentilla* inflorescences were cut at the bud stage and kept in a closed laboratory room until pollination. As a control of the maternal forms fertility, they were crossed intraspecifically with varieties having perfect flowers (Ostara, Redgauntlet). Pollination was considered successful when as its result the receptacle grew and at least one achene developed. The seeds were obtaind by blending the fleshy fruit in large amounts of water and rinsing on a sieve. Then, in the second half of July, all of the seeds were planted in boxes with peat, previously disinfected with captane. For more precise observations, some of the seeds in 1979 and 1980 were germinated under sterile conditions in Petri dishes. Dry seeds were disinfected for 10 min. in 50% sulfuric acid, then for 10 min in 4% cal-

Table 1

A list of Potentilla species used in intergeneric crosses along with their number of chromosomes

Potentilla species	2n number of chromosomes acc. to atlases	2n number of chromosomes determined by this author
P. adscharica	42, 56	
P. ambigens	?	
P. andicola	42	
P. argentea	14, 28, 35, 42, 56, 63	
P. arguta Pursh	14	14
P. aurea	14, 35, 42, 56, 70	
P. chrysantha	28, 42, 56	
P. clusiana	42	
P. fragiformis Willd.	42, 56	42
P. fruticosa L.	14, 28, 42	14
P. geoides Bieb.	14	14
P. glandulosa Lindl.	14	14
P. hippiana	42, 70, 77, 84, 98	
P. nepalensis	14, 42	
P. nitida	14, 42, 32	
P. norvegica	42, 56, 70	
P. purpureoides	?	14
P. rupestris L.	14	14
P. thuringiaca	?	

cium hypochlorite and rinsed several times with bisterilized water. The disinfected seeds were placed on moist filter paper in sterile Petri dishes and taped closed with Parafilm. Observations were run for up to 2 months. The seedlings, which had retained sterility, were transferred to LS medium (Linsmayer and Skoog 1965) at pH 5.6, jelled with agar and enriched with 30 $g \cdot dm^{-3}$ sucrose. After growing roots, they were planted into pots and transferred to a bed, where they wintered under a layer of leaves. Well developed seedlings were transplanted to the ground, covered with black plastic and studied during their first and second years of growth.

RESULTS

SEED DEVELOPMENT AND NUMBER

The results of the study are presented in two tables. In Table 2, data on the parental forms is given, whereas in Table 3, the results of all of the intergeneric pollinations done during successive years of this study in comparison with the results of intravarietal pollinations with the same maternal form, are given. On the basis of the observations from two years (1978, 1979) it was calculated that successful pollinations in intergeneric crosses amount to 42.5—64.2%. This indicates, that under the influence of *Potentilla* pollen, an average of one-half of the pollinated flowers develop fleshy fruit. The number of fleshy fruits, and, indirectly, the number of pollinations (after multiplying by two), was the lowest in 1977 (Table 3). The data from 1978 and 1979 is similar (over seven hundred) and 1980 is the best due to the fact that many pollinations were possible and that the combinations could be chosen in a specific direction. Also in 1980 the highest number of seeds per fruit was obtained in addition to the record number of fruits. The lowest number, an average of 19 achenes on a fleshy fruit, was obtained in 1977 and 1979, which could have been caused by unfavorable atmospheric conditions during pollination. In 1977 the average day and night temperatures were exceptionally low (slight ground-level frosts and a few days with an average day and night temperature of 4-6° C), and exceptionally high average day and night temperatures during this same period in 1979 (20-25° C).

The results of the controls (Table 3) in 1978 and 1980 show that the number of developing seeds on the plants from the studied varieties when crossed within the species exceeds the number of seeds obtained through intergeneric crosses. The differences, however, in 1978 an average of 7.6, in 1980, of 25.4 seeds per fruit, are not as high as could be expected. It could then be regarded that *Potentilla* pollen, especially *P.*

Table 2

The number of seeds and seedlings from the individual parental forms during the four years of experiments

Forms used in the crosses	A	B	C	D	E
F. x *ananassa* maternal forms:					
Freja	516	51,3	26 445	155	0.59
Sonja	752	41.4	31 126	231	0.74
Mieze Schindler	576	24.8	14 256	95	0.67
Reine des Precoce	134	22.1	2 966	78	2.62
Dir. Wallbaum	678	27.6	18 709	111	0.59
F. virginiana "Scheldon"	112	17.4	1 948	28	1.40
Pozdnaya Slodkava	57	23.6	1 344	9	0.67
Pozdnaya from Kubań	36	16.4	590	29	4.90
Others	28	64.6	1 810	3	0.17
Total	2889	34.3	99 194	739	0.75
Potentilla paternal forms:					
P. geoides	687	53.9	37 055	183	0.49
P. rupestris	1439	32.2	46 358	230	0.49
P. fruticosa	326	23.8	7 762	97	1.25
P. glandulosa	110	16.5	1 812	60	3.31
P. andicola	87	20.8	1 788	1	0.06
P. arguta	18	18.2	328	18	5.49
P. aurea	6	23.3	140	1	0.70
P. purpureoides	96	19.3	1 850	41	2.21
P. fragiformis	49	28.7	1 404	43	3.06
P. clusiana	7	7.2	50	—	—
P. chryzantha	5	5.7	34	2	5.88
P. thuringiaca	6	24.7	88	56	63.60
P. ambigens	34	11.6	394	1	0.25
P. argentea	6	5.8	35	2	5.70
P. atrosanguinea	10	8.0	80	—	—
P. nitida	1	10.0	10	1	10.00
P. nepalensis	1	6.0	6	3	50.00
Total	2889	34.3	99 194	739	0.75

A — number of fruits, B — number of seeds per fruit, C — total number of seeds, D — number of germinating seeds, E — percent of germinated seeds.

geoides, *P. rupestris* and *P. fragiformis*, stimulates well the development of pistils and later, seeds (comp. Table 2). It should be emphasized once more that in unpollinated flowers, growth of the receptacle or development of seeds were never observed. The most regular and even growth of fruits was obtained in combinations with *P. geoides*, which indicates that the development of almost all of the pistils in the flower was stimulated. The highest number of developing seeds was obtained in the maternal forms of the Freja (51.3 seeds per fruit) and Sonja (41.4 seeds per fruit).

Table 3

The sum results of intergeneric and intravarietal (control) crosses in 1977-1980

Years	Number of		Total number of seeds	Number of seed-lings	Percent of germinated seeds	Number of one-year plants	Percent of	
	fruits	seeds on 1 fruit					one-year--old plants	surviving plants
Intergeneric crosses								
1977	126	19.1	2 402	47	1.96	6	0.25	12.77
1978	781	25.2	19 657	148	0.75	39	0.20	26.35
1979	729	19.5	14 222	290	2.04	—	—	—
1980	1253	50.2	62 913	254	0.40	133	0.21	52.36
Total	2889	34.3	99 194	739	0.75	178	0.16	24.09
Intervarietal crosses (control)[a]								
1978	137	32.8	4 493	534	11.89	289	6.43	54.12
1980	256	75.6	19 352	1241	6.41	787	4.07	63.42

[a] Results of crossing the same female *F.* x *ananassa* varieties, which were used in the intergeneric crosses, with *F.* x *ananassa* varieties with perfect flowers.

GERMINATION OF SEEDS

The germination of seeds during the four years of this experiment was, in general (Table 3), very poor and amounted to 0.75% of all of the seeds, which is about 700 seedlings from the 99 000 planted seeds. The best germination results were achieved in 1979 and 1977 (2.04% and 1.96%, respectively), whereas the percentage of germinating seeds in the remaining years was lower (0.75 and 0.40%), when, in turn, seed development was exceptionally good.

When analysing the effect of the parental forms on the germination of seeds, it can be noticed that (Table 2) using certain, both maternal and paternal, forms in the crosses could have been the cause of obtaining a very high percent of germinating seeds. These were, however, usually only the results of one year, and so, from not too large a number of seeds. Such a result, achieved probably due to favorable coincidences, cannot be the basis for a generalization. In those combinations in which large numbers of seeds were obtained, the percentage of germinating seeds was lower then 1%.

Looking at the various combinations, pairs which guarantee exceptionally high numbers of germinating seeds can be singled out. To these combinations, Freja x *P. geoides* (both forms are very early and therefore often paired), Sonja x *P. glandulosa*; Sonja x *P. fragiformis* and Mieze Schindler and Dir. Wallbaum pollinated by both *P. glandulosa* and *P. fruticosa*, can be assigned.

VIABILITY OF SEEDLINGS

For future studies, not so much the number of seedlings, as the number of one or two year old plants, that is, plants which have reached full generative or vegetative development, is important (Table 4). On average, after the first year of growth (after the first winter) only about 25% of the plants remain, from which it can be concluded that a greater part of the seedlings is not capable of development in field conditions. In the individual years, the numbers of surviving plants ranged from 0 to 52% (comp. Table 3), and was the highest for the plants obtained in 1980. The control plants, from intravarietal crosses, survive on average much better, although too, slightly less than one-half of them die.

From among the maternal forms, in respect to the viability of seedling offspring, decidedly best are the varieties Sonja (46.3%) and Freja (31.6%), from the paternal, *P. purpureoides* (73.2%), *P. rupestris* (45.6%), *P. fragiformis* (23.3%) and *P. fruticosa* (12.4%). In spite of the good results in the number of seeds obtained when pollinating with *P. geoides*

Table 4

Survival of seedlings obtained from the individual parental forms during the 4 years of experiments

Maternal forms	Number of			Percent of surviving plants
	seeds	seedlings	plants	
F. x *ananassa* varieties:				
Freja	26 445	155	49	31.6
Sonja	31 126	231	107	46.3
Mieze Schindler	14 256	95	3	3.2
Reine des Precoce	2 966	78	—	—
Dir. Wallbaum	18 709	111	13	11.7
Pozdnaya Slodkaya	1 344	9	3	33.3
Pozdnaya from Kubań	590	29	—	—
Others	1 810	3	—	—
F. virginiana "Scheldon"	1 948	28	3	10.7
Total	99 194	739	178	24.1
Potentilla paternal forms:				
P. geoides	37 055	183	13	7.1
P. rupestris	46 385	230	105	45.6
P. fruticosa	7 762	97	12	12.4
P. glandulosa	1 812	60	3	5.0
P. andicola	1 788	1	—	—
P. arguta	328	18	5	27.8
P. aurea	140	1	—	—
P. purpureoides	1 850	41	30	73.2
P. fragiformis	1 404	43	10	23.3
P. clusiana	50	—	—	—
P. chrysantha	34	2	—	—
P. thuringiaca	88	56	—	—
P. ambigens	394	1	—	—
P. argentea	35	2	—	—
P. atrosanguinea	80	—	—	—
P. nitida	10	1	—	—
P. nepalensis	6	3	—	—
Total	99 194	739	178	24.1

and *P. glandulosa*, the plants obtained survive in an unproportionally low percent (7.1 and 5%). In total, 178 plants were obtained which survived the first winter and ground growth conditions, and from them, 159 plants remained which could be cytomorphologically examined in their second year of growth (Table 5).

In general, it can be said that, in our climatic conditions, in order to obtain 100 offspring plants from intergeneric crossing of *F.* x *ananassa* with *Potentilla* spp., two thousand pollinations in the combinations previously singled out as good (see Table 4) must be done. An exceptionally high number of young plants die during their first year of growth, in their second year, however, few die (Table 5).

Table 5

Comparison of the number of offspring obtained from individual combinations in intergeneric crosses during the first and second years of growth

Year	Combination	Symbol	Number of plants	
			after the 1st year of growth	after the 2nd year
1977	Sonja x *P. fruticosa*	MR 45	1	1
	Mieze Schindler x *P. geoides*	MR 39	1	1
	Dir. Wallbaum x *P. fragifor.*	MR 33	1	1
	F. virg. x *P. fruticosa*	MR 38	3	3
	Total		6	6
1978	Sonja x *P. rupestris*	MR 3	2	2
	Sonja x *P. purpureoides*	MR 1	24	22
	Sonja x *P. fragiformis*	MR 2	5	
	Meize Sch. x *P. geoides*	MR 6	1	1
	Meize Sch. x *P. rupestris*	MR 7	1	1
	Dir. Wallbaum x *P. rupestris*	MR 5	1	1
	Dir. Wallbaum x *P. arguta*	MR 4	5	—
	Total		39	31
1980	Freja x *P. geoides*	MR 8	8	7
	Freja x *P. rupestris*	MR 9	41	40
	Sonja x *P. rupestris*	MR 3	60	56
	Sonja x *P. fruticosa*	MR 14	8	7
	Sonja x *P. fragiformis*	MR 2	4	3
	Sonja x *P. glandulosa*	MR 10	3	2
	Dir. W. x *P. purpureoides*	MR 13	6	6
	Pozd. Slod. x *P. geoides*	MR 15	3	1
	Total		133	122
Totals from 1977, 1978, 1980			178	159

1979 — no plants were obtained during the first year of growth.

DISCUSSION

In this study, octoploid varieties of *F.* x *ananassa* were used as maternal forms. However, as was suggested by Prof. B. Kubicki, only female varieties were employed; this allowed a high number of pollinations to be done in field conditions (troublesome emasculation was avoided) and guaranteed the purity of pollinations (self-pollination was never observed in the controls). Strawberry varieties with female flowers: Freja, Sonja, Mieze Schindler, Dir. Wallbaum, Reine des Precoce and Pozdnaya Slodkaya had not been used by other authors for intergeneric crosses. From among the paternal forms of the genus *Potentilla*, the best turned out to be the diploid varieties, from among which *P. fruticosa*,

P. glandulosa and *P. rupestris* had been successfully used by other authors, and others, equally good, such as *P. geoides* and *P. purpureoides*, were not used until now for intergeneric pollination. It is interesting that *P. geoides*, *P. glandulosa* and *P. rupestris* belong to one taxonomic group, *Rupestris* subsection *Closterostylae* (according to Wolf's classification 1908), in which all of the genera are diploid, and, in addition, their places of origin are usually considered to be areas not covered by the last glaciation. *P. fruticosa* belongs to the subsection *Rhapalostylae*, (with a relatively low number of species) and is, in a certain sense, an exception among herbaceus species of *Potentilla* because it is a shrub. *P. fragiformis*, which belongs to the subsection *Conostylae*, is a hexaploid species, morphologically similar to *F.* x *ananassa*. It can be supposed that it forms gametes of different ploidy, since it has different sized pollen grains. This species has not been used in crosses with *Fragaria*. The diploid genus, *P. purpureoides*, is somewhat of a puzzle. It was obtained under that name from some unknown botanical garden. A genus of that name does not figure in available lists of *Potentilla* species, for example in the Index Kevensis (1946) plus Suppl., Flora USSR (1941), Flora Europea (1968) etc. and it is also lacking in Wolf's monography (1908).

The observations of pollen from *F. ananassa* and *Potentilla* genera: *P. geoides*, *P. rupestris* and *P. fragiformis*, carried out at the Interdepartmental Electron Microscopy Laboratory of SGGW-AR, did not reveal any basic differences in the design and sculpture of the pollen grains of these species.

It was known, on the basis of the paper by Hughes and Janick (1974), that 1-2% of the seeds obtained after crossing *F.* x *ananassa* and *Potentilla* spp. varieties, germinated. In the studies by this author, a similar result was obtained in individual years (0.4-2.0%), the 4-year average being 0.75% germinating seeds. It was noted that species like: *P. geoides*, *P. rupestris* and *P. fragiformis*, which produce large amounts of pollen, stimulate the development of numerous ovaries, which, in turn, does not disfigure the fleshy fruit too much. It could also be observed (Table 3) that in those years during which seed development was good (1978 and 1980), the percentage of germinating seeds was lower, and, conversely, in the years when seed development was worse, the percentage of germinating seeds was higher. On the other hand, it is known that the degree of intensification of apomixis, for example, in the apple tree, depends on the atmospheric conditions of a given year (Radionienko and Smychenko 1972).

The analysis of the number of seedlings obtained from individual paternal forms allows the finding of a certain repetitiousness in their formation, especially with a large number of seeds, and also, the singling out of the relatively best parental combination.

A separate problem, for which there is no data from literature, is the question of seedling survival. Most of the authors dismiss their final results by just stating that the seedlings were not capable of further development. From the studies of this author it is shown to be true that many of the seedlings do die, especially during the first winter. Only an average of 24% of the plants obtained surive (Table 3). From the maternal forms, insofar as the seedling survival is concerned, the following varieties are best: Sonja (46%) and Freja (32%). Good, paternal forms are, in this respect: *P. purpureoides* (72.3% — but only in two combinations), *P. rupestris* (45.6% — in many combinations), *P. fragiformis* (23.3%) and *P. fruticosa* (12.4%). All in all, the best combination seems to be Sonja x *P. rupestris*, which is good both in respect to obtaining seeds and their germination and, most of all, in respect to the survival of the seedlings. The survival of the plants obtained as the result of pollination with *P. geoides* as the paternal form, is low (7%), although the seed development is very good. A certain explanation of this fact can be the high proportion of abnormal embryos, which develop into unviable seedlings (Niemirowicz-Szczytt 1984).

On the basis of the experimental data, taking into account that approximately half of the pollinations are successful, an average of 0.75% of the seeds germinate and 24% of the plants obtained from them survive, it can be planned to obtain an appropriately high number of offspring by choosing the appropriate combinations.

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Wyniki zapylenia międzyrodzajowego Fragaria x ananassa Duch. i Fragaria virginiana Duch. gatunkami Potentilla

Streszczenie

Zapylano 9 odmian żeńskich *Fragaria* × *ananassa* i początkowo 1 klon żeński *F. virginiana* "Scheldon" dziewiętnastoma gatunkami *Potentilla* w ciągu czterech lat (1977-1980). Otrzymano w sumie 99 194 nasion i z nich 739 siewek (0,75%). Po pierwszym roku wzrostu przeżyło 24% siewek. Po drugim roku otrzymano 159 roślin w pełni rozwoju wegetatywnego. Za najlepszą kombinację tj. dającą najwięcej przeżywającego potomstwa, uznano *F.* × *ananassa* cv. Sonja × *P. rupestris*.