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Prezygotic Endogenous Barriers to Interspecific Hybridization in *Prunus*

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Abstract. **Eighteen species of *Prunus* and 4 interspecific hybrids from the 3 main subgenera were used to ascertain the prezygotic mechanisms that maintain reproductive isolation. The percentage of pollen germination of pure species was very high (82% to 97%), and ranged from 1% to 97% for interspecific hybrids. Pollen tube growth rates differed greatly among species and ranged from 3.8 to 8.7 mm/day in vitro, and from 3 to 12 mm/day in vivo. These values were highly correlated with pistil length ($r = 0.90$) and pollen volume ($r = 0.91$). Evidence was obtained suggesting the existence of additional incompatibility mechanisms, the 1st preventing interspecific fertilization in the subgenus *Cerasus*. In *P. avium* L., the pollen tubes of some species are inhibited and finally arrested before they reach the first half length of the style. In crosses involving *P. cerasus* L. and *P. serotina* Ehrh., the use of the latter as the seed parent showed a 10-fold increase in fruit set when compared to the reciprocal. Secondly, differences in pistil length and in pollen tube growth rate among species provide a sound basis for explaining the phenomenon of unilateral incompatibility in *Prunus*. The use of male-sterile genotypes of *P. persica* L., which had a prolonged period of receptivity, gave increased fruit set and showed increased potential for overcoming the prezygotic incompatibility barriers.**

Prunus is a large and greatly diverse genus that includes more than 250 species (7, 17) separated into 5 well-differentiated subgenera (30) on the basis of morphological features and geographical distribution. The species are woody perennial trees and shrubs with great differences in phenology, ploidy level (2× to 22×), and mating systems (1, 19, 24, 30, 34). Self-incompatibility (SI) is a common feature of some species in the 3 main subgenera and is expressed by a marked inhibition of self-pollen tube growth within the style (6, 10, 29, 35).

Selection pressure for adaptation to local conditions, geographic isolation, polyploidy and interspecific hybridization have played important roles in the evolution of the genus and account for the origin of some of the most important cultivated species (26, 27, 31, 34). Under natural conditions, time-space barriers generally are very efficient in maintaining reproductive isolation of the different species. In breeding programs, however, there is a great range of success in obtaining interspecific F_1 hybrids that seems to depend upon the species and genotypes involved, pistil receptivity period, pistil length, and ploidy level (5, 15, 25, 26).

Reports of interspecific hybridization can be traced back to the works of Vavilov and Luther Burbank (33) and more recently those by Jones (16) and Kester (18) in California, Bernhard (2) in France, and Gruppe (8) in Germany.

It has been suggested that the genes which govern SI also are responsible for the governance of interspecific incompatibility (5, 10) and that the methods proven to be useful in breaking SI also could be used to overcome interspecific barriers. Some of those techniques, however, such as the use of immunosuppressants, growth regulators, shortening the length of the pistil, etc., have failed in *Prunus* (5).

Many hypotheses have been postulated to explain how endogenous barriers prevent fertilization (5, 10, 14, 20, 28); however, they generally are derived from experiences obtained with other genera and are speculative and contradictory when applied to *Prunus*.

The objective of this work was to detect and describe the main components of the endogenous barriers to interspecific hybridization in *Prunus* that operate during the postmating-prezygotic stage.

Materials and Methods

The field research in these experiments was conducted on mature trees growing on the Arkansas Experiment Station Fruit Substation, Clarksville.

Pollen germination and pollen tube growth. In vitro pollen germination and pollen tube growth rates of all species and hybrids were determined by using a freshly prepared agar (0.8%) medium containing 15% sucrose, 100 ppm B, 300 ppm $Ca(NO_3)_2 \cdot 4H_20$, 200 ppm $MgSO_4 \cdot 7H_20$, and 100 ppm KNO_3 (4, 12). A sample of recently collected pollen was deposited on the medium surface with a brush. After 4 and 8 hr at room temperature, 4 counts (fields) were made for each pollen sample (about 500 pollen grains) to determine the percentage of germination, using a light microscope (×40 and ×100). The average length of the 15 to 20 longest pollen tubes was determined as the growth rate for that particular species.

In order to study pollen tube growth rates in vivo, more than 300 flowers of each receptor species were emasculated and pollinated with each pollen source, and 10 to 30 pistils were removed at different periods of time (4, 8, 12, 24, 48, 72 and in some cases 90 to 134 hr after pollination), and immediately fixed in 1 formalin : 1 acetic acid : 8 ethanol (by volume). After a minimum of 24 hr, they were rinsed in distilled water, transferred to a nearly saturated (8 N) NaOH solution for 24 hr, and then placed in distilled water for 20 to 24 hr. The styles then were stained in a 0.1% solution of water soluble aniline blue in

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0.1N K_3PO_4 for 4 hr, placed on glass slides in 1 or 2 drops of glycerine, and squashed by gently tapping the cover slip (21, 22).

All observations were made in a darkened room using a light microscope with a fluorescent light source (340–370 nm) passing through filters UG1 and BG 38. The average length of the 10 longest pollen tubes in each treatment was determined as pollen tube growth rate.

In 1984, the number of species used to observe pollen tube growth rate in vivo was increased. Most of the species were selfed and crossed in order to observe the performance of pollen tubes within an increased range of genotypes. The branches were brought from the field at the end of the dormant period and forced in a controlled environment (diffuse light, at 19 ± 2°C) with their bases in water. Every other day, the basal ends of the branches were recut and the water was replaced. The flowers were emasculated, pollinated, and collected as described.

Morphology. Pistil length was measured in 15 to 25 randomly chosen flowers from 4 genotypes of each species. Pollen volume was calculated by depositing a sample of dry pollen on a glass slide and measuring under a light microscope (×200). The long and short axes of 20 randomly chosen grains were averaged, and the volume was calculated by using the formula for a prolate spheriod ($V = 4/3\pi ab^2$). Regression analysis was performed on these data together with pollen tube growth rate in vitro.

Period of pistil receptivity. The period of receptivity of 2 genotypes of *P. persica* L. (A-306 and 'Redhaven') and 1 of *P. armeniaca* L. ('Goldcot') was determined during the spring of 1983 by emasculating 600 flowers of each clone and pollinating 45 to 55 pistils each day for 12 days with a compatible pollen source of its own species. Temperatures during this period fluctuated between a maximum of 16.8° to 21.6°C during the day and a minimum of 4.7° to 9.5° at night. The percentage of fruit set occurring after the June drop then was related to the time of pollination after emasculation.

Interspecific hybridization. In 1982, *P. persica* 'Redhaven' was selfed and used as the female parent in crosses with 10 species and 2 interspecific hybrids. Two hundred pollinated flowers for each cross were used to determine fruit set.

During 1983, some species were not used as pollinizers but others were added. 'Redhaven' and a male-sterile selection of *P. persica* (A-306) were compared in their role as female parents. In 1984, the species involved as pollen acceptors were chosen from the 3 main subgenera:

- *Amygdalus*: 3 male-sterile selections of *P. persica* L. (2 peaches and 1 nectarine).
- *Prunophora: P. cerasifera* Ehrh.
- *Cerasus: P. avium* L., *P. cerasus* L. and *P. serotina* Ehrh.

Results and Discussion

Pollen germination and pollen tube growth. In vitro pollen germination was high for all species (82% to 97%), but the interspecific hybrids generally showed a low germination percent (1% to 40%), with the exception of *P. persica* x *P. kansuensis* and 'Nemaguard' (Table 1). These results may reflect the phylogenetic relationships among the species involved as progenitors and/or the degree of genetic differentiation. Even though pollen germination alone is not a complete measure of the fertility of a given genotype, it is an important component. We recognize that pollen germination on an artificial medium may not be the same as on the stigmatic surface of a receptive species, and that the exogenous requirements (temperature, light, relative humidity, nutrient qualities and quantities) may be different for each species. The techniques used in this work have been reported to be optimum for other species of *Prunus* (12) and for other genera (4).

Table 1. Pollen germination percentage and pollen tube growth rate in vitro of some *Prunus* species and their hybrids.

Species	Pollen germination (%)	Pollen tube growth rate (mm/day)
P. angustifolia Marsch.	92	4.2
P. armeniaca L. (cv. Goldcot)	95	7.2
P. avium L. (cv. Hedelfingen)	90	7.2
P. besseyi Bailey (IR-2 264-1)	95	4.8
P. cerasifera Ehrh.[y]	91	4.5
P. cerasus L.[y]	88	7.5
P. davidiana (Carr.) Franch (ALF 15-54)	95	7.2
P. domestica L.	96	7.3
P. ferghanensis (Kost et. Rjab) Kov. et Kost.	97	7.8
P. hortulana Bailey (IR-2 330-3)	90	4.4
P. kansuensis Rehd.	96	7.8
P. mexicana Wats.[z]	89	4.8
P. persica (L.) Batsch. (cv. Redhaven)	97	8.7
P. salicina Lindl.	87	4.4
P. serotina Ehrh.[z]	96	3.8
P. spinosa L. (IR-2 331-3)	82	4.6
P. tomentosa Thunb.	85	4.8
Hybrids		
Nemaguard[x] (*P. persica* x *P. davidiana*)	97	—
GF-677 (*P. amygdalus* x *P. persica*)	40	—
Plumcot (*P. salicina* x *P. armeniaca*)	2	—
P. persica x *P. kansuensis*	90	—
Marianna 2624 (*P. cerasifera* x *P. munsoniana*?)	1	—

[z]Pollen collected from trees in wild stands.
[y]Pollen collected from trees growing as ornamentals on Univ. of Arkansas campus.
[x]Believed to be an interspecific hybrid, but shows little segregation when seed propagated and has pollen germination as high as that of pure species.

Good fertility is a necessary characteristic of fruiting cultivars of *Prunus* and also in some rootstock clones used for seed production purposes. Even moderate percentages of pollen germination may be sufficient to ensure a moderate to high fruit set, however, which is also influenced by such other factors as ovule fertility and pollen tube growth rate.

Pollen tube growth rate in vitro exhibited a wide range of variation and seemed to be a constant feature for each species. It ranged from 3.8 to 8.7 mm/day and closely resembled the growth rates observed in vivo when peaches, plums, and some cherries were used as pollen acceptors (Table 2; Fig. 1, 2).

Pollen germination and pollen tube growth rate in vivo depend on the particular combination of species involved. When *P. persica*, *P. armeniaca* L., and *P. persica* x *P. kansuensis* (Kost. and Rjab.) hybrid were used as pollen acceptors, the same general trend of parallel pollen tube growth in vivo and

Table 2. Pollen tube growth rate (mm/day) in vivo of various parental combinations of *Prunus* species.

Pollen donors	Pollen acceptors									
	P. persica	*P. armeniaca*	*P. cerasifera*	*P. mexicana*	*P. domestica*	*P. subhirtella*	*P. besseyi*	*P. avium*	*P. cerasus*	*P. serotina*
P. angustifolia	3–4	3–4	3–4	2–3				2–3		
P. armeniaca	7–9	10–12	4–5	3–4		5–6		inhibited or 4–5		
P. avium								7–8		
P. besseyi	3		3–4		3–4		4–5	4–5	3	
P. davidiana	*7–8*									
P. domestica					8–9			4–6	4	
P. cerasifera	2–3		3–4		4–5	4–5		3	4	
P. cerasus					*4–5*			*4–5*	*5–6*	
P. ferghanensis	5–6					5–6	4–5	4–5		
P. hortulana	*4–5*									
P. mexicana	3		3–4	2–3						
P. persica	9–10	8–9	4–5	3–4	5–6	5–6	4–5	4–5	4–5	3–5
P. persica x *P. kansuensis*	7–9	8–9								
P. salicina	3–4	4–5	4	3–4						
P. spinosa	4–5									
P. serotina							3–4	3–4	4–5	3–4
P. tomentosa	3–4									

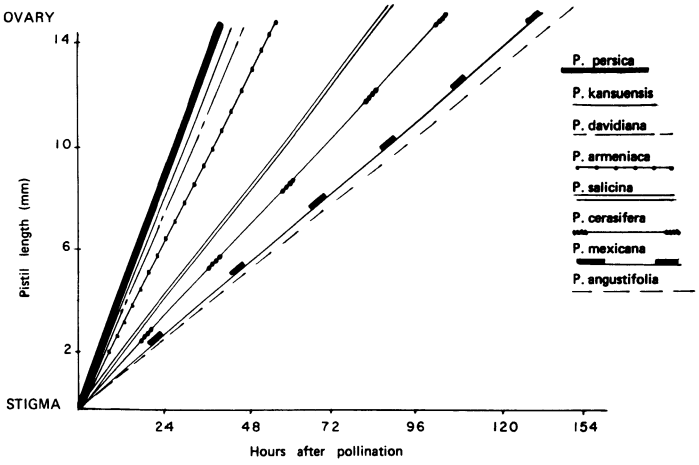

Fig. 1. Average pollen tube growth of different species growing in the styles of *P. persica* 'Redhaven' and A-306.

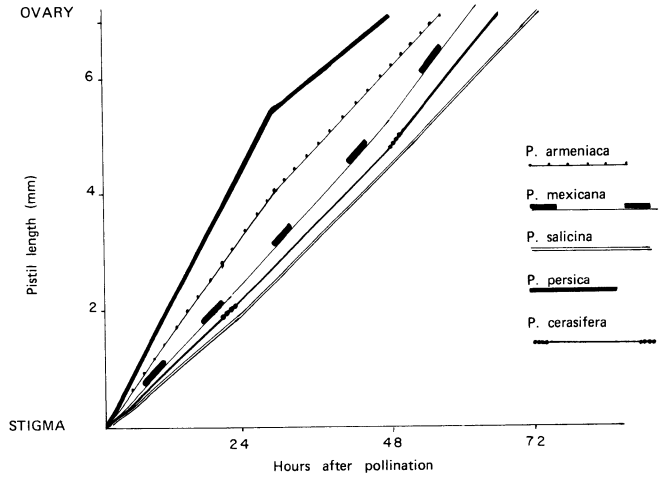

Fig. 2. Average pollen tube growth of various *Prunus* species within the styles of *P. cerasifera* (red leaf ornamental).

in vitro was observed, and it was easy to separate the species in groups of "fast" and "slow" growers. Pollen of all species in this research germinated and grew on the stigmas of *P. persica, P. mexicana* Ehrh., *P. davidiana* (Carr.) Franch, *P. armeniaca,* and *P. cerasifera*, but the most striking difference was their rate of growth (Table 2). Pollen from species in the subgenus *Amygdalus*, when deposited on *P. persica* stigmas, grew at slightly lower rates than *P. persica* pollen tubes, but 2 to 3 times faster than species belonging to the subgenera *Prunophora* and *Cerasus*, with the exception of *P. domestica* L. and *P. armeniaca* which also grew at fast rates, similar to *P. kansuensis* and *P. persica* itself (Table 2).

When *P. armeniaca* was selfed, its pollen tubes reached the ovary only 30 to 35 hr after pollination, but when crossed with *P. persica, P. davidiana* and *P. kansuensis,* those pollen tubes required from 35 to 50 hr to reach the ovaries. This was the same time required for those species to reach the ovary of their own pistils. In fact, it took the same amount of time to observe pollen tubes at the ovary of *P. kansuensis* when selfed and when pollinated with *P. armeniaca, P. persica* or the *P. persica* x *P. kansuensis* hybrid.

Other species of the subgenus *Prunophora*, such as *P. mexicana, P. angustifolia* Marsch., *P. cerasifera*, and *P. salicina* Lindl., grew at slower rates, and 72 hr after pollination their pollen tube tips were found slightly beyond three-fourths the length of the style, but growth did not stop there as suggested by previous workers (5, 6, 10, 20, 28). Instead, the pollen tubes reached the base of the style 90 to 134 hr after pollination. These results were obtained under a controlled environment, and it is expected that daily fluctuations in temperature could increase variation under field conditions. However, similar results were observed in 1982 and 1983 when the samples were collected directly from the orchard.

According to the data collected during the 3 seasons, all of the species used to pollinate *P. persica* germinated well and grew at rates similar to those observed on artificial media. This suggests that the incompatibility observed in crosses between *P. persica* and other species may be due partly to the inherent potential for growth rate of each pollen source.

When *P. armeniaca* was used as a pollen acceptor, the same trend that was observed in peach occurred with similar pollen tube growth rates in vitro and in vivo, except that its own pollen tubes reached the ovary before those of *P. persica, P. davidiana,* or *P. persica* x *P. kansuensis*. Other species included within the same subgenus (*Prunophora*), such as *P. mexicana, P. spinosa* L., *P. cerasifera*, and *P. salicina*, grew at slower rates and required 80 to 90 hr to reach the base of the styles.

An interesting result was observed when reciprocal pollinations were performed. When *P. spinosa* was selfed and crossed with the interspecific hybrid (*P. persica* x *P. kansuensis*), the pollen tubes of the latter reached the ovary when the self pollen tubes had traveled only half that distance. The same results were obtained when *P. cerasifera* and *P. mexicana* were selfed or pollinated with *P. persica* and *P. armeniaca*. The pollen tubes of the latter 2 species reached the base of styles before those of the self pollens. However, even though pollen of *P. persica* and *P. armeniaca* grew faster than the self pollen, their growth rates were still considerably lower than those observed in vitro (Table 1) and when selfed in vivo (Table 2). It may be that genetic-biochemical factors, such as self incompatibility genes, were limiting their potential growth rate, or it may have been due to mechanical constraints offered by the tissues of the recipient species.

Four species within the subgenus *Cerasus* were used as pollen acceptors in order to obtain a broad view of the prezygotic incompatibility systems operating in the genus. Three of the 4 genotypes of *P. avium* used, in which a self incompatibility system is known to exist (5, 6, 10, 11, 19, 33) permitted pollen germination and growth of all species except its own. However, when *P. avium* 'Hedelfingen' was selfed and crossed with *P. armeniaca, P. domestica* L., and *P. tomentosa* Thunb., the pollen tubes stopped growing soon after germination and appeared twisted, thick, and sometimes bifurcated. This result may be explained in terms of the hypothesis proposed by De Nettancourt (10) and supported by Coyle (5), that whenever a self-incompatible species is used as a female parent, it will inhibit the pollen tubes of other species that are self compatible, in the same way that it does with its own. However, when 'Hedelfingen' was pollinated with *P. persica, P. ferghanensis, P. cerasifera* and *P. besseyi* Bailey, their pollen tubes were found at the base of the styles 50 to 60 hr after pollination. Also, when 'Lambert' was used to confirm the results obtained with 'Hedelfingen', pollen tube tips of the same 4 species were seen at the ovarian region 72 hr after pollination. Even though some pollen tubes seemed to be inhibited or slowed in the apical regions of the style, a few always were observed in the basal region.

No strong inhibition of pollen tube growth was evident when pistils of *P. subhirtella* and *P. cerasus* were pollinated with *P. persica, P. cerasifera, P. besseyi, P. ferghanensis,* and *P. avium*. Pollen tubes of all were seen at the base of the ovary 60 to 90 hr after pollination, except for *P. cerasus* x *P. avium*, in which the pollen tubes had reached only half that distance. Since *P. cerasus* and *P. avium* are closely related (11, 26, 30), and some crosses between them have been reported (5, 19, 27), these results were unexpected, and there is no explanation, except that they may be due to the particular genotypes and/or to the conditions in which this work was performed.

In some instances, when *P. domestica, P. subhirtella,* and *P. cerasus* were pollinated with *P. avium*, the pollen tubes were twisted and grew only to the 1st quarter of the style length. This observation was not consistent, however, because when other genotypes of *P. cerasifera* were pollinated with *P. avium*, we did not observe the same type of inhibition. These observations were made only during the 1984 season, and even though more than 1000 pistils were pollinated with 9 species, this section of the work should be repeated. These data suggest that more than one kind of barrier is preventing fertilization. Also, there may be interactions among these barriers that complicate the analysis and do not allow the clearcut observation of each individual mechanism, such as that described for *P. persica* and *P. armeniaca*, and some of the other species combinations.

P. serotina, the wild blackcherry, also was selfed and pollinated with *P. persica*, and the pollen tubes of both were found at the base of the style 48 hr after pollination. The pollen of *P. serotina* also was used to pollinate *P. cerasus* and *P. avium*, and it did not appear to be inhibited in either of them, as it grew at a rate similar to its growth in its own styles and in vitro.

Thus, there are differences among in vivo pollen tube growth rates for all species that seem to be species specific or related to the particular species combination involved. However, in all instances the pollen tubes were observed to grow all the way to the base of the styles after a certain period of time, with the exception of some species combinations involving *P. avium,* either as a pollen acceptor or as a pollen donor.

Morphology. In an attempt to explain the differences in pollen tube growth observed, morphological differences and similarities among the species were studied, with the main emphasis on reproductive structures (Table 3). One character observed was the variability in pistil length, which showed a high positive correlation with pollen tube growth rate in vitro ($r = 0.90$) (Fig. 3). Pollen volume also was highly correlated with pollen tube growth rate ($r = 0.91$). All 3 variables showed a high multiple correlation ($r = 0.92$), and a linear regression curve fits the data better than a quadratic or cubic curve. Therefore, the larger the pollen volume, the greater its potential tube growth rate.

However, in the subgenus *Cerasus*, the interaction with genetic and anatomical incompatibilities may influence and even mask this relationship. The influence of factors such as temperature, genotypic differences within species, and the direction of the cross also may be expected to complicate the situation even further. Therefore, the probabilities of success for future

Table 3. Some characteristics of the different species of *Prunus*.

Species	Pistil length (mm)	Pollen volume (μ^3)	Pollen tube growth rate *in vitro* (mm/day)
P. serotina	4	4779	3.8
P. besseyi	5	13478	4.8
P. salicina	5	10646	4.4
P. angustifolia	5	12905	4.2
P. hortulana	7	16357	4.4
P. tomentosa	7	15057	4.8
P. cerasifera	8	14730	4.5
P. mexicana	8	16286	4.8
P. ferghanensis	10	28289	7.8
P. cerasus	11	22584	7.5
P. davidiana	11	28282	7.2
P. avium	12	20120	7.2
P. domestica	13	27811	7.3
P. kansuensis	14	26428	7.8
P. armeniaca	14	18630	7.2
P. persica	14	32715	8.7

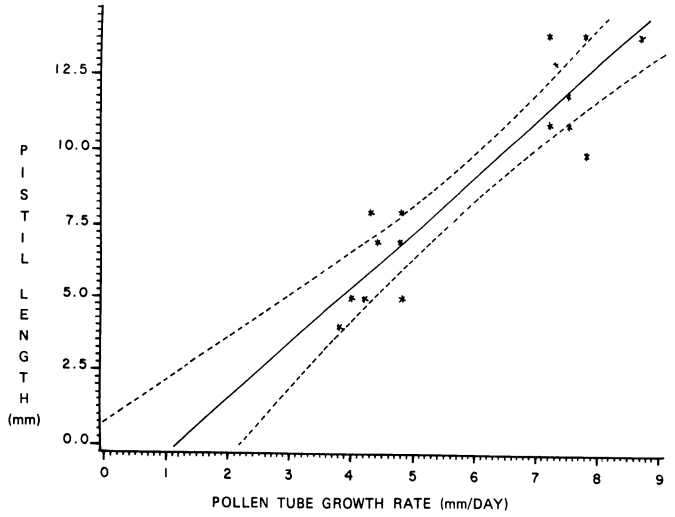

Fig. 3. Regression pistil length and pollen tube growth rate in vitro for 16 species of *Prunus* from 3 main subgenera.

work in this area should be increased if such variables are considered both separately and interacting.

Length of the receptivity period. The data indicate that the length of the pistil plays a determinant role in preventing pollen tubes from reaching the ovary while it is still receptive. Since we found little published information related to the effective time that flowers remained receptive after anthesis, a pollination test, involving 2 genotypes of *P. persica* and 1 of *P. armeniaca*, was conducted in 1983.

P. persica 'Redhaven' and *P. armeniaca* had about the same period of receptivity (5–6 days), but the *P. persica* male-sterile genotype remained receptive for a period that was at least twice as long (Table 4). Toyama (32) reported that pistils of the pollen sterile peach cultivars 'J.H. Hale' and 'Earlihale' remained receptive up to 12 days after anthesis. This prolonged receptivity may explain the high degree of success reported with interspecific crosses involving male-sterile genotypes of *P. amygdalus* and *P. armeniaca* (16) and *P. persica* (2, 3, 9, 18, 23). These researchers reported their results without providing a basis for the differences observed in fruit set between male-sterile and normal genotypes.

It also has been observed (18) that when interspecific hybridizations between *P. persica* and *P. amygdalus* are performed, the success is enhanced if *P. amygdalus* is used as the female parent; but again, no explanations for the results were given. From other studies (13, 29) it is known that *P. amygdalus* has a rate of cross compatible pollen tube growth of about 3 mm/day, considerably less than we found for *P. persica*. Therefore, the cross *P. amygdalus* x *P. persica* should have a higher probability of success than its reciprocal. In fact, this has been reported several times (3, 18, 19).

Interspecific hybridization. In 1982, more than 2000 pollinations were performed using *P. persica* 'Redhaven' as the female parent with 10 species and 2 interspecific hybrids as pollinators, but no seeds were obtained.

In the spring of 1983, trees of 'Redhaven' were covered with plastic cages to create a greenhouse environment. About 2000 pollinations were made, but again, no fruit set was obtained.

Also in 1983, a male-sterile genotype of *P. persica* (A-306) was used as a female parent with the idea that it would remain receptive for a prolonged period of time. With this genotype, a very high proportion of pistils from all crosses swelled initially, but, 24 to 35 days after anthesis, their rate of growth decreased in comparison to the controls. Then, over a time period that ranged from 48 to 57 days after pollination, all of the fruit abscised. In 1984, 3 different male-sterile *P. persica* genotypes (A-263, A-224, and A-306) were used as female parents, and over 5000 flowers were pollinated with 8 species. Again, as in 1983, a very high proportion of fruit set was observed initially, but most eventually abscised.

Of 150 flowers of *P. cerasifera* pollinated with *P. persica* 'Redhaven', *P. armeniaca*, and *P. salicina*, 22%, 27%, and 35%, respectively, were retained after the 2nd drop, but 32 to 41 days after pollination they all abscised (Table 5).

When *P. avium* was crossed with *P. besseyi, P. hortulana, P. persica, P. armeniaca, P. mexicana,* and *P. cerasifera*, some of the pistils appeared wilted and dull green after only 9–15 days, and a very small percentage were retained and continued growth after the 3rd week from anthesis. However, the growth of these fruit was observed to be at slightly lower rates than the controls (80% to 90%).

P. serotina showed a slightly different response when pollinated with *P. avium* and *P. cerasus*. Six to 10 days after pollination, some pistils abscised (1st drop), but 26% to 32% were retained and continued to grow. Two weeks later, after the 2nd drop, only 2% to 4% were retained and growing at similar rates as the controls that had 50% to 70% fruit set. On the other hand, when *P. cerasus* was pollinated with *P. serotina*, only 1% of the pistils were retained and were swelling after the 1st drop (2 weeks after anthesis), only 0.4% after the 2nd drop (23 to 37 days after anthesis). This differential result illustrates the 1st type of incompatibility barrier suggested for the subgenus *Amygdalus;* when the female parent has a longer pistil (11 mm in *P. cerasus*) the probabilities for fertilization are likely to be lower than if the reciprocal cross to the shorter pistilled species is made (4 mm in *P. serotina*).

The results obtained from these experiments suggest that the endogenous barriers that maintain reproductive isolation among *Prunus* species should be separated clearly into pre- and post-

Table 4. The percentage of fruit set in 2 genotypes of *P. persica* and in *P. armeniaca* from pollinations at successive days after anthesis.

	Days after anthesis												
Genotype	0	1	2	3	4	5	6	7	8	9	10	11	12
P. persica													
'Redhaven'	54	65	62	57	62	48	17	1	0	1	0	0	0
A-306 (male-sterile)	62	81	72	77	72	76	65	67	43	34	16	18	9
P. armeniaca	79	85	68	70	68	42	28	5	1	0	0	0	0

Table 5. Percentage fruit set of various interspecific crosses of *Prunus* following the 2nd drop.

	Female parent				
Male parent	*P. persica*	*P. cerasifera*[z]	*P. cerasus*	*P. avium*	*P. serotina*
P. persica	63	22		2.8	
P. ferghanensis	41				
P. armeniaca	52	27			
P. mexicana	43			1	
P. salicina	31	35			
P. cerasifera	28			1.4	
P. angustifolia	26			1.3	
P. tomentosa	16				
P. avium					3
P. cerasus					4
P. serotina			0.4		50–70
P. besseyi				3	
P. hortulana				4	

[z]All of these fruit abscised during the 3rd drop (6 weeks after anthesis).

zygotic, and that they may or may not be correlated. In order to have an embryo developing, the 1st set of barriers must be breached, and once this is achieved, fertilization and zygote formation will likely face another series of blocks that may prevent embryo development.

Further research in this area should concentrate on the effect of environmental conditions (especially temperature) on the prezygotic stage, as well as on growth and development of interspecific embryos, and on techniques of embryo rescue and culture under artifical conditions.

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Rescuing Abortive *Impatiens* Hybrids through Aseptic Culture of Ovules

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Abstract. **Aseptic culture of ovules resulted in the germination and development of abortive hybrids in 38 of 66 interspecific crosses among *Impatiens*. In some crosses, 6- to 11-day-old ovules had significantly higher percentages of germination than 3- to 5-day-old ovules. An average of 19.9% of the germinated seedlings was rescued, and the rest were lost through abnormal development in vitro. About 90% of the abnormal seedlings developed into hard, callus-like masses of varying sizes and shapes, and the rest developed into imperfect seedlings without roots or shoots, albinos, and soft calli. Seedlings were rescued in 25 crosses, and in 13 crosses none of the germinated seedlings was rescued. The 25 rescued progenies were derived from 8 crosses among African species, 1 among Indian species, 4 between African and Indian species, and 12 between African and New Guinea-Indonesian species. All but 2 of the progenies are new and are being reported for the 1st time. The hybrids were usually distinct and easily identified by inspection of leaves and flowers. All were sterile.**

Except for crossses within the New Guinea-Indonesian group (1), interspecific pollinations among *Impatiens* have not been successful (2). In the difficult crosses, fruit usually aborted within 2 weeks after pollination, about 4–5 weeks before the normal harvest date (2). Embryo culture was considered as a possible means of rescue, because ovules of the abscissed fruit occasionally contained embryos in various stages of development (2). Ovule culture was adopted for this purpose when it became evident that the young embryos were extremely difficult to isolate for culture in vitro (3, 6). The early experiments with ovule cultures resulted in the rescue of some seedlings in several crosses, but most crosses did not respond (3). The objective of the present experiments was to rescue abortive embryos of various interspecific crosses using a modified version of the procedure used previously (3).

Materials and Methods

Parental species. Some of the species used here were described earlier (3). Those not mentioned before were C6 and C35 from Celebes, *I. verticillata* Wight, an Indian species obtained from Longwood Gardens, *I. sultani* Hook 'Elfin White', an African species from commercial sources, and the New Guinea 'N3–2', 'N4', 'N24–2', 'N(x47)', 'N175', 'N194', 'N414', 'N514', 'N568', and 'Pele'. *I. marianae* Reich., an Indian species, incorrectly reffered to as an African species in an earlier paper (2), that did not bloom before was used when it flowered in the summer of 1981 and 1982. The species *I. auricoma* Baill., *I. thomassetii* Hook., and *I. tuberifera* Humbert have been, and are included, under the African group (2) although they are from

Table 1. *Impatiens* crosses that did not germinate in vitro.

Crosses	Ovules cultured: Age[z]	Ovules cultured: No. explanted
I. auricoma Baill. (Africa)		
x *I. campanulata* Wight (India)	6	88
x *I. repens* Moon (India)	6–9	774
x *I. thomassetii* Hook. (Africa)	5	123
I. campanulata		
x *I. flaccida alba* Arn. (India)	7–10	168
x *I. repens*	6–7	89
I. congolensis G. M. Schulz & R. Wilczek (Africa)		
x *I. auricoma*	6	113
x *I. epiphytica* G. M. Schulze (Africa)	6	100
I. epiphytica		
x *I. niamniamensis* Gilg. (Africa)	6	156
x *I. sultani* Hook. 'Elfin White' (Africa)	7–12	168
I. flaccida alba		
x *I. auricoma*	7–10	369
x *I. campanulata*	6–9	473
x *I. herzogii* K. Schum. (New Guinea)	7	66
x *I. marianae* Reichb. (India)	2–7	163
x *I. thomassetii*	6–7	110
x *I. uguenensis* Warb. (Africa)	6–11	271
x *I. verticillata* Wight (India)	6–11	372
I. hookeriana Arn. (India)		
x *I. niamniamensis*	7–10	263
x *I. repens*	7	147
I. marianae		
x *I. repens*	4–11	263
x *I. sultani* 'Elfin White'	11	32
I. repens		
x *I. auricoma*	6–11	95
x *I. flaccida alba*	7–8	66
x *I. uguenensis*	4–9	248
I. sultani 'Elfin White'		
x *I. campanulata*	6–9	963
x *I. marianae*	3–5	463
x *I. repens*	5–9	644
x *I. tuberifera* Humbert (Africa)	5–7	275
x *I. verticillata*	5–7	406

[z]Number of days after pollination.

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