

SEED ABORTION IN *PONGAMIA PINNATA* (FABACEAE)¹

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In *Pongamia pinnata* only one of the two ovules develops into a seed in most of the pods. Since pollen was not found to be limiting and reduced fertilization could not completely explain the observed frequency of seed abortion, it implied an effect of postfertilization factors. Aqueous extracts of developing seeds and maternal tissue (placenta) did not influence abortion in vitro, suggesting that abortion may not be mediated by a chemical. Experimental uptake of ¹⁴C sucrose in vitro indicated that both the stigmatic and the peduncular seed have similar inherent capacities of drawing resources, but the peduncular seed is deprived of resources in the presence of the stigmatic seed. This deprivation of the peduncular seed could be offset by supplying an excess of hormones leading to the subsequent formation of two seeds in a pod. The prevalence of single-seeded pods in *P. pinnata* seems therefore to be a result of competition between the two seeds for maternal resources. The evolutionary significance of single-seeded pods in *P. pinnata* is discussed with respect to possible dispersal advantage enjoyed by such pods.

Key words: Fabaceae; *Pongamia pinnata*; resource competition; seed abortion; seed set.

In most flowering plants, only a few of the flowers and ovules that are initiated form fruits and seeds (Lloyd, 1980; Stephenson, 1981; Bawa and Webb, 1984; Lee, 1988). Abortion of flowers and fruits occurs at various stages of development, and even in fruits that finally reach maturity not all the ovules give rise to seeds. Factors affecting seed formation could act before or after fertilization and can be determined by analyzing the characteristics of ovules in the fruits that attain maturity (Hossaert and Valero, 1988).

One of the important factors that act in the prefertilization phase is the limitation of pollen grains (McDade, 1983; Garwood and Horovitz, 1985; Wheelan and Goldingay, 1989). Lack of fertilization of all the ovules despite a large number of pollen grains being deposited on the stigma is known for various plant species (Mogensen, 1975; Guth and Weller, 1986; Cruzan, 1989). Some theoretical studies also suggest that female reproductive success is not limited by pollen availability but by the quantity of resources required for fruit and seed formation (Bawa, 1979; Charnov, 1979; Willson, 1979; Lloyd, 1980). This is confirmed by data from species like *Catalpa speciosa* (Stephenson, 1979) in which artificial pollination failed to increase fecundity, while in a few species of *Asclepias* like *A. verticillata* (Willson and Price, 1980), addition of resources increased fecundity.

Nonrandom or selective abortion can influence the quality of fruits and seeds (Janzen, 1977; Charnov, 1979; Stephenson, 1981; Casper, 1984; Lee, 1984; Bawa and Webb, 1984; Stephenson and Winsor, 1986; Uma

Shaanker, Ganeshiaiah, and Bawa, 1988), and such selective abortion could result from competition among the developing fruits (Lee and Bazzaz, 1982a, b), seeds (Uma Shaanker, Ganeshiaiah, and Bawa, 1988; Bawa et al., 1989; Arathi et al., 1996), or a combination of both.

Competition among seeds for limited resources that could lead to selective seed abortion can be conveniently studied in leguminous plants since they have linear fruits, which exhibit two gradients (Hossaert and Valero, 1988). One gradient is that of the maternal resources that flow from the peduncular to the stylar end and the other gradient is that of the pollen deposited on the stigma, which moves in the opposite direction. Here we report the pattern of seed set in *Pongamia pinnata* (L.) (Syn. *Derris indica* (Lam.); Family: Fabaceae) and suggest proximate causes for the observed patterns of seed abortion, which lead to a large percentage of single-seeded pods in this species.

MATERIALS AND METHODS

This study was conducted at the Botanical Garden of the University of Agricultural Sciences, Bangalore, India. *Pongamia pinnata* is a woody perennial, which flowers between March and May. The papilionaceous flowers are borne in racemose inflorescences and are open pollinated. The ovary has two to three ovules, of which almost always only one develops to maturity. The fruit is a dry indehiscent pod.

Ovule and seed number—Ovaries of fully developed flower buds picked from several inflorescences in 20 trees were dissected, and the number of ovules in each ovary was counted. The number of seeds in randomly picked mature, dry pods from 50 trees were also counted.

Pollen grain number and ovule fertilization—The number of pollen grains on the stigma of mature, persistent, open-pollinated flowers was counted by staining the excised stigma with 1% acetocarmine for 10 min. The number of pollen grains that were germinating was also recorded. Ovaries of flowers fixed 48 h after anthesis were processed as described in Kho and Baer (1986), stained with 0.5% aniline blue for 12–16 h and observed under UV microscope for fluorescent pollen tubes entering the ovules.

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TABLE 1. Number of pods with stigmatic or peduncular seed.

Age (days after anthesis)	Number of pods with stigmatic seed	Number of pods with peduncular seed	$\chi^2 P$
2	16	14	0.45 > 0.05
3	22	18	1.82 > 0.05
5	21	19	1.46 > 0.05
10	27	14	4.12 < 0.05
25	30	16	4.26 < 0.05
40	33	14	7.67 < 0.05

Position of seeds in pods of different age classes—Pods at different stages after anthesis (1, 2–4, 6–8, 10–12, 15–20, 25–30, >40 d and mature, dry pods) were dissected. The number of large, healthy ovules (considered as developing seeds) and shrunken, brown ovules (considered as aborted seeds) was counted and their position in the pods was noted.

Effect of different extracts on ovule abortion in vitro—Water extracts of developing seeds and placenta (maternal tissue) were prepared separately by grinding equal masses of the tissues in 0.25 mL distilled water. The extracts were added to plastic wells of 0.5 cm diameter and 0.5 cm height filled with 0.5% agar. In each well, ten ovules from flowers collected 48 h after anthesis were implanted and incubated at 25°C. The number of ovules aborted (indicated by shrinking and browning of the ovules) was counted at 12-h intervals. Distilled water was added to the control and four replications were maintained for each treatment and the control.

Resource uptake by developing seeds in vitro—Developing pods from open-pollinated flowers were collected 5 d after anthesis from different trees. Plastic wells of 0.5 cm diameter and 0.5 cm height were filled with 0.5% agar and inundated with 16 μ L of 14 C sucrose solution. In one treatment, 15 pods with only the peduncular seed intact (stigmatic seed excised by cutting the pod in half) were implanted in the 14 C agar blocks. In another treatment, 15 pods with both the seeds intact were implanted. The pods in both the sets were retained in the agar block for 48 h, washed in water, and the amount of 14 C taken up by the seeds was measured. The pod coat was removed and the seeds were ground with Bray's medium (naphthalene [60 g], 2,5-diphenyl oxazole [4 g], 1,4 bis-2,4 methyl-5 phenyl oxazole [0.2 g], methanol [100 mL] and ethylene glycol [20 mL] made up to 1L using 1,4 dioxin). Whatman filter paper discs were dipped in this extract and floated in 2 mL of Bray's medium in 50-mL scintillation vials. The radioactivity emitted by these discs was counted using a liquid scintillation counter at 1190 V to determine the amount of 14 C taken up by the seeds. There were five replicates for each treatment, and the mean 14 C taken up by the pods in each treatment was used for the statistical analysis.

Effect of hormones on the number of developing seeds in situ—Twenty inflorescences, with open-pollinated flowers, in two trees were sprayed with hormones daily for a period of 2 wk. A combination of benzyl adenine (BA), gibberellic acid (GA), and naphthalene acetic acid (NAA), 2000 ppm each, was used as the treatment and double-distilled water was used as the control. The sprayed fruits were dissected 30 d later, and the position of the developing seed was noted.

RESULTS

Ovule and seed number—The ovaries of *P. pinnata* mostly contained two ovules (95.04% of the flowers) and occasionally three (4.96%), with a mean of 2.05 ± 0.22 ovules per ovary ($N = 121$). A majority of the pods (90%) had only one seed (1.10 ± 0.42 ; $N = 267$).

TABLE 2. Number of ovules aborted in response to extracts of developing seed and maternal tissue, and comparisons between each treatment and the control ($N = 40$ for each treatment and control) using the chi-square test of independence.

Time (h)	Control (no. aborted)	Extract of developing seed			Extract of maternal tissue		
		No. aborted	χ^2	P/p	No. aborted	χ^2	P/p
12	0	0	—	—	0	—	—
24	2	6	—	$p = 0.11^a$	2	—	$p = 0.21^a$
48	5	7	—	$p = 0.07^a$	2	—	$p = 0.11^a$
60	17	11	1.97	>0.05	10	2.74	>0.05
80	20	21	0.05	>0.05	18	0.20	>0.05

^a The probabilities refer to those calculated from the Fisher's exact probability test used when the expected values are less than 5, which may result in biased estimates of χ^2 .

Pollen grain number and ovule fertilization—Mature persistent flowers had a mean of 96.08 ± 26.53 ($N = 37$) pollen grains per stigma, of which 26.76 ± 13.8 were found to be germinating and growing through the style. Aniline blue staining indicated that, out of the 23 pods screened, 14 (60.87%) pods had both the ovules fertilized, three (13.04%) had only the stigmatic ovule fertilized, two (8.7%) had only the peduncular ovule fertilized, while in the remaining four pods the pollen tubes were not visible clearly.

Position of seed set—Within a pod, the seeds of *P. pinnata* exhibited a position-dependent abortion. The proportions of seeds aborted at the peduncular and the stigmatic ends were not significantly different up to the 5th d. However, from the 10th d onwards the proportion aborted at the peduncular end was significantly higher than that at the stigmatic end (Table 1).

Effect of different extracts on ovule abortion—In all the treatments, the abortion of ovules increased with time after implantation. However, throughout the course of observation, the extracts of either the developing seed or the maternal tissue did not differ significantly from the control in causing abortion (Table 2).

Resource uptake by developing seeds—The amount of 14 C sucrose taken up by the peduncular seed differed significantly depending on the presence or absence of the stigmatic seed. In intact pods, the peduncular seed took up significantly less 14 C sucrose than did the stigmatic seed. The peduncular seed in such intact pods took up significantly less 14 C sucrose (4.78 ± 2.2 CPM [counts per minute] per seed) than the peduncular seed (13.11 ± 8.5 CPM per seed) in pods with the stigmatic seed excised (Wilcoxon's matched pairs signed ranks test, $T_s = 0$; $N = 5$; $P < 0.05$). However, the amount of 14 C sucrose taken up by the peduncular seed in pods with the stigmatic seed excised did not differ significantly from that taken up by the stigmatic seed (9.70 ± 5.6 CPM per seed) in the intact pods.

Effect of hormones on the number of developing seeds—Spraying of BA, GA, and NAA on inflorescences resulted in more two-seeded pods. Six of the 31 pods (19.35%) sprayed with a combination of BA, GA, and

NAA had two seeds compared to two of the 66 pods (3.03%) in the control ($\chi^2 = 7.41$; $df = 1$; $P < 0.01$).

DISCUSSION

These results suggest that the reduced seed set in *P. pinnata* could be a result of competition for maternal resources between the two seeds in a pod. Pollen limitation and lack of pollen germination are prefertilization factors that can lead to ovule abortion as reported in *Polemonium* (Zimmerman and Pyke, 1988), *Phytolacca americana* (Mikesell, 1988), and *Telopea speciosissima* (Wheelan and Goldingay, 1989). However, these factors cannot completely explain the high levels of abortion seen in *P. pinnata*. Pollen grains were abundant on the stigma and most of them were germinating through the style, but only 60% of the pods had both the ovules fertilized. This indicates that reduced fertilization could be responsible for the low seed set as is the case in *Epilobium canum* (Snow, 1986), *Klienhowia hospita* (Uma Shaanker and Ganeshaiyah, 1989), and *Erythronium grandiflorum* (Cruzan, 1989). However, in *P. pinnata*, although both the ovules were fertilized in 60% of the pods, more than 90% of them were single seeded. Therefore, reduced fertilization also does not completely explain the large proportion of single-seeded pods in this species.

It appears, therefore, that single seededness in *P. pinnata* could be a result of postfertilization seed abortion. Such an abortion could be brought about by chemically mediated seed death with the chemical either being secreted by the other developing seeds as in *S. cuminii* (Arathi et al., 1996) or by the maternal parent as in *Dalbergia sissoo* (Ganeshaiyah and Uma Shaanker, 1988). However, in *P. pinnata* neither the extracts of the maternal tissue nor the developing seeds induced abortion of the implanted seeds, suggesting that no chemical was involved in abortion.

Seed abortion could also be a result of competition for maternal resources (Stephenson, 1981; Lee and Bazzaz, 1986; Uma Shaanker and Ganeshaiyah, 1988; Uma Shaanker, Ganeshaiyah, and Bawa, 1988) with a few seeds siphoning most of the resources to themselves. Such differential capacity to draw resources by some seeds could result from a difference in their time of fertilization as in the case of *Iris fulva* (Carney, Hodges, and Arnold, 1996) in which the seeds are linearly arranged in the fruit. A difference in the time of fertilization could lead to the first fertilized ovule beginning development earlier and therefore having a higher sink capacity than the later fertilized ones. In *P. pinnata*, as in other legumes, the seeds are linearly arranged in the pods, as a result of which the stigmatic ovule is likely to be fertilized first and have a head start in development. This head start could make it possible for the stigmatic seeds to draw most of the resources to themselves and cause the abortion of the deprived peduncular seeds. Such a phenomenon also possibly explains the high frequency of pods with stigmatic seeds in *Oxalis magnifica* (Guth and Weller, 1986), *Lathyrus latifolius* and *L. sylvestris* (Hossaert and Valero, 1988), and in *Cassia fasciculata* (Lee and Bazzaz, 1982a, b).

The peduncular seed that is not able to draw as much resources as the stigmatic seed in an intact pod was seen

to be capable of drawing resources when the stigmatic seed is removed. This emphasizes that although the peduncular seed is not inherently incapable of drawing resources, the stigmatic seed can deprive it of resources possibly by virtue of its position in the pod. The single seededness observed in the pods of *P. pinnata* could therefore be a result of such position-dependent seed abortion due to competition for limited maternal resources as has been suggested in *C. fasciculata* (Lee and Bazzaz, 1986). Further studies are necessary to confirm this possibility in *P. pinnata*.

If both the stigmatic and the peduncular seeds are equal in their inherent abilities to draw resources, it is possible that the observed differences between the two seeds in the intact pod could be an influence of plant growth hormones since such hormones are known to influence the resource drawing ability of seeds (Weins, 1984). Supplying an excess of hormones to the developing seeds often resulted in development of both the seeds in the pod, suggesting that such an excess can offset the head start and the consequent higher siphoning ability of the stigmatic seed.

It seems, therefore, that the stigmatic seed in *P. pinnata* is able to draw most of the maternal resources to itself due to its position in the pod, which enables it to have a head start over the peduncular seed. The possible advantage of single seededness observed in *P. pinnata* could be the increased dispersal success of such pods due to reduced wing loading. There is no information about the mode of dispersal of the seeds of *P. pinnata*. However, the pod features represent the syndromes associated with water dispersal (Ridley, 1930) and the pods stay afloat in water for more than two months (personal observation). Therefore, reduced wing loading by decreasing seed number could be hypothesized as a selection towards increased dispersal efficiency. Wing loading of one-seeded pods ($0.29 \pm 0.09 \text{ g/cm}^2$; $N = 20$) was observed to be less than that of two-seeded pods ($0.33 \pm 0.04 \text{ g/cm}^2$; $N = 20$), though the dispersal advantages of one- over two-seeded pods have not been tested (Arathi, 1990). Such decreased wing loading leading to dispersal advantages has been interpreted as an evolutionary strategy in other species like *Platypodium elegans* (Augspurger, 1984), *Cryptantha flava* (Casper and Weins, 1981), *Dalbergia sissoo* (Ganeshaiyah and Uma Shaanker, 1988), *Cassia fasciculata* (Lee and Bazzaz, 1986), and *K. hospita* (Uma Shaanker and Ganeshaiyah, 1989).

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