Naturalia *(eISSN:2177-0727) Rio Claro, v.33, p. 127-135, 2010*

POLLINATION OF ORCHIDS WITH A POLLEN MIXTURE

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ABSTRACT

Self-pollination and cross-pollination were carried out simultaneously in species of the genus *Laelia* Lindl. and of the genus *Cattleya* Lindl. (Orchidaceae). These experimental procedures increased the production of embryos with seeds compared to self-fecundation alone and reduced it compared to cross-pollination alone.

Intraspecific pollination together with heterospecific pollen and intraspecific pollination with recently collected pollen and pollen stored for one year (inactive pollen) were also performed. In the former case there was a reduced production of seeds with embryos and in the latter no seeds with embryo were formed.

Key words: Orchidaceae, seeds with embryos, self-pollination, cross-pollination, heterospecific pollen, inactive pollen.

INTRODUCTION

Most orchid species are hermaphrodite, with the anther and stigma arranged in the column or gynostemium. This arrangement of the reproductive structures may cause the occurrence of self-fecundation. However, the presence of the rostellum and the action of mechanical or physiological factors prevent or impair the occurrence of self-pollination in these plants adapted to cross-pollination. Stort & Galdino (22) and Stort & Martins (23) observed that experimental self-fecundation reduced the production of seeds with embryos in most *Cattleya* Lindl and *Laelia* Lindl species studied. Expceptions were detected in species that normally reproduce by self-fecundation. In these species, incompatibility mainly manifests at the time of fertilization (21, 22, 23).

Under natural conditions, orchids are pollinated by insects and birds that usually are specific in their visits to the flowers. The adaptation that exists between the floral structures and the pollinators permits the intraspecific transfer of pollinia. Because of the movement of pollinators on the flowers, the possibility of contact between pollinia and the stigma of the same flower cannot be ruled out, with the occurrence of self-pollination, together with pollination with pollinia coming from another flower (cross-pollination). On the other hand, since insects in general visit various flowers, there may be sporadic simultaneous transfer of pollinia of orchids and of pollen from other plant families (detected in personal observations) or transfer of pollinia of different orchid species.

In order to determine the effect of these types of pollination on the fertility of orchid seeds, we performed experimental pollinations in species of the genus *Laelia* Lindl. and of the genus *Cattleya* Lindl.

MATERIAL AND METHODS

The pollinations were performed in the following orchid species: genus *Cattleya* Lindl.: *C.amethystoglossa* Lindl. & Reichb.f., *C.aurantiaca* (Batem.) P.N.Don., *C.dormaniana* Reichb. F., *C.intermedia* Graham., *C.mossiae* Park. ex. Hook, *C.warnerii* Moore ex. Warner; genus *Laelia: L.anceps* Lindl., *L.grandis* Reichb.f., and *L.tenebrosa* Rolf. The orchid specimens used were collected in their natural habitat and were cultured on appropriate lattices on the premises of the Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba.

Self-pollinations performed together with cross-pollination

Self-pollinations, together with cross-pollinations, were performed manually using a wood stylus for the transfer of pollinia from the anther to the stigma of the tested flower. Two pollinia for self-pollination and two cross-pollination were used for the species of the genus *Cattleya*, and four pollinia for self-fecundation and four more for cross-pollination were used for the genus *Laelia.*

Pollinations with inactive (dead) pollen together with pollinia of a recently opened flower (intraspecific pollinations)

The crosses were performed in *C.amethystoglossa* using two inactive pollinia and two pollinia of a recently opened flower of the same species. A pollinium becomes inactive after storage for one year or more. These pollinia, according to the tests performe, germinate well on the stigma, reach the ovary, and induce macrosporogenesis but are sterile, i.e., they do not perform fertilization.

Intraspecific pollinations together with heterospecific pollen

Two types of tests were performed: in the first, pollinations with pollen of the same species and of another orchid species (heterospecific pollen) were performed. In the second, pollinations were performed with intraspecific pollen plus pollen from another plant family. The following tests were performed: *C. amethystoglossa* pollinated with a pollinium of *C. amethystoglossa* and with pollen of *Gladiolus communis* (Iridaceae), *C. loddigesii* pollinated with a pollinium of *C. loddigessi* and pollen of *G. communis; L. purpurata* pollinated with a pollinium of *L. purpurata* and pollen of *Amaryllis belladonna* (Amaryllidaceae).

All pollinations were performed with replicates. Five seed samples were removed from the fruits and mounted on slide using 1% acetic carmine to contrast seeds with embryos against seeds containing no embryo. The seeds were counted under a common light microscope. The results of the counts are presented as percentages of seeds with embryos. These data were compared to control values by the Mann-Whitney U test.

RESULTS

Table 1 lists the mean percentages of seeds with embryos found in the fruits formed after cross-pollination plus self-pollination and the control values, i.e., the values obtained with cross-pollination and self-pollination performed separately. The table also shows the U values for the statistical comparison of the values of seeds with embryos detected in the treatments and in the controls.

TABLE 1. Means of seeds with embryo set from self plus cross pollination performed in one flower (test) and seeds with embryo set from crosses pollinations and self-pollinations (control).

Species	Means of seeds with embryo		U in Mann-Whitney test	Mean of seeds with embryo	U in Mann-Whitney test
	⊗ **X**	**X**		⊗	
C.amethystoglossa	36.36	70.31	22 n.s.	5.85	9 n.s.
C.aurantiaca	92.50	62.82	0 s.	79.39	0 s.
C.dormaniana	15.71	42.45	17 n.s.	25.22	16 n.s.
C.intermedia	24.08	64.85	1 s.	13.64	14 s.
C.mossiae	0.00	36.00	-	0.00	-
C.warnerii	5.09	36.73	16 s.	0.00	20 s.
L.anceps	51.78	45.00	9 s.	18.54	0 s.
L.grandis	14.94	42.48	3 s.	8.74	0 s.
L.tenebrosa	20.66	46.55	12 s.	12.95	10.5 s.

Significant at the 5% level
s. – significant
n.s. – not significant

Simultaneous self-pollination and cross-pollination

The mean percentages of seeds with embryos varied according to species and were lower than those obtained with cross-pollination. Exceptions are shown for *C. aurantiaca* and *L. anceps*. The greatest reductions were recorded for *C. intermedia* (40.74%), *C. amethystoglossa* (33.95%) and *C. warnerii* (31,65%). No seeds with embryos were found in *C. mossiae* after self-pollination and self-pollination plus cross-pollination.

Seeds with malformed embryos were detected in *C. dormaniana* (1.53%) and *C. intermedia* (1.28%). These values were low in relation to the remaining results. The mean percentages of seeds with embryos obtained in the treatments (cross-pollination together with self-pollination) were superior to those detected in the self-pollinations. Only in *C. dormaniana* was this value lower than control.

No seeds with embryos were detected in *C. mossiae*, but in *C. aurantiaca* self-fecundation produced more seeds with embryos.

The mean time between pollination and fruit opening varied according to species but not according to treatment or to the number of seeds with embryos.

Intraspecific pollinations performed with dead pollen together with pollinia from a newly opened flower (live pollen)

Intraspecific pollinations with dead pollen and live pollen did not produce seeds with embryos (Table 2).

TABLE 2. Means of seeds with embryo set from pollination with mixture of pollen (test) and intraspecific pollination (control).

Species	Mean of seeds with embryo		U in Mann-Whitney test
	Test	Control	
C.amethystoglossa x *C.amethystoglossa* plus pollen of *C.shroederae*	61.27	70.31	16 n.s.
C.amethystoglossa x *C.amethystoglossa* plus pollen of *C.communis*	63.53	70.31	17 n.s.
C.loddigesii x *C.loddigesii* plus pollen of *C.communis*	62.32	68.69	16 n.s.
L.purpurata x *L.purpurata* plus pollen of *C.belladona*	62.44	68.20	6 n.s.
C.amethystoglossa x *C.amethystoglossa* (active pollen) (plus dead pollen)	0.00	70.31	-

n.s. not significant
Significant at the 5% level.
Control refers to intraspecific crosses with pure pollen

Intraspecific pollinations performed together with pollen from other species

The results presented in Table 2 show lower percentages of seeds with embryos in the treatment, although the U values obtained by statistical comparison of the treatment and control values did not differ significantly (Table 2).

DISCUSSION AND CONCLUSION

Self-pollination and cross-pollination performed in the same flower

The mean values of seeds with embryos obtained in the fruits formed after the tests (self-pollination plus cross-pollination) were lower than those obtained with cross-pollination alone. Exceptions were observed in *C. aurantiaca* and *L. anceps.*

Comparing with the values obtained in self-pollination the mean production of seeds with embryos increased in seven of the nine species studied. Thus, in most species self-pollination was responsible for the reduced production of seeds with embryos. The absence of embryos was due to the lack of fertilization, while non-fructification was due to the non-germination of pollinia or to insufficient growth of pollen tubes, which only reached the upper portion of the ovary. When the pollinia germinated and the pollen tubes grew normally there was fructification.

Studies by Vander Kloet (24, 25) on *Vaccinium corymbosum* showed that self-fecundation pollen germinates as well as allocrossing pollen and better than compatible pollen from distant species. The author observed that when a mixture of self-fecundation pollen and pollen from cross-fecundation of the same population or with distant populations, the time needed for fruit maturation and seed production is significantly reduced compared to pollination with compatible pollen. This relation was not observed in the present study. Also, we detected no relation between the quantity of seeds with embryos produced per fruit and the time elapsed between pollination and fruit maturation. Similarly, we observed no relation between pollination system and maturation time of the fruits.

Competition may occur in pollinium germination and pollen tube growth between structures from the same flower and from a different one, with the latter having an advantage. However, the different time of growth of pollen tubes does not seem to have an important effect since in orchids there is a period between pollination and fertilization during which macrosporogenesis occurs. In most species, fertilization occurs on average 30 days after pollination, and this time is sufficient for all pollen tubes, even those of slowest growth, to reach the entire ovary (21).

The outcrossing rate of a species determines the genetic load that is reached when selection balances mutation (2). This mechanism creates differences in level of genetic load among populations (11) and among species (12).

Ferdy et al (9) founded in a rare deceptive orchid (*Dactylorhiza praetermissa* (Druce) Soo) evidence of inbreeding depression at a small geographic scale. Also, demonstrated that the position of a flower in an inflorescence affect the type of cross.

Deceptive species do not provide any reward to their pollinators which visit few flowers per plant (1, 17, 26). This reduces the transfer of pollen between flowers of the same individual. The pollinator has a tendency to visit a plant that is quite away. Thus, the pollen of a deceptive inflorescence is transfered over long distance (18) yielding high outcrossing rates.

Johansen (13), working with *Dendrobium secundum*, observed that simultaneous pollination with compatible (mentor pollen) and incompatible (self-pollination) pollen only led to the formation of capsules when more compatible than incompatible pollen was used. According to Johansen (13), compatible pollen must produce a quantity of "compatibility factor" that will compensate for the incompatibility factor represented by incompatible pollen. The auxin contained in the pollinia of orchids seems to be the factor that causes the reaction of incompatibility. According to Linskens (15), self-incompatibility is a system that inhibits inbreeding. The author distinguishes two types of incompatibility: sporophytic and gametophytic. In the former, the action of the genes of the pollen tube and of the stylus tissue is determined by the sporophyte of the mother, whereas in the latter each incompatibility gene acts independently on the haploid pollen and on the diploid stylus tissue. According to the author, the incompatibility reaction includes two stages, i.e., recognition and rejection, which affect pollen germination, pollen tube growth and fusion of male and female nuclei.

According to Nettancourt (16), the self-incompatibility system of orchids is not well known but it is a gametophytic, polyalletic and homomorphic system with pollen inhibition by the stigma or pollen tube inhibition by the stylus. Johansen (13) did not confirm this statement in his study on *Dendrobium*. Stort & Galdino (22) and Stort & Martins (23) observed that in species of the genus *Cattleya* and the genus *Laelia* self-incompatibility manifests during fertilization, thus preventing the union of male and female nuclei.

The stigma of orchids contains secretory cells denoted utricles (3) or eleuterocytes (13) which have a different aspect according to whether the pollen that contacts the stigma is compatible or incompatible, of the same species, or of a different species.

Pollination with inactive (dead) pollen together with live pollen

Intraspecific pollinations performed in *C.amethystoglossa* with pollen from a recently opened flower and with inactive (dead) pollen produced fruits but did not produce seeds with embryos. Pollinium germination, pollen tube growth and macrosporogenesis did occur, but there was no fertilization. The same occurred in preliminary tests carried out only with dead pollen. It is possible that dead pollinia and the pollen tubes originating from them release one or more substances that prevent the union of female gametes with male gametes originating from recently collected pollinia. Dead pollen induces incompatibility responses, while compatible live pollen plays the role of mentor, affecting flower abscission.

Johansen (13), working with *Dendrobium* species, determined the effect of dead pollen during the initial fructification phase. *Dendrobium* flowers pollinated with compatible dead pollen suffered abscission as if they had been pollinated with incompatible pollen. According to the author, compatible dead pollen contains an incompatibility factor, while live compatible pollen produces a substance that may prevent flower abscission.

In some self-incompatibility systems, compatible dead pollen acts as mentor pollen, aiding the incompatible pollen (20). In other self-incompatibility systems which prevent pollinium germination and pollen tube growth, dead pollen may not show any incompatibility reaction. According to Johansen (13), compatible dead pollen contains an incompatibility factor. Only live compatible pollen has the potential to serve as mentor, whereas dead pollen induces incompatibility responses.

Intraspecific pollinations carried out together with heterospecific pollen

Data collected on the pollination with intraspecific and heterospecific pollen have shown the negative effect of heterospecific pollen on seed fertility. In general, this process did not affect fructification or the time needed for fruit production and maturation. Galern & Gregory (10) observed that flowers of *Polemonium viscosum* treated with pollen of *Mirtensia* or *Castilleja* did not suffer alteration in the capacity for adherence of co-specific pollen. Heterospecific pollen, however, significantly reduced the germination of co-specific pollen and the success of pollination.

In nature, the infidelity of the pollinator reduces the reproductive success of *P.viscosum*. Pollinators that forage indiscriminately among various plant species may transfer pollen between species, reducing the amount of pollen that reaches co-specific flowers. They may also reduce the dispersal of pollen to a distance, the allocrossing and the reproductive success (10).

Several observations have shown that pollen flow is lower in areas with mixtures of species than in areas with pure populations. Also, a reduction in pollen dispersal is expected in plant populations in which the pollinators are inconstant (4). Orchid pollinators are specific in their visits to flowers (6, 7, 8, 19). This does not mean that these pollinators will not visit other plant species, bringing a pollen mixture to the recipient flower, with a consequent possible reduction of the amount of seeds with embryos produced by the plant. A species may suffer loss of reproductive ability when the heterospecific pollen interferes with pollen adherence and germination and with fertilization of the ovule by co-specific pollen (4, 5, 27).

In this study, the presence of heterospecific pollen did not change the adherence of pollinia to the stigma surface or the space available for the deposition of co-specific pollen. The stigma surface of orchids withstands well the pollen mixture used. There may be interference of the foreign pollen when the pollen load is large or when the heterospecific pollen deposition occurs long before the arrival of the co-specific pollen (28). In the present study the co-specific and heterospecific pollinia were placed simultaneously in the tested flower. Johansen *et al*. (14) observed that pollen mixtures (of the same or of different species) reduce the formation of ovules.

In orchids there is an excess of ovules in relation to the number of pollen tubes that reach the ovary. This occurs because many ovules are not fertilized. In these cases the embryo sac degenerates, leading to the formation of seeds without embryos. Thus, even though reduced ovule production may occur, this would not alter the quantity of seeds with embryos produced by pollination with a mixture of co- and heterospecific pollen.

In view of the results described, we recommend to orchid breeders who perform manual pollination of orchids in their production and breeding work to avoid the use of a pollen mixture, so that pure self-fecundation with a mixture of pollen from another flower from nearby or distant populations will not occur. These precautions should be double when hybrid plants are used since hybridization, of itself, reduces the production of fertile seed (21).

ACKNOWLEDGMENTS

The authors wish to thank the Department of Genetics of Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, for authorizing the use of the plants employed in the present study. They are also grateful to CNPq and CAPES for the fellowship granted to the author Elias Andrade Cortez.

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