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REPRODUCTIVE BIOLOGY IN SPECIES OF THE GENUS *NOTHOFAGUS*

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Riveros M., Parades M. A., Rosas M. T., Cardenas E., Armesto J., Arroyo M. T. K. and Palma B. *Reproductive biology in species of the genus* Nothofagus. Environmental and Experimental Botany **35,** 519–524.—The reproductive systems of three species of *Nothofagus, N. dombeyi* (Mirb.) Oerst., *N. nitida* (Phil.) Krasser and *N. obliqua* (Mirb.) Oerst. (Fagaceae), were studied in their natural forest habitats in Valdivia, Chile, using controlled manual pollination, as well as auto- and cross-pollination methods. The three species, which are monoecious and anemophilous, flower and form fruit between September and February. They are highly self-incompatible and the formation of seeds under natural conditions is infrequent. In cross-pollination tests, however, the frequency of seed formation could be increased. The results of these tests have also been analysed in relation to climatic factors.

Key words: *Nothofagus*, reproductive system, self-incompatibility.

INTRODUCTION

Species of the genus *Nothofagus* are important components of the temperate rain forest in the south of Chile. Its trees have been intensively exploited because of their wood and as a consequence, numbers and distribution have been seriously reduced in recent years. Althought various aspects of the biology of *Nothofagus* species have been studied, e.g. their physiology,[1,12,17,22] phytosociology,[15,16,23] ecology[26] and genecology,[7,8,9,10] their reproductive system is still unknown. For example, knowledge of the fertility of the various species is important for propagation and reforestation of decimated areas, and also for protecting the biodiversity in the existing forests. Fruit development in *Nothofagus* is near 100% (i.e. every flower results in a fruit); however, the majority of fruit has been found to lack a viable seed.[19] Moreover, when seed germination was studied,[6,14] it was found that, for *N. dombeyi*, rates of germination were low, even after using pre-treatments known to increase germination percentages.

Nothofagus species are anemophilous.[13,25] Accordingly, the transfer of pollen between individuals can have low rates of success since anemophily is an aleatoric and potentially ineffective system of pollination.[11,27] Although wind can carry pollen great distances, the lack of wind can, by default, provoke large-scale self-pollination. Only those anemophilous species which are self-incompatible will be

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able to form viable (and heterozygous) seeds. Donoso[8,10] has reported the ocurrence of hybrids among species of *Nothofagus* (e.g. between *N. nitida* and *N. dombeyi*) when the distribution of these species has been restored by reforestation. According to Donoso,[10] these fertile hybrids can introduce new genetic variation by means of introgression. In general, however, the proportion of these hybrids in a population is low.

Determination of the reproductive success of three species of the genus *Nothofagus*, *N. obliqua*, *N. dombeyi* and *N. nitida*, native to the Valdivian forests of Chile, was the main aim of this study. The phenology of these species was also determined, with an emphasis on the flowering and fruit-forming periods. The frequency of success in interspecific pollination between *N. dombeyi* and *N. obliqua* was also assessed.

MATERIALS AND METHODS

Individuals of three species of *Nothofagus*, *N. dombeyi*, *N. obligua* and *N. nitida*, were used in this study. They were located in the Botanical Gardens of the University of Chile, Santiago, and in the forests in the vicinity of Valdivia (39°48′S; 73°14′W). The three species have inflorescences consisting of yellowish-green flowers. The female inflorescence has three flowers (with the exception of *N. nitida* which has between three and seven in each inflorescence) which are surrounded by a persistent green cupule. The gynoecium has between four and six seminal primordia. The clusters of fruits develop as nuts, each containing one seed. The male flower presents numerous stamens.

The recording of data for the flowering phase started in September 1991; *N. obliqua* was the first to flower, *N. nitida* was the last to flower. The fruit-forming phase started in September–October. Data were collected every 15 days, using the method described by Arroyo[2] and Riveros.[18] The duration of flowering was determined by marking 10 flowers in three to five trees of each species. The three species are characterized by their irregular flowering, i.e. they do not flower every year. For example, of the 20 individuals of *N. obliqua* recorded in 1991 and 1992, only seven flowered; of 10 individuals of *N. dombeyi*, and of 12 individuals of *N. nitida*, only five produced flowers in each case.

The monthly averages for rainfall and temperature, in the Valdivia region, for 1991 and 1992 during the periods of flowering and fruiting are shown in Fig. 1.

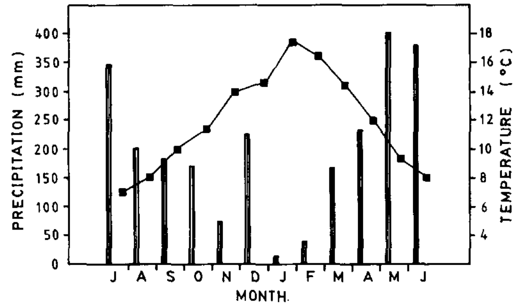

Fig. 1. Monthly average rainfall (bars) in mm and monthly average temperature (squares) in °C in the region of Valdivia.

Self-incompatibility, genetic, autogamic and agamospermic tests were carried out using a crossing programme developed by Ruiz and Arroyo.[21] The experimental tests were as follows:

I. *Manual self-pollination.* This was done using receptive flowers which had been previously isolated with paper bags at the bud stage. Pollination was performed with pollen from the same tree.
II. *Manual cross-pollination.* Receptive female flowers which had been isolated were pollinated with pollen obtained from other individuals. Afterwards they were bagged until the flowers withered and fruit development began.
III. *Autogamous self-pollination.* Another group of flowers, isolated within paper bags from the bud stage, was left covered without external intervention until they reached the fruit-forming stage.
IV. *Agamospermy.* To observe the formation of fruits and/or seeds in the absence of male gametes, buds which had previously been emasculated were isolated until they reached the fruit-forming stage.
V. *Natural pollination.* The number of fruits which developed under natural conditions of pollination was quantified. To do this, branches with a known number of flowers were marked from the flowering up to the fruit-forming stages.

Tests for inter-specific pollination were carried out

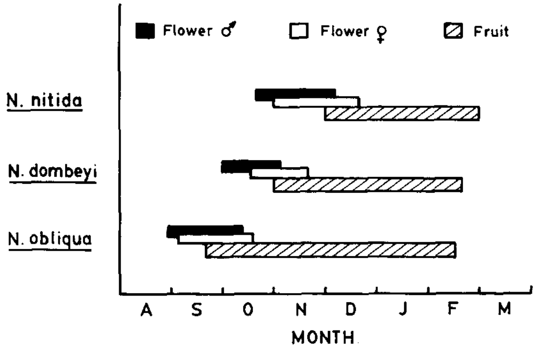

Fig. 2. Flowering periods observed for three species of *Nothofagus* during 1991 and 1992. Filled bars indicate the period of male flowering, open bars correspond to female flowering and the hatched bars correspond to the period of fruit development.

on branches of *N. dombeyi*, isolated from the flower bud stage, which were manually pollinated with pollen from *N. obliqua*. The subsequent development of fruits was observed.

The development of seeds was assessed in each type of crossing and fertility indices, F, were estimated. The relative fertility (F_r) of each species was calculated according to the ratio of the frequency of seeds developed in natural conditions of pollination (F_n) and of the frequency of seeds from individuals pollinated manually (F_c) (i.e. $F_r = F_n/F_c$).

RESULTS

Nothofagus obliqua was the first species to flower (Fig. 2). In 1991, the flowering time extended from 28 August until 20 October (a total of 54 days), with September being the month of maximum flowering. *Nothofagus dombeyi* started to flower on 30 September and continued for a period of 52 days until 20 November. Flowering of *N. nitida* starded on 20 October and continued until 30 December, a total of 81 days. Between *N. obliqua* and *N. nitida*, the overlap of flowering was smaller (in proportion to the total flowering time of either species) than that observed between the other two species (Fig. 2). There was an overlap of 1 month (from mid-October to mid-November) between *N. nitida* and *N. dombeyi*. In these monoecious species, the male flowers matured earlier than the female ones, sometimes with a difference of 15 days, as in the case of *N. nitida*. The opening of the female flowers took place in parallel with leaf- and bud-break.

The fruit-forming phase lasted 4–4.5 months, reaching its peak between February and the beginning of March. Although *N. obliqua* was the first species to flower, its fruits ripened in February also. The shortest fruit-forming period (2.5 months) is that of *N. nitida*, with ripe fruits again occurring towards the end of February and beginning of March (Fig. 2).

The three species studied were found to possess a highly self-incompatible reproductive system (Table 1) because the results of manual cross-pollination test showed considerably higher frequencies of seed formation (F_c) than those following self-pollination. The former values (expressed as fruits per 100 flowers) were 56.3%, 64.8% and 35.1% for *N. dombeyi*, *N. nitida* and *N. obligua*, respectively, where as frequencies from manual self-pollination were

Table 1. *Results of three types of pollination experiments performed on* N. dombeyi, N. obliqua *and* N. nitida. *Test I: controlled manual self-pollinated flowers; II: controlled manual cross-pollinated flowers; III: bagged flowers observed for spontaneous, autogamous self-pollination.* n: *the number of trees used for each set of observations*

Species	Test	n	No. flowers	No. fruit	No. seeds	Seeds per fruit
N. dombeyi	I	1	119	119	6	0.050
	II	5	1393	1393	785	0.563
	III	4	1887	1887	74	0.039
N. obliqua	I	2	353	353	6	0.016
	II	2	757	757	266	0.351
	III	5	1564	1564	132	0.084
N. nitida	I	4	954	954	21	0.022
	II	4	165	165	107	0.648
	III	1	1061	1061	10	0.009

Table 2. Results of agamospermy test (Test IV) performed on N. dombeyi, N. obliqua *and* N. nitida

Species	No. of flowers	No. of seeds	Seeds per flower
N. dombeyi	576	7	0.012
N. obliqua	2676	36	0.013
N. nitida	177	7	0.005

< 5.0%. All species showed a low percentage of agamospermy; the formation of seeds (per 100 flowers) in the absence of the male gamete reached, at most, only 1.3% (Table 2). However, the possibility exists that some of these seeds, because of the characteristic anemophily of the species, developed as a result of contamination by pollen entering the bagged inflorescence.

Seed formation under natural conditions (F_n) was relatively infrequent, despite the physical proximity of the individuals of a given species (branches may touch each other). In *N. dombeyi* and *N. obliqua*, F_n values of 12.6% and 16.4% (seeds per 100 flowers), respectively, were recorded, whereas in *N. nitida* the F_n value was lower (5.1%). However, all these values of F_n were lower than those (F_c) from experimental cross-pollination tests and indicate that natural fertility in the three species is low. As for the relative fertility index, F_r, *N. obliqua* presents the highest value (0.467%) and *N. nitida* the lowest (0.078%) (Table 3). Comparisons between the indices F_n and F_r (natural and relative fertility) indicate that each of the three species has a deficiency of pollen flow, on the one hand, and a high dependence on the pollinating agent, on the other.

Inter-specific pollination using *N. obliqua* and *N. dombeyi* gave low values of fruiting. Only 2.7% of 298 flowers pollinated with pollen from the other species developed fruit. This low value suggests that the formation of hybrids would be even less frequent in nature, where the pollination conditions are different from those used in the present test.

DISCUSSION

The three species of *Nothofagus* studied have a highly self-incompatible system of reproduction, which corroborates the results obtained by Arroyo and Uslar[3] and Riveros[18] for other tree species found in the temperate Chilean forest. The flowering period for the three *Nothofagus* species investigated lasts between 3 and 3.7 fortnights (one fortnight = 14 days). However, the length of the flowering period in each individual species is relatively short. The longevity of the flowers is relatively long, with an average of 7.7 days ($\pm$ 3.8) between opening and withering. In the Valdivian forest near Osorno, Chile, Riveros[18] registered an average of 3.3 ($\pm$ 1.3) fortnights of flowering for 12 tree species. However, for the total number of species (42 in all, including both trees and herbaceous species), the average flowering time was 4.9 ($\pm$ 3.0) fortnights.

Mature individuals of the *Nothofagus* species do not flower every year and when they do, the abundance of flowers also varies. Stiles[24] observed similar variability in the duration of flowering phases in 42 species over a 4-year period in a humid tropical community in Costa Rica (10° 25' N).

All pollinated flowers of *Nothofagus* develop fruits, but only a low percentage of these contain seeds. Experimental results clearly indicate that these species, which are self-incompatible, have a high level of depedence upon the pollinating agent[18,20]

Table 3. Formation of seed in three Nothofagus *species under natural pollination conditions (Test V to give values of the fertility index,* F_n*) and using manual cross-pollination (Test II to give the index* F_c*). The relative fertility index (*F_n/F_c*) was calculated for each species*

Species	Test	No. flowers	No. seeds	Seeds per flower	Relative Fertility Index (F_n/F_c)
N. dombeyi	V(F_n)	567	72	0.127	0.226
	II (F_c)	1393	785	0.563	
N. obliqua	V(F_n)	469	77	0.164	0.467
	II (F_c)	757	266	0.351	
N. nitida	V(F_n)	879	45	0.051	0.078
	II (F_c)	165	107	0.648	

since in the absence of pollen (agamospermy) percentages of seed formation are extremely low. Therefore, the natural efficiency of pollination (by wind) is primarily related to climatic conditions. During rainy or foggy days at one extreme, there can be little or no dispersal of pollen and, moreover, it has been found that under such conditions, the anthers are flooded and in some cases (as has been shown in *N. nitida*) the pollen starts to germinate inside the anthers. For *N. dombeyi* it was observed that, under such conditions, the surface of the stigma has a low number of pollen grains.

According to Bawa[5] and Ayre and Whelan,[4] the low proportion of seeds compared to fruits in many species is due to factors present during the development of each of these structures. These factors operate before and after fertilisation and can affect the development of the embryo and, hence, the fertility of the species. Among the pre-fertilization factors affecting *Nothofagus* is the aleatoric nature of the pollinating agent (wind), as well as associated climatic factors such as rain, fog and high levels of humidity, all of which decrease the efficiency of anemophilous pollen dispersal. It can also be assumed that there are post-fertilization limitations in cases where the genetic constitution of the fertilizing pollen grain is unsuitable, and because of this there is abortion of the embryo at an early stage of its development. Another post-fertilization factor is the physiological and nutritional inadequacy of the developing fruit to maintain a viable seed, as in the case of polyspermic species. In *N. obliqua*, it is possible that its lower seed-forming level (F_n in Table 3) was due to climatic conditions, in this case, long periods of rain during the flowering period (September) (see Fig. 1 and Fig. 2). These rainy conditions were not so prevalent during the flowering periods of the other two species. This hypothesis is supported by the higher percentages of seeds formed after manual cross-pollination where fertilization would be relatively independent of the climatic conditions.

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