Outcrossing in the tropical forage legume *Centrosema brasilianum* (L.) Benth.[1]

BRIGITTE L. MAASS and ALBA M. TORRES

Centro Internacional de Agricultura Tropical (CIAT), Apdo. Aereo 6713, Cali, Colombia

Introduction

Of those *Centrosema* species with potential for pasture improvement in the tropics, *C. brasilianum* is particularly well adapted to the semiarid tropics in regions with acid and infertile soils (Schultze-Kraft and Belalcazar 1988). According to Miles et al. (1990), not a single measure of outcrossing rate in any *Centrosema* species has been recorded. Thus, little is known about the genetic stability of accessions. Although, evidence of some natural outcrossing is provided by the observation of phenotypic variability within germplasm accessions of *C. brasilianum* produced from field-grown seed (Schultze-Kraft and Belalcazar 1988), in the past, germplasm has been handled as if self-pollinated. The present germplasm collection of *C. brasilianum* at CIAT comprises about 260 accessions. Usually, flowers are purple, violet, lilac or, rarely, white. Coloured flowers were shown to be dominant over white flowers (Maass and Torres 1992). In the present study outcrossing rate of *C. brasilianum* was determined under field conditions, utilizing flower colour as genetic marker.

Material and methods

Experiments were carried out at CIAT headquarters in Palmira, Colombia (lat. 03°31'N; long. 76°20'W; alt. 1000 m.a.s.l.; mean temp. 24°C; rainfall 960 mm). Among 24 germplasm accessions established in two rows for seed multiplication in February 1990, the only two white-flowered accessions (CIAT 5305 and CIAT 15918) consisting of two and five plants, were chosen as females. Distance between individual plants was 50 cm, between two accessions in the row 2 m, and between rows 3 m. All adjacent accessions to the white-flowered ones had flowers of different violet tones, according to R.H.S. Colour Chart. Differences in flower size, leaflet size and shape were also observed. 475 seeds of 62 pods and 516 seeds of 89 pods resulting from open pollination of accessions CIAT 5305 and 15918, respectively, were randomly chosen. Plants were established in the greenhouse in February and March 1991, and transplanted two months later to the field marking progeny of individual pods. Characters recorded were: pod size, number of seeds and unfertilized ovules per pod, flowering time, flower colour and size, leaflet shape and size. Statistical analyses were carried out on an IBM 4361 computer utilizing SAS software.

[1] Poster presented at XIII EUCARPIA Congress, 6-11 July 1992, Angers, France.

Results and discussion

Reproductive System. Anthesis ocurred early in the morning and flowers wilted in the late afternoon. Insect visitation was during the whole day. Some insects were determined as *Epicharis violascens, Eulaema fasciata, Eulaema* sp., and *Apis mellifera* of the family *Apidae*; and *Campsomeris servillii* of the family *Scoliidae* all belonging to the Hymenoptera (personal communication M.P. Hernandez). Most insects perched on the standard from the front side and made stamen and stigma leave the corolla by pressing the keel with the help of the dorsal part of their abdomens. While they were sucking nectar, stamens and stigma could touch the dorsal part of their abdomens and pollen was transferred. Pod development took three to ten weeks from anthesis to mature pods. Comparing pods which gave completely white-flowered (autogamous) offspring with those whose seeds resulted in coloured-flowered (allogamous) plants, the tripping and/or pollination by insects produced a higher seed:ovule ratio with overall means of 0.68 for autogamous and 0.73 for allogamous pods (t-test, $P<0.05$). Other pod and seed characters recorded did not differ significantly according to the mode of fertilization (Table 1).

Table 1. Some characteristics of autogamous and allogamous pods in two open-pollinated *Centrosema brasilianum* accessions at Palmira, Colombia.

Character	Offspring	
	white-flowered (autogamous)[a]	coloured-flowered (allogamous)
Accession CIAT 5305 (n)	30	32
pod length (cm)	10.3a	10.6a
seeds per pod	13.9a	15.2a
unfertilized ovules per pod	4.1a	3.9a
seed:ovule ratio	0.77a	0.80a
Accession CIAT 15918 (n)	31	58
pod length (cm)	14.4a	14.1a
seeds per pod	12.5a	13.4a
unfertilized ovules per pod	8.9a	7.1b
seed:ovule ratio	0.58b	0.65a
Total seed:ovule ratio	0.68b	0.73a

[a] Means within offspring of the same accession and the same character followed by the same letter do not differ significantly from each other by t-test ($P<0.05$).

Escobar and Schultze-Kraft (1990) showed that insect visitation increased seed production

in *C. macrocarpum*. The slightly higher seed:ovule ratio in allogamous pods demonstrates the positive effect pollinating insects also have on seed multiplication in *C. brasilianum*. Nevertheless, seed:ovule ratios of 0.77 and 0.58 obtained in the autogamous offspring of both accessions, respectively, suggest the species also to be self-compatible, which supports earlier results of 20% self-compatibility (Battistin 1983).

Outcrossing Rate. Only 48.4 and 34.8% of pods of both accessions resulted in completely white-flowered progeny, respectively (Table 2). The offspring originating from individual allogamous pods, showed various violet tones from lilac to purple, and even white. Only in 1.6 and 6.7% of the allogamous pods gave progeny uniform for lilac flowers. Overall results in the offspring of open pollination were 148 coloured-flowered plants of 475 in CIAT 5305 and 276 of 516 plants in CIAT 15918, which indicates 31.2 and 53.5% outcrossing rates, respectively (Table 2). These rates deviate from hypothesized 20% outcrossing rate for both accessions (Chi-square-test, $P < 0.05$). Comparing accessions, the rate of allogamy differed significantly (t-test, $P < 0.05$). Other plant characters recorded, showed continuous variation over a wide range (Table 3). Leaflets were longer and wider in progeny of allogamous pods, while flowers of allogamous offspring were larger only in one accession. Flowering time was earlier for allogamous progeny in both accessions.

Table 2. Offspring of two open-pollinated *Centrosema brasilianum* accessions and proportion of outcrossing (percentage (%) in parenthesis) at Palmira, Colombia.

Variable	Accession			
	CIAT 5305		CIAT 15918	
White-flowered (no. pods)	30	(48.4)	31	(34.8)
Mixed coloured-flowered (no. pods)	31	(50.0)	52	(58.4)
Uniformly coloured-flowered (no. pods)	1	(1.6)	6	(6.7)
White-flowered (no. plants)	327	(68.8)	240	(46.5)
Coloured-flowered (no. plants)	148[a]	(31.2)	276[a]	(53.5)

[a] Chi-square were 9.43 ($P < 0.005$) and 223 ($P < 0.005$), respectively.

Frequent insect visitation led to high outcrossing rates in two white-flowered open-pollinated accessions. Narrow violet lines in the center of the standard petal occurring in accession CIAT 15918 may explain its higher rate of allogamy. Probably, these lines serve as nectar guides as in other wild, insect-pollinated species (Penny 1983). In addition, violet is a colour heavily visited by bees and other insects (Kevan and Baker 1983). This also supports the observation in the present study that white-flowered accessions were not preferred by the visiting insects. Epperson and Clegg (1987) also reported that white flowers of *Ipomoea purpurea* were visited less often by the primary

pollinating insects, and they had lower outcrossing rates than coloured flowers when they were in the minority.

In addition, three reasons may even have caused outcrossing rates to be underestimated: first, because of the marker used, cross-pollination among individual plants of the same accession could not be recorded. Second, cross-pollination between both white-flowered accessions could have occurred without being detected. Or, third, if the adjacent lilac-flowered accessions were heterozygous, their recessive gene for white flower could have increased the occurrence of white-flowered offspring. Even so, the allogamy estimated, which is the first record for *C. brasilianum*, suggests that this species should be regarded as partially cross-pollinating. Although, differences in outcrossing can be expected because of genotype and environmental conditions, future seed multiplication of *C. brasilianum* germplasm has to take special precaution to isolate individual accessions so as to conserve their genetic identity. The approach of CIAT's Genetic Resources Unit to multiply germplasm of various species and even genera randomly distributed in a single plot seems to be appropriate.

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