DEVELOPMENT OF A MULTICROSS CULTIVAR OF STYLOSANTHES SPP.

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ABSTRACT
A synthetic population, referred to as multicross, has been developed from an interspecific mixture of *Stylosanthes capitata* and *S.macrocephala* germplasm. This basic population evolved in a farming situation subject to uncontrolled, continuous, heavy grazing. Natural selection and systematically programmed intercrossing among anthracnose-resistant Venezuelan and Brazilian genotypes of *S.capitata* have played major roles in the subsequent development of this heterogeneous population. Eight anthracnose-resistant Venezuelan and two Brazilian accessions of *S.capitata* were introgressed into the basic population. Genetic diversity was further increased by adding seed of four selected accessions of *S. macrocephala* to the synthetic which had already included three accessions from the basic population. The composite was advanced through five generations of bulk seed production to improve its stability, synchronise harvest maturity, and primarily to facilitate natural intercrossing among *S.capitata* genotypes. The multicross population displayed anthracnose-resistance in the presence of susceptible, infected accessions in field trials conducted over the years since its initial synthesis in 1990.

KEYWORDS
Pasture legume, Cerrados, anthracnose-resistance, heterogeneous populations, interspecific mixture

INTRODUCTION
Several species of the genus *Stylosanthes* have potential or actual economic application for legume-based pastures in the Cerrados of Brazil. *S. capitata Vog.* and *S.macrocephala* M. B. Ferr. *et* S. Costa are native species of savanna habitats. The agronomic potential of these species for the Cerrados and Llanos ecosystems has been demonstrated only relatively recently. Both species are well-adapted to low fertility quartz sands. *S.capitata* has a discontinuous pattern of distribution in the eastern tropics of Brazil and Venezuela. *S. capitata* is self-compatible with considerable outcrossing ability. In Colombia, the natural outcrossing rate in *S.capitata* ranged from 12.4% to 47%, with a mean of 19.5% outcrossing recorded (Miles, 1983). *S.macrocephala* is native only to a limited region of central and eastern Brazil where it overlaps with *S.capitata.* Accessions of *S. macrocephala* have proved the most resistant to antracnose, as tested in the Brazilian Cerrados (Thomas and Grof, 1986).

The disease anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc.) limits the potential of *s.capitata* in the Cerrados. Lenné (1988) recorded variation in anthracnose reaction among progenies of native populations of *S.capitata* in Brazil. Although the majority of progenies were susceptible to a virulent race of anthracnose, a small proportion of progenies were highly resistant and immune. According to Lenné (1988) the persistence and stability of native *S. capitata* populations in Brazil is caused by their genetic heterogeneity. The specific objective of the *S.capitata* improvement project at CNPGC is to develop a cultivar using a mixture of productive anthracnose-resistant accessions of *S. capitata* and *S. macrocephala*.

METHODS AND MATERIALS
The basic population of *S. capitata* and *S. macrocephala* was discovered in 1990 near an abandoned forage species agronomic evaluation trial site on an acid (pH 5), infertile, quartz sand (87% sand; 0.7% OM;1.7 ppm P; 14.3 ppm K; 6 me Ca and Mg/100ml; 1.2 me Al/100ml). This type of soil occupies 17%, or 34 million ha of the Brazilian Cerrados, and there are 6 million ha of quartz sands in the state of Mato Grosso do Sul, where these studies have been conducted. *S. capitata* and *S. macrocephala* were the only two of 21 legume accessions which had survived under conditions of uncontrolled, continuous, heavy grazing. All *S. capitata* accessions included in the original agronomic trial were of Brazilian origin.

Some 150 accessions of *S.capitata* and eight accessions of *S. macrocephala* have been evaluated during the course of these studies at CNPGC on an acid oxisol (pH 4.7). A major source of anthracnose-resistance and variability was a range of *S. capitata* accessions of Venezuelan origin. In a preliminary field experiment 17 accessions were evaluated for anthracnose-resistance using susceptible plants in the border rows as an inoculum source. Four Venezuelan accessions and a Brazilian line showed the best tolerance with maximum damage of 6% to leaf and stem area. (Fernandes *et al.* 1993). In three follow-up experiments 133 *S. capitata* accessions were evaluated. Selection criteria were : forage dry matter yield, seed yield and reaction to anthracnose.

Dry matter (DM) and seed yields of advanced generations of multicross and its *S. capitata* components were determined in row plots 50m and 12.5m long in the fourth and fifth generations, respectively. In each case, sample yields were harvested from $1m^2$ of each of eight randomly located sites and DM yields (oven-dried) and seed yields (cleaned seed-in-pod) were recorded. Seven accessions of *S. macrocephala* were evaluated in a separate small plot experiment. The treatments were arranged in 5 m long row plots spaced 1m apart in four randomised blocks.

In order to produce new variability, selected accessions of *S. capitata* were introgressed into the basic population. The selections were sown in four randomised blocks between rows of the original composite. The resulting heterogeneous population was advanced through five generations of bulk seed production to improve the stability of the population, synchronise harvest maturity, and primarily to facilitate natural intercrossing among *S. capitata* genotypes. The sixth generation contains eight Venezuelan accessions of *S. capitata* and two Brazilian accessions. Seed of four selected *S. macrocephala* accessions was added to the final composite. The multicross contains approximately 20% by weight of *S. macrocephala* seed, which includes three *S. macrocephala* accessions from the originally sampled population as well as the four recently selected lines.

Both multicross and its component accessions were tested for their anthracnose response in the glasshouse using virulent local and introduced isolates. The fourth and fifth generation multicross and its components were assessed for disease severity in the field using the rating scale of CIAT. The six-point scale has provision for half-point increments between the major points: 0, no disease; 5, plant dead.

RESULTS AND DISCUSSION
Dry matter yields of multicross and components. Accumulated dry matter yields of the fourth and fifth generations multicross and its components were harvested at the end of the wet season in the pre-flowering stage on three occasions. Although the mean dry matter yield of fourth generation multicross was significantly higher

than the mean value of its components, this yield difference was not maintained in the fifth generation. There was no significant difference between the dry matter yield of the fifth generation multicross and the mean yield of its component accessions (Table 1).

Considerable phenotypical changes were observed in the progenies of Venezuelan accessions. These progenies were produced in alternate rows with Brazilian accessions of the original population and strongly resemble the erect, robust, large leaved Brazilian accessions. Apparently, a high rate of outcrossing has occurred among accessions in the crossing plots. Consequently, the progenies of these Venezuelan accessions are regarded as putative hybrids of the two groups of diverse geographical origin.

Dry matter yields of *S.macrocephala* accessions. An accession from Bahia, GC 1506, produced the highest dry matter yield. GC 1506 outyielded cv. Pioneiro and two other accessions also from Bahia (Table 2).

Seed yields. Yield differences between the fourth generation multicross and its component accessions were not significant. The overall yield of 230.9 kg/ha of seed-in-pod was considered quite satisfactory.

Response to anthracnose. The incidence and severity of anthracnose in the fourth and fifth generation multicross and its component accessions was recorded in early April, at the end of the wet season, when the cumulative effect of anthracnose is normally in evidence. In the fourth and fifth generations, disease incidence or the proportion of plants infected was <1% and <0.5% of leaf area was affected by anthracnose. In field trials there was a slight but non-significant difference between the mean values of multicross and its components. Disease symptoms were observed only on *S.capitata*, whilst *S.macrocephala* accessions, totalling seven in the fifth generation, were wholly symptomless. Artificial inoculation with a virulent isolate of the pathogen caused only light infection on multicross with no significant difference between the composite and seven of its components. Only one component accession showed significantly ($P<0.05$) higher anthracnose severity than seven other components and the multicross.

The multicross population has displayed several attractive forage traits. All components are well-adapted to low fertility acidic soils. They are prolific seeders and readily regenerate from self-sown seed. The multicross population has been observed to be resistant to anthracnose in field trials conducted over the years since its initial synthesis in 1990. This resistance is presumably due to the inherent genetic resistance of component genotypes as well as to inter-and intraspecific genetic heterogeneity. Progenies of widely outcrossed Venezuelan accessions showed heterotic advantage indicated by dry matter production levels similar to the generally more productive Brazilian accessions. Paradoxically, Venezuelan accessions were noted for early flowering and low dry matter production in the past and were discarded in early agronomic evaluation trials (Thomas and Grof, 1986). In spite of gross phenotypic changes in the *S.capitata* population, flowering and harvest maturity dates of the multicross have been successfully synchronised. This was achieved by mid-season (last week of June - first week of July) harvesting of five successive seed crops since 1991.

Table 1
Accumulated wet season yields (DM kgha^{-1}) of two generations of multicross and their components.

	Germplasm					
Harvest	Multicross 4	Components	Lsd (5%)	Multicross 5	Components	Lsd (5%)
1	3057.5	1824.4	1034.7	-	-	-
2	-	-	-	3786.7	3454.5	768.5
3	-	-	-	3593.6	3230.1	923.0

Table 2
Accumulated wet season yields (DM kg/ha) of accessions of *Stylosanthes macrocephala*.

Accession (CNPGC nº)	DM (kg/ha)
1506	5361.0 a*
1582	4395.8 a b
1509	3887.3 a b
1507	3799.8 a b
c.v. Pioneiro (control)	2835.8 b
1508	2792.8 b
1572	2731.3 b
Mean	3686.2

* Means followed by a common letter are not significantly different at the 5% level by DMRT.

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