GIS AND GENETIC DIVERSITY - CASE STUDIES IN *STYLOSANTHES*

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ABSTRACT
We present a new technique for mapping the potential occurrence of wild germplasm based in climate data and show its application to six important *Stylosanthes* species. The method can be used to develop hypotheses as to the distribution for purposes of collection and/or *in situ* conservation. It can also be used to investigate genetic diversity with a species. We present some first results based in isozyme data from *S. guianensis*.

KEYWORDS
characterization, climate, conservation, distribution, forage legume, isozymes, genetic resources, mapping

INTRODUCTION
Stylosanthes is the tropical legume genus most widely used for pasture and soil improvement. Collection of germplasm and its conservation in *ex situ* germplasm banks have thus been of high priority over the last 20 years. Among the species represented by large collections are *S. guianensis* (Aubl.) Sw., *S. scabra* Vog., *S. capitata* Vog., *S. viscosa* (L.) Sw., *S. humilis* H.B.K., and *S. hamata* (L.) Taub., which all have their centres of diversity in tropical and subtropical America. The potential importance of these species new to agriculture was identified during the process of characterization and agronomic evaluation carried out mainly by CIAT and the Australian Commonwealth Scientific and Industrial Research Organization (CSIRO) (Edye, 1987; Schultze-Kraft *et al.*, 1984). Variation has been found in inter- and intraspecific susceptibility of *Stylosanthes* to the fungus *Colletotrichum gloeosporioides*, which causes anthracnose disease, and whose centre of diversity is also in the Americas (Cameron *et al.*, 1996). Geographic information systems (GIS) are being applied to study this genus' genetic diversity (Jones *et al.*, 1996b).

METHODS
Passport data of six sets of accessions of *Stylosanthes* species from the germplasm banks of CIAT and CSIRO were selected. The climate of the collection sites were estimated from the CIAT climate database (Jones, 1991), which were then used as a calibration set to estimate a statistical model of the multidimensional space occupied by the accession sample. Climates vary in two quite distinct ways; the form of the rainfall and temperature functions throughout the year and the timing of the seasonal variation. To remove this timing effect, twelve monthly rainfall totals, mean temperatures and diurnal temperature ranges were standardized to align the seasons by applying a 12 point fourier transform to convert the data to frequencies and amplitudes (Jones, 1987). After retransforming the data to produce a rigid rotation of the original value, a principal component analysis was performed on the calibration set. The first four principal components were used to map the climate characteristics throughout the continent. The probability was calculated that each 10 minute pixel in the climate files could have drawn from the climate distribution defined by the calibration set. For details of this analysis see Jones *et al.* (1996a). The climate probability maps generated have been validated by examples of *Phaseolus vulgaris* L. (Jones *et al.*, 1996a) and *S. hamata* (Jones *et al.*, 1996b).

RESULTS AND DISCUSSION
Climate probability maps: As many *Stylosanthes* species are pioneers that occur in disturbed habitats, climate and the fact of habitat disturbance may be more important for their natural occurrence than edaphic conditions. In fact, the probability maps generated for the occurrence of *S. guianensis* and *S. scabra* match quite well the natural distribution described by Williams *et al.* (1984).

Species consist of different populations: *S. guianensis* has a wide natural distribution in tropical America; it occurs naturally from Mexico to Argentina (Williams *et al.*, 1984), and has been widely collected. Considerable collecting has been done in Colombia, but these collection points do not all fall in high probability areas, whereas most of the points in Brasil do. Are there in fact two or more populations of the species with different climatic requirements?

Natural variation of *S. guianensis* has been described by Burt (1984), who specified the occurrence of different botanical varieties of *S. guianensis*. Except var. *guianensis*, which is sympatric with all other varieties over part of their range, most of these relatively heterogeneous varieties are confined to certain environmental conditions (Burt, 1984). This is supported by the example of *S. guianensis* var. *pauciflora* accessions that were singled out in a climate group by this study. When subsequently only var. *pauciflora* accessions were used to predict occurrence, the probability map generated was very similar to that of *S. capitata*. This points at the similar environmental conditions of natural occurrence of these two taxons, as described in the biogeography by Costa and Schultze-Kraft (1993).

Fitting a single multivariate normal distribution to the calibration set when the set is a composite population may cause spurious regions of high probability where no accessions exist, or accession sites in areas of low probability. To investigate this, the calibration set climates were clustered to see if they fall into clearly separate groups. The first four principal components were used to construct a similarity matrix for 499 accessions. The hierarchic cluster procedure of Genstat (Genstat 5 Committee, 1987) was used with the average link method to produce 6 distinct clusters at 93% similarity or 10 clusters at 95% similarity. The question is now whether these climate groups are really different from an ecotype point of view.

Climate and genetic groups: Fingerprinting of almost 600 *S. guianensis* accessions by isozymes and seed protein by polyacrilamide gel electrophoresis (PAGE) has been carried out at CIAT (C.H. Ocampo and B.L. Maass, unpublished data). Data on three isozymes (α- and ß-esterase, and diaphorase) are being analysed using similarity indices to identify the structure of genetic diversity in the species.

We used these data with a genetic algorithm to identify specific isozyme band combinations that were found significantly more often in some climate groups of this study than in others (Table 1). This indicates that isozyme groups match some of climate groups of *S. guianensis* determined by the cluster analysis.

CONCLUSIONS
This mapping technique seems to hold considerable promise in identifying key areas for collecting or for *in situ* conservation. There are indications that the climate grouping might be identifying genetically distinct populations. Further work with isozyme data and genetic markers, such as RFLP or AFLP, should clarify the situation,

particularly for the smaller outlying climate classes. If it can identify and map diverse populations it will be a valuable aid to the conservation of natural biodiversity.

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Table 1
Frequency of combinations of α-esterase bands 8 and 10 in selected climate groups.

Climate	Number of accessions in bands				
Group	Neither	Only 8	Only 10	Both	Total
1	3	23	2	6	34
2	1	3	0	10	14
5	20	37	14	81	152
6	9	63	6	70	147
7	5	65	2	16	88