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Stomatal area as an anatomical criterion for the determination of chromosome number in the *Eragrostis curvula* complex

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ABSTRACT

Twenty stomatal areas of each of 55 *Eragrostis curvula* (Schrad.) Nees plants were determined. An increase in polyploid level is shown to be moderately correlated with an increase in stomatal area. However, the extent of overlap in stomatal areas between different polyploid levels is too great to use this character for the determination of the polyploid level above the diploid level. All diploid *E. curvula* plants have an area of less than 280 μ^2, whereas the tetraploid plants have areas greater than 320 μ^2. It is therefore possible to identify diploid *E. curvula* plants on the basis of their stomatal area.

INTRODUCTION

A correlation exists between cell size and the ploidy level of the cell as polyploid cells are usually larger than the cells of their diploid counterparts (Stebbins, 1950; Allard, 1960). This fact has led cytogeneticists to search for suitable cells which could be used to determine chromosome number accurately without a study of the chromosomes. Blakeslee & Avery (1937) used pollen size to distinguish between different polyploid levels in *Datura stramonium*. De Wet (1954) used stomatal length as a criterion for a similar study in the genus *Danthonia*. These studies have not always given the desired results. The most successful results have always been obtained from induced polyploidy where the chromosome number of a single plant has been altered by the use of chemicals such as colchicine (Blakeslee & Avery, 1937; Biswas & Battacharyya, 1976).

The aim of the current investigation was to determine to what extent this method could be used for predicting chromosome numbers in the *Eragrostis curvula* (Schrad.) Nees complex.

MATERIAL AND METHODS

Plants of the *E. curvula* complex were collected in the field throughout South Africa and were transplanted in the Pretoria National Botanical Garden (Vorster, 1978). The chromosome numbers of these cultivated plants were determined by Vorster (1978).

Leaves of 55 of these plants (Table 1) were sampled during March 1981. After 24 hours fixation in F.A.A. fixative, epidermal scrapes of the abaxial epidermis were prepared following the method of Metcalfe (1960) with slight modifications. Safranin and Methylene Blue were used for staining the epidermal scrapes.

Twenty stomata from each plant were measured. Measurements of the stomatal area were made from photomicrographs. A Kontron MOP–AMO2 image analyser was used for determining the stomatal area.

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RESULTS AND DISCUSSION

The average stomatal area of each plant is shown in Table 1. These stomatal areas were plotted graphically (Fig. 1). A correlation factor of 0,6774 was found to exist between chromosome number and stomatal area for each plant. This correlation is represented graphically in Fig. 1.

From Table 2 it is clear that the only homogeneous group in this study was the diploid (2n = 20) group. This group showed a standard deviation of 21,79. From Fig. 1 it is evident that the diploid group is the only group with almost no overlap with any other group. The only overlapping that occurred was between the diploid group and one pentaploid plant (*Vorster* 334). During a cytogenetical re-examination that followed this anatomical study, it was found that this plant was indeed a diploid and not a pentaploid. As the Vorster collection was made and transplanted before 1978, the possibility exists that this plant could have reverted to a diploid plant or that the plant was wrongly numbered.

A meiotic analysis of the unpaired chromosomes of *Vorster* 334, showed an average of 5,75 unpaired chromosomes per cell (Vorster, 1978). Unfortunately no embryosac analysis was done on this plant during the present study. It is clear, therefore, that this is a very unstable plant and, although the degree of apomictic reproduction is not known, the chromosome number of this plant could have been altered in various ways. From these results it appears that it can be predicted with a high degree of confidence that any *E. curvula* plant with a stomatal area of less than 280 μ^2 will be diploid.

An unexpected high standard deviation was encountered in the tetraploid (2n=40) group. This results from the fact that the tetraploid sample can be divided into two groups and a big gap of more than 50 μ^2 exists in the area measurements between *Vorster* 422 and *Vorster* 989. The first group corresponds with the regression line drawn in Fig. 1. The second group (*Vorster* 989, 750, 303, 797 and 210) have very large stomatal areas. The average stomatal area (502,37 μ^2) of the second group is almost as large as the average stomatal area (510,41 μ^2) of the octoploid (2n=80) group. It is possible that this might be a case of divergent evolution occurring, or that two different sources exist from which these two different groups have evolved. It

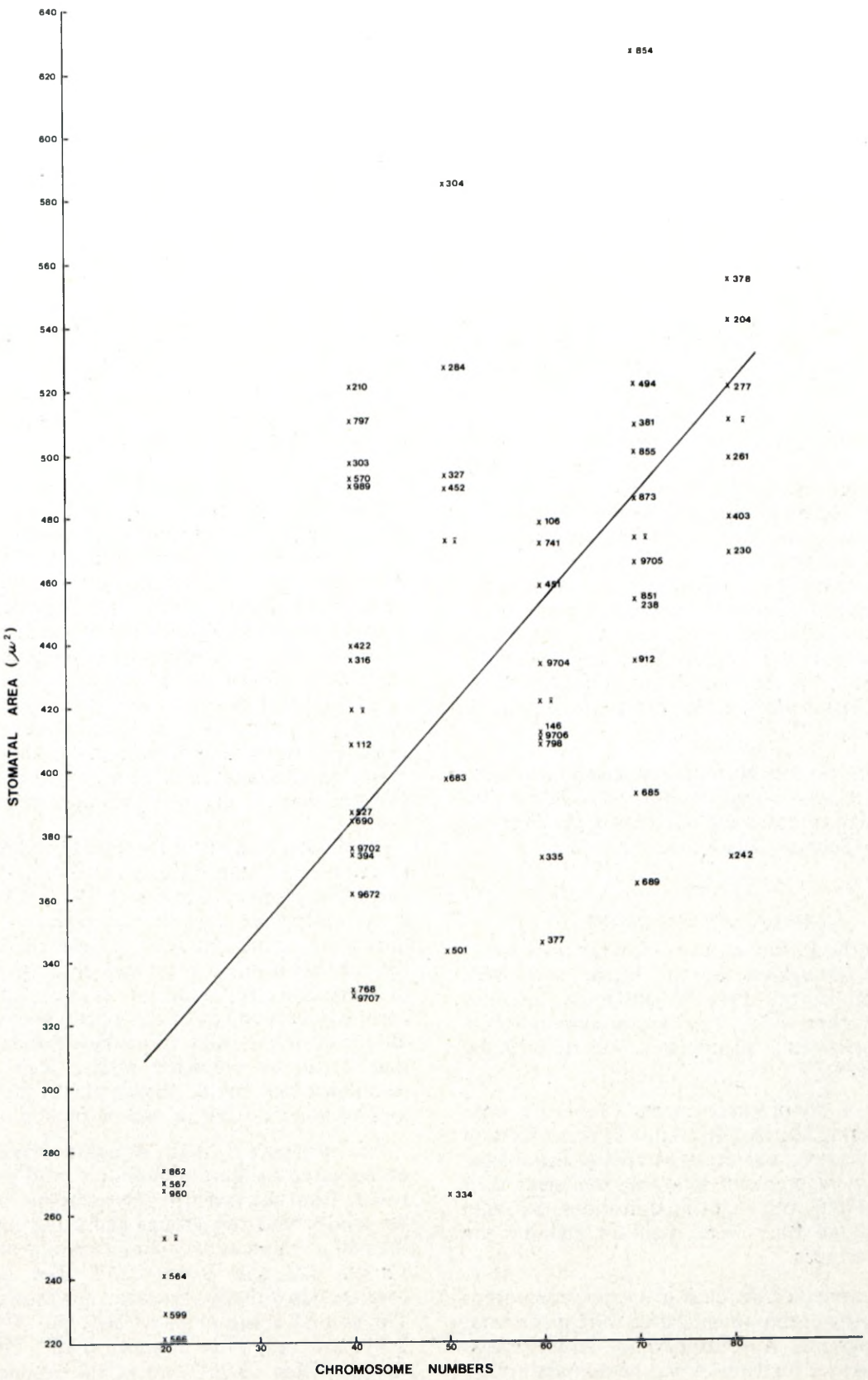

FIG. 1.—Correlation between chromosome numbers and stomatal areas in selected representatives of the *Eragrostis curvula* complex.

TABLE 1.—List of plants used for this study

Locality	Collectors no. (Vorster)	Chromosome no. (2n=)	Stomatal area (μ^2)
2217CA	422	40	439,17
2523BD	564	20	241,64
2524CA	566	20	221,07
2524CA	567	20	270,02
2530BD	683	50	397,41
2530DB	685	70	392,78
2531DD	960	20	268,12
2623BB	559	20	229,61
2625CD	146	60	410,19
2628DD	451	60	458,84
2629CA	452	50	489,76
2630AA	690	40	384,26
2630AB	689	70	364,51
2725BB	106	60	478,98
2728CD	277	80	520,17
2730BB	261	80	498,14
2730DD	204	80	541,82
2823DA	527	40	387,24
2824BD	112	40	408,86
2825BC	501	50	343,49
2826CB	494	70	521,86
2828DA	284	50	526,42
2829CB	238	70	452,31
2829DB	242	80(40)*	372,79
2830DB	210	40	521,1
2924CC	750	40	492,48
2926BB	304	50	585,65
2927AA	303	40	497,35
2929CC	989	40	490,1
2929DA	230	80	468,97
3023DB	768	40	330,64
3025BD	316	40	435,31
3025CB	741	60	471,82
3027AA	327	50	493,11
3027CB	334	50(20)*	266,37
3027DA	335	60	372,94
3028BD	394	40	374,55
3029CD	398	60	408,42
3127AD	381	70	509,54
3128BD	403	80	479,32
3128CA	378	80	554,04
3128CD	377	60	345,66
3224BA	797	40	510,8
3321BD	912	70	434,1
3324BB	862	20	274,34
3324DD	851	70	453,57
3324DD	854	70	627,64
3325CC	855	70	500,82
3418BB	873	70	485,52
Transkei	9702**	40	375,94
,,	9703**	40	329,91
,,	9704**	60	433,36
,,	9705**	70	465,67
,,	9706**	60	410,09
,,	9672**	40	361,97

* These plants were re-examined cytogenetically at a later stage and it was found that *Vorster* 242 had 40 chromosomes and *Vorster* 334 had 20 chromosomes (see text)

** Collected by De Winter

should prove interesting to have a closer look at morphological differences between these two tetraploid groups. It may also be significant to note that all plants in this 'abnormal' group were originally collected in mountainous areas.

A large degree of variation (standard deviation = 87,99) exists in the pentaploid (2n=50) group. Because the pentaploid group tends to be genetically unstable (Vorster, 1978), it will be necessary to undertake a cytogenetical study on this group again, to determine whether chromosome instability is responsible for this excessive variation.

The abnormally large stomatal areas found in some of the tetraploid and pentaploid plants are responsible for the fact that the hexaploid group has a relative lower average stomatal area than both above-mentioned groups. The hexaploid (2n=60) group is relatively homogeneous with regard to the stomatal area (standard deviation = 44,59) and this fact is also reflected in their relative normal meiotic behaviour where an average of 1,64 unpaired chromosomes per cell were found (Vorster, 1978).

With the exception of *Vorster* 854, the heptaploid (2n=70) group shows relatively little variation. This fact corresponds with the observation that heptaploid plants in this study have an average of 4,52 unpaired chromosomes for each meiotic cell (Vorster, 1978). Pentaploid plants showed an average of 5,71 unpaired chromosomes per cell. The heptaploid plants are, therefore, genetically more stable than the tetraploid plants. This fact is clearly reflected in this study by the more limited variation of stomatal areas of the heptaploid plants in comparison with the pentaploid plants.

In the octoploid (2n=80) group only one deviation from the normal acceptable distribution was found. This plant (*Vorster* 242) was studied cytogenetically and found to be tetraploid (2n=40). This might be due to secondary haploidization or a wrong number might have been used somewhere during different collecting and transplanting operations.

CONCLUSIONS

From the results of this study, it is clear that an increase in stomatal area is correlated with an increase in polyploid level (Fig. 2). However, the extent of overlapping of stomatal areas between different polyploid levels of *E. curvula* is too great for the prediction of the polyploid level by this means. The only exception in this regard is the diploid group. All diploid plants had areas of less than 280 μ^2, whereas the other polyploid levels had values above 320 μ^2.

The only published example where cell size has been used directly to determine polyploid level, is with induced polyploidy (Blakeslee & Avery, 1937; Biswas & Battacharyya, 1976). The present results indicate, therefore, that it might be possible to use cell size as a criterion for determining chromosome numbers, but only when working with very closely related plants exhibiting little morphological divergence and originating from similar environments. The De Winter collection included in this study, was sampled from a small area and consists of morphologically almost identical plants but having different chromosome numbers. This group shows a correlation coefficient of 0,953. It might therefore be possible to use this method successfully if the following selection criteria are strictly applied:

1) Collect only morphologically similar plants
2) Collect only in one geographical area

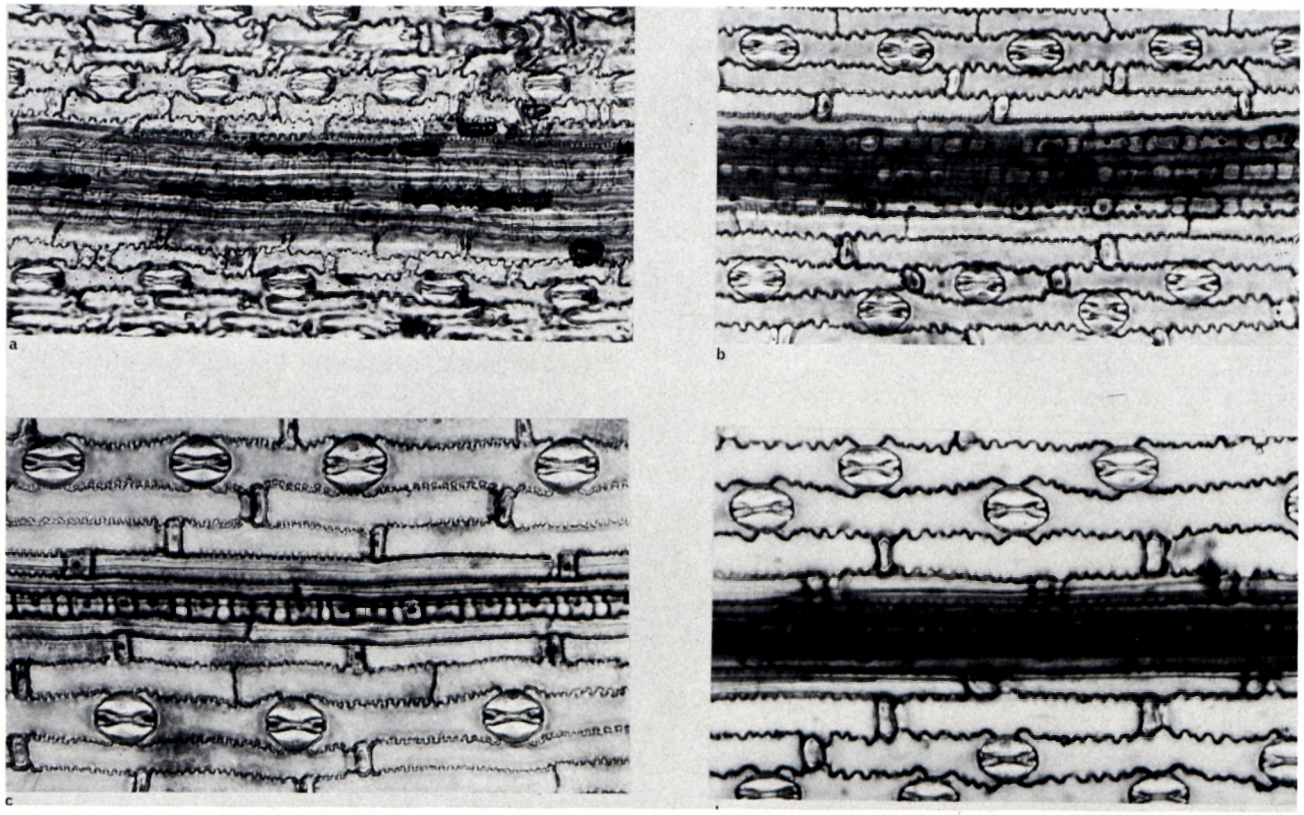

FIG. 2.—Photomicrographs of stomatal areas of *Eragrostis curvula* (×1860) a) 2n=20 b) 2n=40 c) 2n=60 d) 2n=80.

TABLE 2. — Average stomatal area (μ^2) of each plant for the polyploid levels of the *Eragrostis curcula* complex

2 n	20	40	50	60	70	80
Average stomatal area of each plant (micron^2)	221,07	329,91		345,66	364,51	
	229,61	330,64	343,49	372,94	392,78	468,97
	241,64	361,97	397,41	408,42	434,1	479,32
	266,37	372,79				
	268,12	374,55	489,76	410,09	452,31	498,14
	270,02	375,94	493,11	410,19	453,57	520,17
	274,34	384,26	526,42	433,36	465,67	541,82
		387,24	585,65	458,84	485,52	554,04
		408,86		471,82	500,82	
		435,31		478,98	509,54	
		439,17			521,86	
		490,1			627,64	
		492,48				
		497,35				
		510,8				
		521,1				
$\bar{x}$	253,02	419,53	472,64	421,14	473,48	510,41
S.D.	21,79	65,01	87,99	44,59	70,1	34,17

UITTREKSEL

Twintig huidmondjie oppervlaktes per plant van 55 Eragrostis curvula *plante is bestudeer. 'n Verhoging in poliploïede vlak is noemenswaardig gekorreleer met 'n toename in huidmondjie oppervlakte. Die mate van oorvleueling in waardes vir huidmondjie oppervlaktes is egter te groot om hierdie eienskap te gebruik vir die bepaling van die poliploïede vlak bo diploïed vlak. Alle diploïede* E. curvula *plante het 'n oppervlakte van minder as 280 μ^2 gehad. Dit is dus moontlik om diploïede plante te identifiseer op grond van hulle huidmondjie oppervlaktes.*

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Destruction of the *Phoenix/Hibiscus* and *Barringtonia racemosa* Communities at Richards Bay, Natal, South Africa

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ABSTRACT

The destruction of the *Phoenix/Hibiscus* and *Barringtonia racemosa* Communities described by Venter in 1972 on the southern shores of Richards Bay is reported. The cause was the artificial opening of a new mouth about 5,5 km south of the original mouth, which increased tidal range and salinity. These swamp communities occupied a narrow band about 6 ha in area behind the *Bruguiera gymnorrhiza* Community. An estimated 95% of the communities was affected and only on the landward border were some isolated remnants of species such as *Acrostichum aureum, Hibiscus tiliaceus* and *Phoenix reclinata* detected. Young stands of *Phragmites australis*, seedlings of *Bruguiera gymnorrhiza* and *Avicennia marina* and epipelic algae are recolonizing the affected area.

INTRODUCTION

The construction of Richards Bay Harbour resulted in the Richards Bay Lagoon (28°49' S and 32°05' E) being divided into a harbour zone and a natural sanctuary by the construction of a berm wall and the opening of a new mouth for the nature reserve during 1975. Begg (1978) reports on the many abiotic and biotic changes occurring after the habilition of the harbour. This work focuses on one of these changes affecting vegetation.

Tree mortality along the Zululand Coast has been described previously. Breen & Hill (1969) reported on a mass mortality of mangroves of the Kosi Estuary (Natal) in 1965 following natural closure of the mouth for five months. Bruton & Appleton (1975, in Begg, 1978) described the death of a mangrove swamp at Mgobezeleni Lake (near Sodwana Bay) following the construction of a bridge that impeded tidal flow and caused the swamps to be flooded.

During studies on Natal estuaries (Ward, MS) and on the conservation priorities between Richards Bay and Mlalazi Mouth (Weisser, MS) the vegetation of the southern shores was remapped, sampled with eight relevés and compared with findings of Venter (1972), who studied the vegetation before the harbour development.

FIG. 1.—Remnants of *Phoenix/Hibiscus* and *Barringtonia racemosa* Communities on southern shore of Richards Bay destroyed by increased tidal range and salinity following opening of new mouth at Richards Bay. In background landward side of *Bruguiera gymnorrhiza* Zone.

RESULTS

During mapping, the almost complete destruction of 6 ha of the *Phoenix/Hibiscus* and *Barringtonia racemosa* Swamp Communities (*sensu* Venter, 1972) in the Richards Bay Sanctuary Area was observed. Dead trees of up to 12 m tall were found and most of the trunks of the bigger trees were still upright (Fig. 1). At high spring tide water completely flooded the dead forest.

The opening of the new mouth in 1975 about 100 m

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TABLE 1.—Eight relevés of the *Bruguiera gymnorrhiza* and *Phoenix/Hibiscus* Communities in the study area. The values correspond to Braun-Blanquet cover-abundance estimates (Braun-Blanquet 1964). The plots 25 m^2, were approximately level, with muddy substrate and usually with stagnant water (dated 1980.11.20–24)

Relevé No.	429	428	427	441	442	443	446	445
Total cover %	80	50	60	10	6	15	70	7
Bruguiera gymnorrhiza	5	4	4	1				1
Avicennia marina			+		r			
Phragmites australis				2	2	2	4	+
Acrostichum aureum							2	1
Phoenix reclinata							1	1
Barringtonia racemosa								+

away from the *Phoenix/Hibiscus* and *Barringtonia racemosa* Communities clearly resulted in the death of these communities. It caused the pre-harbour development tidal range of 0,35 m to increase to 1,63 m in April 1977 (Begg, 1978) and the water salinity to increase, consequently the death of the plants may be attributed to a combination of these two factors. A few, isolated survivors and resprouting plants of *Phoenix reclinata* and *Acrostichum aureum* were found to occur near the southern corner of the bay, on slightly higher parts farthest from the sea (Table 1, relevé 445). A plant that has lately increased in the affected area is *Phragmites australis* which, in places, forms dense stands between the dead trunks and debris (Fig. 2 and Table 1, Relevés 441, 442, 443, 446). *Bruguiera gymnorrhiza* seedlings and *Avicennia marina* saplings were observed to be colonizing the area (Table 1, Relevés 441 & 445). This suggests a landward extension of the Mangrove Community. Open parts of the destroyed zone were colonized by epipelic (= growing on mud) algae.

Venter (1972) described the *Phoenix/Hibiscus* Community prior to its destruction as occupying a small zone immediately behind the *Bruguiera gymnorrhiza* Community at the south-eastern shore of Richards Bay. Landward it bordered on the *Barringtonia racemosa* Community (see Fig. 3 in Venter 1972) and on primary or secondary Dune Forests. It was a dense thicket formed mainly by the palm *Phoenix reclinata, Hibiscus tiliaceus* and the fern *Acrostichum aureum.* Other species present were *Rapanea melanophloeos, Ficus trichopoda, F. capensis, Syzygium cordatum, Bruguiera gymnorrhiza* and *Avicennia marina.* The last two species were in poor condition and were probably remains of the neighbouring *Bruguiera gymnorrhiza* Community which, in the course of time, was apparently displaced by the *Phoenix/Hibiscus* Community.

Climbers were *Mikania cordata, Ipomoea cairica, I. congesta, Dioscorea sylvatica* and *Rhus nebulosa,* the first two species being particularly common. Field layer species recorded were *Acrostichum aureum, Nidorella auriculata, Phragmites australis, Typha latifolia* subsp. *capensis, Cyperus alternifolius, Scadoxus magnificus* and *Blumea lacera.*

Many *Phoenix reclinata* trees were cut by Zulu people living nearby to tap the sugar-rich sap. The damaged plants coppice freely from the base to form dense thickets.

The *Barringtonia racemosa* Community was quantitatively studied by Venter (1972), who took samples at three places nearby: at Mzingazi Lake, west

FIG. 2.—View across part of destroyed zone. In this area *Phragmites australis* and *Bruguiera gymnorrhiza* adapted well to changed conditions and are now increasing.