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16. Summary/Notes

Plant and soil samples were collected from Vazante and Morro Agudo, MG. Chemical and anatomical analyses were made, and the plants and soils from mineralyzed and unmineralyzed areas were compared. Differences of plant morphology and chemical contents of soils and plants were noted.

17. Remarks

CHAPTER I

INTRODUCTION

In recent years the studies of using remote sensing technique to detect the geobotanical anomaly for mineral exploration have caused considerable interest. These techniques are based on the assumption that the root systems of vegetation act as a powerful sampling mechanism which may penetrate heavy overburden to bedrock and meet geochemical anomalous zones. If the metal ion in the soil solution is in an available form it may enter the plant's circulatory system through root tips by cation exchange reaction (Fig. 1). However, for plant nutrition, a minor amount of all the metal ions may be necessary for plants' normal growth. An excess amount in nutritional supply may be subtle and cause physiological or morphological changes of the plants (Table 1). The toxic effect of the metals accumulated in the surface soil through biogeochemical cycle (Fig.2) may disturbing the normal distribution pattern in the region of mineralization. This is because metal toxicity tolerance for each plant variates. Plants which can tolerate high metal concentration in the supporting soil may grouped into three categories:
1. Plants which grow on any soil and absorb a large amount of metallic ions when rooted in mineralized zone are called accumulator plants; an ideal accumulator plant is one that is widely distributed and in which the plant tissue contains the characteristic element proportional to the amount of minerals in the soil and in quantities easily determined analytically. 2.An indicator plant is one that grows only on soil directly over deposits; it may or may not have any of the characteristic elements in its tissue.

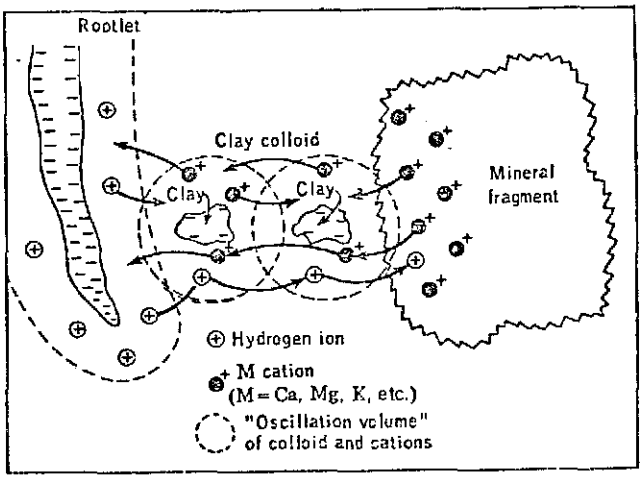


Fig. 1 - Diagram illustrating cation exchange reactions near root tips.

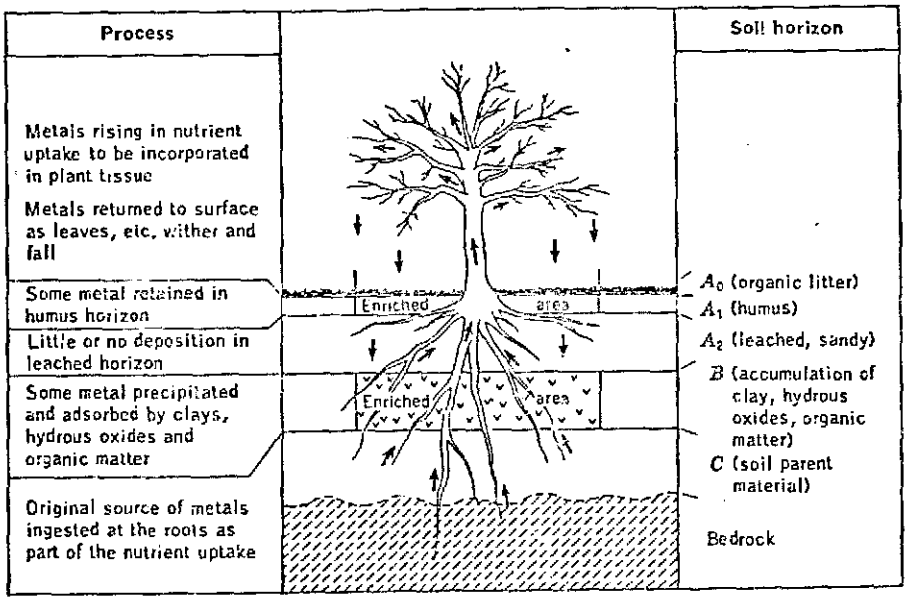


Fig. 2 - The biogeochemical cycle.

TABLE 1

PHYSIOLOGICAL AND MORPHOLOGICAL CHANGES IN PLANTS DUE TO METAL TOXICITIES

ELEMENT	EFFECT
Aluminum	Stubby roots, leaf scorch, mottling
Boron	Dark foliage; marginal scorch of older leaves at high concentrations; stunted, deformed, shortened internodes; creeping forms; heavy pubescence; increased gall production
Chromium	Yellow leaves with green veins
Cobalt	White dead patches on leaves
Copper	Dead patches on lower leaves from tips; purple stems, chlorotic leaves with green veins, stunted roots, creeping sterile forms in some species
Iron	Stunted tops, thickned roots; cell division disturbed in algae, resulting cells greatly enlarged
Manganese	Chlorotic leaves, stem and petiole lesions, curling and dead areas on leaf margins, distortion of laminae
Molybdenum	Stunting, yellow-orange coloration
Nickel	White dead patches on leaves, apetalous sterile forms
Uranium	Abnormal number of chromosomes in nuclei; unusually shaped fruits; sterile apetalous forms, stalked leaf rosette
Zinc	Chlorotic leaves with green veins, white dwarfed forms; dead areas on leaf tips; roots stunted

Source: Cannon

3. Convertor plants are those not only capable of converting insoluable metal compounds into an available form but also of returning them to the surface soil. Plants of these three categories are useful for mineral exploration.

With increasing world population, new mineral resources are badly needed. Remote Sensing of "metal stressed plants" or the pattern of vegetation distribution provides geochemical information concerning the location of the buried ore without actually visiting the area on the ground; this is an important consideration for areas of difficult access such as Amazon and rest of the prospecting area of the world. The geobotanical methods have great advantages over other geochemical methods of prospecting in which the results of the survey are immediately available without further treatment of the samples. The toxic effects of metals on vegetation, have long been known, the earliest practical observation being in the fifteenth century (NASA). The systematic use of botanical methods of prospecting began in the fifties in U.S.S.R., French and U.S.A.. More recently experiments investigating the changes in reflectance of metal stressed vegetation have been contributed to the series of international symposiums on remote sensing of the environment. Howard et al. carried out measurements of the visible and near infrared spectra of foliage taken from Pinus ponderosa growing in a copper-rich area. They observed an approximately 25% greater reflectance at 800 µm from foliage of trees from anomalous sites, than from the foliage of trees from background sites. However, these results were not observed in the laboratory.

This seems to suggest that the mineral uptake by the foliage primarily influences the foliage density and foliage pattern on the trees and does not affect the internal structure of the needles sufficiently to change their spectral reflectance characteristic. Yost and Wenderoth made a two-year experiment on a group of red spruce (Picea rubens) and balsm fir (Abies balsamea) which grew upon and adjacent to a copper molybdenum anomaly at Catheart Mountain, Maine, U.S.A.. They analyzed the reflectance spectra at 26 wavelengths between 380 and 1250 μm, and found a high degree of correlation between soil geochemistry and the percent of directional reflectance of the trees. These results confirmed that significant spectral differences existed between mineralized and non-mineralized tree groups. Until now, there are several discoveries as a direct result of remote sensing and geochemical investigation of vegetation conditions; the most interesting one is the Kalengwa copper deposit in Zambia (Ellis and McGregor).

The objective of this project is to evaluate the remote sensing techniques in the recognition of plant communities and of physiographic and geological features to assist mineral exploration.

For data interpretation we have to bear in mind that the minerals in the soil and those in the plant present a very complicate problem, because the zinc available to the plant is dependent upon the following soil factors: PH, phosphorous level, organic matter and clay contents. Numerous studies have shown that the zinc availability to the plants decreases as zinc forms precipitates (presumably of $Zn(OH)_2$ and $CaZn(OH)_4$) of

low solubility with increased soil PH (Clark and Graham), . Shaw and Dean observed at soil PH 6.5 or less, there were few occurrences of zinc deficiency if the soil had 0.5 ppm zinc or more; at soil PH above 6.5 zinc deficiency occured even with 2.7 ppm zinc. Zinc also has an antagonistic effect on phosphor and is tenaciously absorbed by organic matter and clays.

In the case of zinc toxicity, the symptoms of chlorosis or stunting occur. Zinc is not a component of chlorophyll but it has antagonistic effects with Fe ion which is an important co-enzyme for chlorophyll synthesis. The chlorotic leaves combine with the zinc-induced damaged root system causing stunting of the plant. For the above reasons, the soil analysis has to include PH and organic matter determination. For plants, in addition to observation in the field, the anatomical analysis has to be carried out to see if zinc caused anatomical deformation of the plant.

A Kodak 2443 infrared aerofilm will be used for photographing the Vazante and Morro Agudo areas. This infrared film is superior to color and panchromatic film because 750 ∿ 900 μm is an accurate guide to plant vigor; The thicker and heavier the vegetation canopy (higher leaf area index; LAI) the greater the infrared reflectance. So, even through a plant may still have enough leaves on it to appear green on a color aerial photograph, an infrared aerial photograph might show a lower tone than usual because of a reduction in the number of leaves on the metal stressed plant. A flight over the areas is planned to be carried out in Oct. 1975. Here, the preliminary report is emphasized on geobotanical and geochemical investigations from Nov. 1974 to June 1975.

CHAPTER II

SAMPLE PREPARATION

2.1 - FOR ANATOMICAL ANALYSIS

Leaves which were fixed in FAA (formalin-acid-alcohol) solution were cut in small pieces approximately one centimeter on either side of the mid-rib, dehydrated with a tertiary butanol series, embedded in paraffin, stained with Delafield's Haematoxylin, and transversally microtomed at 10 μ thickness. Photographs were obtained with an American Optics Photomicroscope.

2.2 - FOR ATOMIC ABSORPTION SPECTROPHOTOMETER

The 50 mg soil samples were oven-dried, sieved to -80 mesh and digested with acid solutions (1 ml aqua regia and 3 ml HF) and then made up to 100 ml with distilled water for element determination.

For plant samples 1 g, oven-dried leaf samples were dissolved in 50 ml of a mixture of sulfuric, nitric and hydrochloric acids (2:2:1). This mixed solution was warmed on a 80°C hot plate for 1 hour and then added up to 100 ml with distilled water. This 100 ml solution was further diluted (1:10) for element determination.

2.3 - SOIL PH MEASUREMENT IN WATER

A weight of 20 g of soil was added to a 50 ml beaker with 20 ml distilled water, stirring the suspension several times during the next 10 mins. Let the suspension stood for 30 mins. to settle down. PH was measured by a Hath model 2075 PH meter with the eletrode immersed into the semi-clear supernatant solution.

CHAPTER III

RESULTS

Owing to the limited time and personnel, only one or two soil or plant samples were collected from each area. Nevertheless, these results gave us good indications for future field work.

3.1 - OF ANATOMICAL ANALYSIS

Plant's scientific names could not be identified because they were in vegetative state last Nov. when collected. A numeric number was assigned to each different plant for distinguishment (see photos). The location map of the areas which have been visited is annexed to this report.

Plant 1 (Gomphrena sp.). Owing to the pubescent leaf of this plant, the microtome sectioning was not successful, but the free-hand sectioning gave good cuts for microphotographs (Fig.4). This plant has dorsiventral leaf with heavy multicellular hairs which were branched in dendroid manner on both sides of the leaf surface. Extraordinary large calcium oxalate druses were found in the mesophyll. No comparison could be made on Gomphrena from mineralized and unmineralized area, because area 1 was the only area covered by this plant. Some limited studies have been done on Gomphrena sp. . Handro showed that the number of druses were increased when Gomphrena prostrate Mart. was treated with additional amount of $CaCO_3$,

and this plant was very tolerant to the concentration of mineral nutrients.

Plant 2. This is a typical cerrado plant with multiseriate palisade tissue and no clearly defined sponge cells. The outermost layer was the longest while the innermost (closer to the abaxial side) was the shortest, and also the less palisade-like. The upper leaf surface (Adaxial side) with hypodermis had no stomata and covered only by ordinary hairs. However on the lower side (abaxial side) stoma were easily located between papillae. Both ordinary and gradular hairs were found. The plant hair types and the mesophyll structure make us believe that plant 2 is a species of the genus Bauhinia.

Plants which were collected from mineralized (area 1, 3, 5, 7, 10 and 11) and unmineralized (area 8) area were compared anatomically. The differences that were noted are:

1. The mesophyll structure of the leaves from mineralized areas was more loosely arranged, and had more inter-cellular spaces than the leaves from unmineralized area. Whether this will cause any difference on relfectance in situ has to be checked by future field work.
2. The leaves from mineralized areas had more fibers in their bundle sheaths and were more fragile after alcohol dehydration than the leaves from unmineralized area. Thus, after microtoming the outermost cells were not kept intact. This is probably because mineralized plants are physiologically "under stress" thus making the leaves more sensitive to chemical process.

3. The plants from mineralized areas seemed to have smaller leaves. Galls were also found on the twigs of these plants. Whether these were sampling errors, or an effect of the high mineral content of the soil (which made the plant less resistant to insect attacking?) have to be further studied.
4. The palisade perenchyma of the leaf from unmineralized area had more chloroplasts than that of the anamolous areas by microscope observation. To verify this, a chlorophyll determination and the reflectance measurement at the chlorophyll absorption bands (0.45 to 0.65 mμ) have to be done.

An assumption of the above observation is that the comparison is made on the leaves of the same age and under same environmental conditions. Thus, different development and solar radiation received will not affect the mesophyll structure, the fiber, and the chlorophyll content in the leaves.

Plant 3. This plant had very compact mesophyll with one layer of palisade cells and 2 or 3 layers of short, round, and palisade-like cells. Little intercellular space was observed.

Plant 4. This plant had one layer palisade cells and several layers of sponge cells. Intercellular spaces could be easily seen.

Plant 5. A typical dorsiventral leaf with clearly different-

iated palisade and sponge cells. The chlorosis of this plant had been noted in the field, but whether this was caused by anomalous zinc content in the soil has to be proved.

3.2 - OF CHEMICAL ANALYSIS

As the normal soil contains zinc from 10 to 300 ppm, we put the threshold at 300 ppm. Areas with zinc content above 300 ppm is called a mineralized area, and below 300 ppm is called a unmineralized area. The results of soil and plant chemical analysis are listed in table 1. Unfortunately the complete soil analysis from ESALQ (Escola Superior de Agricultura Luiz de Queiroz) is not terminated, otherwise more information would be available for data interpretation. Table 2 shows that all the areas except area 8 have extraordinary high zinc content in the soil. Area 1 with 5.4% zinc in the soil is definitely an outcrop. The only vegetation that had been found in this area was Gomphrena which not only survived but also grew vigorously. No Gomphrena sp. was found out of this area. It is interesting to do a green house work to see what is the tolerant range of this plant to zinc.

Cannon reported that the average zinc content of grasses (above ground), herbs (above ground), and shrubs (leaves) growing in unmineralized ground were 850, 666, and 1585 ppm respectively, but these ppm were based on ash weight of the plants. Comparing Cannon's data with table 1 which was obtained using wet digestion method we may still see that the zinc

TABLE 2

CHEMICAL ANALYSIS OF PLANT AND SOIL

AREA Nº	PLANT Nº	PH IN WATER	SOIL ppm DRY WT.		PLANT ppm DRY WT.	
			Zn	Pb	Zn	Pb
1	1	5.6	54.000	14.960	21.670	969
1	2	5.6	4.200	1.560	860	367
3	2	6.2	8.320	2.960	730	3.841
3	3	6.2	8.320	2.960	530	468
5	2	5.4	2.120	800	270	300
7	2	5.5	880	1.880	270	300
8	2	4.9	220	3.360	n.d	367
9	4	5.6	1.040	1.040	4.280	367
9	5	5.6	1.040	1.040	3.870	534
10	2	5.9	960	160	1.540	368
11	2	6.4	480	440	1.940	534

content of those plants from anomalous areas are unusually high. The only plant which we might compare the mineralized with the unmineralized situation is Bauhinia sp. The data show that this plant did not absorb zinc proportionally to the amount in the supporting soil. Area 10 and 11 had less zinc in the soil and were less acidic compared to areas 1, 3 and 5, but more than two times the amount of zinc was detected in the leaves. This suggests that more samples are needed for statistical analysis. Also, the twigs and stems probably are better parts for sampling than the leaves.

ACKNOWLEDGEMENT

The author wishes to thank Prof. A. de Souza Grotta and Dr. Aderbal C.Corrêa for their suggestions in the preparation of this report. Mr. N.F. Koffler, E. Crepani and R.P. da Cunha whose help in collecting the samples are also gratefully appreciated.

Fig. 3 - Plant 1 (Gomphrena sp.).

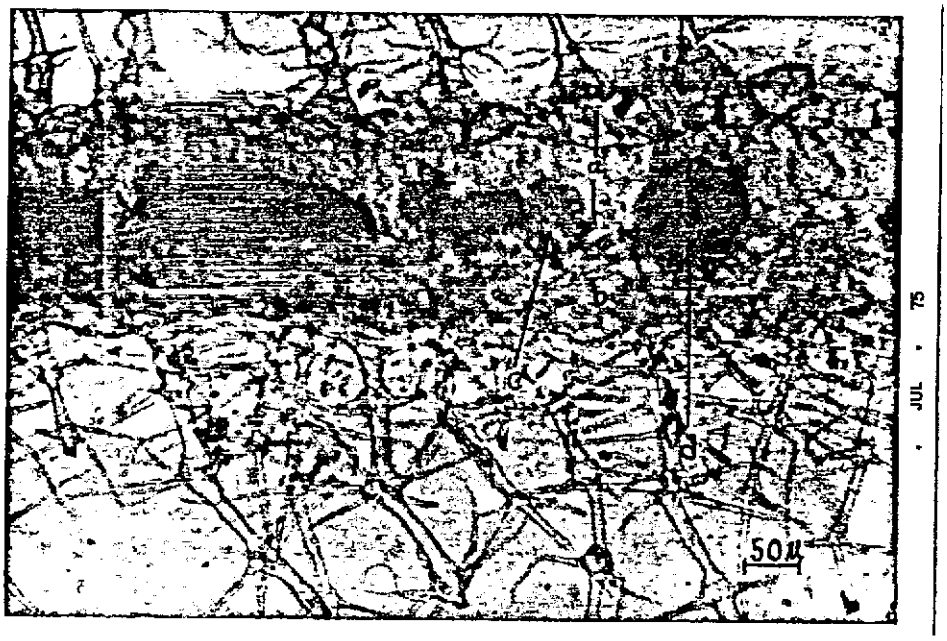


Fig. 4 - Free-hand transection of plant 1 leaf.

a. palisade parenchyma.
b. spongy parenchyma.
c. druse.
d. vascular bundle.

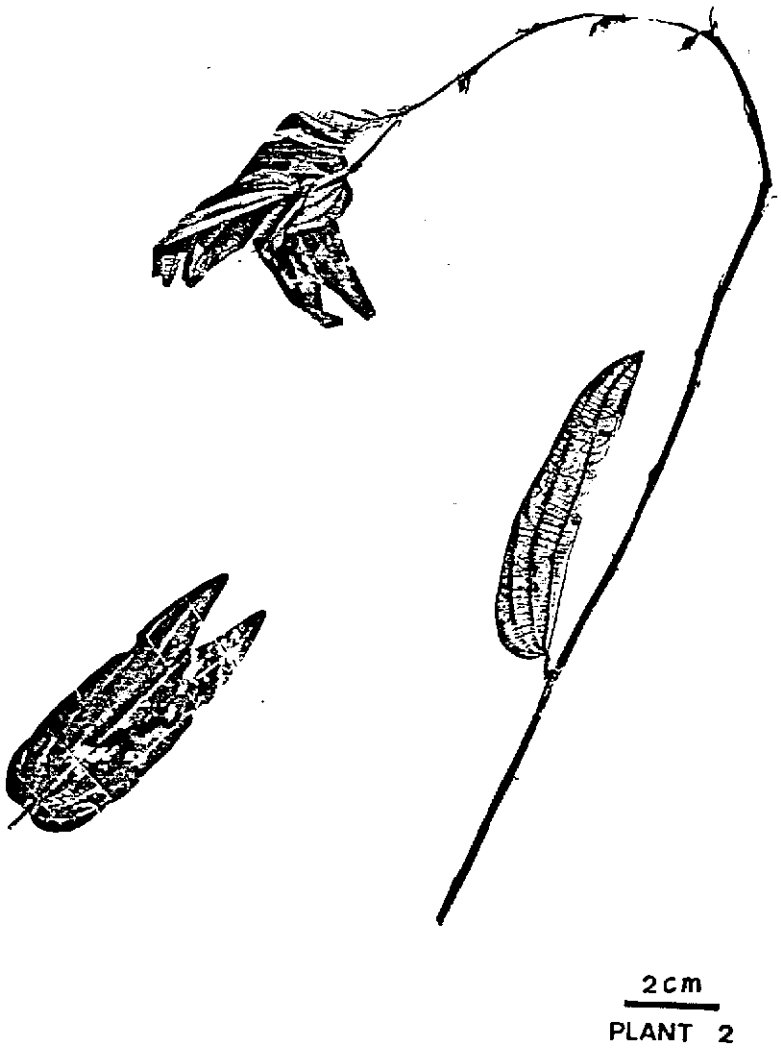
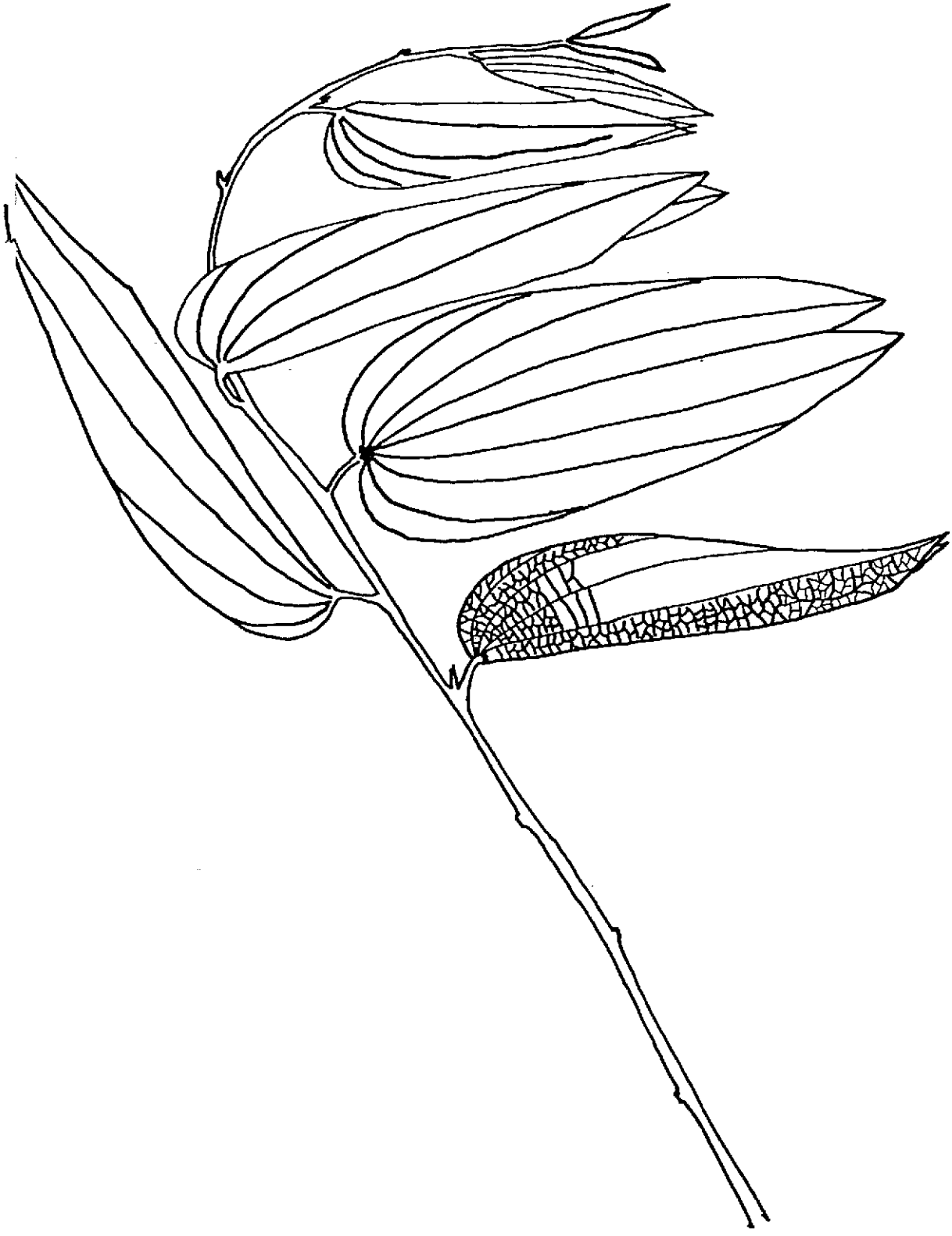


Fig. 5 - Plant 2 (Bauhinia sp.).



2 cm

PLANT 2

Fig. 6 - Transection of plant 2 leaf from area 8 (background area)

a. mesophyll.
b. gradular hair.
c. papilla hair.
d. vascular bundle of lateral vein.

Fig. 7 - Mesophyll of plant 2 from area 1 (mineralized) showing hypodermis, first layer of palisade parenchyma, and the less palisade-like cells toward the abaxial side of the leaf.

Fig. 8 - A close-up of Fig.7 showing intercellular space.

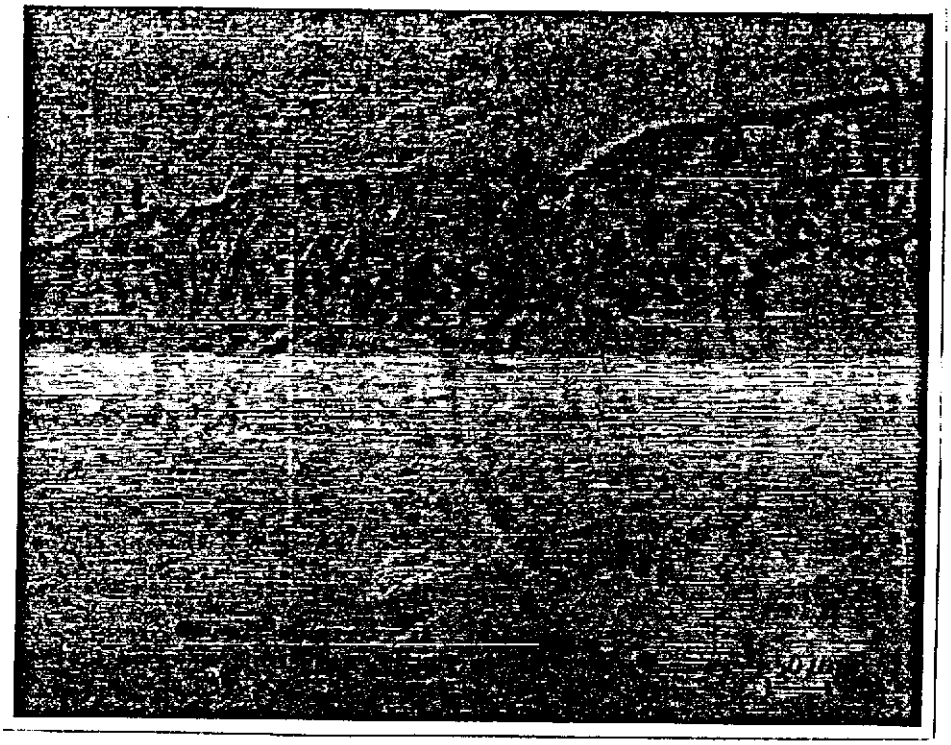


Fig. 9 - Transection of plant 2 leaf from area 1 (mineralized area).
a. mesophyll.
b. vascular bundle.
c. fiber.

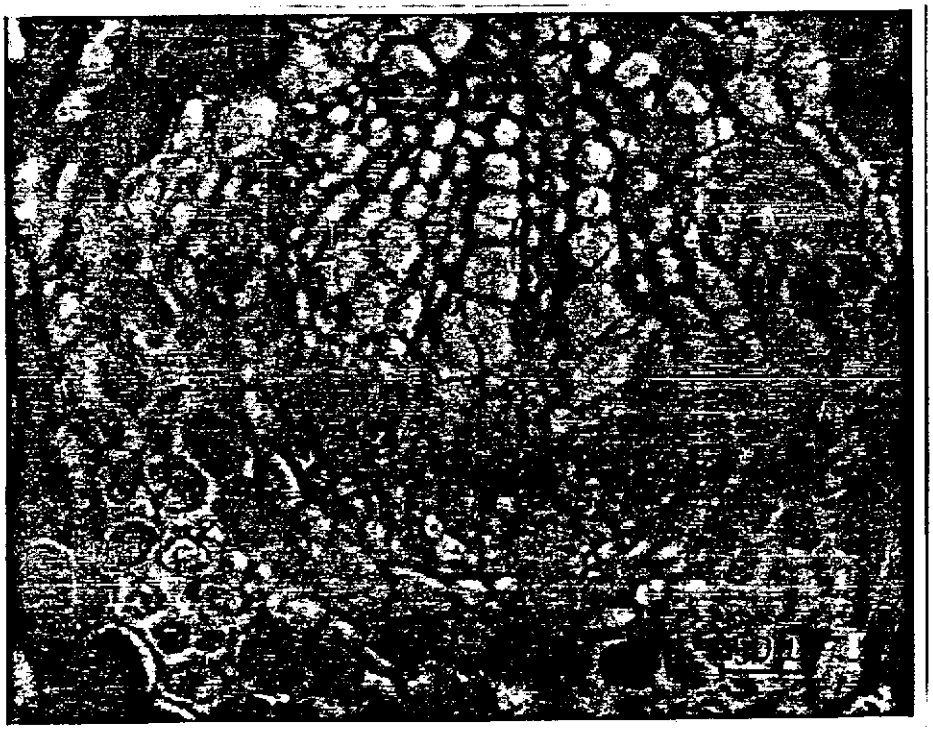


Fig. 10 - Transection of plant leaf from area 1 showing vascular bundle and fibers.

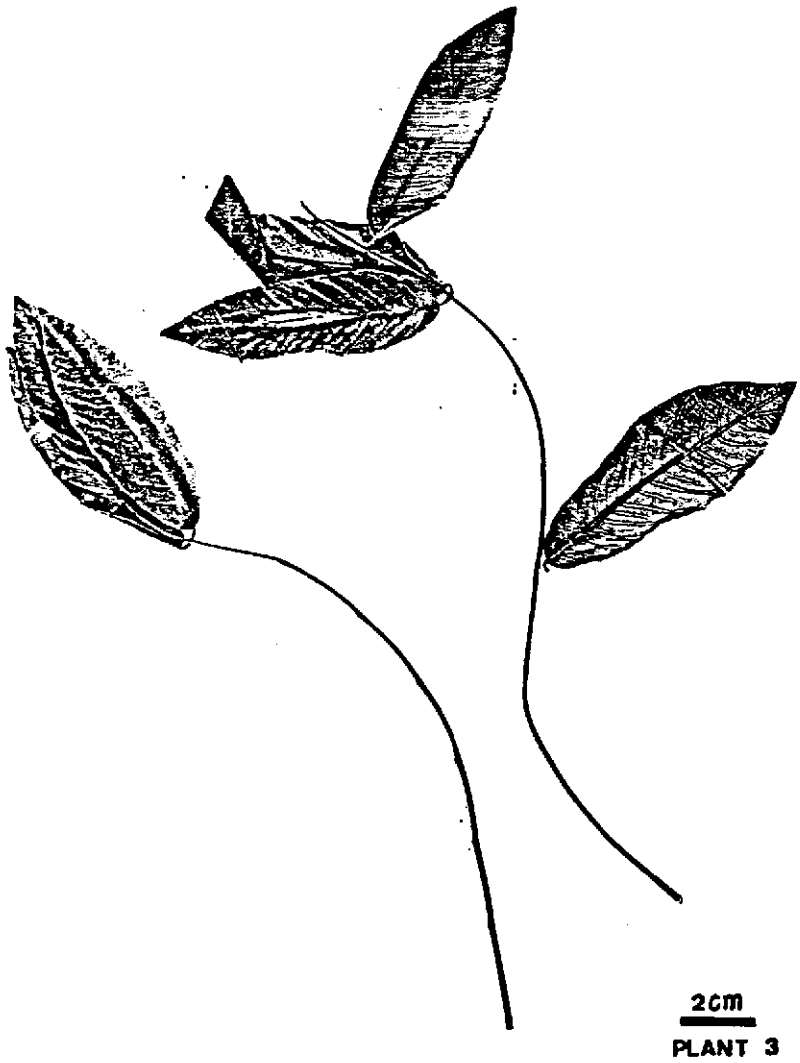
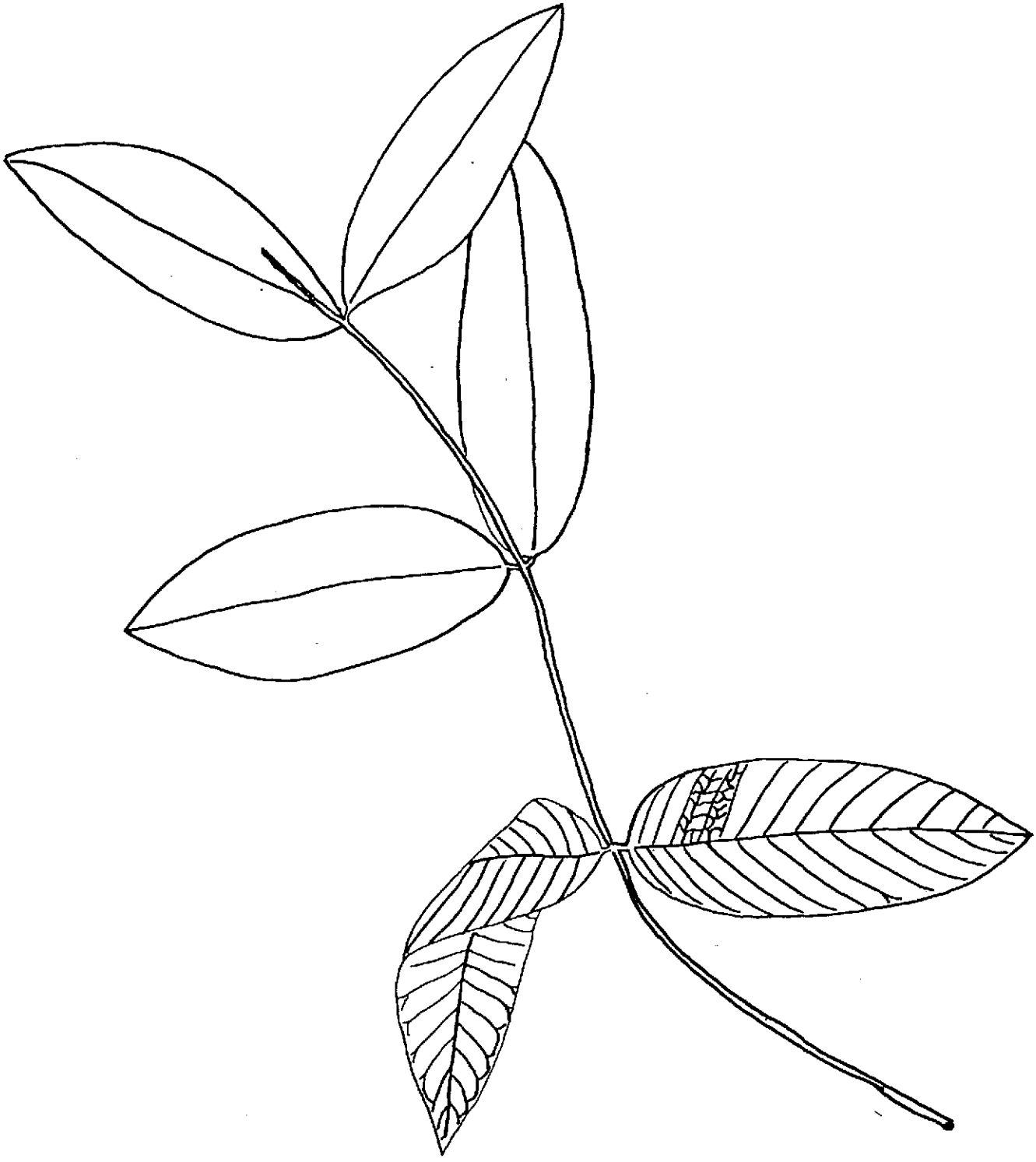


Fig. 11 - Plant 3.



2 cm

PLANT 3

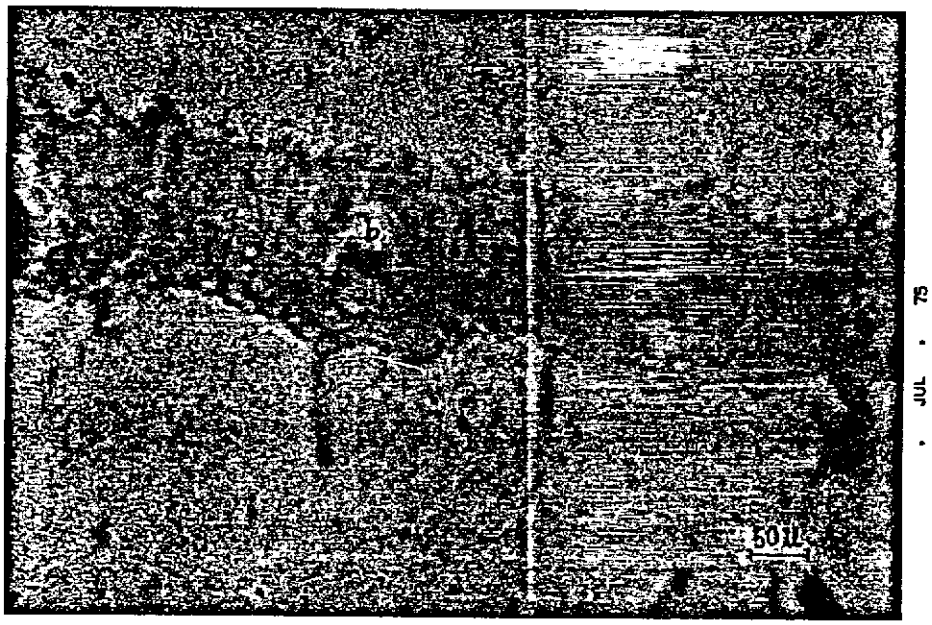


Fig. 12 - Transection of plant 3 leaf.

a. Mesophyll.
b. Vascular bundle.

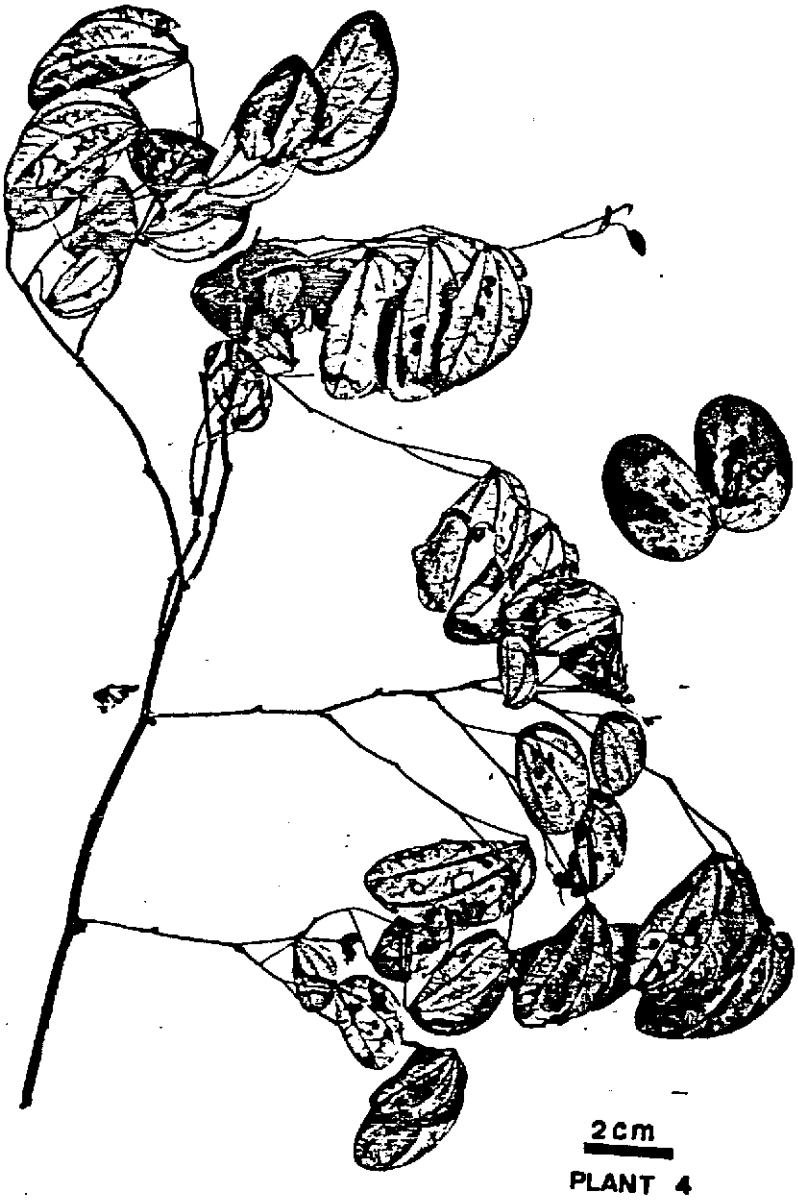
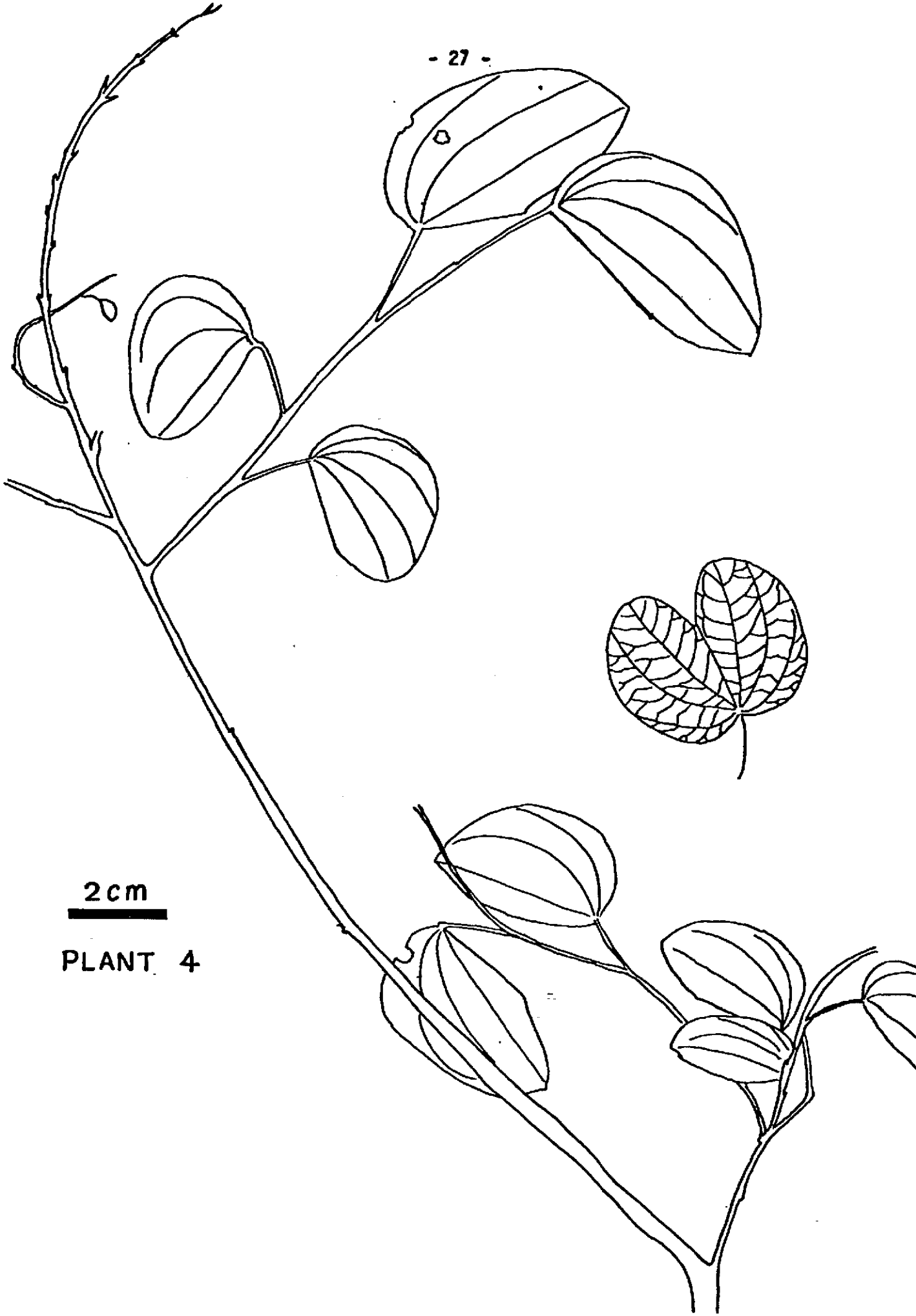


Fig. 13 - Plant 4.



PLANT 4

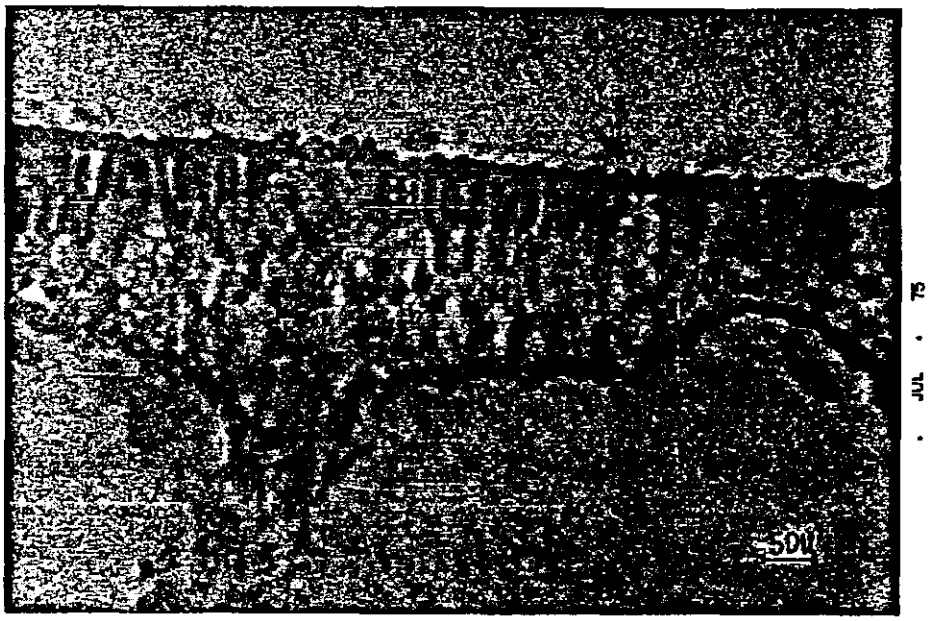


Fig. 14 - Transection of plant 4 leaf.

a. palisade parenchyma.
b. spongy parenchyma.

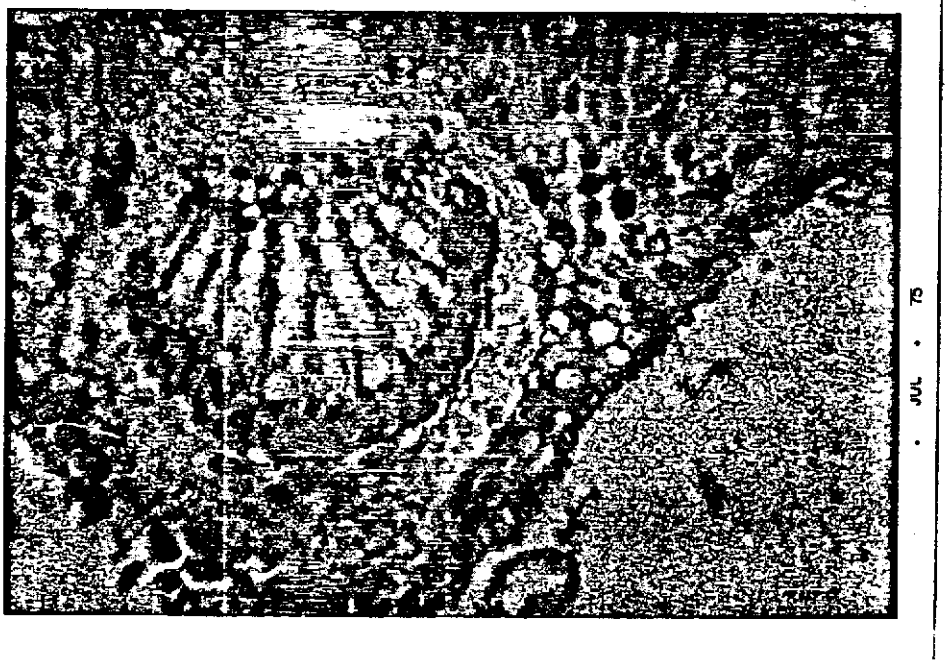


Fig. 15 - Vascular bundle of plant 4, note fibers (f) around it.

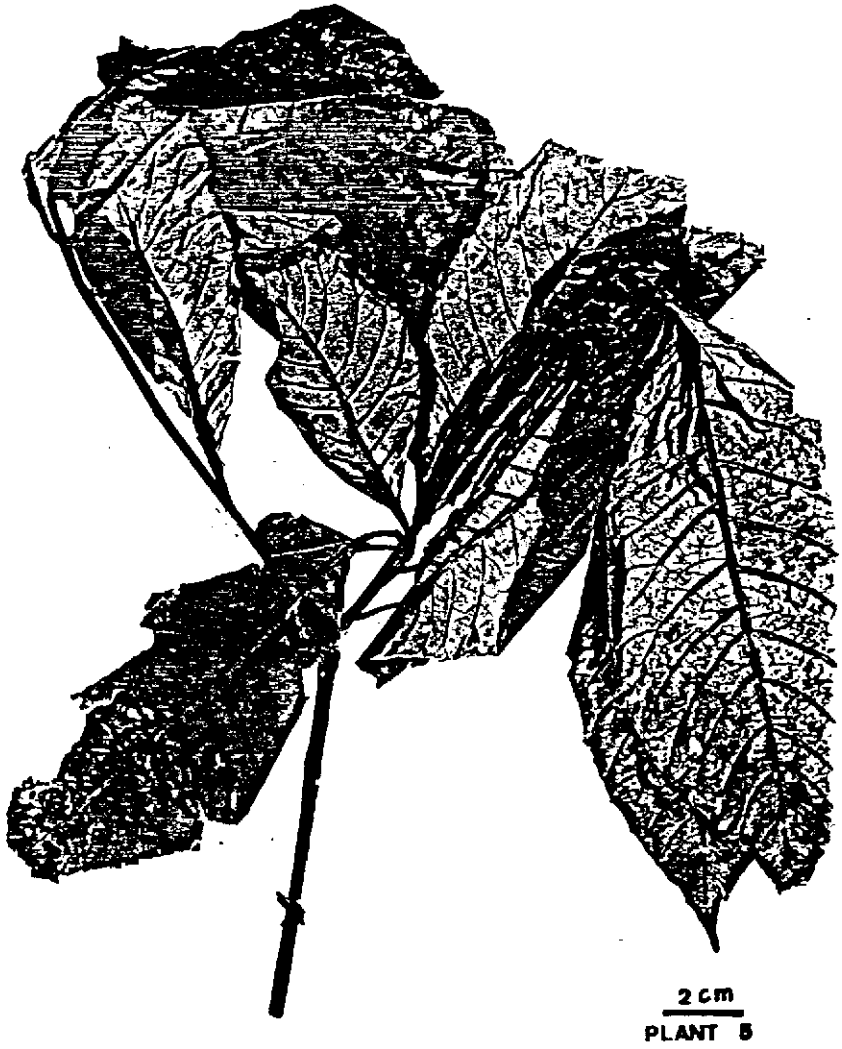
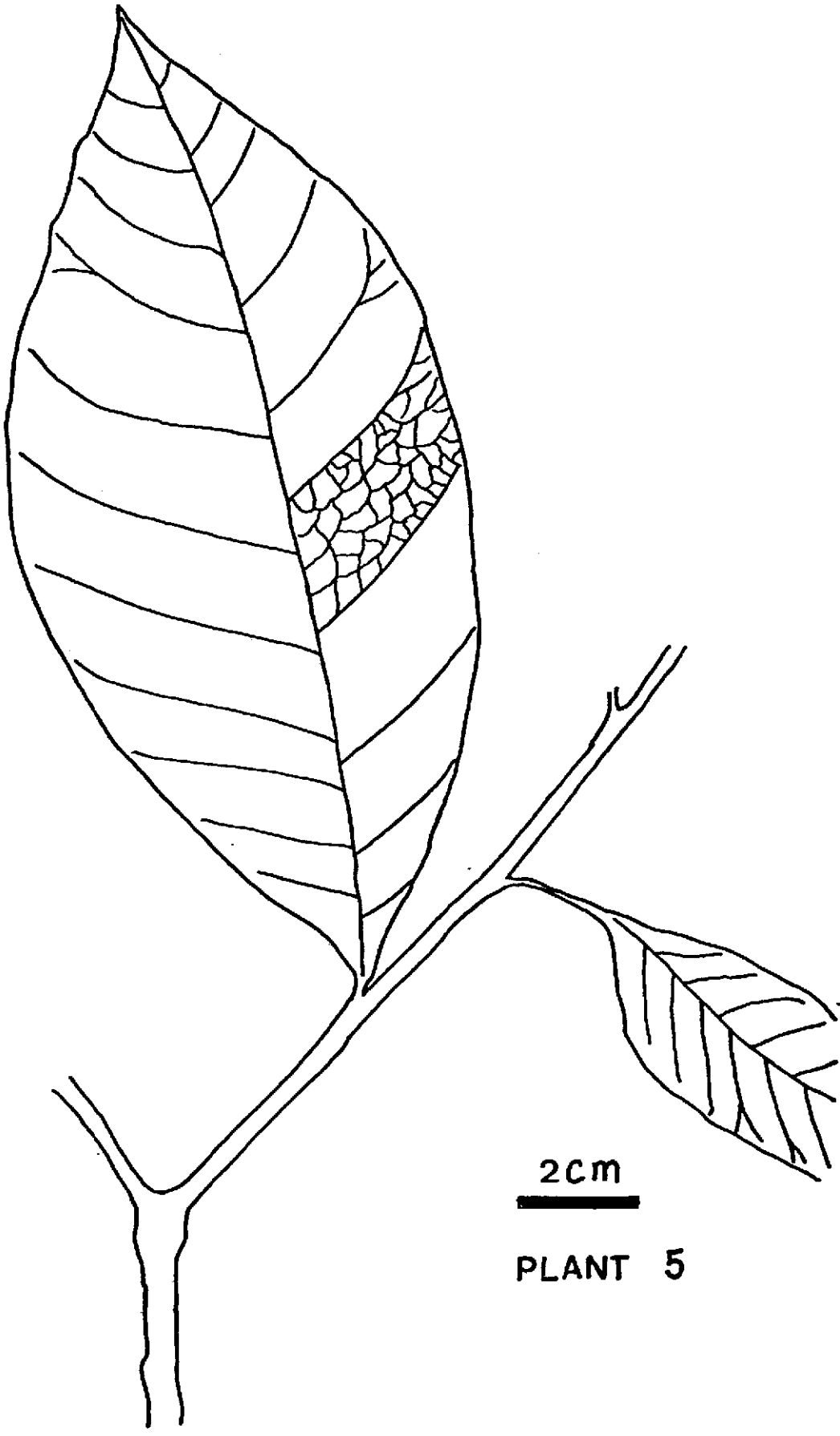
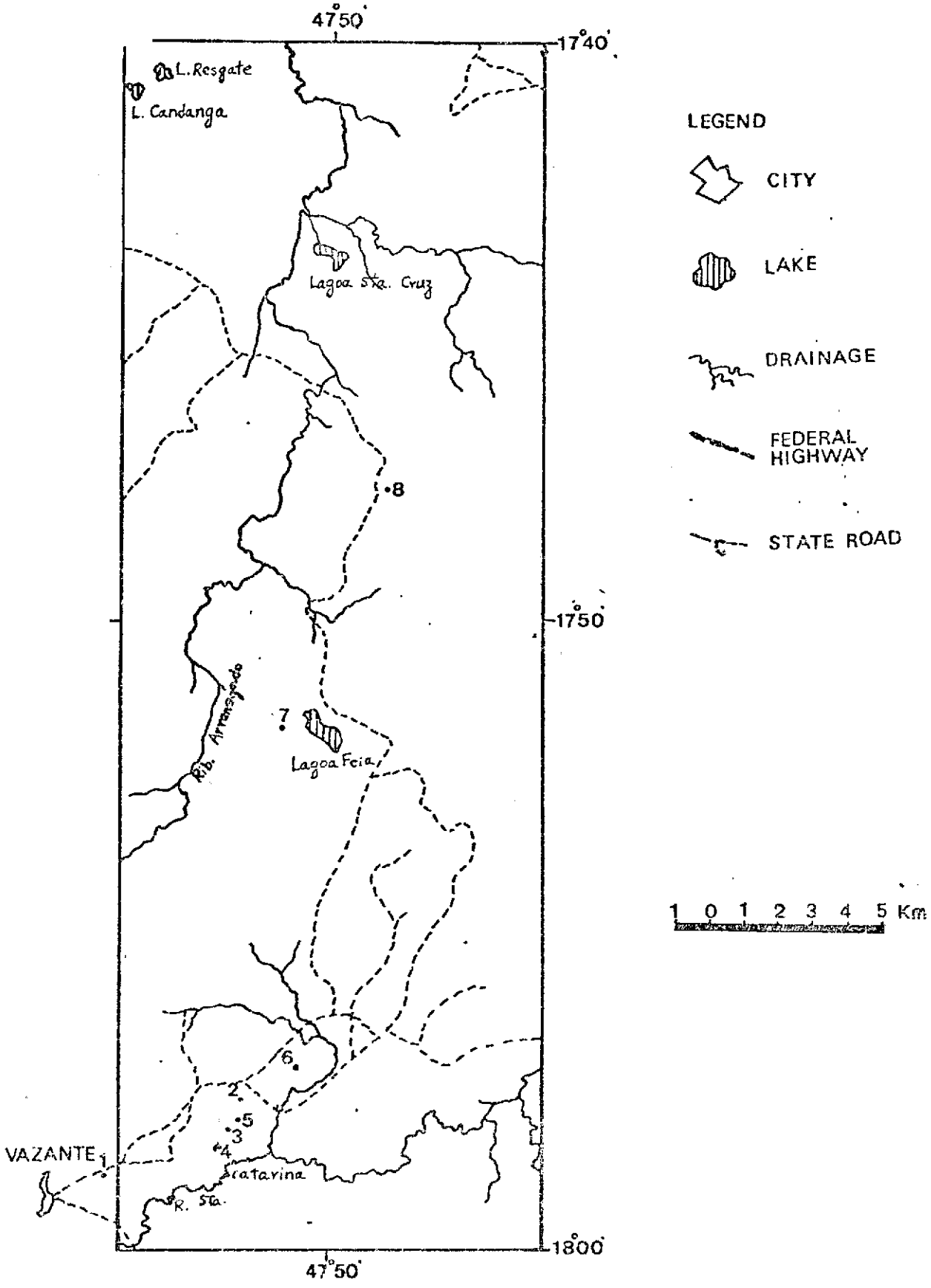


Fig. 16 - Plant 5.

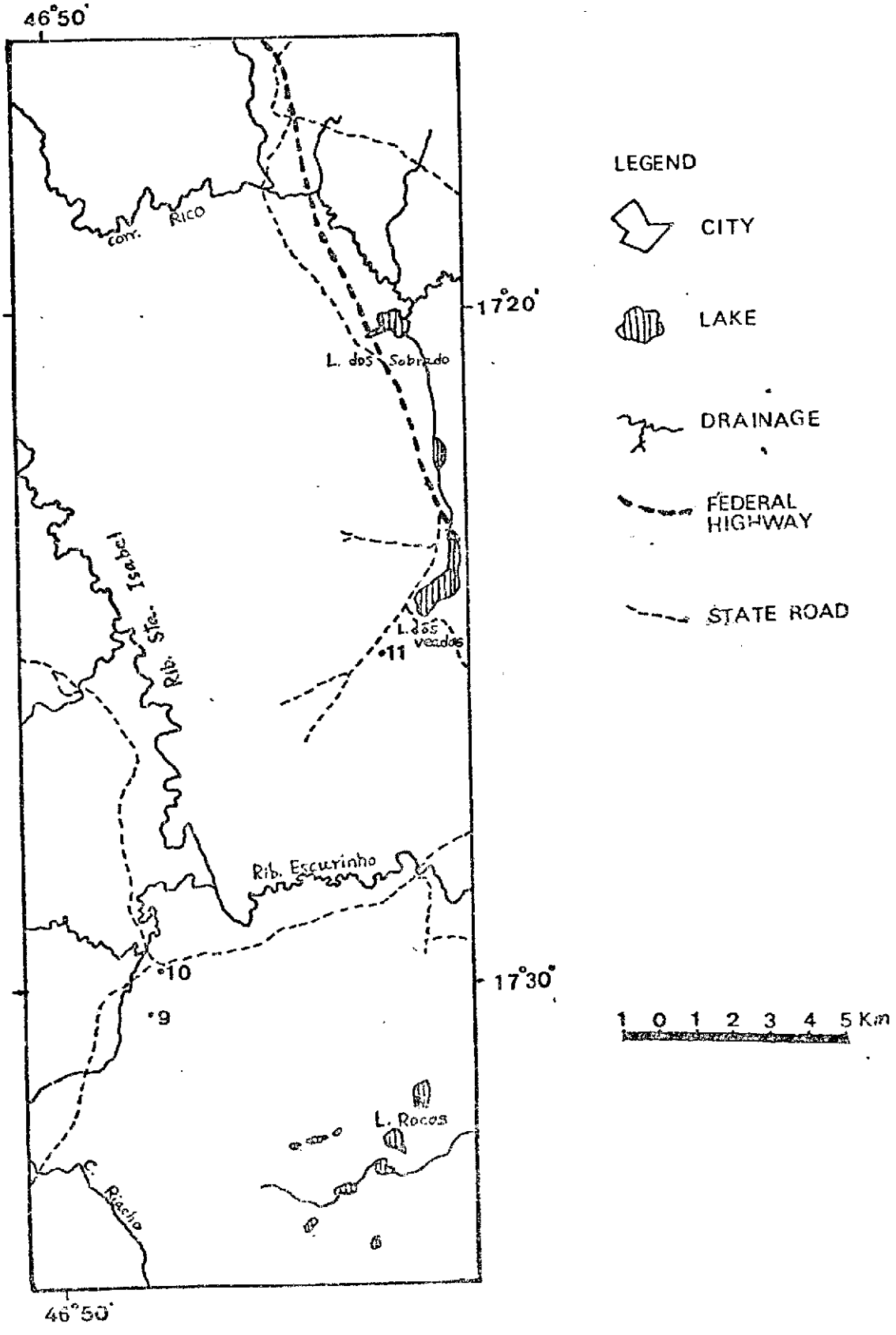


PLANT 5

LOCATION MAP OF VAZANTE AREA



LOCATION MAP OF MORRO AGUDO



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