SOME ASPECTS OF THE BIOLOGY OF THE AFRICAN WALNUT
(TETRACARPIDIUM CONOPHORUM (MÜLL.ARG.) HUTCH. & DALZ.)

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A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (BOTANY) OF THE UNIVERSITY OF LAGOS

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CERTIFICATION

THIS IS TO CERTIFY THAT THE THESIS -

SOME ASPECTS OF THE BIOLOGY OF THE AFRICAN WALNUT (TETRACARPIDIUM CONOPHORUM (MULL. ARG.) HUTCH. & DALZ).

SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES UNIVERSITY OF LAGOS FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY (BOTANY).

IS A RECORD OF ORIGINAL RESEARCH CARRIED OUT BY **OLUWOLE, SURUKITE OPEOLU**

IN THE DEPARTMENT OF BIOLOGICAL SCIENCES.

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D E D I C A T I O N

To EL - SHADDAI, the Almighty God and to the memory of my father, Chief J.O. Opaneye.

ABSTRACT

Studies into some aspects of the biology of the African walnut Tetracapidium conophorum (Euphorbiaceae) - were carried out in the laboratory and in the field.

The germination and growth of the species were investigated to determine the response of the species to such ecological factors like light and dark (shade), soil moisture conditions viz dry, wet and water-logged conditions, soil types viz humic, red earth, clay and sand, pH - 3.5, 5.5 and 7.0, soil depth- in the range of 0 - 10cm, temperature of 15°C, 31°C and 41°C, salinity in the range of 0 to 50% sea water and mineral nutrients specifically nitrogen,phosphorus and potassium.

Studies on the morphology, phenology and anatomy of the species were also carried out considering the dearth of information on these aspects of the species.

The results of the various experiments on the germination response of the species show that the seeds exhibit innate dormancy. The dormancy was broken artifically by physical and chemical scarification. The physical scarification was by scrubbing with iron sponge; while the chemical one was by soaking the seeds in 1% copper sulphate solution for 4 - 5 hours. Scarification reduced germination from about 8 weeks to 3 weeks.

Light significantly enhanced germination better than darkness. When seeds which have been kept in the dark were later brought to light, further germination occurred after about 5 weeks. Wet soil condition also significantly enhanced germination better than dry soil condition; there was no germination under water-logged condition. Humic soil produced the best germination, there was significant difference when it was compared with the other soil types tested. While there was no germination at pH 3.5, germination was significantly better at pH 7.0 than at pH 5.5. Also, as planting depth increased up to 2.5cm, so did germination, thereafter germination decreased. No germination occurred at 15^{o}C, but it increased as the temperature increased up to 31^{o}C; thereafter germination decreased. Germination significantly decreased with increase in salinity to the extent that there was no germination above 30% sea water concentration.

For growth, the seedlings responded similarly to both light and shade in terms of mean dry weight, leaf area and leaf area ratio all of which increased with time (harvest) in both treatments. Like in the germination experiment, there was no growth in the water-logged condition and reduced growth was observed as the soil moisture decreased; the wet soil condition produced significantly better growth than dry condition. The results also show conclusively that of all the four soil types used, humic soil significantly favoured growth. The humic soil also has the highest mean values of dry weight and leaf area for the three harvests. It was only at the third harvest that the red earth significantly favoured growth better than the clay soil while there were significant differences in both red earth and clay compared to the sandy soil at the 2nd and 3rd harvests.

There was a decrease in both the mean dry weight and leaf area with time at pH 3.5 and pH. 5.5 though the growth at pH 5.5 was significantly higher than at pH 3.5 at the two harvests. The best growth was observed at pH 7.0 which had an increase in both the mean dry weight and leaf area with time. The leaf weight ratio and the shoot: root ratio decreased with time at each of the pH treatment, but the reverse was observed for the root weight ratio. The growth response under different salinities show that as salinities increased, growth in terms of total dry weight and leaf area decreased. The seedlings hardly survived at 40% sea water and above. Also the leaf weight ratio and shoot: root ratio decreased with increase in salinity while the reverse was the case for the root weight ratio and leaf area ratio. Growth at 0% sea water was significantly better than that at 10% sea water.

The absence of any of the three inorganic nutrients (nitrogen, phosphorus and potassium), alone or in combination with each other from the growth medium had significant effect on the growth of the species. Significant reduction was observed in the mean dry weight and mean leaf area when the species lacked any of the nutrient or all of the nutrients as compared to when all the nutrients were present.

Morphological studies show that the first pair of true leaves is opposite while the others are alternately arranged. It was observed that the fruit is generally four winged with ridges between the wings but three or two winged fruits also occur. The proportion of the four-seeded fruits was about 50% of the total seeds in the two populations studied. The three-seeded fruits constituted about 40%, the two seeded 9% while the one seeded was 1%. For the phenological studies, flowering occurs from late November to early January while fruiting thereafter occurs till September. There were reproductive failures in late 1989/1990 at the two populations studied. Anatomical studies of the transverse section of root, stem, petiole and leaf of the species showed that they look like the normal sections of root, stem, petiole and leaf of dicotyledonous plants.

The results are discussed in relation to how these environmental factors could affect the distribution of the species and the use to which the results could be put to increase its productivity because of its many economic uses.

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CHAPTER ONE

GENERAL INTRODUCTION

The difference in the current rates of increase in population (3.4% per annum) and food production (2.0% per annum) (Lagos State Agriculture Report, 1984) reinforces the need to step up food production in order, to meet the demands of the people. Although, attempts are being made to rectify this imbalance, yet it is still not possible to reverse the trend. This may be due in part to the fact that:

(i) There is less space for farming as more farmland and vegetation are being converted for industrial uses.

(ii) The inherent and concomitant problems which arise from some methods used in increasing productivity. One of such is the application of fertilizers which are mainly used to correct the problem of soil infertility created as a result of continuous cropping on the same land.

Another problematic method is irrigation practices when the soil is supplied with water to supplement insufficient rainfall and thus enable the crops to mature or increase their yield. The problem is that, with irrigation the land becomes salinised with time. Generally, the water used is often of poor quality so that evapotranspiration leads to the concentration in the soil of salt during irrigation (Raheja, 1966).

At least 50% of all irrigated lands is damaged by secondary salinisation and/or sodification and water logging and at least one-third is salinised (Van Aart, 1971). Salinization of soil leads to reduced productivity of the conventional crops. These salinised soils have to be reclaimed or they will be lost to agriculture. Recently, efforts are being made to grow halophytes or salt tolerant crops and varieties on such lands.

(iii) There is concentration of efforts on the popular food items. The result (output) do not appear to match the input as it seems we might be close to the limits of both genetic and ecological manipulation of the popular and conventional crops.

The alternative it seems, is to turn to the lesser known foods and those not already used as food, and bring them to the same level as the conventional foods.

In Southern Nigeria, one of such food crops is the African walnut, - Tetracarpidium conophorum (Muller. Arg). Hutchinson & Dalziel. It is a tropical plant that belongs to the family Euphorbiaceae. The family includes trees, shrubs and herbs. The family occasionally contains milky juice. The family is found mainly in the tropics and subtropics. T. conophorum is a perennial climbing shrub which branches profusely and does not contain milky juice. The species is monoecious with separate male and female flowers on the same plant and they are arranged alternatively on the axis of the receme inflorescence. The species is known to grow from the seed to maturity i.e. up to the first flowering and fruiting stage in 5 - 6 years; subsequently flowering and fruiting occurs almost annually. Flowering normally occurs from late November to early January and fruiting starts as from March or April till September. The fruit of the species T. conophorum is generally four-seeded but fruits with 1, 2 or 3 seeds do occur. Please see chapter two for details of the morphology and anatomy.

The species is restricted only to the rainforest zone of Nigeria where rainfall is heavy (3,000mm/100 inches) and there is uniformly high temperature ($27^0C/80^0F$) throughout the year. In the rainforest, plant growth is continuous throughout the year - seeding, flowering, fruiting and decaying do not take place in a seasonal pattern. When viewed from the air, the tropical rainforest appears like a thick canopy of foliage, broken only where it is crossed by large rivers or cleared for farming (Adeleke & Leong, 1981). The rainforest in Nigeria covers vast areas including parts of Lagos State (Badagry), Ogun, Oyo, Ondo, Edo, Delta, Imo, Abia, Akwa Ibom, Cross Rivers and Rivers States. T. conophorum is generally found in the evergreen i.e. the wetter part of the forest. The Yorubas call it asala/awusa while the Ibos call it ukpa.

The economic importance of the species includes the edibility of the seeds (which contain copious oils in the endosperm) by people in Southern Nigeria, Zaire and Sierra-Leone. It is usually eaten with maize as food supplement since the fruits are produced at the same time as the early maize. The seeds are known to yield up to 59% of a rapidly drying oil which is whitish-yellow in colour. The oil is edible and is suggested as suitable for soap making and as a substitute for linseed oil in the varnish and lacquer industries (Hutchinson & Dalziel, 1954). The biochemical analysis of the seed shows that it contains 58.3g/100g of fats/oil, 20.5g/100g of proteins and 10.2g/100g of carbohydrate. (Oyenuga, 1971). The deffated oil from the seeds are known to contain protein which has been used in baking bread made from composite flours (Ogusua, 1986). The seed cake after oil extraction contains over 45% of protein and is used as animal feed (Irvine 1961, Oyenuga, 1971). The leaves are also applied locally for head-ache in Southern Nigeria and the shells or hard testa are used for fueling.

Despite its great economic importance, to date, very little report if any at all, is available in the literature on the biology/ecology of <u>T. conophorum</u>. Consequently, the purpose of the research is to study some aspects of the biology of the species.

The period, manner and sequences of flowering and fruiting are of considerable importance in food supply situation. Since T. conophorum is known to take between 5 - 6 years to fruit for the first time form seed, one of the aims of this research is to carry out vegetative propagation studies with the view if possible, to reduce the number of years it takes the plant to produce fruit for the first time. Similar work was done by Okafor 1976, 1978a who successfully propagated Treculia africana Decne ex Trecul vegetatively by budding the stem cutting. These processes reduced not only the period to fruiting from an average of 12 years to only 4 years but also the fruiting height from 10 metres to about 2 metres. Okafor (1978b) has indicated that differences in fruiting times of varieties of a species can be exploited to increase the period of fruit availability.

Vegetative propagation has been carried out successfully on some edible woody plants so as to incorporate them into the traditional farming systems. Other species that have been so successfully propagated vegetatively include Irvingia gabonensis and Chrysophyllum albidium (Okafor 1971, 1973, Okafor and Okolo 1974 and Okafor 1978a). An attempt was therefore made with T. conophorum to see if it can be propagated this way since this species is normally raised from the seed.

In many ecological studies it has been found that one of the problems encountered is the failure of otherwise viable seeds to germinate. Physiologically, a seed which does not germinate when provided with adequate water, sufficient oxygen for normal aerobic metabolism and suitable temperature within physiological limits is said to be dormant (Berrie, 1984). Three types of dormancy have been recognized. Some seeds are born dormant, some achieve dormancy and some have dormancy thrust upon them (Harper, 1957, 1977). These types of dormancy are also referred to as innate, induced and enforced dormancy respectively. When seeds have surmounted the various hazards which attend their ripening, dispersal and dormancy phases, they are ready to germinate provided they encounter the appropriate environment (Fenner, 1985). Thus, the distribution and abundance of a species in nature depends not only on the efficient seed dispersal but also on the occurrence of good sites which provide essential requirements for the successful colonisation (Okusanya, 1976).

Many stringent demands are placed on the seeds that arrive in a particular habitat if they are to be able to complete satisfactorily the rest of the life cycle (Harper, Lovell and Moore, 1970) and conversely the habitat should be capable of satisfying the requirements of the new arrivals (Gleason, 1939). Thus seed germination is a complicated process influenced by many factors (Cone & Spruit, 1983). It is also a critical stage in the life of a plant since without it, seedling growth and establishment cannot occur (Okusanya, 1976). A variety of physiological mechanisms are known to exist to ensure safety in these critical phases: these include the fact that the germination requirements for each species or population should keep the seeds in safer pre-germination phases when the probability of successful seedling establishment is low. Therefore seed germination has been assumed to depend upon sites offering the required conditions for germination in which case they are termed 'safe sites' (Harper, 1977).

Pemadasa and Amarasinghe (1982) working on the ecology of a montane grassland in Sri Lanka found that the germination of the predominant grasses namely Eulalia trispicata, Pennisetum polystachyon, Themeda tremula and Cymbopogon nardus is influenced by light, temperature, soil moisture concentration, soil surface heterogeneity and the depth of seed burial. The timing of natural germination was determined by innate and enforced dormancy mechanisms and is synchronous with the monsoon rains. The monsoon rains start in March and the soils moisture increases and the grasses begin to germinate rapidly. The inability of these species to germinate soon after seed set is of ecological significance for the young seedlings would be exposed to a dry period which would cause considerable mortality. Therefore it is necessary for any meaningful biological/ecological study to include the effects of various environmental factors on germination of T. conophorum.

The next most important stage in the life of a species after seed germination is seedling establishment and growth which is the end result of the interaction of numerous physiological processes. After a seed has germinated, it gives rise to a seedling whose growth is largely dependent at least for a time on its own stored food reserves. As the growth of the shoot and root proceeds, dependence on internal resources is gradually reduced and an external supplies of carbon and minerals are exploited until the seedling is fully established (Fenner, 1985).

It is necessary to know how the basic processes which control growth are affected by the environment so as to understand why plants grow differently under various environmental conditions and cultural treatments. Klebs (1913, 1918) pointed out that environmental factors can affect plant processes and conditions. For example, light and temperature may determine both the onset and cessation of growth as well as the rate of growth during the vegetative period (Bannister, 1976). Also Harper & Sagar (1953) illustrated the establishment of Ranunculus seedlings whose response to different water tables is related to their distribution in the field i.e. adapting to the conditions of the environment in which they are normally found. Likewise the development and timing of many plant functions (phenology) is strongly influenced by both temperature and the length of the day. It is therefore necessary to carry out studies on the effect of various environmental factors on the growth of the species.

The need to use local examples to teach in our schools has been stressed by successive governments. While most of the textbooks used in our schools are foreign, the few local ones do not carry local examples.

With this type of study, it was thought necessary and useful to report the details of the morphology, anatomy and phenology of the species for use in our schools.

Consequently, this thesis is divided into five chapters. The first is this chapter which deals with general introduction. The second deals with morphology, anatomy, phenology and vegetative propagation studies while the third is on germination studies. The fourth deals with growth studies while the fifth is general discussion and conclusion.

CHAPTER TWO

MORPHOLOGY, ANATOMY, PHENOLOGY AND VEGETATIVE PROPAGATION STUDIES

INTRODUCTION

A morphological character is one inherent in or manifested by a structural component of the plant (Lawrence, 1951). External morphology has traditionally provided a primary and evident source of distinguishing characters and are therefore relied upon for diagnostic purposes and as a basis for classification. Morphological features permit ready observation, determination and correlation of characters. They are easily quantified and subjected to comparative analysis to a greater degree than can characters from most other studies.

According to Dilcher (1974), the gross form of angiosperm leaves, including such features as size, shape, nature of margins, form of the apex, base and petiole, positioning of glands and nature of venation, has always been very important in the description of fossil and extant leaves.

The morphologic aspects of T. conophorum in all the localities sampled are presented and illustrated below.

The application of anatomical characters in plant classification dates back to Bureau (1864), who for the first time used them for delimitation of taxa at various levels within the family Bignoniaceae. Since then the guiding principles of systematic anatomy have been laid by

Bailey (1951) and Metcalfe (1954) among others. Anatomical evidences can be useful to systematics in several ways. They might enable us identify fragmentary materials, like a piece of wood. It can be used in the preliminary identification of herbarium materials when the morphological characters would not help and they can also inform us on the evolutionary trends and interrelationships of taxa at and above the species level.

Leaves, probably anatomically the most varied organ of angiosperms (Carlquist, 1961), constitute a valuable source of taxonomic characters. Hickey (1973) and Dilcher (1974) have proposed detailed terminologies for the various architectural features of dicotyledonous leaves. Cuticular characters like the epidermis and stomata have been of great value in the taxonomy of several taxa (Stace, 1965).

The innumerable variations in foliar venation patterns have provided taxonomic information since very early times. In fact one of the characters on which the basic classification of angiosperms into two subclasses viz: Dicotyledonidae and Monocotyledonidae is based in leaf venation pattern. The detailed terminologies are provided by Hickey (1973). With a few exceptions reticulate and parallel venation patterns are characteristic of dicotyledons and monocotyledons respectively. The number of primary and secondary veins, and the nature of tertiary and higher order of venation can provide valuable classificatory criteria and clues to evolutionary directions. Angiosperm leaves are unique in having 'vein islets'. The number of such veinlets in areoles vary within narrow limits and incombination with other characters are of taxonomic importance.

Epidermal characters of some genera of the Euphorbiaceae to which T. conophorum belong have been studied by Kakkar and Paliwal (1974) and Raju and Rao (1977). However no anatomical work has been reported for the genus Tetracarpidium and so far only one species, T. conophorum, is still known.

In view of the economic importance of this species, the present study, which incorporates morphology, phenology and anatomy, was undertaken to provide additional new sources of data which may improve our understanding of the biology of this little-studied species.

MATERIALS AND METHODS

Observations and measurements of leaf characters were made on matured plants grown in the biological garden of the University of Lagos, Akoka ($6^{o}30'N$, $3^{o}29'E$) and those collected at Ikenne ($6^{o}52'N$, $3^{o}43'E$) and Ikole-Ekiti ($7^{o}48'N$, $5^{o}31'E$). Anatomical studies were also carried out on the stem and root cuttings.

Morphological Data

The general morphology of matured plants from each of the three localities was studied. The descriptive terminology of the morphological features is based on Dilcher (1974).

CUTICULAR STUDIES

The descriptive terminology for the cuticular studies is mainly based on stace (1965) and Dilcher (1974).

Vegetative Anatomy

The methods of Sass (1961) and Bradbury (1973) were adopted with slight modifications. Plant materials (leaf lamina, petioles, stem and root pieces) were processed for microtomy.

Vegetative Propagation Studies

Seedlings and cuttings of T. conophorum were used for these experiments; and they were treated with growth hormones which include kinetin, indole-acetic-acid and gibberelic acid at various concentration levels.

Seedlings

i. Seedlings that still bear cotyledons were cut at soil level and placed in a beaker of tap water half immersed until all cuttings were made.

ii. The cuttings were placed in a beaker through perforated disks of paraffin coated and cooled cardboard with 1ppm in 50 ml of gibberellic acid.

iii. They were kept and observed for 6-8 days (Kaufman et al 1975).

Cuttings

Forty-five day old plants and older plants were used. About 6ft long stems were cut under water from lower portions. Smaller cuttings were made which include 3-4 nodes (about 1 cm from the cut-end) where the stem will root. The cuttings were dipped in various concentrations viz (0.02mgl/l - 0.10 mg/l, 10^{-4} M, 10^{-5} M) of kinetin, Indole-acetic-acid and gibberelic acid. Some of the cuttings were then planted in humic soil (watered once a day) while some were left to root in distilled water (Arora et al, 1959, Miller 1961 & Kaufman et al 1975).

RESULTS

MORPHOLOGY

<u>Vegetative Features</u>

<u>Habit</u>

T. conophorum is a perennial climbing shrub with a main stem that has many branches. It is restricted to the moist rain forest zone in Nigeria (Fig.1).

<u>Root</u>

The root system consists of a main tap root which penetrates deeply into the soil. The roots branch profusely giving rise to secondary and tertiary roots. The primary root persists and gives rise to the main tap root.

<u>Stem</u>

The stem twines around a support in its vicinity especially trees. The stem often branches laterally.

<u>Leaves</u>

The leaves are green, simple and ovate with a serrated margin. The apex is acuminate while the base is rounded. The surface is glabrous with a reticulate venation (Fig.2). The leaves are arranged alternately but the first pair of true leaves is opposite. This phenomenon has never been previously reported for *T. conophorum*.

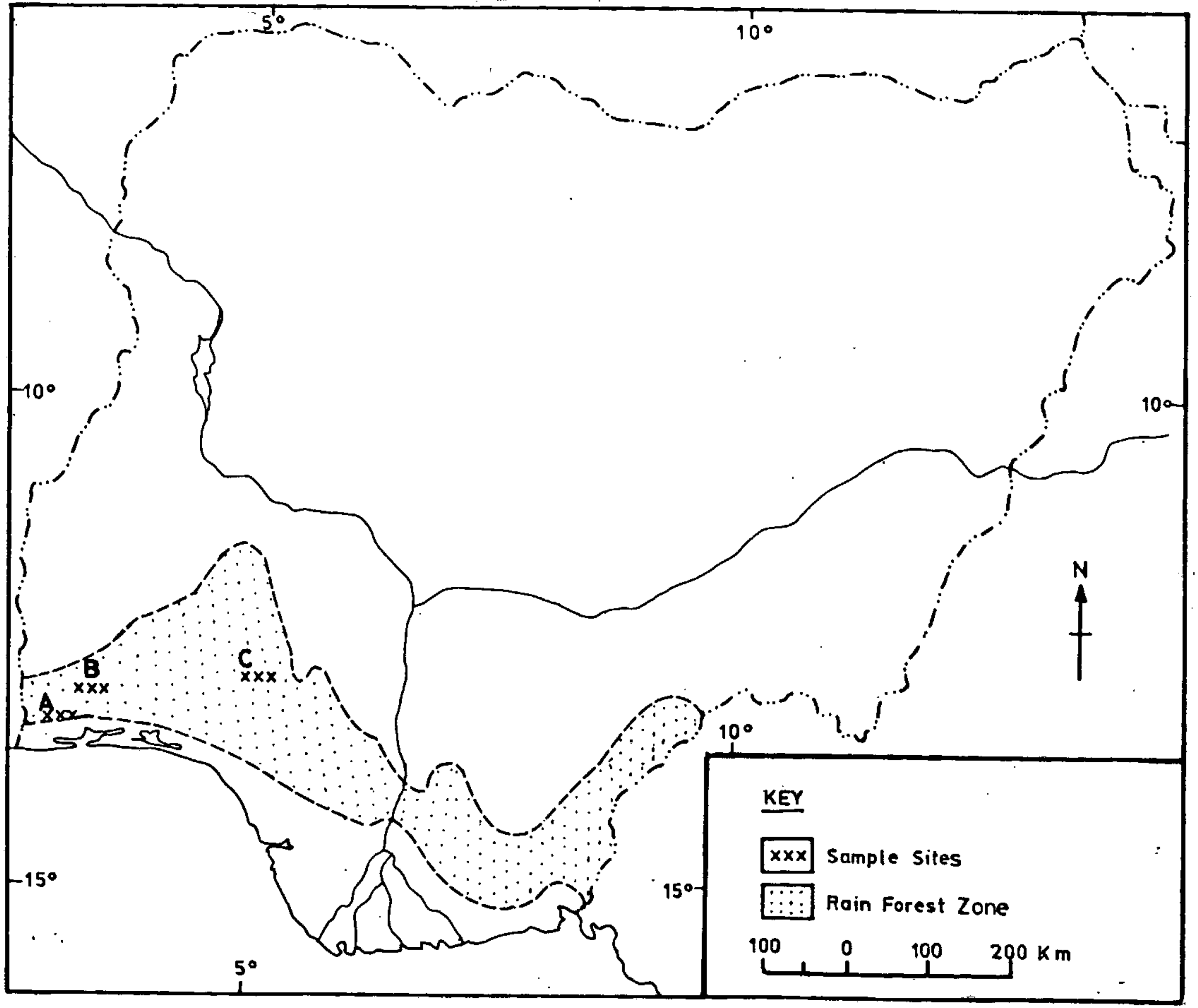

5°
10°
10°
10°
A
B
C
xxx
N
10°
15°
15°
5°
KEY
xxx Sample Sites
Rain Forest Zone
100 0 100 200 Km

Fig 1: Map of Nigeria showing the rainforest zone and the sampled sites.

A - Lagos, Lagos - State
B - Ikenne, Ogun - State
C - Ikole-Etiti, Ondo State.

Fig.3: Drawing of the raceme inflorescene of *T. conophorum*. mag.xi

Reproductive Morphology

Flowers

T. conophorum is monoecious and the flowers are small and unisexual. The male flowers which are about one hundred are on top with one to two females at the base (Fig.3). The male flowers which are yellowish green are deciduous leaving the females at the base of the raceme. The male flowers of T. conophorum have no petals and the sepals are five in number (Fig.4) and are glabrous. The male flowers consist of about forty stamens. The female flower generally has four-spreading stigmas with a stout style, and the ovary is four-lobed (Fig.5) Pollination is by insect especially ants which are commonly seen around the flowers.

Fruits and seeds

The fruit of T. conophorum is a simple dry indehiscent capsule measuring 7.60 + 0.10 cm across and usually contains four subglobose seeds. Fruits with less than four seeds (3, 2 & 1) also occur. The fruit is generally four winged with ridges between the wings. Three or two winged fruits also occur due to one or two lobes of the ovary being aborted (Fig.6). The fruit is green in colour at maturity but as it decays having fallen to the ground turns brown, then black.

The seed which has a hard dark-brown testa is 2.22 + 0.07cm across. It is a dicotyledonous endospermous seed.

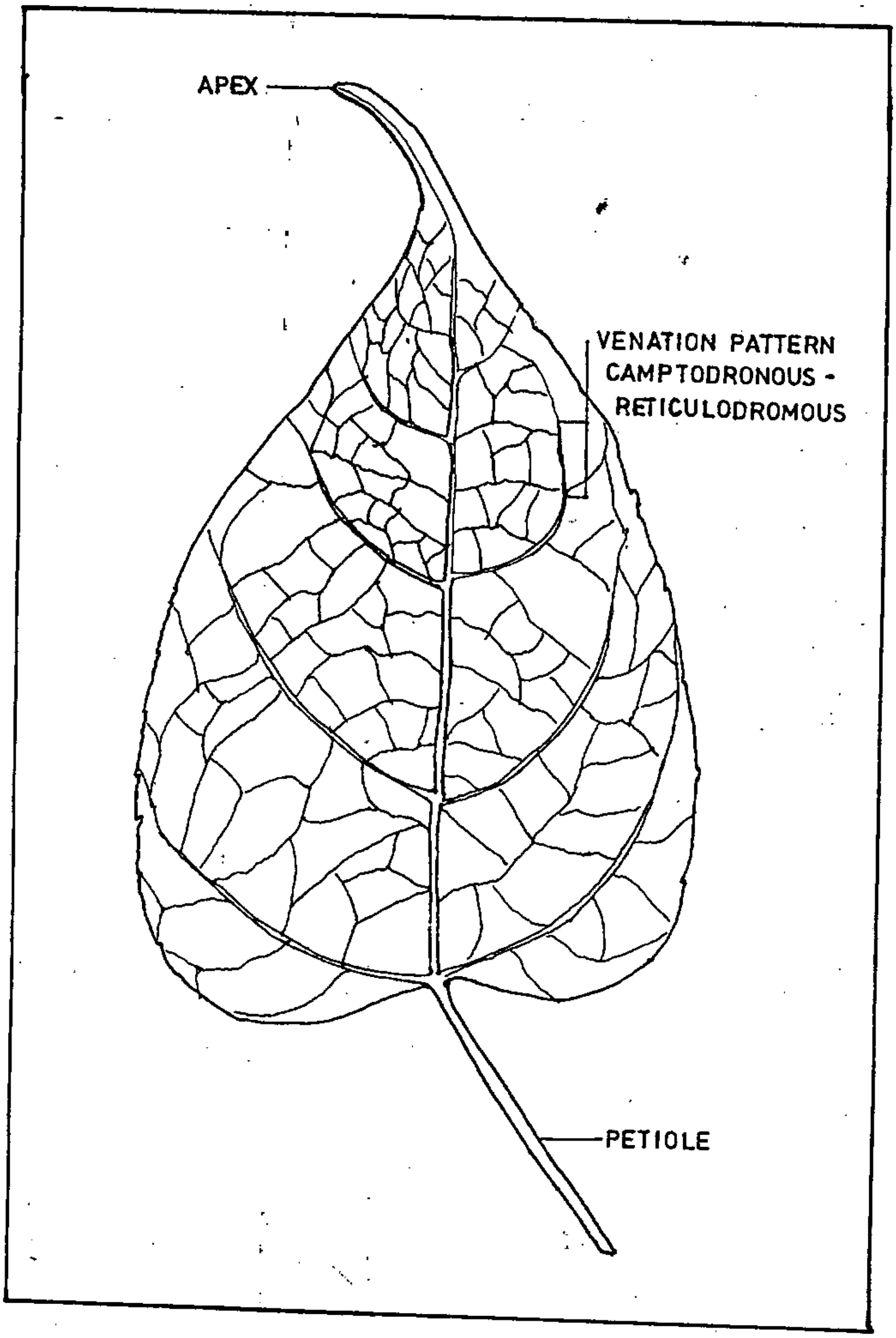

APEX
VENATION PATTERN
CAMPTODRONOUS -
RETICULODROMOUS
PETIOLE

Fig 2: Leaf morphology of *T. conophorum*., ventation pattern is camptodronous - reticulodromous. mag. XI

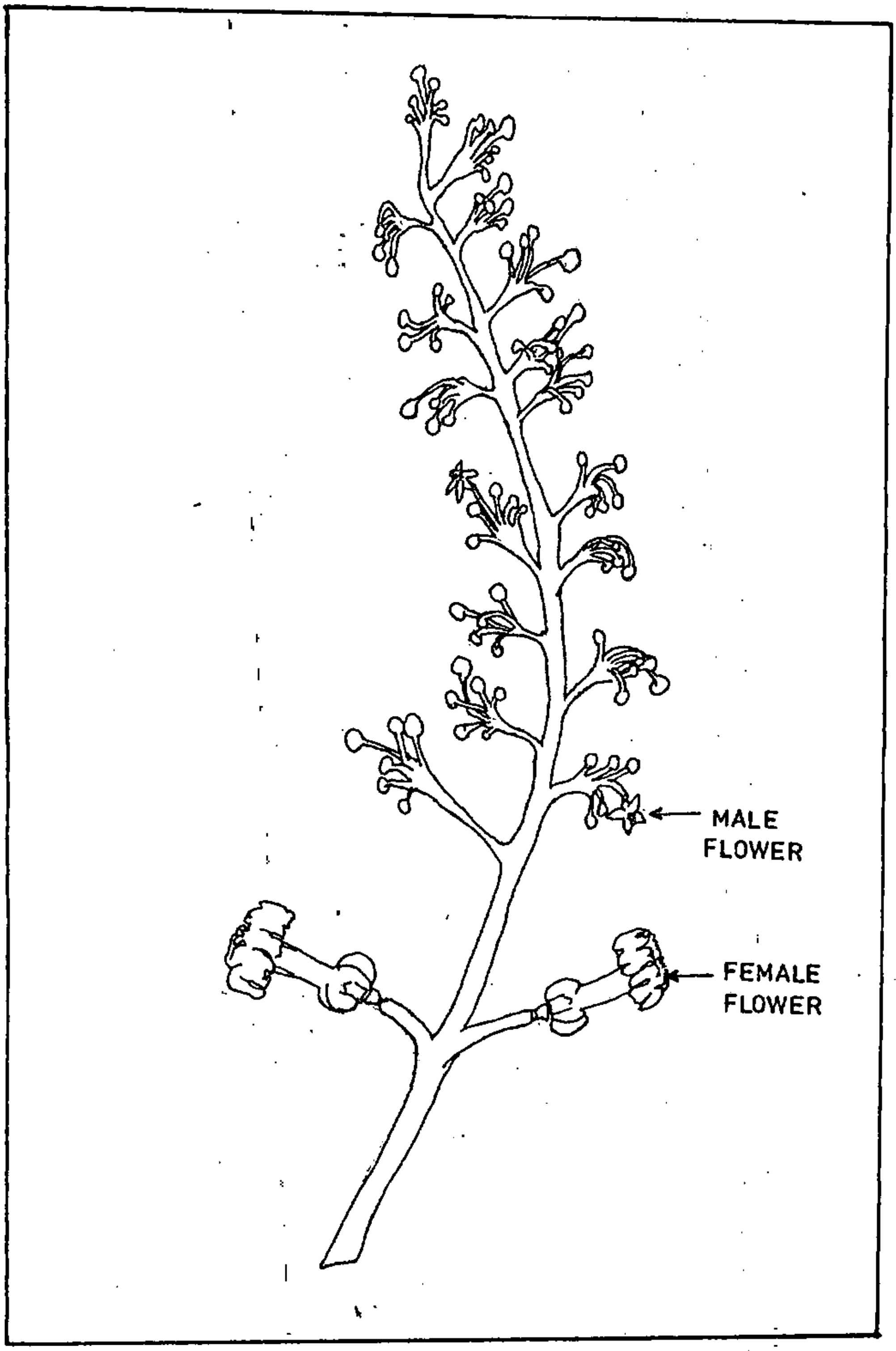

MALE
FLOWER
FEMALE
FLOWER

Fig: 4: T. conophorum male flower consisting of five sepals and many stamens. mag. x10

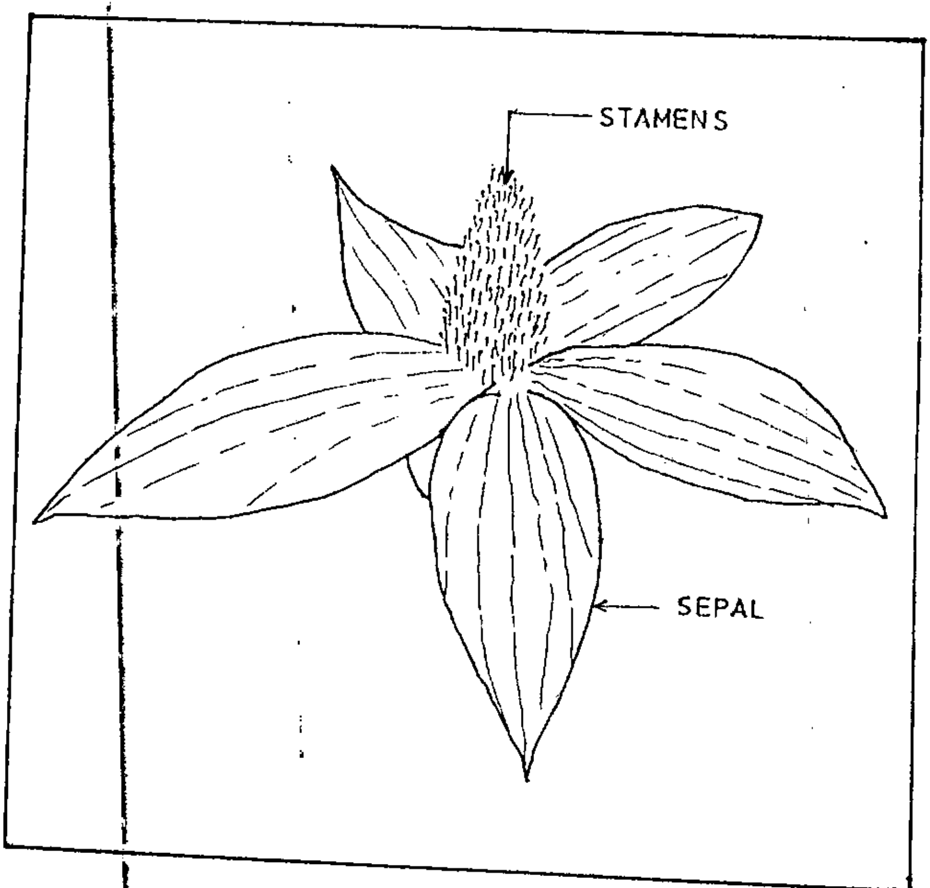
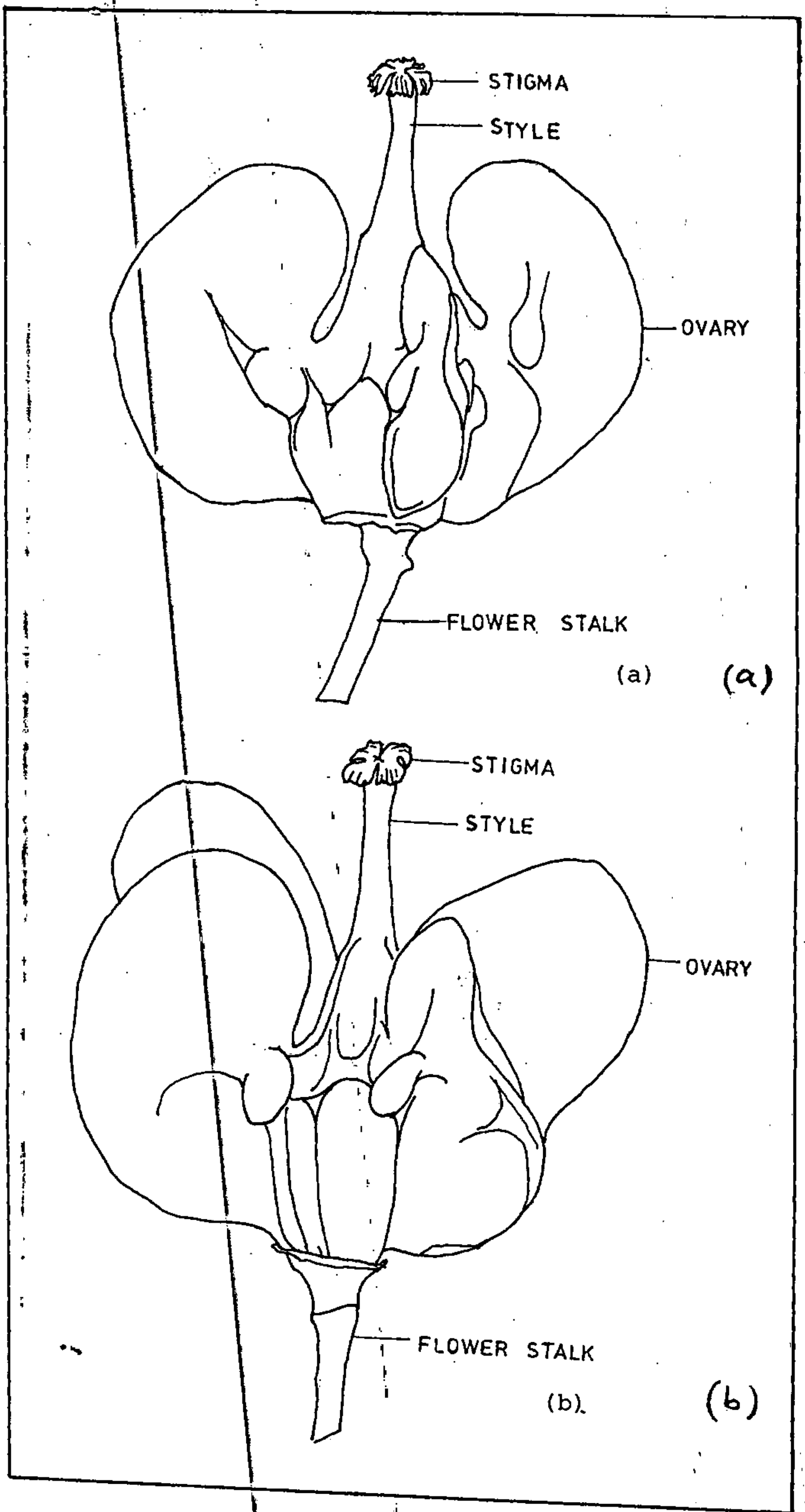

STAMENS
SEPAL

Fig: 5: *T. conophorum* female flowers showing four spreading stigmas and (a) three-wings with seeds, (b) four wings with seeds. mag. x2


STIGMA
STYLE
OVARY
FLOWER STALK
(a)
(a)
STIGMA
STYLE
OVARY
FLOWER STALK
(b).
(b)

The testa is rough to touch (it has fibres). The endosperm (kernel) is off-white in colour and the embryo which is leaf-like is sand-witched within the cotyledons.

LEAF EPIDERMAL MORPHOLOGY

Epidermal cells are irregular in shape. The anticlinal walls are curved or undulate on both surfaces of all specimens irrespective of their locality (Figs 20 a & b). The epidermal cells on the adaxial surface vary from 28.45um + 1.07 in University of Lagos population to 30.19um + 1.03 in Ikole-Ekiti species on the adaxial surface (Tab 1). For the Ikenne species the epidermal cells on the adaxial surface measured was 29.28um + 1.02 and 28.25 + 1.01 at the adaxial surface.

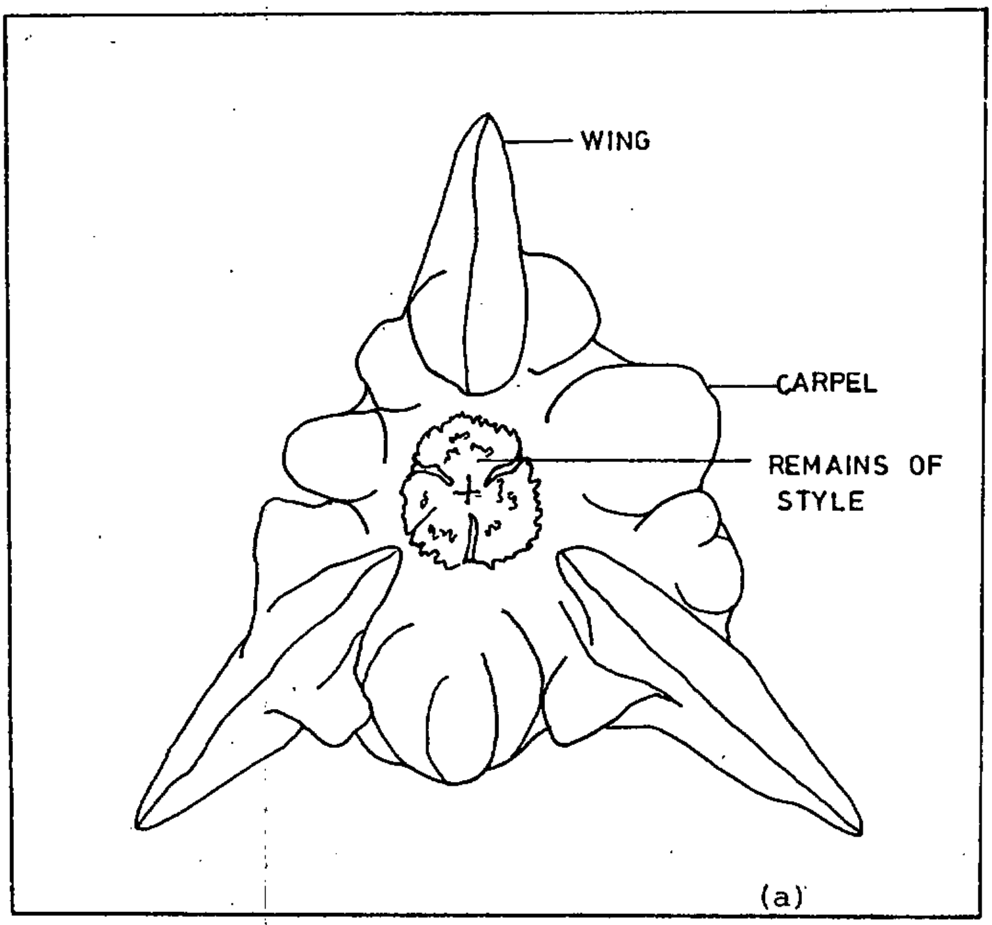
All specimens examined are hypostomatic. Paracytic stomata occur in all specimens - the stomata being enclosed by one pair of subsidiary cells whose common walls are parallel to the guard cells (Fig.20b). The variation in stomata number is either 9 or 10 recorded in University of Lagos and Ikenne species, and Ikole-Ekiti species respectively. The size of the stomata is relatively uniform in all specimens. The variation is between 13.70 x 7.72 um in University of Lagos population to 13.95 x 8.12 um in Ikole-Ekiti population (Table 1). Biserrate, tapered and pointed hairs occur in the abaxial surface of all specimen (Plate 1) but the adaxial surface is completely glabrous.

Fig 6: *T. conophorum* showing

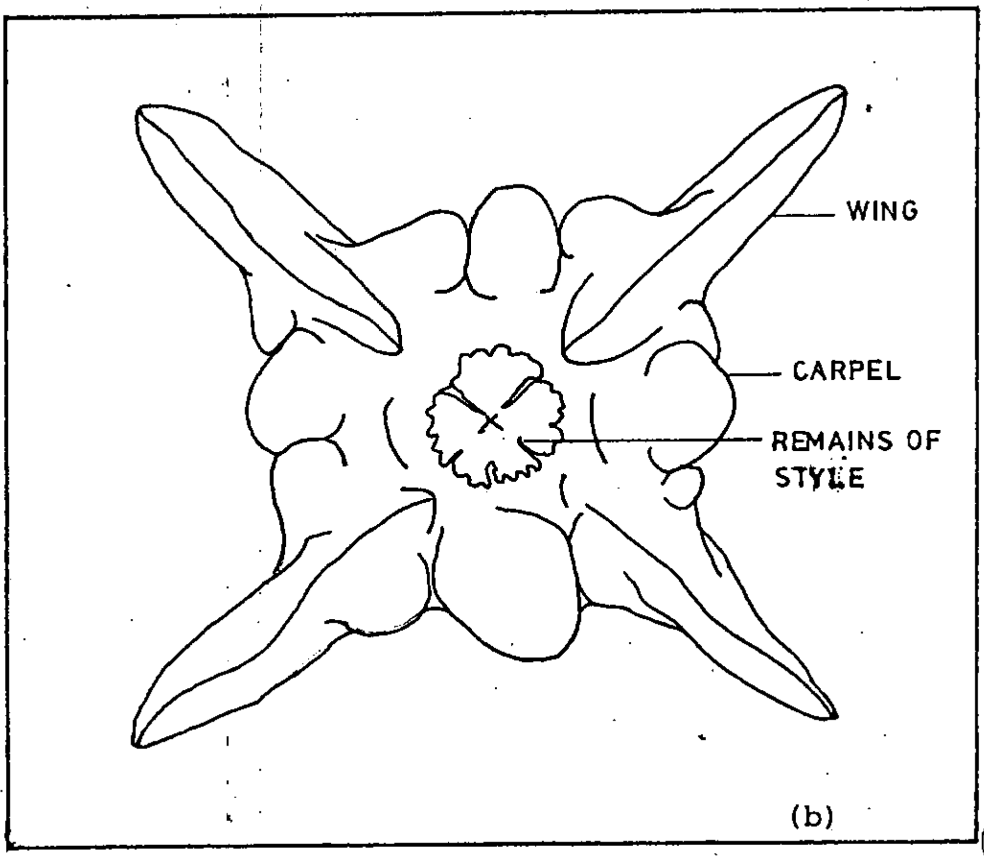
(a) three-seeded fruit and

(b) four-seeded fruit.

mag. x2


WING
CARPEL
REMAINS OF
STYLE
(a)

(a)


WING
CARPEL
REMAINS OF
STYLE
(b)

(b)

TABLE 1

Leaf Epidermal Characters of *T. conophorum*

Location	Epidermal Cell Size min (mean+s.e) max.	No of Cells Per Field	Cell Shape	Wall Pattern	Stomatal Complex: Length min (mean+s.e) max	Stomatal Complex: Width min (mean+s.e) max	No of Stomata per field	Trichomes
Biological Garden Unilag	up. 21.92(28.46±1.07)34.25	58	Irregular	Curved/ Undulate	up -	-	-	Glabrous
	lw. 15.07(27.07±0.89)36.99	67	Irregular	Curved/ Undulate	lw. 10.96(13.70±0.19)15.07	5.48(7.72±0.27)10.96 Paracytic	9	Pubescent
Ikole-Ekiti	up. 23.85(30.19±1.03)36.18	62	Irregular	Curved/ Undulate	up -	-	-	Glabrous
	lw. 17.71(29.48±0.91)38.71	70	Irregular	"	lw. 11.01(13.95±0.18)15.18	5.73(8.12±0.31)11.48 Paracytic	10	Pubescent
Ikenne	up. 22.97(29.28±1.02)34.97	57	Irregular	Curved/ Undulate	up. -	-	-	Glabrous
	lw. 16.09(28.25±1.01)37.51	69	Irregular	"	lw. 10.98(13.78±0.20)15.11	5.65(8.03±0.29)11.25 Paracytic	9	Pubescent

Sample size = 30

Fig 7: Leaf epidermal features of *T. conophorum*. Mag x400

A - Upper epidermis showing curved anticlinal walls.

B - Lower epidermis showing paracytic stomata and anticlinal walls.

Plate 1 Biserrate, tapered and pointed hair found on the abaxial surface of the leaf of *T. conophorum* Mag. x 1,0000.—

Plate 2 Venation pattern of the leaf of *T. conophorum*. Mag. x 400

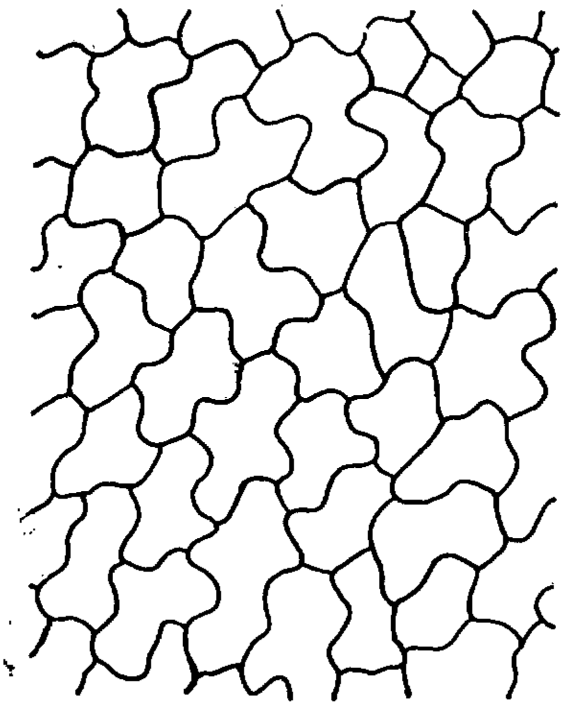


Fig 7(A)

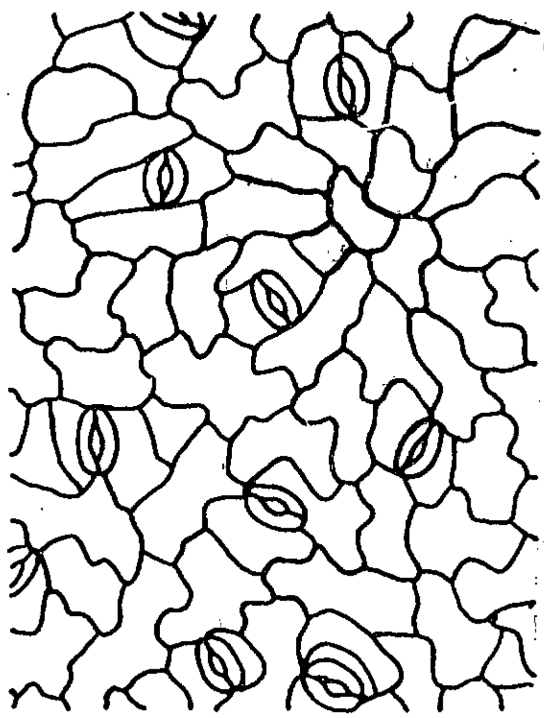


Fig 7(B)

VENATION PATTERN

Leaves are pinnate (single primary vein runs through the lamina), camptodronous - reticulodromous. The veins do not terminate at the margin (camptodromous) and the secondary veins lose their identity towards the leaf margin by repeated branching into a vein reticulum (reticulodromous). Course of the primary vein is straight. The angle of divergence is at right angle while the variation in the angle is uniform. The course of the secondary vein is uniform while the inter-secondary loop is composite (Plate 2). The tertiary vein pattern is random reticulate, that is the tertiary veins anastomosing with other tertiary veins or with the secondary veins, and the angles of anastomoses vary.

The highest vein order—of the leaf is 6^{o} while the highest vein with branching is 5^{o}. The development of areoles is the incompletely closed meshes in which case one or more sides of the mesh is not bounded by a vein, giving rise to anomalously large meshes of highly irregular shape. The arrangement of the areoles is random, the shape is irregular and the veinlets branched once.

ANATOMY

The results of the anatomical study are shown in Plates 3, 4 & 5 and described separately below for each of the organs.

Plate 3 *T. conophorum*, T. s. of root. Mag. x100

Plate 4a *T. conophorum*, T. s. of young stem Mag. x100

Plate 4b *T. conophorum*, T. s. of old stem. Mag.x400

Plate 5 *T. conophorum*, T.s. of petiole showing xylem vessels. mag. x 400.

Plate 3

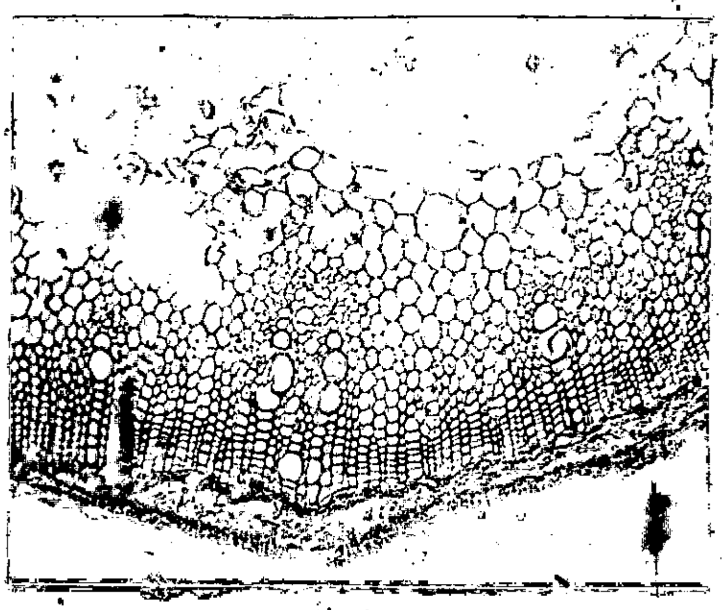


Plate 4a

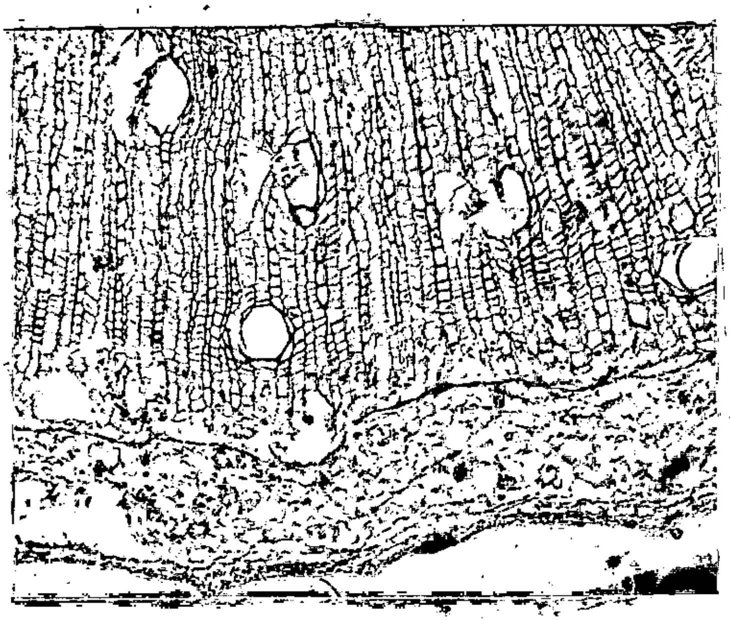


Plate 4b

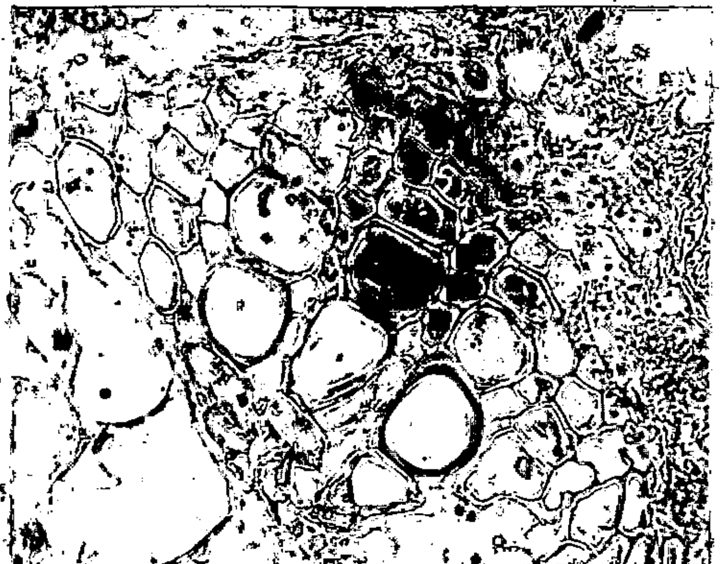


Plate 5.

Root

The piliferous layer consists of a single row of cells which bear numerous root hairs. The cortex is 5-6 rows of cells thick. The cells are rounded or spherical in shape with intercellular spaces. The vascular bundle consists of the xylem and phloem tissues and are both bounded by a thin layer of endodermis (Plate 3). The xylem tissue consists of prominent vessels which are polygonal in shape. The phloem cells lie above the xylem forming a cap on it. The pith consists of parenchymatous cells which are spherical in shape. They are loosely packed with numerous intercellular airspaces between them. It comprises the major part of the inner cylinder.

Stem

The epidermis is made up of a single row of cells which are rectangular in shape. The cortex is 8-10 rows of cells thick. The cells are irregular, polygonal or spherical in shape with no air spaces. The endodermis is made up of thin cells and is only a layer thick. It bounds the inner cylinder or stele. The vascular bundle is concentric and closed with the xylem at the central position (amphivasal). The phloem is made up of 4-6 rows of cells that continues with the xylem cells without any line of demarcation. The xylem vessels are very prominent and are polygonal in shape. The pith consists of parenchymatous cells which are rounded or spherical in shape (Plates 4a & b).

Petiole

The epidermis consists of a single row of cells which are isodiametric or rectangular in shape. The epidermis is glabrous. The cortex is 2-3 rows of cells thick with intercellular air spaces between them. The vascular bundles are usually 4-6 in number, and restricted to the peripheral region of the petiole. The vascular bundle is collateral and closed. The xylem consisting predominantly of vessels, which are spherical or polygonal in shape (Plate 5). The pith occupies major part of the petiole and comprises of parenchymatous cells which are rounded or spherical, and loosely packed together.

Leaf

The upper epidermis consists of a single layer of cells with a thick cuticle. The cells are rectangular in shape without stomata. Similarly, the lower epidermis has a single layer of rectangular cells interspersed with numerous stomata. The palisade mesophyll consists two layers of elongated cylindrical cells that are loosely packed and at right angles to the epidermis. The spongy mesophyll consists of irregularly shaped cells, scattered on the ground tissue. The vascular bundle consists mainly of the xylem and phloem tissues. The xylem vessels which are spherical or polygonal is shape made up the major part of the bundle, while the phloem formed a semi-circle round it.

Vegetative propagation studies

After repeating the experiment three times, no rootings were observed on both the seedlings and stem cuttings experiments. So far this has not been positive but experiments will continue to be performed to see if this species can be propagated vegetatively.

Phenology

T. conophorum grows from seed to maturity i.e. flowering/fruiting stage, in 5-6 years. In subsequent years flowering and fruiting occur independently usually annually, as previously observed independently by Omoleye and Adeilo. However, flowering may not occur in a particular year only to resume the subsequent season. This was confirmed by field visits to the two populations studied. There was no flowering in the year 1990 (i.e. late 1989/1990) at both the Ikenne and Ikole populations.

Flowering occurs from late November to early January. Sometimes late flowering can occur e.g. from December 1988 to early February 1989. From middle to late February all the male flowers would have died or dropped as they are deciduous, leaving the females that have been pollinated at the base of the inflorescence. These later develop into fruits. All plants observed are pollinated by small insects such as ants which are commonly seen around the flowers.

Fruiting occurs from February till September. This period falls within the raining season when most crops are planted. During this period the soil is wet or moist and some of the fruits that fall are buried by litter and eventually germinate.

The distribution of the 1,2,3,& 4 seeded fruits were studied for 2 years on two plants in the Ikole-Ekiti populations and the shell/seed ratio for 50 seeds was also calculated. The results show that there was a proponderance of the four-seeded fruits which constituted about 50% of the total fruits produced each year. The three-seeded fruits constituted about 40% of the total fruits set. The two-seeded fruits constituted about 5-10% and the least was the one seeded fruits which constituted about 1%.

Tetracarpidium _conophorum_ percentage seed set

1st _T_ _conophorum_ climber plant _Number of fruits_

	1st yr.	%total	2nd yr	% total
Four seeded fruits	97	48.5	104	50.0
Three " "	85	42.5	83	39.9
Two " "	17	8.5	20	9.6
One " "	1	0.5	1	0.5
	200	100.0	208	100.0

Tetracarpidium _conophorum_ percentage seed set
2nd _T_ _conophorum_ climber plant _Number of fruits_

	1st yr	%total	2nd yr	% total
Four seeded fruits	89	54.9	79	53.7
Three " "	61	37.7	60	40.8
Two " "	10	6.2	6	4.1
One " "	2	1.2	2	1.4
	162	100.0	147	100.0

The shell/seed ratio calculated for 50 seeds was 1.16 ± 0.002.

DISCUSSION

The observation that the first pair of true leaves are opposite on germination is being reported for the first time in _T_. _conophorum_. All other leaves produced subsequently are alternately arranged. Generally in the family Euphorbiaceae the leaves are alternate and rarely opposite, except in _Mallotus_ _oppositifolius_ where the leaves are opposite (Olorode, 1984).

In the family Euphorbiaceae the flowers are usually small, unisexual and mostly monoecious though some are dioecious. In _T_. _conophorum_ the flowers are small, unisexual and monoecious.

The results obtained for the anatomy reveal a number of interesting features. The species is mostly confined to the rain-forest with relatively high mean annual rainfall and high humidity. The very slight variations in epidermal cell size, number of cells per field and stomatal complex within the 3 populations are not wide enough to justify differences within the taxa. Rather, it substantiates the fact that there is a single species in Nigeria (Hutchinson & Dalziel, 1954). This fact is further substantiated by the constancy of other characters such as the cell shape, anticlinal wall pattern and the distribution of the trichomes on both surfaces. The presence of curved or undulate wall in the taxa is in conformity with the suggestion of Stace (1965) who stated that this character is a mesomorphic character. In this respect also these characters agreed with the work of Metcalfe and Chalk (1979) that suggested irregular or polygonal shape, and curved or undulate, rarely straight for members of the family Euphorbiaceae.

The presence of veins that are pinnate, camptodromous reticulodromous with primary veins that are straight makes the taxa closely related to the type Genus Euphorbia of the same family as these characters were also recorded by Daramola (1991). However differences occur in other characters such as the angle of divergence of the secondary veins that is acute in most species of Euphorbia but at right angle in T. conophorum. Although, right-angled secondary veins were also recorded in E. hyssopifolia and E. convolvuloides making the taxa closely related to these species. Also, it resembles members of Euphorbia by having the tertiary veins that are random reticulate, which more than half of the species possessed (Daramola, 1991).

The appearance of cork in the epidermis is a characteristic feature of some members of the family Euphorbiaceae. In respect of this, the taxa resembles Acalypha. The xylem is in form of a continuous cylinder transversed by narrow rays in all genera of Euphorbiaceae. However, the vessels of T. conophorum have simple perforations and scalariform plates making it similar to species of Drypetes and Mercurialis (Metcalfe and Chalk, 1979).

The fact that the male flowers withered after pollination is not suprising as this type of behaviour had been observed in some other angiosperms e.g. _Citrullus lanatus_, _Curcurbita maxima_, _Cucumis sativus_, _Momordica charantia_ and _Trichosanthes cucumerina_. This may thus be a genetic factor in the plants that such occur.

The late flowering that occured in 1990 could be due to a number of factors. It is known that _T. conophorum_ occurs in the evergreen of the rainforest, this therefore means that if there is drought and the species gets less water, its flowering and fruiting will be affected i.e. could be delayed or there may be no flowering and fruiting for that year.

Other factors that could be responsible for reproductive failure may include other physiological phenomena such as

(i) Gametophytic isolation - in which case cross pollination takes place, but the pollen tube fails to germinate or fails to reach and penetrate the embryo sac of the female parent.

(ii) Gametic isolation - In this case the pollen tube releases the male gametes into the embryo sac, but gametic fusion and or endospermic fusion does not take place.

(iii) Seed incompatibility - The zygote or immature embryo ceases development, so that a mature seed is not formed. (Stace, 1980).

Generally as the name suggests, this species is expected to be four seeded. The occurence of 3, 2 and 1 seeded fruits therefore is as a result of 1, 2 or 3 ovules being aborted during the development of the fruit. Curiously enough, the local people in the farm has a myth surrounding this observation. It is that, if one picks the fruit with either 3 or 2 fingers or 1 finger before sowing the seeds then after germination and growth, the plant will give rise to seeds/fruit corresponding to the number of finger used to pick the fruit.

The study of the biology of a species involves a lot of areas, all of which cannot be covered in this thesis. However, in view of the dearth of information on the morphology, anatomy and phenology of Nigerian species, this aspect is considered important in this study.

CHAPTER THREE

GERMINATION STUDIES

INTRODUCTION

Germination is the resumption of growth of the embryonic plant in a seed. It is a critical stage in the life of a plant since without it, seedling growth and establishment cannot occur (Linhart, 1976, Okusanya, 1976). Each species has its own characteristic set of germination requirements. The responses shown by seeds to the great variety of conditions to which they are subjected can be regarded as adaptation for maximizing the likelihood of surviving in a patch of unpredictable environment. To this end, the seeds need to be able to recognize the potentially safe sites (Fenner, 1985). Thus, the continued existence of a species in a given habitat is possible if the environmental factor complex of the habitat lies within the limits of the physiological tolerance of the species (Okusanya, 1976).

Seed germination is a complicated process influenced by many factors (Cone and Spruit, 1983). It can be divided into three phases - imbibition, transition and growth. The imbibition phase is a passive movement of water into the seed due to the matric potential of the seed. The transition phase involves the process of rehydration, and 'priming' of biochemical pathways while the growth phase involves the start of cell expansion and/or division (Stumpf et al, 1986.

Germination responses to factors of the environment such as moisture, light and temperature vary among and within populations of plant species (Edwards, 1975). Since water is one of the major factors necessary for germination, seeds of many species have evolved ways to prevent desiccation of the embryo in response to the moisture regime of the habitat. Harper and Benton (1966) have shown that the failure of some plant species to germinate was the result of delayed or insufficient hydration of the seeds - a condition brought about by the level of water table in the soil. Water uptake and available water have been shown to be two important aspects of moisture requirements of many plant species (Lazenby, 1955, Toole et al, 1956).

The germination of viable, non-dormant seeds is generally known to be high when water is freely available and seeds which are characteristic of plants from a range of moisture regimes usually all show optimal germination in very moist soils (Bannister, 1976). As such, failure of water present to reach the embryo may result in seed dormancy as in such seeds that posses hard or impervious or glossy testa etc. The seeds of T. conophorum do have hard shells and are also known to be fairly dormant. Also, since this species occurs in moist tropical rainforest and its seeds have hard shells, moisture might be a factor controlling its germination. An experiment was therefore set up to determine the effects of different soil moisture regimes on the seed germination of the species.

In the field, seeds may become buried in wind-borne sand, leaf litter etc. and the depth of burial might influence their germination and seedling emergence (Pemadasa and Lovell, 1975). T. conophorum being a climber, usually drops its fruits under the climbed trees whose leaves and twigs fall on and cover the seeds. Some seeds may even be carried by squirrels into their holes while others may be dispersed to other parts of the forest. As such, depth of burial is a significant aspect of the germination behaviour of this species. Consequently, an investigation was carried out to determine the effect of depth of seed burial on the germination of the species.

Light plays a vital role in the germination of seeds and survival of plants. When seeds are dispersed, they are either half or completely buried in soil or they lie on the surface and are covered with litter. They could also occupy cracks or crevices in the soil or they could be completely exposed on the rock surface thus obtaining different amounts of light (Cavers and Harper, 1967, Edwards, 1975). Light often has pronounced effects on germination; it may either inhibit or promote (Kolier et al, 1962). Since the responses of seeds to light are related to the red: far-red phytochrome reaction; the red light in normal daylight induces the production of a phytochrome (Pf) sensitive to far-red light. Prolonged exposure to far-red light may inhibit germination because of the conversion of phytochrome to the inactive germination - inhibiting form in the dark.

It was therefore necessary to determine the effect of light and dark regimes on germination of the seeds of the species.

The soil may be defined as the material in which plants root and from which they draw their water supplies and essential nutrients (Russel, 1968). The detailed microtopography of the surface of the soil determines the density of safe-sites within a gap and so is an important feature in regulating regeneration. The effect which the microrelief has on germination is largely controlled by the amount of water content which exists between the seed and the soil surface, and on the tension with which water is held in the soil (Fenner, 1985).

Seeds often possess highly specific germination requirements and when dispersed onto soil surfaces, the number and proportion of species establishing may be determined by the sort of microenvironment in which each seed lands (Harper, Williams and Sagar, 1965). Some of the common soil types in Nigeria are sand, gravels, red earth, loam and clay soils with significant differences in physical and chemical composition (Table 2). The effects of four soil types namely humic soil, red earth, sand and clay and various pH regimes on the germination of T. conophorum were therefore investigated.

Table 2

The analytical data of the soils used in the various germination and growth experiments

Soil Types	pH	Organic matter (g/100g dry soil)	Soil moisture (g/100g dry soil)	K^+ (me equiv/100g dry soil)	Na^+ (me equiv/100g dry soil)	Ca^{2+} (me equiv/100g dry soil)	Mg^{2+} (me equiv/100g dry soil)	Total Nitrogen (%)	Chloride ppm
Humus	7.8±0.1	19.±1.5	22.1±0.4	9.24	2.10	29.62	8.16	0.141	3×10^{-5}
Red earth	5.6±0.2	16.2±0.9	8.7±0.3	0.00	2.30	0.00	2.80	0.040	4×10^{-5}
Clay	5.7±0.4	15.5±0.5	30.4±0.3	0.51	2.60	0.25	3.6	0.050	4×10^{-5}
Sand	6.9±0.05	13.7±0.5	15.9±0.6	2.04	2.40	0.77	2.94	0.189	2×10^{-5}

Temperature is another factor which plays an important role in controlling germination. It has two major influences upon germination. The first is a pretreatment which allows dormancy to be broken while the second is a direct influence upon the rate and amount of germination (Bannister, 1976). The minimum and maximum temperatures at which germination can occur vary considerably from one species to another. The temperature in the tropics is high while it is relatively lower and fairly constant in the forest. As the seeds of <u>T. conophorum</u> may be dispersed to open areas in the forest, while others are under the tree canopy or buried under litter, the effect of temperature on germination was therefore investigated.

The germination of seeds may be affected by salinity through osmotic or specific ion toxicity or both (Uhvits, 1946). These effects may slow down or completely inhibit germination depending on the level of salt in the growth medium (Ayers & Hayward, 1948). The precise salinity concentration causing a delay and reduction in the number of seed germination depends upon the salt tolerance of each individual species. Though forest soils are usually low in salinity, less than 0.5% sea water (Okusanya <u>et al</u>, 1991), an experiment was set up to determine the effect of salinity on the germination of the seeds of <u>T. conophorum</u>.

In all, the germination responses of <u>T. Conophorum</u> to (i) soil moisture, (ii) soil depth, (iii) light and dark regimes (iv) soil types, (v) pH, (vi) temperature and (vii) salinity were investigated and reported in this chapter.

Physiologically, a seed which does not germinate when provided with adequate water, sufficient oxygen for normal aerobic metabolism, and surrounding temperature within physiological limits is said to be dormant (Berrie, 1984). Dormancy may be defined as a state in which growth is temporarily suspended (Mayer & Poijakon-Maber, 1963). It is also a delaying mechanism which prevents germination under conditions which might prove to be unsuitable for seedling establishment (Fenner, 1985). This delay may last for a variable period of time under constant conditions and in some cases may continue indefinitely until some conditions are fulfilled; i.e. the embryo may need to undergo physiological and in some cases morphological changes before germination occurs (Stokes, 1965, Villiers, 1972). And as long as the seed remains viable, the possibility exists that it may eventually find itself more favourably placed to germinate (Fenner, 1985).

Three types of dormancy have been recognized, they are innate, enforced and induced (Harper, 1977). Innate or primary dormancy is caused by the genetical constitution of the seed. A seed that is innately dormant will not germinate even under conditions suitable for germination until a specific period after it has been dispersed from the parent plant. This inability to germinate may be due in certain species to the embryo being immature at the time of dispersal; or in other cases the seed possesses hard or glossy testa which prevents the entry of water or oxygen.

In enforced or imposed dormancy, seeds do not germinate because they are being deprived of their requirements for germination, for example, by the absence of sufficient moisture, oxygen, light or a suitable temperature. There is no special physiological mechanism being involved and the seeds may be properly considered mere quiescent e.g. seeds lying deep in the soil are probably prevented from germination by lack of oxygen or adequate temperature. Induced or secondary dormancy is a condition caused by the external factors to which the seeds are exposed, like failing to meet suitable conditions for germination. For example, Wesson and Wareing (1969) found that freshly collected seeds of a range of herbaceous species germinate readily in both light and dark, but seeds of the same species which had been buried in the soil for sometime would only germinate in the presence of light.

Depending on the type of dormancy, various methods could be used in the laboratories and in nature to overcome it. The breaking of dormancy does not in itself constitute germination, but is a necessary prerequisite of it. Thus, a seed may need to experience some environmental conditions which act as a trigger for germination, but which would be quite unsuitable for germination as such.

Preliminary experiments show that the seeds of _T. conophorum_ may be fairly dormant. Consequently, some experiments were carried out to attempt to break the dormancy of its seeds and achieve faster germination.

MATERIALS AND METHODS

The fruits of T. conophorum which had fallen from the mother plant were collected from a population in a farm at Ikenne-Remo, Ogun State of Nigeria. The seeds were extracted from the decaying pulp by hand before being used.

1. Experiments to break dormancy

Under natural conditions and in the preliminary laboratory work, the seeds of T. conophorum first germinated between 7 and 8 weeks. Thus experiments to break the dormancy or hasten germination were carried out so as to reduce the time of germination. The experiments to break dormancy involves both physical and chemical treatment. In each treatment,there were 4 replicates of 25 seeds. For each of the chemical treatment, the seeds were soaked for the desired period after which the seeds were washed thoroughly several times in tap water before being tested for germination.

The seeds for each of the treatment were sown in humic soil (2.5cm deep) which completely covered the seeds in seed trays (Pandeya et al, 1969). Each experiment lasted for 10 weeks.

Table 3: Effect of various methods of scarification on the germination of Tetracarpidium conophorum ± SD

Treatment	Period of germination (weeks)									
	3	4	5	6	7	8	9	10	11	12
1% $CuSO_4$ for 1-3 hr.	0	0	45 ±2.5	55 ±2.0	65 ±2.5	70 ±4.0	75 ±3.0	75 ±3.0	75 ±3.0	75 ±3.0
" " 4 hr.	0	50 ±2.0	55 ±2.5	70 ±2.0	75 ±3.0	80 ±2.5	85 ±2.5	85 ±2.5	85 ±2.5	85 ±2.5
" " 5 hr.	50 ±2.0	55 ±2.5	60 ±3.0	75 ±2.5	80 ±3.0	85 ±3.0	85 ±3.0	85 ±3.0	85 ±3.0	85 ±3.0
Rubbing testa with iron sponge (mechanical scarification)	0	0	0	40 ±3.0	45 ±3.0	55 ±2.8	60 ±2.5	70 ±3.0	70 ±3.0	70 ±3.0
10-100% Conc. H_2SO_4 for 1-60min.	0	0	0	0	0	0	0	0	0	0
No scarification (Control)	0	0	0	0	40 ±2.0	50 ±2.5	60 ±3.0	70 ±3.0	70 ±3.0	70 ±3.0

(i) Physical treatment of seeds:

Mechanical scarification - The seeds were scarified with iron sponge to rub off the outer fibers, cleaned and then germinated.

(ii) Chemical treatment

(a) Seeds were soaked in concentrated sulphuric acid (Conc. H_2SO_4) of various dilutions (10 - 100%) and for different periods (one to 60 minutes). The seeds were washed as described earlier and then tested for germination.

(b) Seeds were soaked in 1% copper sulphate ($CuSO_4$) solution for 1, 2, 3, 4 or 5 hours (Pandeya et al, 1969).

II. Effect of Environmental Factors On Germination

The seeds which were mechanically scarified and then treated with 1% copper sulphate solution for 5 hours (from the results of the scarification experiments (Table 3) were subjected to the effects of some environmental factors viz: soil types, soil moisture contents, light and dark regimes, soil depth, temperature, pH and salinity. For each treatment, there were five replicates of twenty seeds in the sowing trays. Each experiment lasted for 10 weeks during which time germination usually levelled off. Watering was done once a day except in the soil moisture and salinity experiments. The time of first germination and the percent germination were recorded. The comparisons of the germination percentage were done using the analysis of variance (ANOVA) as outlined by Clarke, (1980). All experiments except the light and dark effect and temperature ranges, took place in the glasshouse of the biological garden of the University of Lagos. In the biological garden, light was from sunlight with a photoperiod of 12 + 1hr and the temperature averaged 32 + 2°C (day) and 25 + 2°C (night). Relative humidity was about 85% at 0900hrs.

The seeds in the appropriate medium were buried in (2.5cm deep) so that the soil just covered the seeds.

<u>Effect of Light and Dark</u>:- Humic soil was used to sow seeds for this experiment. For the dark effect, the trays containing the seeds were placed in dark cupboards in the laboratory. They remained in the dark throughout the duration of the experiment. Seeds which did not germinate in the dark were later transferred to light to determine whether dark pretreatment had caused permanent inhibition of germination.

Seed trays for the light treatment were supplied with light from six 60 watts fluorescent tubes for 12 hours daily giving a total intensity of about 2,500 lux.

<u>Effect of Soil Moisture Conditions</u>:- Seeds in humic soil in seed trays were subjected to three watering regimes namely (wet treatment) and continuous watering (water-logged). The trays in a plastic bowl with water covering the entire soil

level. Seed trays for the dry and wet treatments had holes at the bottom to facilitate good drainage of water. Dry treatment had 11.6% of soil dry weight, the wet treatment had 24.4% of soil dry weight while the water-logged had 30.2% of soil dry weight.

<u>Effect of Soil Types</u>:- Four soil types designed as humic, red-earth, clay and sand were used. The humic soil was collected from the botanical garden of the University of Lagos and the red-earth was collected at Ilaro road, Papa near Ewekoro in Ogun State. The clay used was from the Access road, University of Lagos while the sand was collected from river Majidun near Ikorodu in Lagos State. The soils were sieved to remove stones and debris before being used. The characteristics of the soils are given in Table 1.

<u>Effect of pH</u>:- One-fifth strength Hoagland and Arnon (1938) solution adjusted to pH 3.5, 5.5 and 7.0 using dilute potassium hydroxide or dilute sulphuric acid and distilled water of pH 7.0 were used. The appropriate solutions were used to wet the seeds in sand in the seed trays.

<u>Effect of Soil Depth</u>:- Seeds were sown at surface, 2.5cm, 5.0cm and 10cm depth in planting trays using humic soil.

Effect of Temperature:- This was done by subjecting the seeds sown in humic soil to the effect of constant temperature regimes of 15, 21, 31, and 41^{o}C in germinating incubators manufactured by Astell Hearson, London. Illumination was provided by four, 15 watt fluorescent tubes giving a light intensity of about 1,500 lux. Lighting in the incubator was regulated to give 12 hours photo-period. Watering was done once a day.

Effect of Salinity:- Sea water concentrations of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% were used. The different concentrations were prepared by mixing different volumes of filtered sea water (salinity 3.2%) collected from the Lagos Bar Beach and one-fifth strength Hoagland and Arnon (1938) solution.

The solutions of the different concentrations were used once a day to wet the seeds in sand soil. Every five days, 1,000 ml of distilled water was used to leach the sand containing the seeds, immediately thereafter, the appropriate sea water solution was then used. This procedure helped to reduce any large scale increase and fluctuation in the various salinities due to evaporation (Okusanya, 1979).

Soil Analysis

In order to know the composition of the soil types used, physical and chemical analysis were carried out.

Three independent determinations were carried out for each sample.

pH: The pH was determined as soon as possible after the collection of the sample usually on the same day. To each sample, distilled water was slowly added and stirred until the soil was a 'thick paste'. The pH was then measured electrometrically by a pH meter (L. Pusl Munchen 15). The values were given to one place of decimal.

Water Content: The soil moisture was determined gravimetrically. Fifty grams of fresh soil was heated at 80°C for 24 hours in an oven, cooled and then reweighed. The results are expressed as weight of water per 100gm dry soil.

Organic Matter: The organic matter content was determined as loss on ignition. The oven dried samples were heated at 375°C for 24 hours in a muffle furnace (carbolite, Sheffield, LMF 3). cooled and weighed. The results are expressed as gram of organic matter per 100gm dry soil.

Total Nitrogen: This was determined by the micro Kjeldahl's method (Black, 1965).

Chloride Determination: Chloride was extracted from air dried soil with distilled water and estimated by electrometic titration with silver nitrate (about 10mE/1). The results are expressed in parts per million.

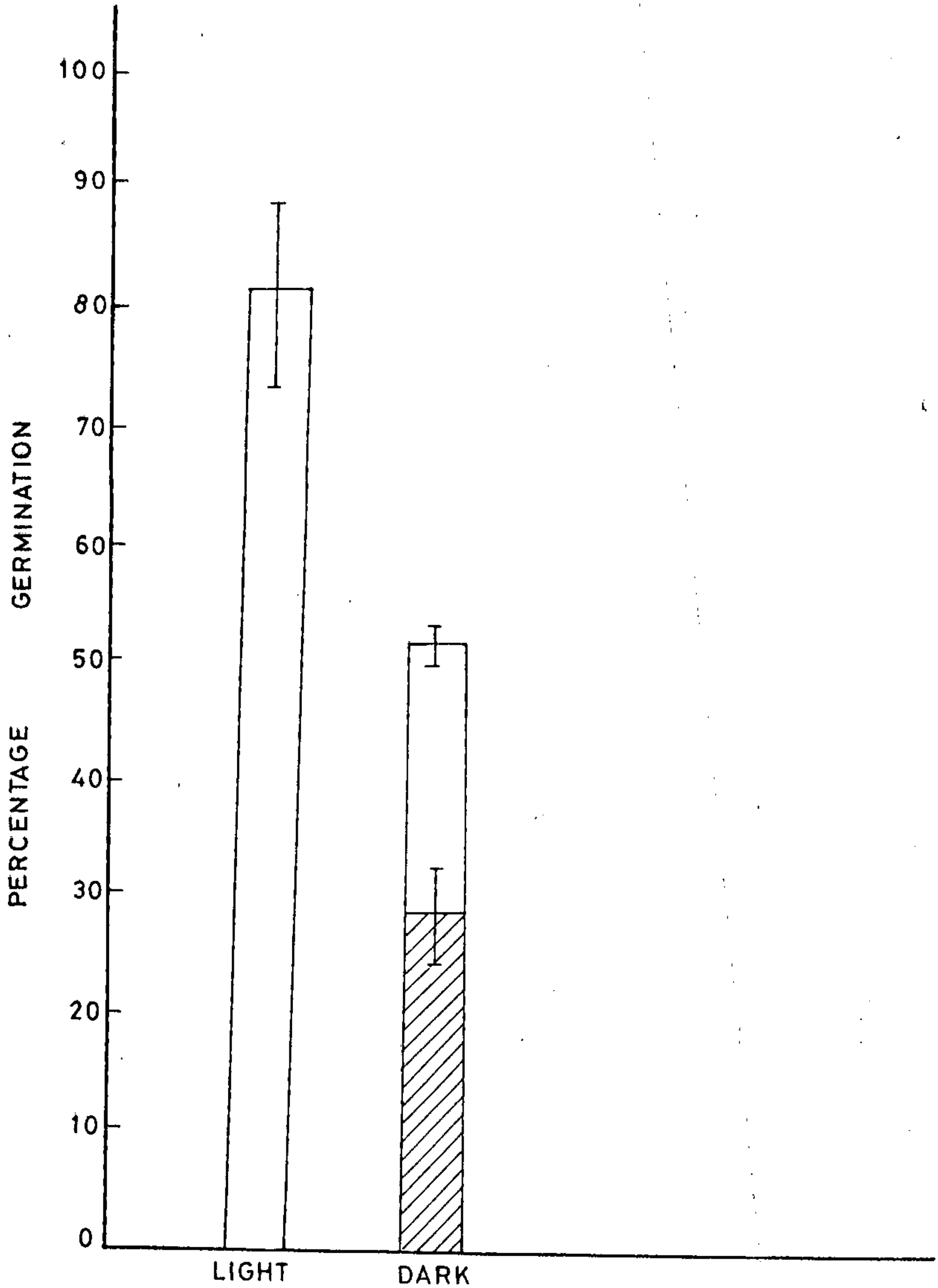
Cation Determination: A known weight of air dried soil was leached with IN neutral ammonium acetate solution. Potassium and sodium were estimated by flame photometry while calcium and magnesium were estimated by atomic absorption spectrophotometry. The results are expressed as milliequivalents of cation per 100gm air dry soil. (Black, 1965, Perkin - Elmer Corp, 1968).

RESULTS

Breaking Dormancy: The results for the dormancy breaking (scarification) experiments are shown in Table 2. When the seeds of T. conophorum were mechanically scarified, they first germinated between 6 and 7 weeks and the final germination was 70% after ten weeks.

There was no germination with the application of various dilutions of concentrated H_2SO_4 for different periods. When seeds were soaked in 1% $CuSO_4$ for 4 - 5 hours, first germination was achieved between 2 1/2 and 4 weeks and total germination was 85%. When treatment was for 1, 2 or 3 hours, first germination occurred at the 5th week and the total germination was 75%. Thus, scarification of seeds with 1% copper sulphate solution for 4-5 hours increased not only the rate of germination, it also reduced the time of first germination from seven weeks in control to about 2 1/2 weeks. Normally, in nature and without scarification the first germination of T. conophorum takes between 7 and 8 weeks. The type of germination in this species is epigeal.

Effect of Light and Dark:- The results of the effect of light and dark regimes on the seed germination are given in Fig. 8. The percentage germination in the light was significantly higher $P<0.001$) than in the dark regime.



PERCENTAGE GERMINATION
100
90
80
70
60
50
40
30
20
10
0
LIGHT
DARK

Fig 8: Germination of Tetracarpidium conphorum in light and in dark. Unhatched area in the dark rnsult represents additional germination when seeds which failed to germinate in the dark were brought to light for six weeks. Bars represent standard deviation (SD).

When seeds which did not germinate in darkness were brought to light, some further germination occurred.

<u>Effect of Soil Moisture</u>:- Fig. 9 shows the results of the effect of soil moisture on germination. There was no germination under the water-logged condition. Germination was first achieved after four weeks under the dry condition as against 2 1/2 weeks in the wet condition. The percentage germination in the dry condition was significantly lower ($p<0.001$) than in the wet condition.

<u>Effect of Soil Types</u>:- Germination was best in humic soil and least in the sand with that in clay and red-earth being in the middle (Fig. 10). There was no significant difference ($p > 0.05$) in the percentage germination between red-earth and clay soil. Germination percentage in sand was significantly lower than in the other three soil types, ($p<0.001$) for humic soil and red-earth and ($p < 0.05$) for clay soil. There was also significant difference in percentage germination in humic soil when compared with the red earth ($p < 0.01$) and clay soil ($p < 0.001$).

<u>Effect of pH</u>:- Fig. 11 shows the response of the germinating seeds to different pH values. It was observed that the seeds germinated better as the pH of the medium increased. Germination was significantly inhibited by acidity as there was no germination at pH 3.5.

Fig 9: Germination of *T. conophorum* under various soil moisture conditions. Bars represent SD. There was no germination in water-logged condition.

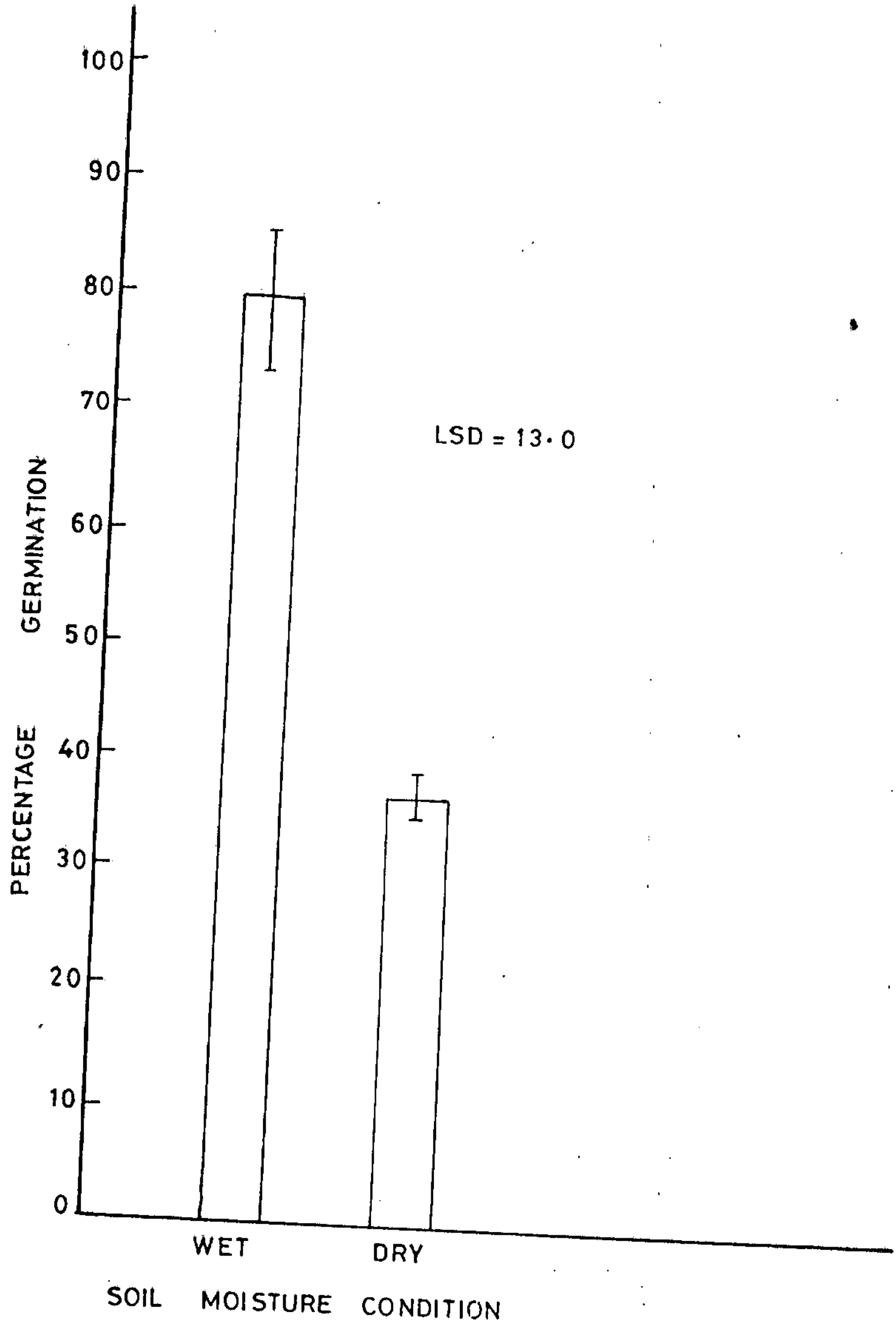
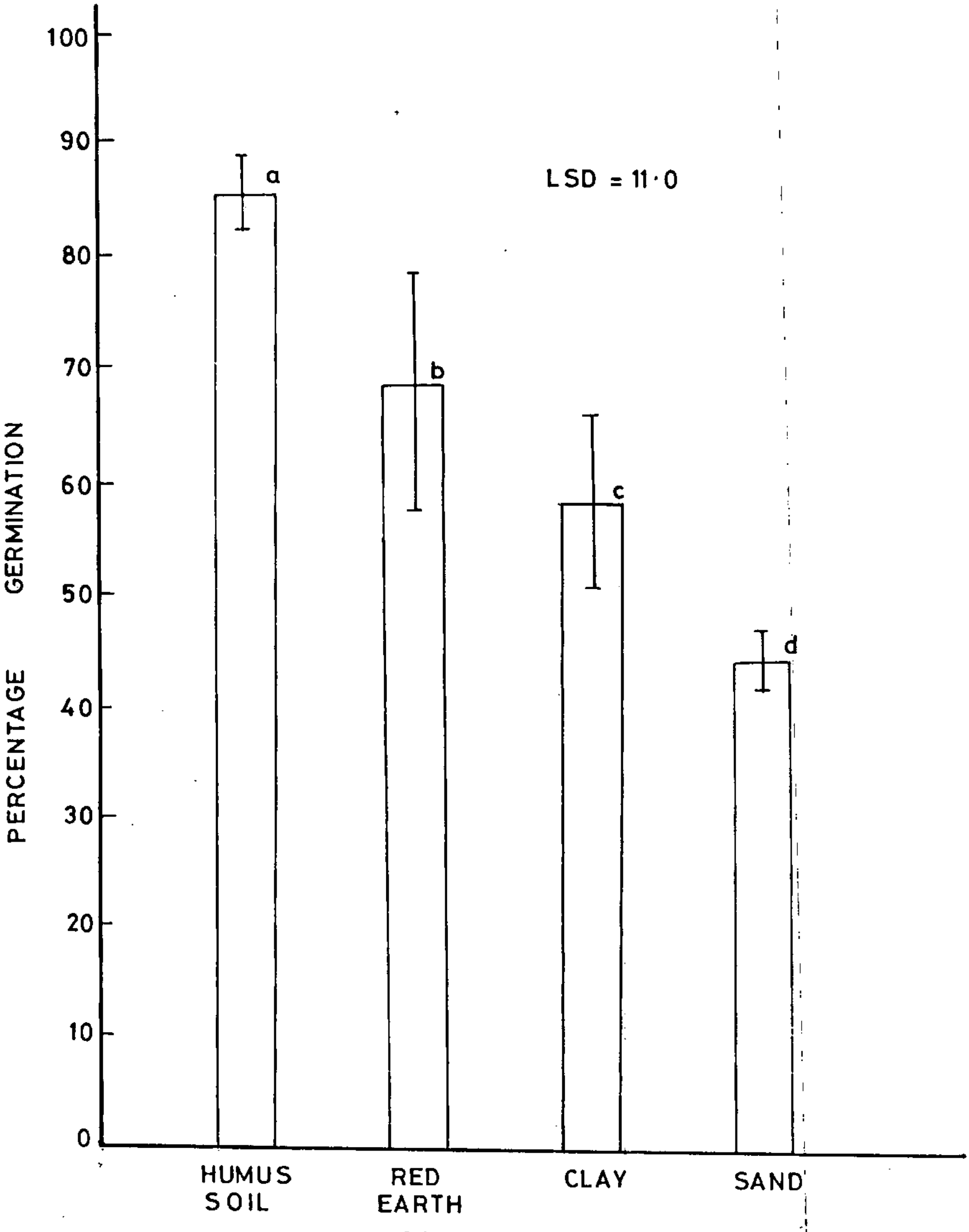

100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
LSD = 13·0
WET
DRY
SOIL MOISTURE CONDITION

Fig 10: The effect of soil types on the germination of _T_. _conophorum_. Values with the same letters are not significantly different at the 5% probability level. Bars represent SD.


LSD = 11·0
a
b
c
d
100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
HUMUS SOIL
RED EARTH
CLAY
SAND
SOIL TYPES

The highest percentage germination occurred in Hoagland's solution of pH 7.0 and this was significantly higher ($P < 0.001$) than in pH 5.5 and distilled water, while the percentage germination in distilled waster was also significantly higher ($p < 0.05$) than that of pH 5.5. First germination in pH 7.0 occurred in 2 1/2 weeks while germination was not achieved until the 4th week in pH 5.5 and distilled water.

Effect of Soil Depth:- There was an increase in percentage germination as soil depth increased up to 2.,5cm, thereafter, germination decreased (Fig. 12). Germination at soil surface was significantly lower ($p < 0.001$) than that at 2.5cm soil depth.

Percentage germination at 2.5cm soil depth was significantly higher ($p < 0.001$) than at 5cm soil depth and at 10cm soil depth. Likewise there was significant difference ($p < 0.05$) in the percentage germination of surface sown seeds and those sown at 10cm soil depth ($p < 0.001$).

Effect of Temperature:- The seeds responded differently to the effect of the various temperature regimes. No germination occurred at 15°C and there was increase in germination as the temperature increases up to 31°C, thereafter there was a decrease (Fig. 13).

Fig 11: Effect of pH on the germination of _T. conophorum_. Values with the same letters are not significantly different at the 5% probability level. Bars represent SD. There was no germination at pH 3.5

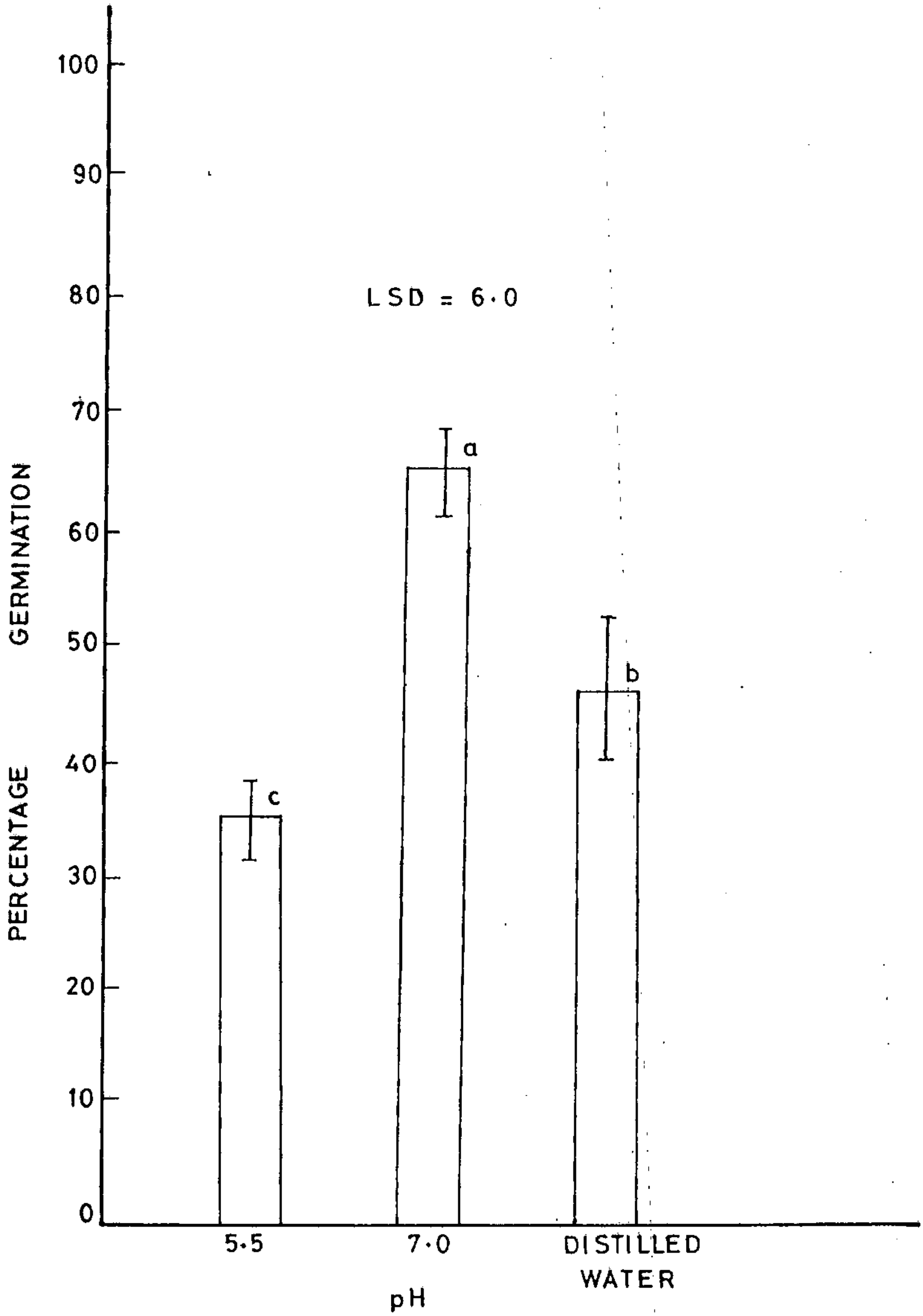
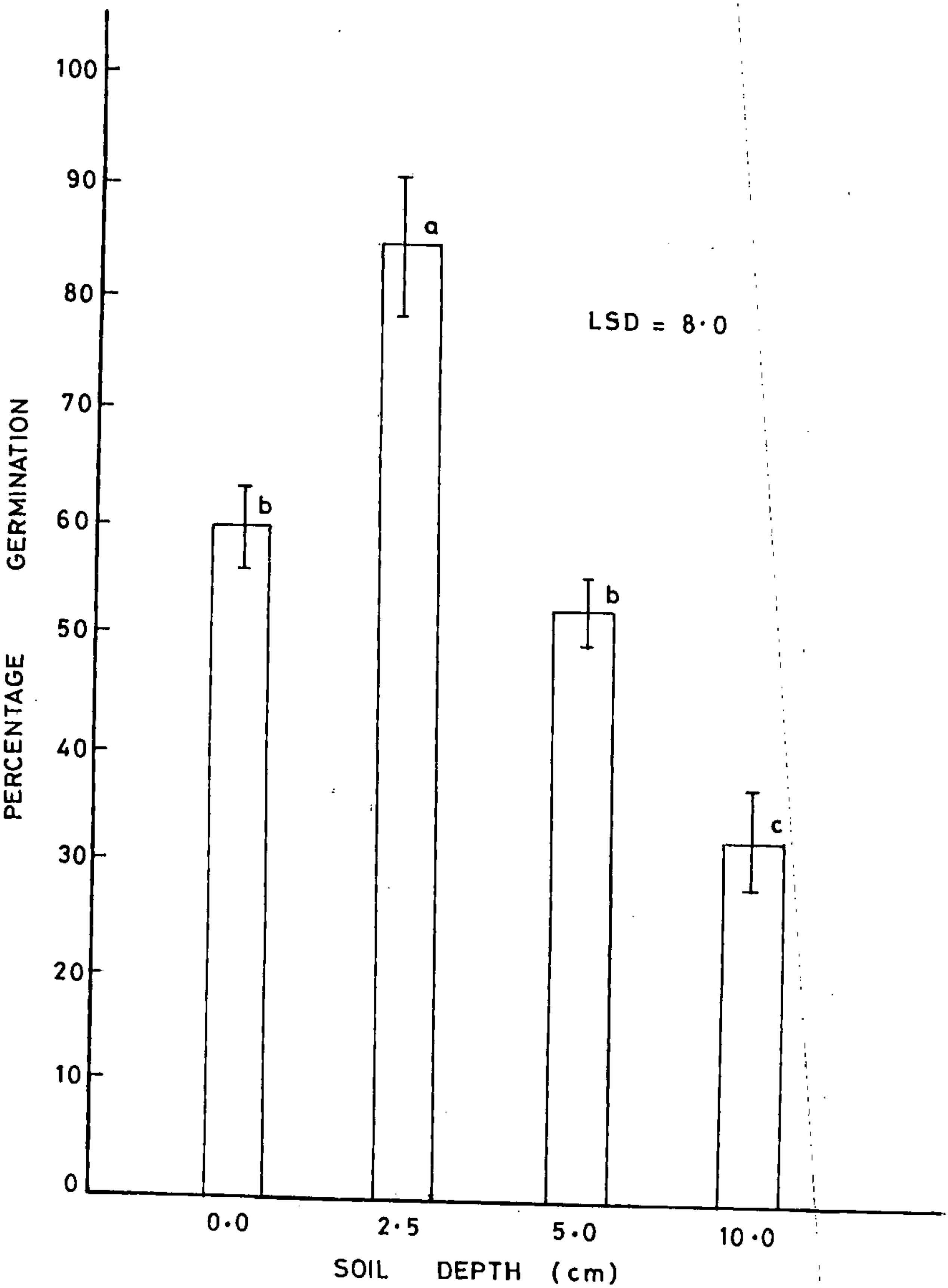

100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
LSD = 6·0
a
b
c
5·5
7·0
DISTILLED WATER
pH

Fig. 12: Germination of _T_. _conophorum_ as affected by depth of sowing. Values with the same letters are not significantly different at the 5% probability level. Bars represent standard deviation.



100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
a
b
b
c
LSD = 8·0
0·0
2·5
5·0
10·0
SOIL DEPTH (cm)

The germination percentage at 31°C was significantly higher ($P < 0.001$) than at 21°C and at 41°C. Also the percentage germination at 21 °C was significantly higher ($p < 0.001$) than at 41°C.

<u>Effect of Salinity</u>:- Fig. 14 shows the result of the effect of salinity on germination. Salinity caused a loss in germination at concentration of 10% sea water and above. There was no germination above 30% sea water concentration. Germination percentage in 0% salinity was significantly higher ($P < 0.001$) than at 10%, 20% and 30% sea water. When seeds which had been at high salinity were returned to distilled water, there was no further germination.

Fig 13: Germination of _T. conophorum_ at various constant temperatures. Bars represent SD.

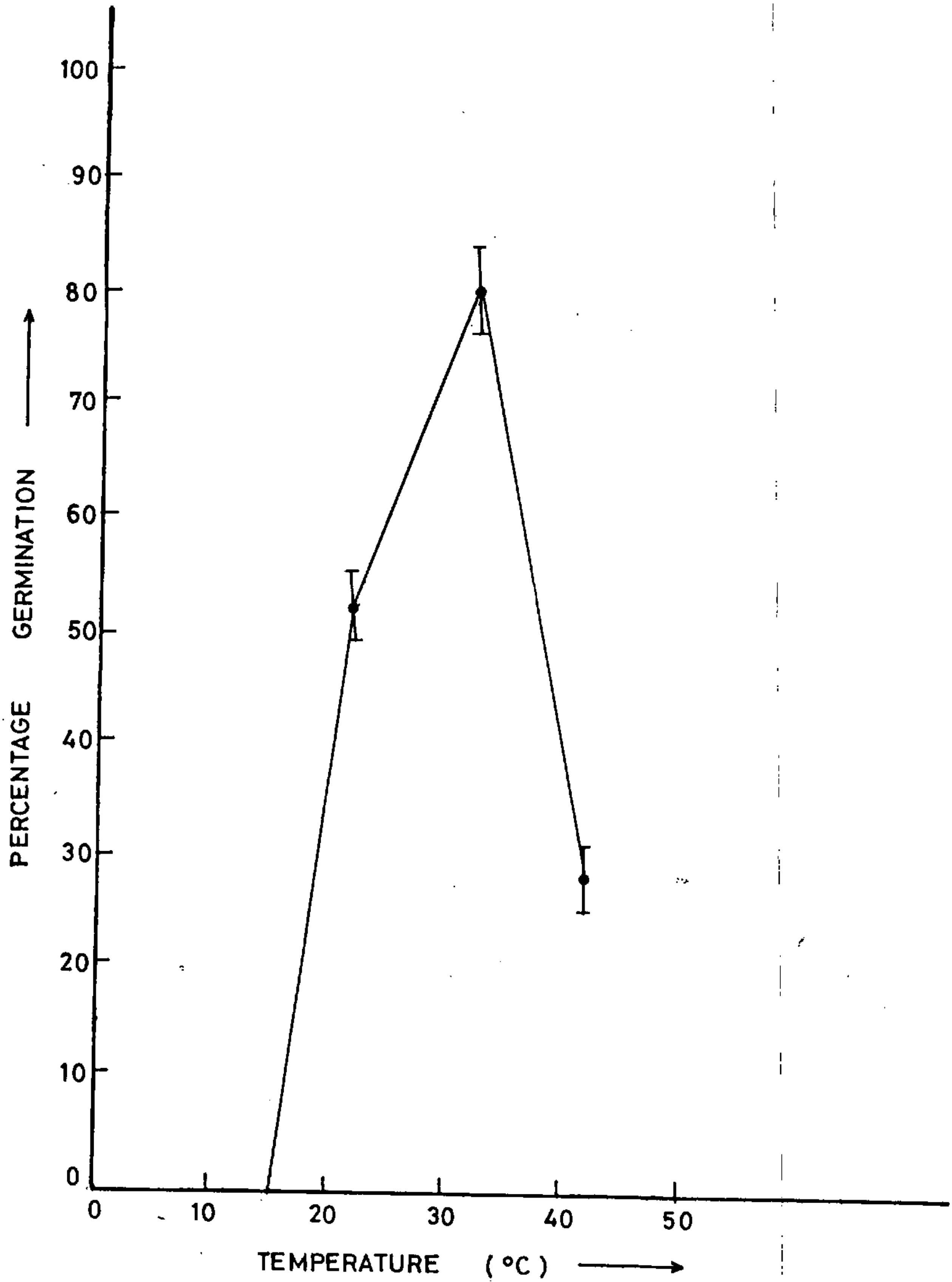
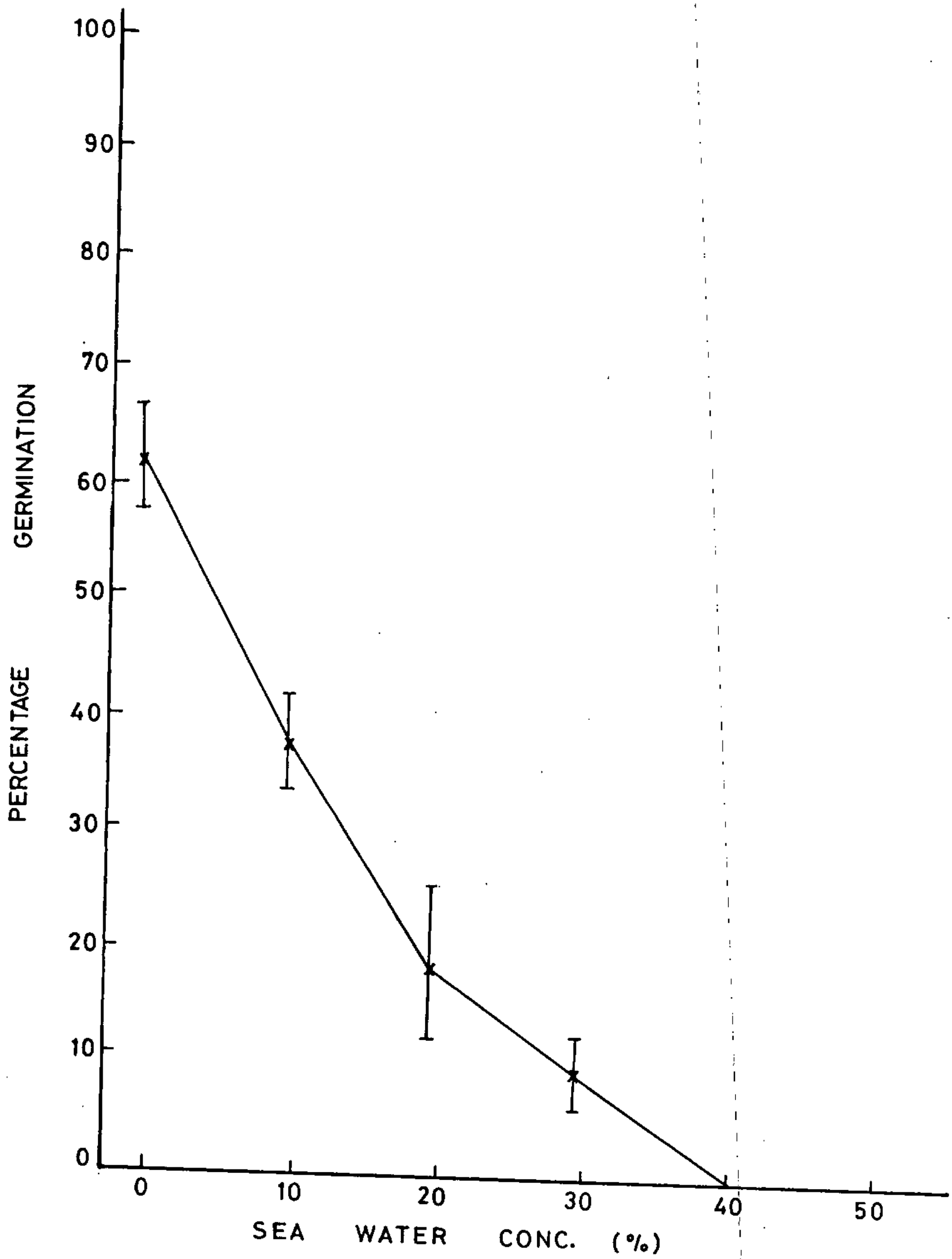

100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
0
10
20
30
40
50
TEMPERATURE (°C)

Fig 14: Germination of _T. conophorum_ at various sea water concentrations. Bars represent S.E.


100
90
80
70
60
50
40
30
20
10
0
PERCENTAGE GERMINATION
0
10
20
30
40
50
SEA WATER CONC. (%)

DISCUSSION

In this study, the result of the mechanical and chemical treatment carried out to break seed dormancy in T. conophorum showed that dormancy can be broken and germination achieved at a faster rate by using 1% $CuSO_4$ solution with or without the mechanical treatment (Table 3). Dormancy in the seeds is possibly due to the hard testa of the species. As such the dormancy breaking action of 1% $CuSO_4$ solution could therefore be related to the weakening of the hard shell which led to an increase in the permeability of the seeds to water and probably air.

The lack of response to the effect of mechanical scarification attests to the hard nature of the testa. It is believed that the impermeable seed coats adapt species very will to tropical climates in which permeable seeds degenerate rapidly leading to massive loss in seedling production (Garrad, 1955).

Although it is known that the presence of inhibitors in the seed coat of some species can be eliminated by soaking the seeds in dilute sulphuric acid (Copeland, 1976), in this experiment with T. conophorum, no germination occurred with different dilutions of concentrated H_2SO_4. This shows that the species is very sensitive to acidity and/or dehydration since concentrated sulphuric acid is both a strong acid and a dehydrating agent. The lack of germination at pH 3.5 (Fig 11) supports the idea of its sensitivity to acidity. Also there are other factors besides hard seed coat which are responsible for dormancy in seeds, these may include the presence of inhibitors/toxic materials in the seed coat. This does not seem to be the case in the present study. Thus T. conophorum appears to be innately dormant possibly because of the hard shell.

When the shells were cracked, the seeds became infested with microorganisms leading to germination failure. Thus the hard shell not only delayed germination, it also prevents infection. In order to confirm that the hard shell is the cause of dormancy in this species, the shell can be completely removed from the seeds and sterilized with 0.1% mercuric chloride before testing for germination in a sterilized medium in the laboratory.

If enhanced germination occurred, then it could be concluded that the testa caused the dormancy. However this experiment could not be carried out now because the seeds are out of season.

The effects of light and dark on germination showed that light is essential for maximum germination in T. conophorum (Fig. 8). Generally, the low percentage germination in the dark may be attributed to the inability of the seeds to produce enough growth promoting substances which will initiate germination processes in the dark (Khan, 1971). Though in nature as described earlier, the seeds are covered by fallen leaves and twigs they may not be completely in darkness, thus a high proportion of seeds will still germinate. Also some seeds may be dispersed to gaps in the forest and they will receive high amount of light and they are likely to achieve better germination. In nature, however the forest floor where the seeds dropped is not in total darkness as in the experiment but only in partial shade - 25 - 35% full sunlight, (Okusanya et al, 1991 in press). Thus one expects better germination in nature than it was in compete darkness (Fig. 8). The fact that some seeds from the dark treatment germinated after some period (about 5 weeks) in the light showed that the dark effect might not have caused a permanent inhibition of germination.

The result also show that many species with large seeds achieve reasonable germination in dark as opposed to those with small seeds which usually do not germinate in dark (Fenner, 1985).

The effect of soil depth (Fig. 12) showed that both germination and seedling emergence were substantially reduced with increasing depth of burial beyond the 2.5cm depth. The low germination in darkness (Fig.8) ties well with that in the soil depth of 5.0 - 10.0cm (Fig. 12). Similar effect of the depth of seed burial on germination has been observed by other workers (Onyekwelu, 1972, Pemadasa and Lovell, 1985, Okusanya 1979). But with Luffa aegyptiaca (Okusanya, 1978), the depth of seed burial affected only the time of germination and not the final percentage germination as reported in T. conophorum. Seed burial however can have a significant inhibitory effect on germination, the deeper the burial, the lower the germination. This indicates that only those seeds which lie near the surface of soil are capable of rapid germination (Pemadasa and Lovell, 1975).

The effect of seed burial on the species might probably be the direct effect of the absence of light as poor germination in the dark may be due to the conversion of phytochrome to the inactive germination - inhibiting form as opposed to the active germination - inducing form in light (Fenner, 1985). Similar results have been reported for other tropical species (Okusanya, 1978, 1980 and Okusanya et al. 1991.

The significant lower percentage germination ($p < 0.001$) on soil surface than at 2.5cm soil depth was possibly the effect of low soil moisture or dehydration and rehydration effect. Fenner, (1985) has indicated that seeds lying on soil surface are exposed to the risk of dehydration and will only germinate if water is absorbed more quickly than it is lost. This is similar to what obtains in the natural habitat of T. conophorum - the forest floor - is a very humid area and is wet to moist throughout most of the year (Keay 1959). Thus moisture may be an ecological requirement for the success of the species. This statement is supported by the significant higher germination in the wet condition than in the dry condition (Fig. 9). Treculia africana which also has soft and decaying fruit pulp like T. conophorum and which inhabits the same ecological zone also shows strong preference for germination in wet-moist condition than for dry condition (Mabo, Lakanmi and Okusanya, 1988).

There was no germination under the water-logged condition (Fig. 9) indicating that oxygen deficit may be responsible for the lack of germination as water logged soils lack air. Water-logged condition can also lead to, (for example), accumulation of ferrous and manganous ions in high concentration which could be toxic to some seeds (Kramer, 1949). In addition, there may be production of gaseous inhibitors which may be product of the seeds metabolism (Wesson and Wareing 1969).

The significantly high percentage germination in humic soil (Fig. 10) could be the result of the physical properties as well as the high nutrient value of the soil (Table 2). while the low percentage germination in the sandy soil (lowest percentage germination of the four soil types) could be attributed to its low nutrient level and its poor water retention ability (Russell, 1968), the significantly low percentage germination in the red-earth and clay when compared to the humic soil may be due to the fact that these two soils have poor aeration, slower percolation, acidic pH and high water-holding capacity which tend to make them water-logged especially after initial watering (Table 2). All these conditions reduce germination. In all, the result of the effect of soil types show that the response of the species fits its ecological requirement in that the forest soil is humic and only slightly acidic (pH 6.5) and it is in this soil type that the best germination occurred.

The germination response of this species to different pH ranges showed that the species is sensitive to acidity and so does not have the ability to germinate in high acidic areas since there was no germination at pH 3.5. Increasing pH thus favoured the germination of T. conophorum (Fig 11). As such, the species may not colonize a wide range of habitats.

Acid pH could affect seed germination by hydrogen ion concentration, low nutrient concentration and high aluminum concentrations (Marschner, 1986). Low nutrient concentration could not be the cause for the total lack of germination of pH 3.5 and the reduced germination at pH 5.5 since there is sufficient mineral elements in the Hoagland and Arnon's solution. Therefore the poor germination may probably be due to high hydrogen ion concentration. It is not unlikely therefore, that the high hydrogen ion concentration might be partially responsible for the failure of the seed scarified with various concentrations of sulphuric acid to germinate (Table 3).

As stated earlier, forest soils where natural germination occurs are only slight acidic (pH 6.5), and this is not expected to appreciably affect germination as shown in the control (Table 3). The significant higher germination of the seeds in Hoagland's solution of pH 7.0 than in distilled water seems to indicate that high nutrient status which abounds in the fruit pulp and in the litter surrounding the seeds, may enhance germination. Similar results have been reported for L. aegyptiaca (Okusanya, 1983). Though the pH of the forest soils is about 6.5 which is near pH 7.0, other factors of nature may also be responsible for better germination than distilled water of pH 7.0.

The failure of the seeds to germinate at 15°C and their subsequent germination with increasing temperature up to 31°C is probably a reflection of its ecological habitat. (Fig 13). The climate in rainforest in southern Nigeria is generally warm and as such a constant temperature of 15°C which is not normally encountered there will not favour germination of the species. The poor germination at 41°C can be explained by the fact that this temperature is also not normally encountered in the natural habitat (forest) of the species. As such, high temperatures may cause an osmotic inhibition of enzymatic processes and consequently enzymes involved with germination might have been denatured. Thus, its germination response to temperature may be enzymatically controlled. (Okusanya & Sonaike 1991).

The result of the effect of soil salinity on the seed germination showed that T. conophorum is a glycophyte which is incapable of tolerating even low salinity since the germination of the species was drastically reduced at 10% sea water concentration and were seriously inhibited above this level (Fig 14). Also the seeds showed the two general characteristics of seeds in saline environment in that as salinity increased, the time of first germination increased and total percentage germination decreased (Macke & Ungar, 1971, Okusanya 1977, 1983). Similar results have been reported for other tropical glycophytes (*T. africana*, Mabo *et al* 1989; *L. aegyptiaca*, Okusanya 1983; *Uraria picta*, Okusanya *et al*. 1991).

Salinity reduces germination through osmotic stress, and toxicity of ion (Levitt 1980). The fact that there was no further germination when seeds at high salinities were returned to distilled water indicates that both effects may be operating. Forest soils are usually low in salinity, less than 0.5% sea water (Okusanya *et al* 1991) except for mangrove forest, thus the response of the seeds to salinity reflects its ecological habitat.

On the whole, it can be seen that the seeds germinated best in moist/wet humic soil in adequate light, with temperature of about 31°C prevailing and at pH 7.0 which was close to the pH of the forest soil. All these conditions are similar to those either in the forest zone or in the forest floor microclimate. Thus the tropical rain-forest appears to be the best ecological habitat for the germination and, of course, the distribution of the species.

CHAPTER FOUR

GROWTH STUDIES

INTRODUCTION

The next most important aspect in the life of a species after seed germination is seedling establishment and growth which is the end result of the interaction of numerous physiological processes.

Growth in a restricted sense, refers to an irreversible increase in size, reflecting a net increase in protoplasm. Development infers differentiation, a higher order of change that involves anatomical and physiological specialization and organisation (Janick, et al, 1974).

The physiological processes of a plant constitute the machinery through which heredity and environment operate to control growth. There is increasing evidence that the distribution and abundance of adults in a plant community is often mediated by events that occur during seedling establishment and growth (Gross and Werner, 1982). Cavers and Harper (1967) and Hume and Cavers (1982) found that population of Rumex crispus are morphologically adapted to their environments such that these affect the subsequent germination and growth responses of the seedlings.

After a seed has germinated, it gives rise to a seedling whose growth is largely dependent, at least for a time, on its own food reserves. A seedling is considered to be fully established when it has become effectively independent of its seed reserves. The hazards faced during the process of establishment comprise the last of the hurdles which the plant has to negotiate in the process of regeneration by seed (Fenner, 1985). Also, as pointed out by Klebs (1913, 1918), environmental factors can affect plant processes and conditions, as such, plants grow differently under various environmental conditions and cultural treatments.

For successful colonisation, the seeds must not only be able to germinate on the different soil types, but the seedlings must also be able to establish and grow in them (Okusanya, 1976). Some of the common soil types in Nigeria are sand, red-earth, loam and clay soils with differences in physical and chemical composition. Consequently the soil environment influences many of the plant functions. The size of the soil particles also has important consequences. Large particles result in large pores, good aeration but poor water retention while small particles result in fine pores, high retentivity of water but poor aeration (Bannister, 1976). The seeds of T. conophorum had different germination percentages in the soil types tested (Fig 10) thus indicating the possibility of seedling establishment and growth in all the soil types ranging in pH from near acid to neutral. And as a forest vine, this species tends to grow on slightly acidic to neutral soils. It was therefore necessary to determine the effects of different soil types and pH on the growth of T. conophorum.

In the salinity germination experiments, germination occurred at 0-30% salinity though there was a decrease of germination percentage as from 10% salinity and no germination occurred above 30% sea water concentration. This result is not surprising because glycophytes are known to achieve their best-growth in non-saline conditions, and their growth is reduced with increasing salinity (Ungar, 1974, Levitt, 1980). Generally at high salinity, plants become salt stressed and this condition may result in toxicity of ions, low uptake of essential mineral ions, reduced enzymatic action, chloroplast damage, necrosis etc., ultimately resulting in reduced plant performance. An experiment was therefore set up to determine the growth response of T. conophorum to salinity.

Water is probably the most important factor limiting plant growth over the land surface of the earth. Plants living on land obtain their water supply from the soil and generally different soil moisture status affect plants in various ways. This is because the water retention capacity of soil and the frequency of amount of water replenishment determine the amount of soil moisture in the soil. This results in some soils being dry while some may be water-logged or just moist. The germination behaviour of this species seeds to the three moisture regimes of dry, wet and water-logged condition differs significantly in terms of percentage germination (figure 9, Chapter 3). It was thus decided to investigate the effect of various soil moisture contents on the growth of this species.

The germination of _T_. _conophorum_ in light and dark differs significantly. As a climber _T_. _conophorum_ may show preference for light than shade since a reduction of light intensity may lead to a lowered potential for photosynthesis. Also it is known that some species with large seeds tend to store enough food to allow their seedlings to grow independently of photosynthesis for quite some time, (Bannister, 1976). _T_. _conophorum_ grows initially under the shade of plants that it will climb. Later on, as it climbs it receives partial light. Finally as it branches profusely, it forms a mass of leaves on top of the tree it climbs where it receives maximum light. It was therefore suspected that the light requirements of this species may change with time of development. Consequently it was thought necessary to investigate the effects of light and shade on the growth behaviour of this species.

All plants have certain basic requirements for mineral constituents from the soil and if these requirements are not met, then the plant is unable to grow properly. Some elements such as nitrogen, potassium and phosphorus are needed in large amounts and this form the major inorganic components while lesser amounts of iron, magnesium, sulphur and calcium are equally essential for the growth of the plant (Bannister, 1976). There is little evidence to suggest that an external supply of minerals is essential for germination but after germination, the seedling becomes much more susceptible to external influences once the seed reserves are exhausted and it will thus become dependent upon external sources of minerals (Larsen & Sutton, 1963). This species living as it were, in the forest with much leaf litter and high rate of mineralization, may have certain mineral requirements. An experiment was therefore set up to investigate the mineral nutrition of _T. conophorum_.

In all, the effects of (i) light and shade, (ii) soil types, (iii) pH regimes, (iv) salinity and (v) soil moisture contents as well as the mineral requirements of the seedlings of _T. conophorum_ were investigated.

MATERIALS AND METHODS

Seeds of *T. conophorum* collected as described in the germination experiment were used in this study. They were germinated in sand moistened with nutrient solution in seed trays and in each experiment, uniform seedlings which were 4 weeks old were transplanted into nursery bags (28cm deep and 18.7cm wide). Nursery bags which were three-quarter filled with the appropriate growth medium were used for the experiment. Two layers of Whatman qualitative filter paper were placed at the base of the nursery bags to prevent the growth medium sieving through. There were six replicates per treatment. Light was from the natural source with about 12 +1hr photo-period. Temperature averaged 32+2^{o}C (day) and 25+2^{o}C (night) except in the shade where the average temperature was about 2^{o}C lower than the rest. Relative humidity was about 85% at 0900 hour.

Seedlings of *T. conophorum* were subjected to the effects of some environmental factors namely light and shade regimes, soil moisture contents, soil types, pH and salinity. In addition, the mineral nutrition of the seedling was determined. This is to know how nitrogen, phosphorus and potassium, alone or in combination with each other affect the growth of the species. Each experiment lasted for six weeks.

All the experiments were carried out in the glasshouse in the Biological garden of the University of Lagos, except the shade experiment which took place in the secondary forest in the Biological garden. The complete randomised experimental design was used for each experiment.

Effect of Light and Shade:- One batch of seedlings in humic soil was placed in the ground of a secondary forest in the Biological garden. This constituted the shade treatment. Another batch of seedlings was placed in the open to receive maximum light in the Biological garden; this constituted the light treatment. The amount of light in the shade was about 40% that of full sunlight. Watering was done once a day.

Effect of soil moisture:- The seedlings also in humic soil were subjected to three watering regimes namely dry treatment achieved by watering the soil twice a week (11.6% of soil dry weight), wet treatment achieved by watering the plants everyday (24.4% of soil dry weight) and water-logged treatment (continuous watering) achieved by putting the nursery bags in plastic containers such that the soil was completely covered by water (30.2% of soil dry weight).

Effect of soil types:- Four soil types designated as humic soil, red earth, clay and sand were used. Analysis of the soil types is given in Table 2. A seedling was transplanted into a particular soil type in the nursery bags. Watering was done once a day.

Effect of pH:- One-fifth strength Hoagland and Arnon (1938) solution adjusted to pH 3.5, 5.5 and 7.0 using dilute potassium hydroxide or dilute sulphuric acid were used. The appropriate solutions were used to wet the seedlings in acid-washed river sand.

<u>Effect of salinity</u>:- Seedlings in humic soil in nursery bags were treated with sea water concentrations of 0,10, 20, 30, 40 and 50%. The various sea water concentrations were prepared by mixing varying volumes of tap water and filtered sea water (3.5% salinity) collected from the Lagos Bar Beach. The soil containing the seedlings were watered with 300ml of the appropriate solutions in the morning. Seedlings receiving high salinity concentrations were brought there in step-wise increments. This was done by watering all the seedlings first with sea water concentration of 0% (distilled water), 2 days later solution of 10% sea water concentration was used to water seedlings for 10, 20, 30, 40 and 50% treatments. This was continued at 2 days interval until the eleventh day when the appropriate sea water dilution was used for each treatment. Once a week, the growth medium was leached with 600ml of distilled water followed immediately by the addition of the appropriate solution. This process helps to prevent the accumulation of ions to toxic levels and to maintain salinity level.

Mineral nutrition

This was done to determine how nitrogen, phosphorus and potassium, alone or with the three elements combined together or their absence in the culture solution would affect the growth of T. conophorum. The following five treatments were used. One-fifth strength Hoagland and Arnon (1938) solution (complete), complete solution minus phosphorus (-P), complete solution, minus potassium (-K), complete solution minus nitrogen (-N) and complete solution lacking nitrogen, phosphorus and potassium (-NPK). The solutions contained the amounts of salts recommended for Hoagland's solution (Hewitt, 1966).

Each seedling was transplanted into a nursery bag which contained acid-washed coarse river sand.

Washing of the sand was done by putting the sand in 2% HCL in a big plastic drum. The sand was left in acid solution for five days being stirred twice a day. The sand was then rinsed with running tap water that was passed continuously through a hose into the drum until it was nearly chloride free. It was further washed in distilled water till completely chloride free. Wetting of the seedlings was done with 300ml of the various solutions. There was an initial harvest, and then two/three other harvests in each experiment. Each harvest was after six weeks.

At the end of each experiment, plants were carefully removed from the soil and the roots were washed with tap water. The plants were then separated into root, stem and leaves. The leaf area was determined by the use of electronic planimeter (PATON and CSIRO Model). Fresh weights of the plant parts were determined, thereafter, the plant parts were dried in an oven at 80°C for 24 hours, cooled and their dry weights determined.

The above data were used to determine the following growth parameters - mean total dry weight, leaf area ratio (LAR) calculated as leaf area/total dry weight, leaf weight ratio (LWR), stem weight ratio (SWR), root weight ratio (RW). (RWR) and shoot: root ratio (S:R). The plant parts dry weight ratios are calculated as percent of total dry weights.

<u>Chemical analysis of dried plant materials</u>

In each treatment, oven dried materials were finely ground in a rotary grinder. Weighed samples ranging from 100-200mg were then placed in a crucible and ashed for 4 hours at 550°C in a muffle furnace (Carbolite, Sheffield, LMF 3). When cooled, the ash was dissolved in 0.1ml, 1M nitric acid and then made up to 10ml volume with 100mM nitric acid. Sodium, potassium, magnesium and calcium contents were determined by atomic absorption spectrophotometry (Pye Unicam SP9). Phosphorus was determined by Colorimetric determination (Vanado-Molybdate Method). While the total nitrogen was determined by the micro-Kjeldahl method. (Black, 1965)

The data were statistically tested by the usual technique of the analysis of variance using the total dry weight of the different treatments to determine whether the treatments had any significant effect on the growth of the species. In those experiments where the treatments had a significant effect on the growth, the least significant difference (LSD) was calculated for comparison of treatment means.

RESULTS

Effect of Light and Shade

Analysis of variance for each of the two harvests (Tables 4a & b) shows that growth in terms of dry weight was equally good in both light and shade (Fig. 15). There was no significant difference ($p > 5\%$) in the growth of *T. conophorum* under the two treatments (Tables 4a and 4b). The results of the growth parameters (Tables 5a, 5b, and Fig 15) show that while the mean dry weight, the leaf area and the leaf area ratio increased with time (harvest) in both treatments, their values were similar in both treatments. For the plant parts, only the root weight ratio increased with time while the leaf weight ratio, stem weight ratio and shoot: root ratio decreased with time in both treatments.

Effect of soil moisture

There was no growth in the water-logged condition. Soon after the commencement of the experiments the leaves became chlorotic and they dropped off. The growth of the plants became stunted and the plants finally died.

Growth of the species as measured in terms of dry weight was significantly better ($P < 0.1\%$) in the moist condition than the dry condition at each harvest (Tables 6a & 6b, Fig. 16). There was an increase in dry weight and leaf area with time under the moist condition, the reverse was the case for the dry condition, such that as the soil

TABLE 4A

Analysis of variance on the mean dry weight on the effect of light and shade on the growth of T. conophorum for the first harvest.

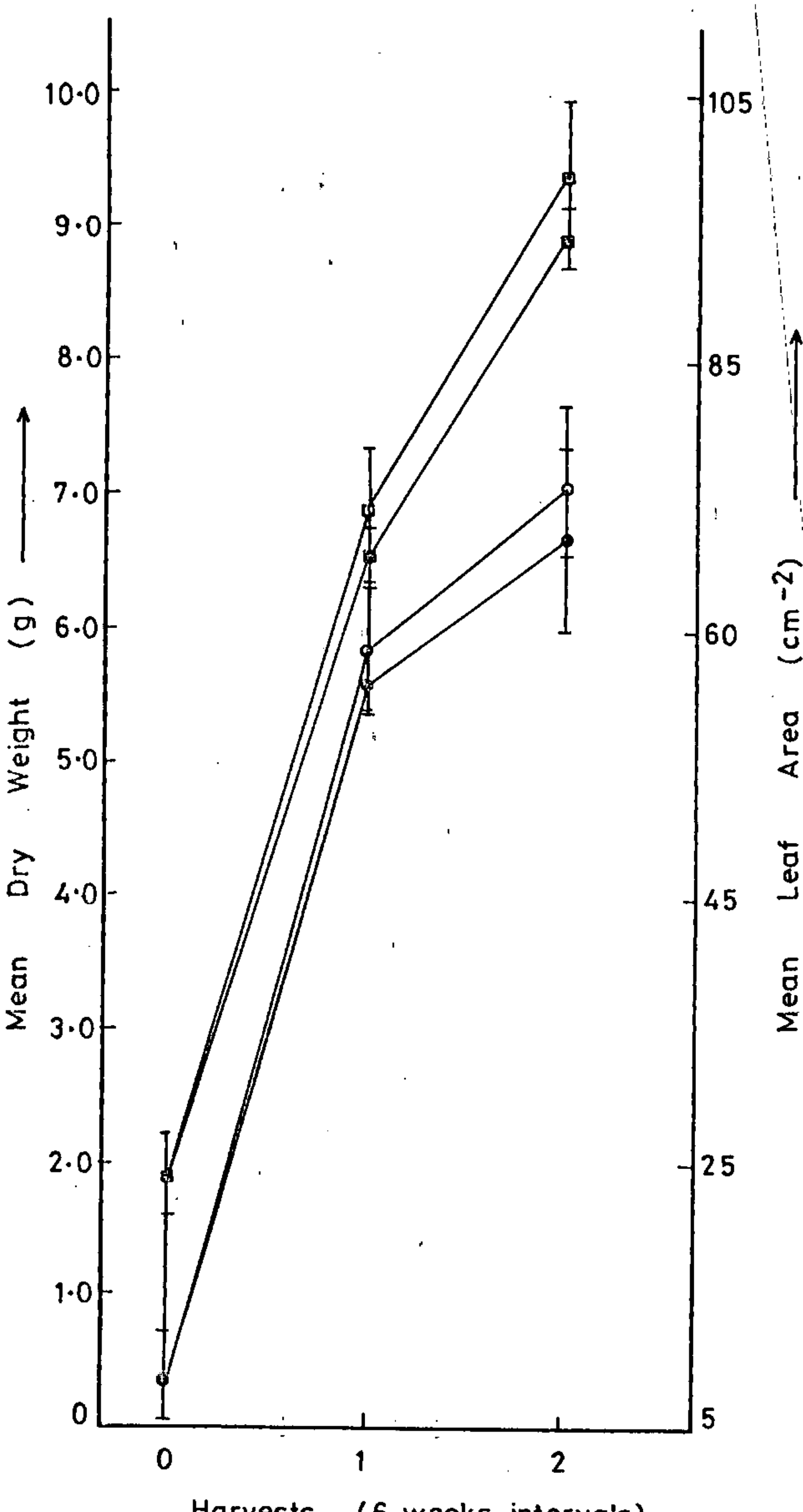
Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F-ratio
Treatment	1	0.34	0.34	
Error	10	1.06	0.106	3.12 N.S.
Total	11	1.40		

TABLE 4B

Analysis of variance on the mean dry weight on the effect of light and shade on the growth of T. conophorum for the second harvest.

Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F-ratio
Treatment	1	0.7	0.7	
Error	10	1.62	0.162	4.32N.S.
Total	11	2.32		

Fig 15: The effect of light and shade on the growth of _T. conophorum_ seedlings showing the mean dry weight □———□ in light, ■———■ in shade and mean area ○———○ in light and ●———● in shade. Bars represent + S.E.



10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
Mean Dry Weight (g)
105
85
60
45
25
5
Mean Leaf Area (cm-2)
0
1
2
Harvests (6 weeks intervals)

TABLE 5A

The effects of light and shade on the growth of *T. conophorum* atr the first harvest. Mean values of six replicates ± S.E.

GROWTH PARAMATERS	LIGHT	SHADE
Leaf area ratio (cm^2/g)	29.62± 1.34	29.78 ± 1.86
Leaf weight ratio(% of total dry weight)	31.06± 1.47	31.20 ± 2.28
Stem weight ratio(%"of total dry weight)	46.32± 1.34	47.14 ± 1.24
Root weight ratio(% Of total dry weight)	22.62± 1.14	22.01 ± 0.99
Shoot: root ratio	3.43	3. 55

TABLE 5B

The effects of light and shade on the growth of *T. conophorum* at the second harvest. Mean values of six replicates ± S.E

Leaf area ratio (cm^2/g)	31.01± 0.99	30.77 ± 1.41
Leaf weight ratio(% of total dry weight)	26.05± 1.43	26.13 ± 1.78
Stem weight ratio(% of total dry weight)	45.22± 0.65	45.66 ± 0.71
Root weight ratio(% of total dry weight)	28.73± 0.94	28.26 ± 1.30
Shoot:root ratio.	2.48	2.55

TABLE 6A

Analysis of variance of the mean dry weight on the effect of soil moisture content on the growth of T. conophorum for the first harvest.

Sources of variation	Degree of Freedom	Sum of squares	Mean squares	F-ratio
Treament	1	12.2	12.2	
Error	10	2.3	0.23	53.04***
Total	11	14.5		

Moist&Dry   Difference between means.
2.02***

Expected LSD at 5% = 0.62, at 1% = 0.89 and at 0.1% = 1.28.

*Significant difference at 5% level
** " " " 1% "
*** " " " 0.1% level.

TABLE 6B

Analysis of variance on the mean dry weighton the effect of soil moisture content on the growth of T. conopnorum for second harvest

Sources of variation	Degree of freedom	Sum of squares	Mean squares	F-ratio
Treatment	1	99.35	99.35	
Error	10	2.72	0.272	365.25***
Total	11	102.07		

Moist&Dry   Difference between means.
5.8.***

Expected ISD at 5% = 0.67
" " " " 1% = 0.95
" " " " 0.1% = 1.38

* Significant difference at 5% level
*** " " " " " 1% "
*** " " " " " 0.1% level.

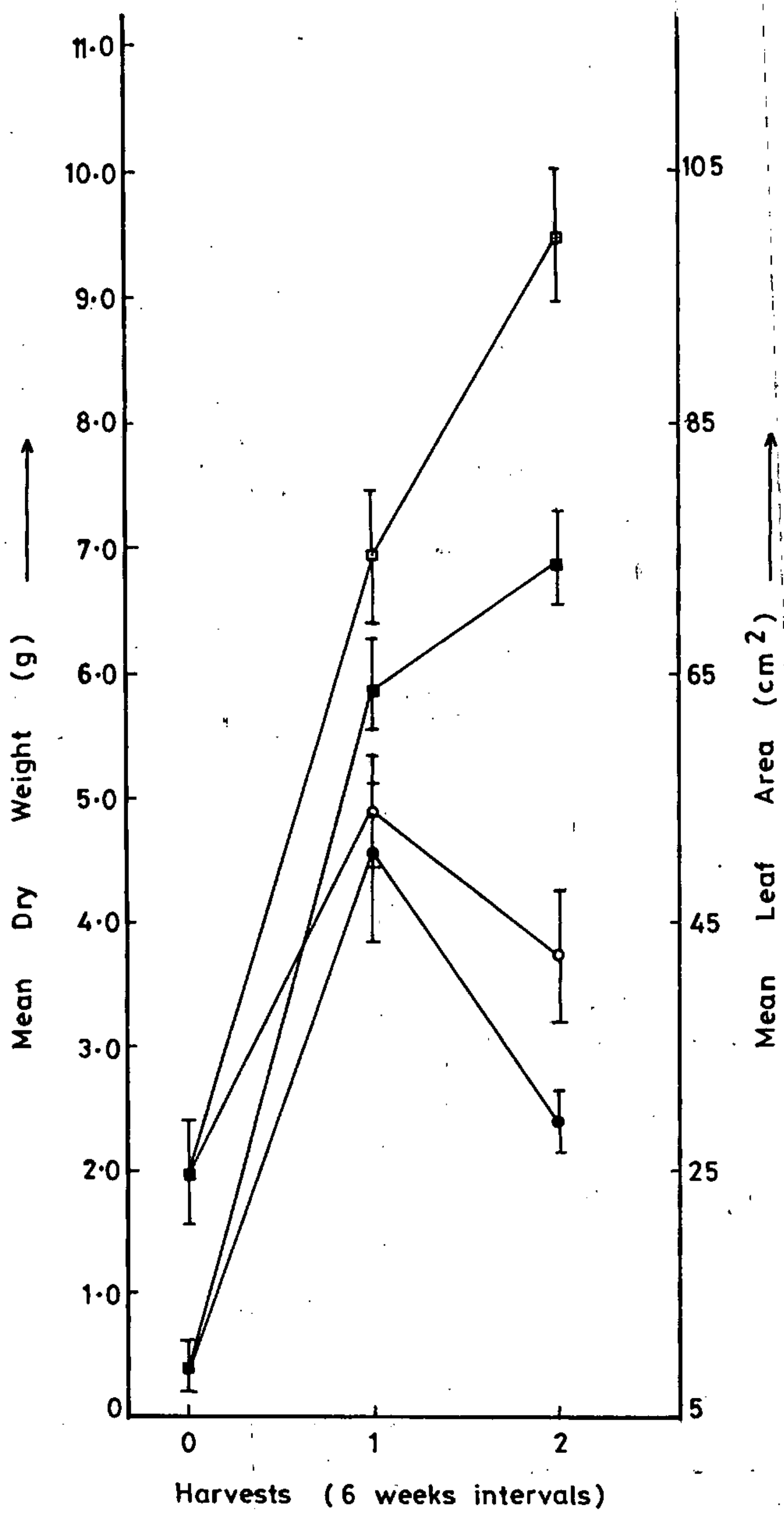
moisture decreases the mean dry weight and leaf area also decreased (Fig. 16).

In comparing the plant part weight ratios of the two treatments, the pattern showed that the root weight ratio was higher in the moist condition though there was an increase under each condition with time. This was not the case with the stem weight ratio which showed the reverse of root weight ratio. The shoot: root mass ratio decreased with time since the root weight ratio (RWR) increased with time in both conditions. However the shoot: root mass ratio was slightly higher in the dry treatments than the moist. In both conditions the leaf weight ratio (LWR) decreased with harvest but the values in the moist condition were higher than in the dry treatment (Tables 7a & 7b).

Effect of soil types:-

Analysis of variance of the mean total dry weight at the three harvests (Tables 8a, b & c) shows a high significant difference in growth in the humic soil ($P<0.1\%$) than the other three soil types as from the second harvest. In the first harvest, there was no significant difference in the mean total dry weight between the treatments (Table 8a). The humic soil also has the highest mean values of dry weight and leaf area for the three harvests (Figs 17a & b), an indication that the humic soil best favours the growth of *T. conophorum*.

Fig 16: The effect of soil moisture conditions on the growth of *T. conophorum* seedlings showing the mean dry weight □——□ in moist condition, ○——○ in dry condition and mean leaf area ■——■ in moist condition and ●——● in dry condition. Bars represent ± S.E. All seedlings under the water-logged condition died before the first harvest.



11·0
10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
Mean Dry Weight (g)
105
85
65
45
25
5
Mean Leaf Area (cm 2)
0
1
2
Harvests (6 weeks intervals)

TABLE 7 A

The effect of soil moisture content on the growth of *T. conophorum* at the first harvest. Mean values of six replicates ± S.E. All seedlings under the water-logged condition died before the first harvest.

GROWTH PARAMETERS.	SOIL MOISTURE MOIST	DRY
Leaf area ratio (cm^2/g)	31.11 ± 3.16	37.34 ± 2.70
Leaf weight ratio(% of total dry weight)	30.09 ± 3.02	27.20 ± 1.73
Stem weight ratio(% of total dry weight)	45.44 ± 0.71	50.01 ± 1.12
Root weight ratio(% of total dry weight)	24.48 ± 2.41	22.79 ± 0.86
Shoot:root ratio	3.12	3.39

TABLE 7 B

The effects of soil moisture content on the growth of *T. conophorum* at the second harvest . Mean values of six replicates ± S.E.

GROWTH PARAMETERS.	SOIL MOISTURE MOIST	DRY
Leaf area ratio (cm^2/g)	30.92 ± 1.18	35.56 ± 3.09
Leaf weight ratio(% of total dry weight)	25.35 ± 0.67	20.15 ± 1.70
Stem weight ratio(% of total dry weight)	44.50 ± 0.73	50.53 ± 2.22
Root weight ratio(% of total dry weight)	30.16 ± 0.21	29.32 ± 1.67
Shoot:root ratio	2.32	2.42

TABLE 8/A

Analysis of variance on the mean dry weight on the effect of soil types on the growth of T. conophorum for the first harvest.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-ratio
Treament	3	19.56	6.52	
Error	20	52.34	2.617	2.49,N.S
Total	23	71.90		

TABLE 8/B

Analysis of variance on the mean dry weight on the effect of soil types on the growth of T. conophorum for the second harvest.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-ratio
Treatment	3	29.91	9.97	
Error	20	4.35	0.22	45.32***
Total	23	34.26		

Soil types	Difference between means
Humus & Red earth	1.81***
Humus & Clay	2.36***
Humus & Sand	2.99***

Expected LSD at 5% = 0.56, at 1% = 0.768 and at 0.1% = 1.04

* = Signficant difference at 5% level
** = " " " 1% "
*** = " " "0.1% "

TABLE 8C

Analysis of variance on the mean dry weight on the effect of soil types on the growth of T. conophorum for the third harvest

Sources of variation	Degree of freedom	Sum of squares	Mean square	F-ratio
Treatment	3	69.76	23.25	
Error	20	5.25	0.26	89.42***
Total	23	75.01		

Soil types	Difference between means
Humus and Red earth	2.73***
Humus and Clay	3.48***
Humus and Sand	4.64***

* = Significant difference at 5% level — Expected LSD at 5% = 0.604

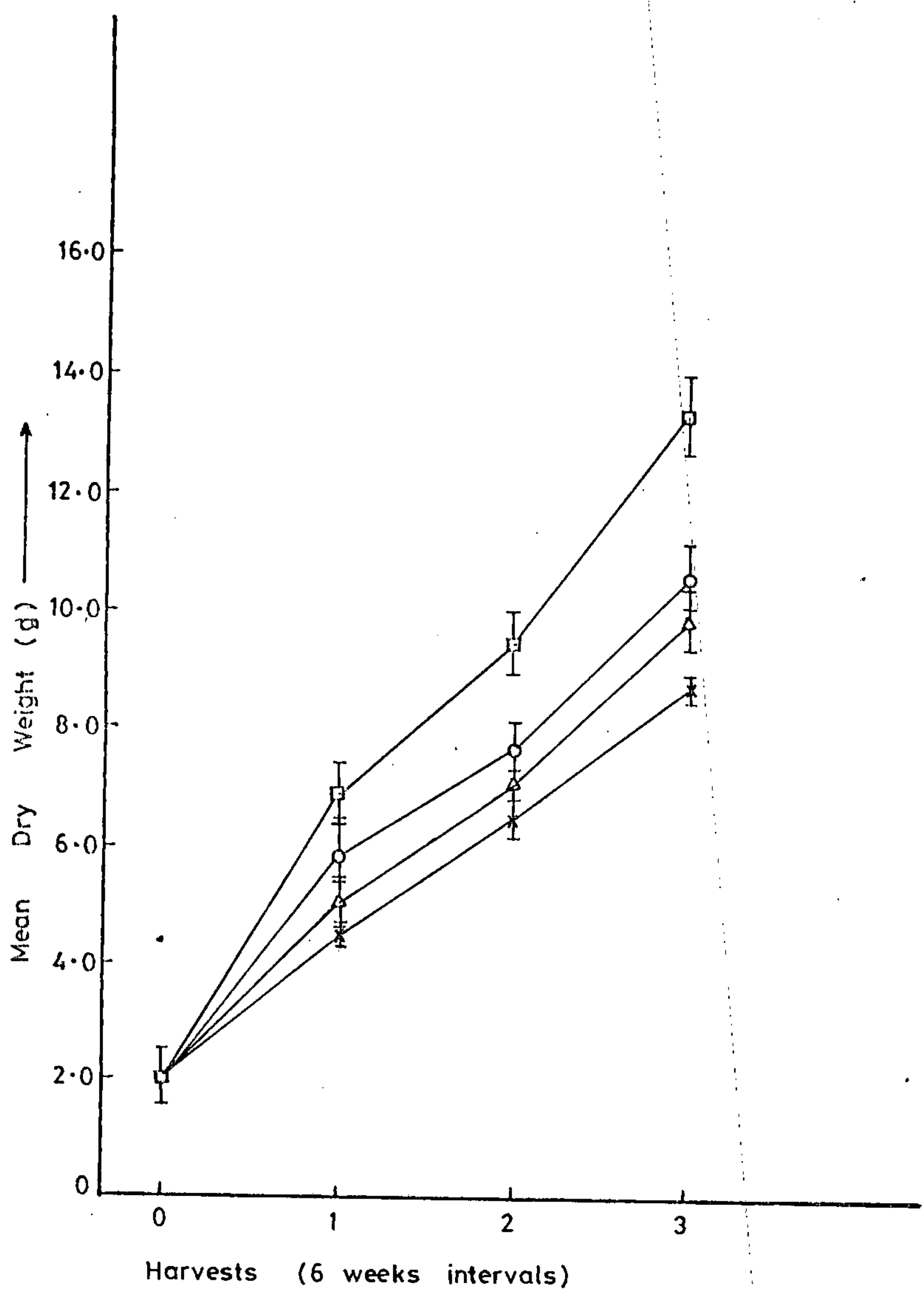
** = Significant difference at 1% level — at 1% = 0.83

*** = Significant difference at 0.1% level — at 0.1%= 1.12

It was only at the third harvest that there was a significant difference ($p<5\%$) in growth in the red earth compared to the clay soil while there were significant differences ($P<0.1\%$) in the two soils (red earth and clay) than in the sandy soil at the 2nd and 3rd harvests. As such, the sandy soil is the poorest of all the soil types used.

Generally, in all the soil types, the mean total dry weight increased with time of growth though at different rates (Fig. 17a). While the leaf weight ratio decreased with time of harvest in all the soil types, on the other hand, the root weight ratio increased with time and this also accounted for the decrease in the shoot: root ratio. The leaf area ratio (Tables 9a, b & c) in the sandy soil increased with time but for the other soil types, there was no definite pattern. The analysis of the various mineral elements in the plants grown in the various soil types (Table 10) showed that the potassium content in the plants was virtually the same for the different soil types. The plant in the sandy soil had the highest sodium content followed by that in clay, the red earth and the least was in humus. The calcium content was highest for plants in humus followed by that in the clay, then in the sand and the lowest was in red earth. The phosphorus content followed the same pattern as calcium.

Fig 17a: The effect of different soil types on the growth of T. conophorum seedlings showing the mean dry weight □———□ in humus, ○———○ in red earth, △———△ in Clay and ×———× in sand. Bars represent ± S.E.



16·0
14·0
12·0
10·0
8·0
6·0
4·0
2·0
0
Mean Dry Weight (g)
0
1
2
3
Harvests (6 weeks intervals)

Fig: 17b The effect of different soil types on the growth of T. conophorum seedlings showing the mean leaf area ■——■ in humus, ●——● in red earth, ▲——▲ in clay and ⊠——⊠ in sand. Bars represent ± standard error (S.E.)

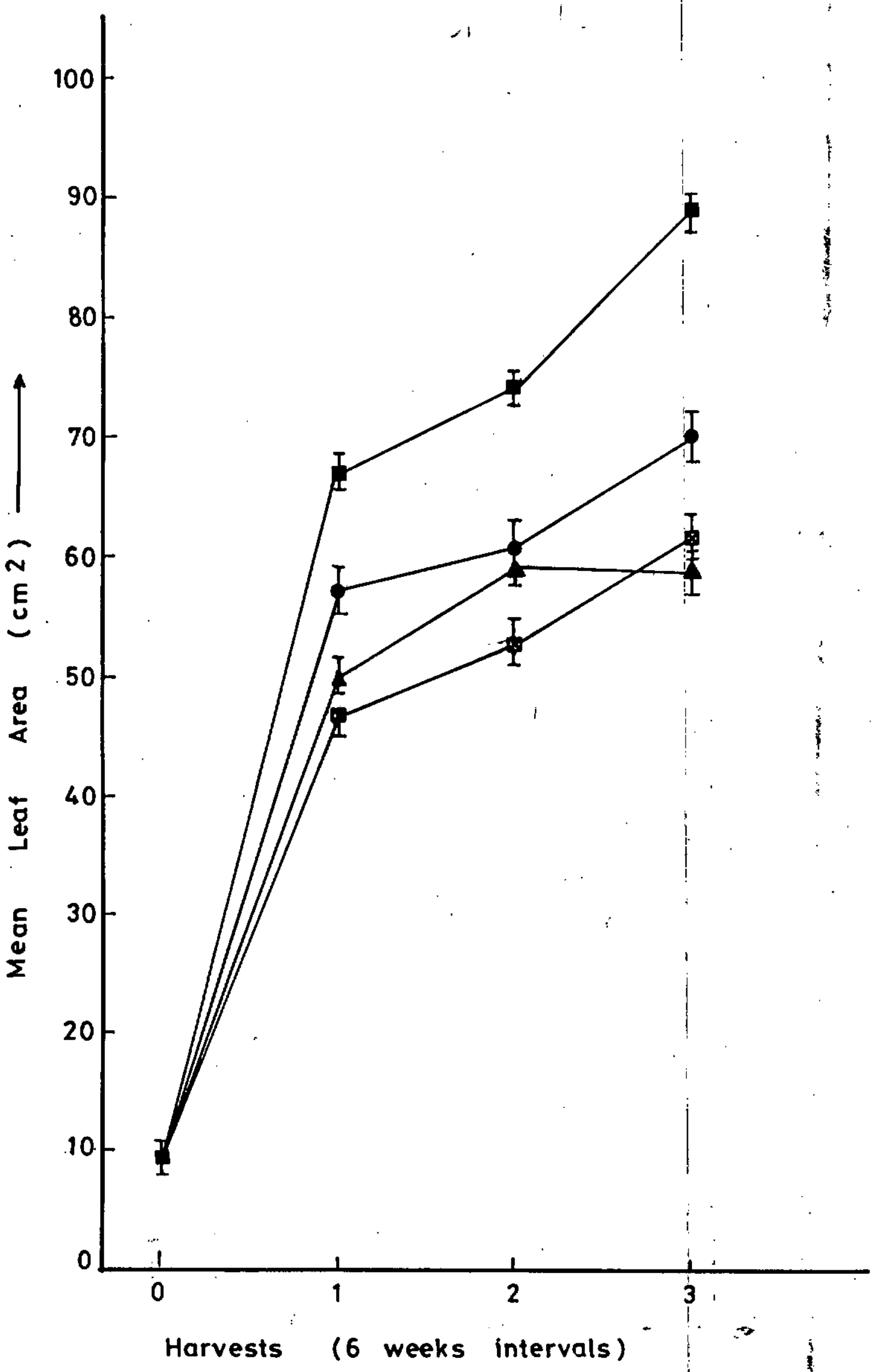

Mean Leaf Area (cm 2)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
Harvests (6 weeks intervals)

TABLE 9 A.

The effects of different soil types on the growth of *T. conophorum* at the first harvest. Mean values of six replicates ± S.E.

Growth parameters	Soil types			
	Humus	Red-earth	Clay	Sand
Leaf area ratio (cm^2/g)	32.01 ± 1.66	35.71 ± 7.36	36.07 ± 4.55	41.35 ± 3.22
Leaf weight ratio (% of total dry weight)	30.20 ± 3.01	28.08 ± 6.84	27.21 ± 3.47	25.07 ± 5.40
Stem weight ratio (% of total dry weight)	45.38 ± 0.77	49.25 ± 4.38	43.15 ± 2.71	46.96 ± 3.15
Root weight ratio (% of the dry weight)	24.42 ± 2.28	22.67 ± 2.54	29.64 ± 1.42	27.97 ± 2.75
Shoot: root ratio	3.12	3.06	2.38	2.60

TABLE 9 B

The effect of different soil types on the growth of _T. conophorum_ at the second harvest. Mean values of six replicates ± S.E.

Growth parameters	Soil types			
	Humus	Red-earth	Clay	Sand
Leaf area ratio (cm^2/g)	31.23 ± 2.30	33.57 ± 4.53	37.61 ± 2.44	42.15 ± 3.53
Leaf weight ratio (% of total dry weight)	25.28 ± 2.57	24.07 ± 5.40	22.06 ± 2.76	19.50 ± 3.84
Stem weight ratio (% of total dry weight)	44.92 ± 1.44	48.53 ± 3.10	41.81 ± 1.93	44.70 ± 2.45
Root weight ratio (% of total dry weight)	29.79 ± 1.64	27.39 ± 3.01	36.13 ± 1.02	35.80 ± 2.18
Shoot: root ratio	2.36	2.69	1.78	1.80

TABLE 9C

The effects of different soil types on the growth of *T. conophorum* at the third harvest. Mean values of six replicates ± S.E.

Growth Parameters	Soil types.			
	Humus	Red-earth	Clay	Sand
Leaf area ratio (cm^2/g)	32.61 ± 0.82	34.20 ± 3.62	35.46 ± 3.71	43.64 ± 1.91
Leaf weight ratio (% of total dry weight)	20.44 ± 1.88	19.43 ± 3.98	16.91 ± 2.14	16.27 ± 2.93
Stem weight ratio (% of total dry weight)	47.05 ± 1.64	52.99 ± 2.51	45.66 ± 1.47	46.25 ± 1.32
Root weight ratio (% of tatal dry weight)	32.52 ± 1.63	27.57 ± ±.60	37.46 ± 0.67	37.47 ± 1.73
Shoot: root ratio	2.08	2.64	1.67	1.66

For the magnesium content and total nitrogen, they were highest in humus, followed by red earth, then sand and least was in clay. In general, seedlings in humus had highest mineral nutrient values.

Effect of pH

The results of the experiment examining growth responses in relation to pH (Figs. 18a & b) show that there was a decrease in both the mean dry weight and leaf area with time at pH 3.5 and pH 5.5 though the growth at pH 5.5 was significantly higher ($P<0.1\%$) than at pH 3.5 at the two harvests (Tables 10a & b). At pH 7.0, there was an increase in both the mean dry weight and leaf area with time. Analysis of variance showed that there was a significant difference ($P<0.1\%$) between pH 7.0 and pH 3.5 for the two harvests, and between pH 7.0 and pH 5.5 only ($P<0.1\%$) at the second harvest (Tables 11a & b). The result of the second harvest (Table 11b) showed that as pH increased, the leaf area ratio decreased.

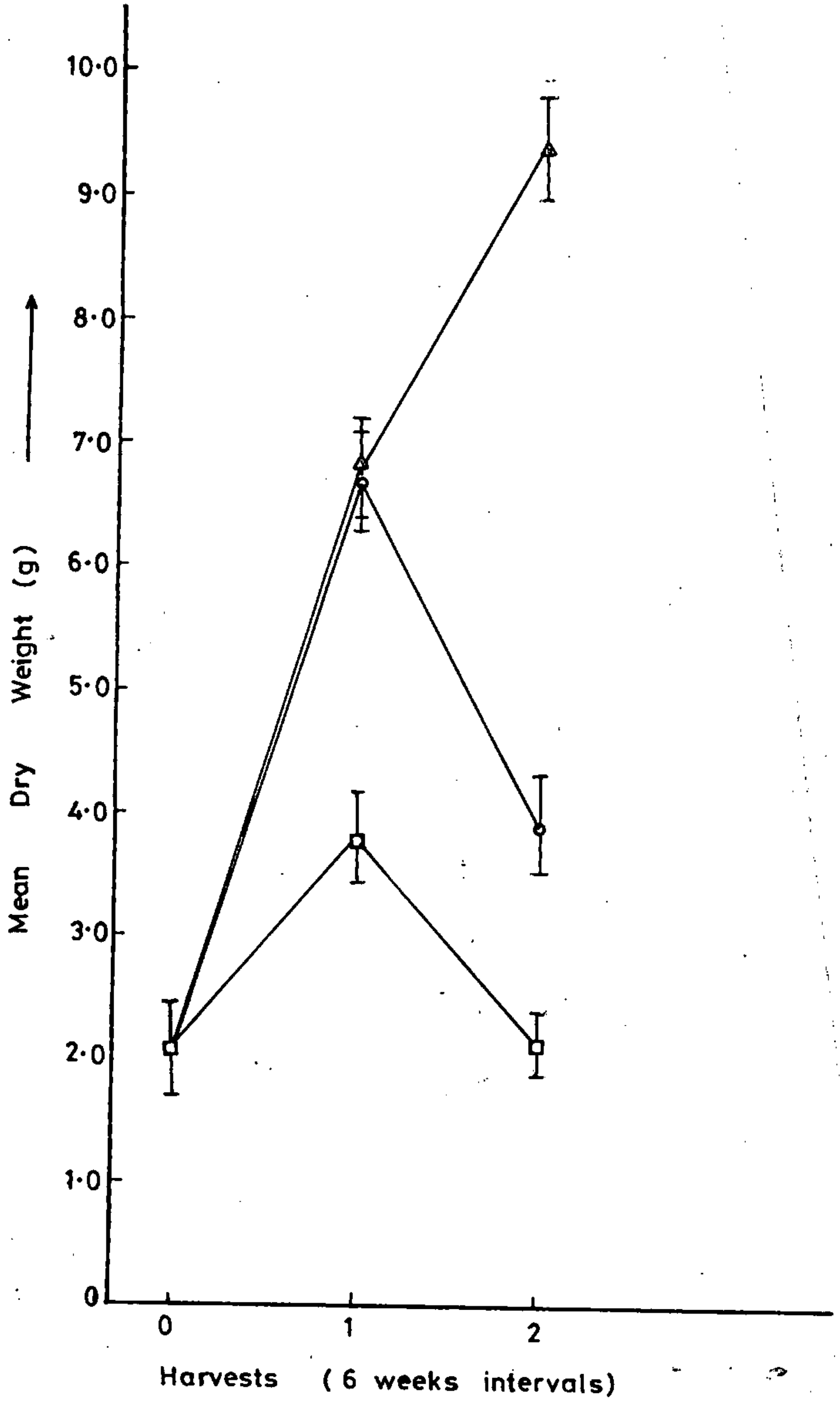
For the plant part ratios, the results showed that the leaf weight ratio and the shoot: root ratio decreased with time (harvest) at each of the pH treatment while the reverse was the case for the root weight ratio. For stem weight ratio, there was a decrease with harvest at pH 3.5, an increase at pH 5.5 and no change at pH 7.0. In general, pH 3.5 has the lowest dry weight, lowest leaf area, leaf weight

TABLE 10

The mineral element content of *T. conophorum* seedlings grown in the various soil types. Mean standard values of six replicates ± standard error. (S.E).

Soil	K^+	Na^+	Ca^{2+}	Mg^{2+}	P	Total Nitrogen (%)
	m equiv/100g dry sample				ppm	
Humus	3.52 ± 0.28	0.235 ± 0.04	3.203 ± 0.20	2.015 ± 0.12	1.257 ± 0.07	15.088 ± 1.31
Red earth	2.99 ± 0.31	0.282 ± 0.03	1.75 ± 0.12	1.596 ± 0.08	0.263 ± 0.05	13.454 ± 1.18
Clay	3.52 ± 0.34	0.864 ± 0.07	2.86 ± 0.30	1.14 ± 0.09	0.588 ± 0.08	1.18 ± 0.07
Sand	3.26 ± 0.31	1.964 ± 0.08	2.44 ± 0.15	1.431 ± 0.09	0.317 ± 0.062	6.746 ± 0.93

Fig 18a: The effect of three pH values □———□ pH 3.5, ○———○ pH 5.5 and △———△ pH 7.0 on the mean dry weight of *T. conophorum* seedlings. Bars represent ± S.E.



10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
Mean Dry Weight (g)
0
1
2
Harvests (6 weeks intervals)

Fig 18b: The effect of three pH values ■——■ pH 3.5, ●——● pH 5.5 and ▲——▲ pH 7.0 on the mean leaf area of _T. conophorum_ seedlings. Bars represent ± S.E.

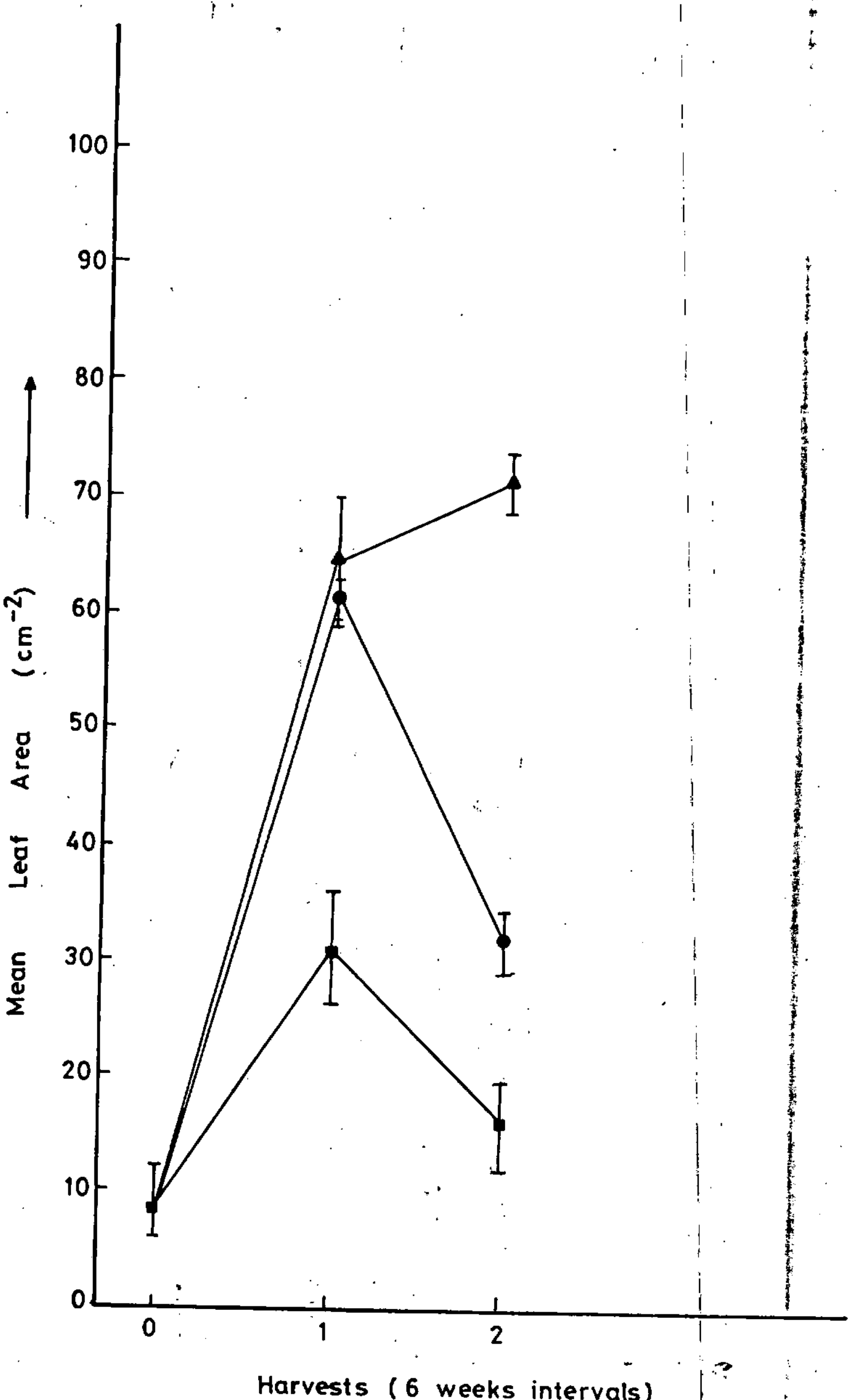

Mean Leaf Area (cm^-2)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
Harvests (6 weeks intervals)

TABLE 11A

Analysis of variance on the effect of different pH values on the growth of T. conophorum for the first harvest.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-ratio
Treatment	2	34.92	17.46	
Error	15	2.24	0.149	116.4***
Total.	17	37.16		

pH values	Difference between means.
pH 7.0 & pH 3.5	3.01***
pH 7.0 & pH 5.5	0.13 N.S

Expected LSD at 5% = 0.477
" " " 1% = 0.660
" " " 0.1%= 0.912.

TABLE 11B

Analysis of variance on the effect of different pH values on the growth of T. conophorum for the second harvest.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F-ratio
Treatment	2	174.66	87.33	
Error	15	2.59	0.173	504.80***
Total	17	117.25		

pH values	Difference between means
pH 7.0 & pH 3.5	7.33***
pH 7.0 & pH 5.5.	5.51***

Expected LSD at 5% = 0.511
" " " 1% = 0.707
" " " 0.1% = 0.978

ratio and root weight ratio for the two harvests followed by pH 5.5 (Tables 12a & b). Thus, the growth of this species was poorest at pH 3.5 and best at pH 7.0.

Effect of salinity

Analysis of variance shows that the effect of salinity (Table 13) on the growth of T. conophorum followed the usual pattern for a glycophyte as there was a significant decrease in both total dry weight and leaf area (Fig 19) as the sea water concentration increased. Seedling survival decreased as salinity increased (Table 14). The leaf area was very low especially as from 30% salinity upwards since most of the plants had lost their leaves. The leaves became chlorotic and fell off. Consequently this resulted in higher leaf area ratio as salinity increased. Another effect was that the leaf weight ratio decreased as the sea water concentration increased while the root weight ratio also increased as the salinity level increased (Table 13). Consequently the shoot: root ratio decreased with increase in salinity level.

In table 15, it can be seen that the mineral element contents i.e. potassium, calcium, phosphorus, magnesium and total nitrogen all decreased in the plants as the salinity level increased. It is only the sodium content that increased with increase in salinity.

TABLE 12A

The effects of different pH values on the growth of T. conophorum at the first harvest. Mean values of six replicates ± S.E.

GROWTH PARAMETERS	pH3.5	pH5.5.	pH7.0
Leaf area ratio (cm^2/g)	33.84±2.29	31.13±1.71	32.83±1.16
Leaf weight ratio (% of total dry weight)	24.25±1.97	29.25±1.84	29.04±1.12
Stem weight ratio (% of total dry weight)	49.36±1.44	44.56±1.25	44.32±1.14
Root weight ratio (% of total dry weight)	26.39±1.18	26.19±1.07	26.64±0.73
Shoot: root ratio	2.73	2.82	2.76

TABLE 12B

The effect of different pH values on the growth of T. conophorum at the second harvest. Mean values of six replicates ± S.E.

GROWTH PARAMETERS	pH3.5	pH5.5.	pH7.0.
Leaf area ratio (cm^2/g)	44.46±2.74	35.11±3.35	30.71±1.64
Leaf weight ratio (% of total dry weigth)	16.62±1.17	23.24±2.38	24.83±0.96
Stem weight ratio (% of total dry weight)	46.04±1.80	48.93±2.01	44.78±0.68
Root weight ratio (% of total dry weight)	32.65±2.28	27.83±2.76	30.39±0.58
Shoot: root ratio	2.10	2.63	2.29

TABLE 13

Analysis of variance on the mean dry weight on the effect of Salinity on the growth of _T. conophorum_.

Sources of variation	Degrees of freedom	Sum of squares	Mean squares	F- ratio
Treatment	5	130.33	26.07	
Error	30	3.82	0.13	200.54***
Total	35	134.15		

Sea water concentrations %	Difference between means
0 and 10	3.6***
0 " 20	4.16***
0 " 30	4.48***
0 " 40	5.39***
0 " 50	5.86***

Expected LSD at 5% = 0.42, at 1% = 0.57 and at 0.1% = 0.76.

Fig 19: Mean dry weight (g) ○——○ and mean leaf area (cm^2) ●——● of *T. conophorum* seedlings at various sea water concentrations. Bars represent ± S.E.

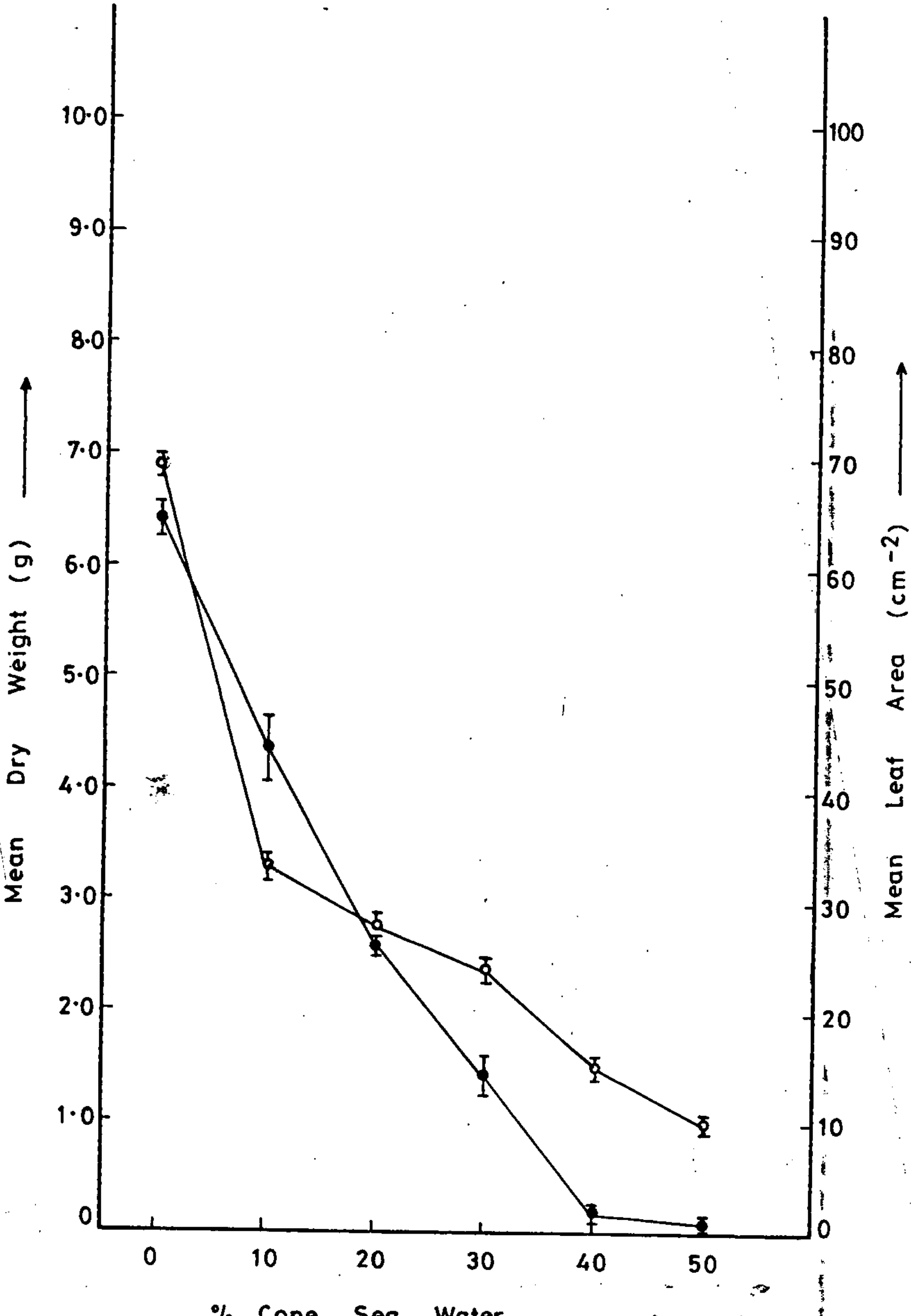

10·0
9·0
8·0
7·0
6·0
5·0
4·0
3·0
2·0
1·0
0
Mean Dry Weight (g)
100
90
80
70
60
50
40
30
20
10
0
Mean Leaf Area (cm -2)
0
10
20
30
40
50
% Conc Sea Water

TABLE : 14

The effects of Salinity on the growth of *T. conophorum*. Mean values of six replicates ± S.E.

Growth parameters	Sea water concentrations (%)					
	0%	10%	20%	30%	40%	50%
Survival (%)	100	70	30	10	10	10
Leaf area ratio (cm^2/g)	31.18 ± 1.15	44.93 ± 5.52	46.31 ± 4.30	47.10 ± 24.62	49.03 ± 54.27	50.31 ± 83.53
Leaf weight ratio (% of total dry weight)	30.11 ± 2.80	25.72 ± 6.71	20.80 ± 4.89	11.30 ± 7.80	0.008 ± 0.014	0.005 ± 0.01
Stem weight ratio (% of total dry weight)	45.43 ± 0.84	35.76 ± 6.37	39.92 ± 6.65	44.18 ± 4.95	34.35 ± 9.15	27.54 ± 15.61
Root weight ratio (% of total dry weight)	24.56 ± 2.13	38.52 ± 7.74	39.29 ± 8.20	44.51 ± 7.94	62.62 ± 11.84	71.95 ± 15.98
Shoot: root ratio	3.10	1.68	1.66	1.32	0.60	0.45

Mineral nutrition

The result of the mineral nutrition experiment of *T. conophorum* showed that the absence of each of the three inorganic nutrients alone or in combination with each other had significant effect on the growth of the species. In all, from the analysis of variance (Table 16) the dry weight of the seedlings receiving all nutrients (NPK) was significantly higher than the others. ($P<1\%$ for -P, and $P<0.1\%$ for -K, -N and - NPK).

There was significant reduction ($P<0.1\%$) in the mean dry weight and mean leaf area when the species lacked any of the nutrient or all of the nutrients as compared to when all the nutrients were present (Figs. 20a & b).

The results (Table 16) also show that seedlings which received nutrient solutions lacking nitrogen, phosphorus and potassium (-NPK) had the poorest growth ($P<0.1\%$) followed by those which received solution lacking nitrogen (-N) ($P<0.1\%$). The growth of seedlings treated with solutions lacking phosphorus (-P) was still significantly higher ($P<5\%$) than those in solutions lacking potassium (-K). The leaves of the seedlings in treatments -NPK and -N became chlorotic and most of the leaves dropped off. This resulted in low leaf area and leaf weight ratio and high leaf area ratio especially that of -NPK i.e. the solution lacking the three nutrients. Also the absence of all the nutrients resulted in the seedlings having a high stem weight ratio and shoot: root ratio (Table 17).

TABLE 15:

The mineral content of *T. conophorum* seedlings grown at various salinities. Mean values of six replicates ± S. E.

Salinity (%)	K^+	Na^+	Ca^{2+}	Mg^{2+}	P	Total N (%)
	m equiv/100g dry sample.				ppm	
0	3.34 ± 0.32	2.481 ± 0.18	2.2 ± 0.16	1.548 ± 0.09	2.22 ± 0.15	11.676 ± 1.12
10	3.25 ± 0.31	3.461 ± 0.19	2.068 ± 0.13	1.428 ± 0.08	1.89 ± 0.053	8.524 ± 1.0
20	3.0 ± 0.29	3.668 ± 0.24	2.06 ± 0.15	1.23 ± 0.04	1.83 ± 0.8	6.104 ± 0.91
30	2.56 ± 0.21	3.716 ± 0.31	1.665 ± 0.09	1.027 ± 0.03	1.214 ± 0.061	5.309 ± 0.93
40	2.07 ± 0.13	3.637 ± 0.33	1.05 ± 0.08	0.713 ± 0.01	0.869 ± 0.03	4.501 ± 0.89

TABLE 16

Analysis of variance on the mean dry weight on the effect of different nutrient regimes on the growth of *T. conophrum*.

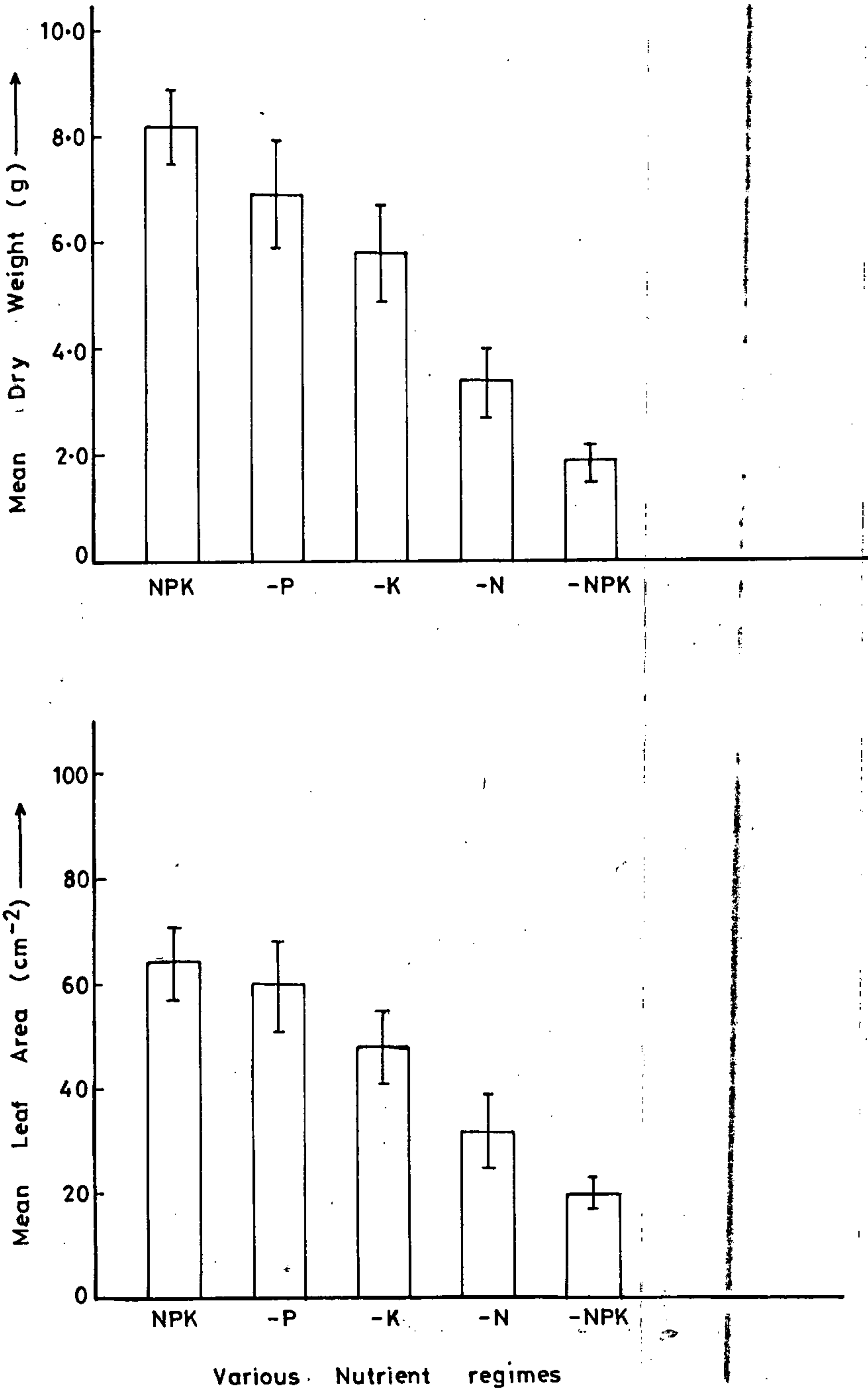
Sources of Variation	Degrees of Freedom	Sum of Squares	Mean Squares	F- Ratio
Treatment	4	161.13	40.28	
Error	25	14.38	0.575	70.05***
Total	29	175.51		

Nutrient regimes	Difference between means
NPK ¢ -P	1.35**
NPK ¢ -K	2.41***
NPK ¢ -N	4.86***
NPK ¢ -NPK	6.37***

Expected LSD at 5% = 0.91 * = Significant difference at 5% level
" " " 1% = 1.23 ** = " " " 1% "
" " " 0.1% 1.64 *** = " " " 0.1% "

Fig 20a: The effect of various nutrient regimes on the mean dry weight of _T. conophorum_ seedlings. Bars represent ± S.E.

Fig 20b: The effect of various nutrient regimes on the mean leaf area of _T. conophorum_ seedlings. Bars represent ± S.E.



10·0
8·0
6·0
4·0
2·0
0
Mean Dry Weight (g)
NPK
-P
-K
-N
-NPK
100
80
60
40
20
0
Mean Leaf Area (cm-2)
NPK
-P
-K
-N
-NPK
Various Nutrient regimes

The highest values of mineral elements were obtained in seedlings grown in complete nutrient solution. When any mineral element is absent the value will be lowest in the seedlings grown in such nutrient solution. Lowest values of the mineral elements were obtained in seedlings that lacked the three nutrients (-NPK) (Table 18).

TABLE 1 7

The effect of nutrient regimes on the growth of *T. conophorum*. Mean values of six replicates ± S.E.

Growth parameters	Nutrient regimes				
	NPK	-P	-K	-N	-NPK
Leaf area ratio (cm^2/g)	42.04 ± 3.55	41.77 ± 2.86	41.63 ± 4.55	65.32 ± 19.67	117.37 ± 12.17
Leaf weight ratio (% of total dry weight)	18.56 ± 1.22	21.15 ± 3.32	20.24 ± 3.37	15.89 ± 5.37	9.25 ± 1.10
Stem weight ratio (% of total dry weight)	50.82 ± 5.58	53.36 ± 6.72	55.15 ± 4.41	54.09 ± 10.38	66.06 ± 10.04
Root weight ratio (% of total dry weight)	30.62 ± 6.02	25.49 ± 9.20	24.60 ± 3.22	30.09 ± 9.83	24.69 ± 10.56
Shoot: root ratio	2.39	3.35	2.24	2.78	3.78

TABLE 18

The mineral content of *T. conophorum* seedlings grown under various combination of nitrogen, phosphorus and potassium. Mean values of six repicates ± S. E.

Various Combinations of Nitrogen, Phosphorus and Potassium.	K^+	Na^+	Ca^{2+}	Mg^{2+}	P	Total N (%)
	m equiv/100g dry sample.				ppm	
NPK	5.92 ± 0.35	3.22 ± 0.24	4.52 ± 0.4	1.716 ± 0.09	2.22 ± 0.12	15.308 ± 1.35
-P	6.48 ± 0.42	1.356 ± 0.04	2.41 ± 0.1	1.308 ± 0.05	0.277 ± 0.02	9.784 ± 1.04
-K	1.59 ± 0.09	2.105 ± 0.14	3.3 ± 0.31	1.74 ± 0.07	0.884 ± 0.05	9.844 ± 1.05
-N	2.95 ± 0.075	0.594 ± 0.08	1.94 ± 0.09	1.32 ± 0.03	0.528 ± 0.03	2.582 ± 0.18
-NPK	1.55 ± 0.07	0.372 ± 0.03	1.53 ± 0.07	0.626 ± 0.04	0.133 ± 0.01	1.939 ± 0.09

DISCUSSION

Many environmental conditions affect the growth and distribution of plants. The factors tested in this study are by no means exhaustive, but they were considered to be the most important to the growth, survival, distribution and cultivation of this species, bearing in mind its ecological habitat.

The response of T. conophorum to light and shade is similar to what obtains in nature at the stage at which these seedlings were harvested. In nature, the species grows under the shade of the trees that it climbs but with age as a climber, the stem twining around a support, it continues to move up and a mass of leaves is formed at the top of the support usually a tree where there is more light. The result indicates that the species is shade tolerant and it is not surprising because it takes T. conophorum quite sometime (some years) before it will finally reach the top of the support where it receives high light intensity. Thus the result obtained in this investigation where there was no significant difference between light and shade fits its ecological habitat.

It is not clear how the species adapt to the shade condition as it is not due to increase in leaf area which will help maximise the little light there is. It may be reduced respiration and efficient use of the little light reaching the seedlings by special arrangement of the leaves on the twining stems. It is likely that the tolerance of shade of this species may change at later stage of growth since it spreads on top of the crown of the climbed trees where it receives maximum amount of light. Whitmore (1984) has indicated that the light requirements of a plant may change with age. The result is similar to that of Oberbauer & Strain (1985) who reported that seedlings of Pentaclethra macroloba a neotropical tree species of Central America when grown in full sun or partial shade (25% sunlight) had similar growth and dry weight values.

This result showing tolerance of shade is in contrast to the results obtained with Luffa aegyptiaca whose growth was poor in shade but responded by increasing stem height and weight and producing many tendrils that will enable it to climb over the shading plants so as to obtain more light (Okusanya, 1978). Other tropical species which are shade intolerant are Treculia africana (Okusanya, Lakanmi & Osuagu 1991) and Uraria picta (Okusanya, Oyesiku and Lakanmi 1992).

Also the possession of large seeds in some species may be an adaptation for establishment in shade (Fenner, 1985). As T. conophorum possesses large seeds (2.5cm in diameter) with a lot of stored food it may also contribute to its shade tolerance.

The response of the species to varying soil moisture (Tables 6a & 6b, Fig. 16) clearly showed the overriding effect of soil moisture in controlling the growth of this species as can be seen by the sharp difference in the dry weight of the species in the wet and dry soil conditions.

T. conophorum performing better in the moist treatment can be attributed to the fact that it cannot thrive well in areas with low rainfall, low relative humidity and high evapotranspiration like the savanna region of Nigeria. This fits the species ecological requirements because the forest soil is wet for nearly 9 months of the year and the remaining period is at least moist (Keay, 1959). The high leaf area value in the moist condition at the end of the second harvest as compared to the low leaf area in dry condition still points to the fact that a decrease in moisture does not favour the species and so the leaves dried up and dropped due to lack of adequate amount of moisture. This is a behaviour to moisture stress since the availability of soil moisture is a function of the forces that hold the water in the soil (Larcher, 1980). The difference in leaf area - the photosynthetic organ may be mainly responsible for the difference in the dry weight.

The death of the plants by the third week under the water-logged condition is not surprising. It is known that high water regimes result in poor aeration (Kramer, 1949) and this will cause oxygen deficiency and accumulation of carbon-dioxide (CO_2). Thus poor aeration will have influence on plant growth, in which case root growth will be hindered since there is reduction in respiration rate and nutrient absorption is reduced because of the high dispersal of nutrients. High soil water also favours microbial reduction-reaction which influence the solubility and availability of nitrates, sulphates, ion and manganese (Russel, 1952).

Growth inhibition brought about by water-logging may also be due to the production of ethylene by the plant, because it has been found that in many crop plants ethylene has been shown to increase upon water-logging or anaerobiosis (Seliskar, 1988, Bradford & Dilley 1978, Jackson, Gales & Campbell, 1978, Newsome, Kozlowski & Tang 1982). Ethylene production is often stimulated by waterlogging and can bring about an inhibition of stem elongation and plant growth (Abeles 1973). All these point to the fact why the species is not found in fresh water, swamps or on river banks.

The experimental results presented for the soil types (Figs. 17a & 17b) show conclusively that of all the four soil types used humic soil is the best for the growth of T. conophorum. The poor performance in sandy soil could be due to the poor nutrient, poor organic content and poor water holding ability of sand in comparison to the humic soil (Russell, 1968). The good seedling growth in terms of mean dry weight and high leaf area in the humic soil can be attributed to high nutrient especially calcium and magnesium, high organic content (Table 10) and consequent high water holding ability. There was no significant difference between the four soil types at the first harvest probably because of the stored food in the seeds, so the effects of the various soil types became significant as from the second harvest when the stored food must have been exhausted. The fact that with time the humic soil showed significant difference (P<0.1%) from the other three soil types may be an indication why the species is restricted to regions with humic soil (the forest). The poorer growth in red earth and clay is similar to the significantly lower germination of this species in the same soil types compared to the humic soil. Normally, these two soil types tend to be water-logged, a condition which inhibits germination and growth of the species (Table 2) (Chapter 3). T. conophorum appears to be high nutrient loving since it grew best in humus with high nutrient content and under the soil type the seedlings had highest nutrient content (Table 10). This statement is strongly supported by the result of the mineral nutrition experiment where the best growth and highest nutrients were in the medium with complete nutrient (NPK) (Tables 17 & 18) (Figs. 20a & b).

Similar results of poor germination and growth in red earth and sand have been reported for Treculia africana another rain forest species (Mabo, Lakanmi & Okusanya, 1988, Okusanya, Lakanmi & Osuagu 1991). The response of T. conophorum thus fits its ecological requirements as the forest soil is humic.

The effect of different pH treatments on T. conophorum shows that this species cannot tolerate acid environment; since pH 3.5 and pH 5.5 had a significant decrease in mean dry weight and leaf area with harvest (Tables 11a & 11b,

Fig. 18). The decrease in growth may be due to the acidity i.e. hydrogen ion concentration and not lack of nutrients because the treatment medium had adequate nutrients. But the pH levels used in this experiment are lower than the forest soils; as in nature, forest soils are known to have pH between 6.0 and 6.5 which is only slightly acidic. Thus the species is expected to do better even in acidic soil which may have adequate nutrients (Okusanya, 1978, Vlamis, 1953).

The effect of pH on this species is unlike that of T. africana another tropical species growing in the forest which survives both in acidic and slightly basic media (Okusanya et al 1991).

The species could not tolerate salinity of 10% sea water and above as the dry weight and leaf area decreased with increasing salinity (Fig. 19). There was the usual pattern of increased leaf area ratio as the salinity increases, since there is proportionally low total dry weight compared to the leaf area. This result is not surprising since glycophytes achieve their best growth in non-saline conditions and their growth is reduced as salinity is increased (Ashby and Beadle 1957, Ungar 1974, Okusanya 1979, 1983). This probably explains why T. conophorum thrives only in the forest soils with very low salt concentrations or no salt at all (Table 14). Similar results were obtained with many other glycophytes whose growth were drastically reduced with increase in sea water concentration (Okusanya 1983, Sonaike & Okusanya 1988, Okusanya et al 1992, Okusanya, Lakanmi & Osuagu 1991).

One of the theories put forward by (Levitt 1980) to explain the causes of reduced growth in plants at high salinity is the inability of the root to absorb mineral nutrients due to competition with sodium ions in the medium. The high sodium content and the reduced mineral nutrients in the seedlings as from 10% sea water concentration (Table 15) attests to this. As such there is a wide range of limits of stress survival among plants. The limit for growth may be as low as 0.3% salinity in some glycophytes though others may grow at somewhat higher salt stresses. The limit may be indicated by a cessation of growth or by actual killing of the tissues (Strogonov, 1964) in the form of a necrosis, followed by a loss of turgor, falling of leaves and finally death of the plant (Kovalskaia, 1958). Such was the case for T. conophorum especially at higher salinity levels (40 & 50% sea water).

The increase in root development with rising salinity in this species is probably a response to water stress. The species may be producing more roots under water stress in a saline medium in an effort to reach more favourable regions (Brouwer 1963, 1968). Similar results were obtained with Crithmum maritimum (Okusanya, 1979).

The experimental results (Table 17) show that this species requires all the three elements tested for good growth. The results also show that nitrogen limits the growth more than either potassium or phosphorus judging from the analysis of variance data (Table 16) as the poorest growth occurs without nitrogen. Langer (1966), has reported that nitrogen has the greatest effect on growth affecting cell number and cell size, phosphorus has similar but less pronounced effects and potassium has the least effect on growth affecting mainly cell size. Similar results were obtained with L. aegyptiaca (Okusanya, 1983) that had fairly good growth in the absence of phosphorus and potassium when compared with results in the absence of nitrogen. Ingestad and Lund 1979, also described nitrogen as primarily influencing the development of leaf area which subsequently controls other growth and metabolic activities.

The increase in stem weight ratio at low nutrient levels, may be due to the fact that when nutrients are in short supply in T. conophorum, more dry matter is allocated to the stem at the expense of the root. This may be because the species is a climber and it would need to increase its stem length to climb. This result is contrary to that of Brouwer (1966), which reported that when nutrients are in short supply, more reserves are allocated for root growth at the expense of shoot growth thus resulting in a high root: shoot ratio at low nutrient levels.

Generally, it was observed that the leaf weight ratio decreased with time in all the parameters even in favourable conditions and so is the shoot: root ratio (Tables 5a, 5b, 6a, 7b, 9a, 9b, 9c, 12a, 12b, 14, 17). This may be due to the fact that as a climber and as it twines round the support, most of the older leaves at the bottom dropped off.

It can thus be concluded that rainforest appears to be the ecological habitat most suited for the growth and survival of T. conophorum as it performed best under those conditions which are similar to those found in the tropical rainforest. However in cultivating the species, because of its many uses, cognisance must be taken of the environmental factors which enhance its growth.

CHAPTER FIVE

GENERAL DISCUSSION AND CONCLUSION

The study of the biology of any species must of necessity include vegetative, reproductive, physiological, phenological, ecological and other aspects. However, with time constraint, only a few could be dealt with if thorough job has to be done.

This type of study is important especially in developing countries to enable us understand the lesser known food crops with a view to applying such knowledge in alleviating the problems of food shortage. The study of T. conophorum is considered important for its many uses most especially as food and the oil from its seeds which can be used as alternative or substitute for linseed oil in the varnish and lacquer industries (Hutchinson & Dalziel, 1954).

In this thesis, the vegetative and reproductive parts have been described in depth greater than hitherto given anywhere. Among the recent finds are the fact that the first pair of true leaves are opposite while the others are alternately arranged. The species is also known to reach maturity for the first time in 5-6 years but in subsequent years flowering and fruiting occurs usually annually but there may be fruiting failure in some years. Also, the fruit of the species is not always 4-seeded as expected rather 1, 2 or 3 seeded fruits occur. These are apart from the anatomy which was reported for the first time.

For seed germination, it is clear that the seeds exhibit innate dormancy due mainly to the hard testa (Fenner, 1985, Harper, 1977). However, this dormancy can be broken artificially by either chemical scarification with 1% copper sulphate or potassium permanganate or physical scarification by rubbing with iron sponge. The effect of scarification appears to be the weakening of the hard testa. Thus if large scale growing of _T_. _conophorum_ is contemplated, the seeds can be germinated much earlier (3 weeks) then in nature (7 weeks). The head start by such seedlings will be of immense value especially for a climbing plant like this species. Ross and Harper (1972), have reported that early emergence of seedlings is of great significance to their subsequent growth rate and the amount of space they occupy. Similar result of early germination was obtained in _U_. _picta_ (Okusanya _et al_ 1991). For _T_. _conophorum_ whose seedling growth is unaffected by shade, the importance may be to quicken the attainment of maturity and consequently early fruiting, the weather/environment permitting. Nonetheless, it is evident that the dormancy exhibited by this species in nature, may be of ecological advantage in that seeds are set as from April and germination then occurs as from June about 8 weeks after seed maturity when rains are heaviest providing the high moisture content needed by the seeds to germinate.

It is also clear that maximum seed germination will be obtained for the species when the seeds are sown in wet, non-saline humic soil of near basic pH, at a depth of about 2.5 cm and exposed to light at a temperature of about 31^{oC}. Since all these factors are present in the tropical rain forest, it is highly probable that the distributional limit of the species may be controlled by these factors. l Hence it is absent from the savanna regions of Nigeria.

The results of the effects of light and shade on seedling growth show that the seedlings are adapted to shade in that they responded similarly to light and shade. It is not clear what these adaptations are but such response and adaptation are not unexpected of a species which must first germinate and establish under the canopy of the tree it climbs. Like in germination, the favourable response of the seedlings to wet, non-saline humic soil and basic pH indicates the possibility of a rigid control of its distribution by these factors all of which occur in the rain forest. Thus, for agronomy purposes, seedlings like the seeds must be grown in wet, non-saline humic soil with near basic pH. The result of the mineral nutrient experiment confirm its requirement for high nutrient. Consequently, in the propagation of the species, fertilizers in the form of NPK should be added to the growth medium of the seedlings for best results.

In conclusion, from the results of these investigations, more detailed knowledge of the morphology and phenology of the species is now known. The anatomy of the root, stem and leaves is reported for the first time and can be used as examples in text books and in the teaching of students in secondary and post-secondary institutions.

Furthermore, with the knowledge gained about methods of breaking seed dormancy in the species, seedlings could be raised early. Also with the knowledge of some environmental factors which aid seedling growth, the plant could be raised not only to reach maturity early but also to give heaviest/highest production for human consumption and for the industry.

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