Rhodes Thesis.

AN ANATOMICAL INVESTIGATION
OF
JOINTED CACTUS (Opuntia aurantica, Lindley)
WITH GERMINATION TESTS, AND OBSERVATIONS
ON THE MOVEMENTS OF THE STOMATA

THESIS

PRESENTED FOR MASTER OF SCIENCE

DEGREE

BY

E. E. A. ARCHIBALD.

3 microfiche

In presenting this thesis I should like to express my thanks to Professor N. J. G. Smith at whose suggestion this subject was undertaken, and under whose direction the work has been carried out. I owe a deep debt of gratitude to Dr. A. C. Leemann, of the Division of Plant Industry, for his kind assistance in obtaining references, for his advice in several technical matters, and for the use of his photographic apparatus with which most of the photomicrographs in this thesis were taken. I am also indebted to Miss L. Britten for several references, and for her guidance during Professor Smith's absence.

I wish to thank Mr E. du Toit, Officer in Charge of Jointed Cactus Eradication, for the material he has supplied, and for his help in arranging field excursions; Dr. Pettey, Officer in Charge of the Cactoblastes Station, Uitenhage; the officers in charge of the meterological station at Grahamstown and Uitenhage; and the Holy Rosary Convent, Port Elizabeth for the loan of a Stevenson Screen, without which the work at Uitenhage would have been seriously handicapped.

The work has been carried out with the help of a University Research Grant, a University Research Scholarship, and a Government Grant from the Division of Plant Industry, for all of which I make due acknowledgement.

CONTENTS.

Page.

Introduction

External Appearance & Plant Habit

Anatomy of Joint

Secondary Growth

Structure & Development of Reproductive Organs

General Physiology

Stomatal Observations

INTRODUCTION

In 1934 the Government started an active campaign on the eradiction of Jointed Cactus. This plant was causing considerable damage to the farms and commonages of the Eastern Province, and was spreading at an alarming rate. The Officer in Charge of Eradication, Mr. E. du Toit, was stationed at Grahamstown which is fairly centrally situated in the infested area. In 1935 Dr. A. Leemann of the Division of Plant Industry made his headquarters at Rhodes. Univ. College

It became evident that a knowledge of the anatomy of the plant, its conducting system and the movements of the stomata might be of use in combating the pest. It was also necessary to know something of the structure and vitality of the seed and its germinating capacity. No previous work of this nature had been done on the Jointed Cactus, and application was made to the Botany Dept. of Rhodes University College, for some student who had time to make such investigations. At Dr. Smith's suggestion I undertook the investigation as a subject for my thesis.

NAME.

At one time the Jointed Cactus was known as *Opuntia pusilla* (28) and as such was proclaimed a noxious weed in the Cape, the Orange Free State and the Transvaal. Lansdell gives her authority as the Agricultural Gazette, N.S. Wales April 1911, for determing it as *Opuntia Aurantiaca* Gilles, and as such it has been proclaimed a noxious weed in the Cape and Natal.

The correct authority for *OpuntiaAurantiaca* is Lindley (29). I am indebted to the Department of Agriculture and

Forestry for confirmation of this.) According to Britton and Rose (2) in their key and description of genera and species of the Cactacae, *Opuntia aurantiaca* is described by Lindley in Edward's Botanical Registrar 1833. *Cactus aurantiacus* Lemaire 1868 (2) is usually cited as a synonym, but this is Gilles manuscript name first published in the Botanical Register 1833 as a synonym of *O. aurantiaca*. In the Index Kewensis (25) *Cactus aurantiaca Gill*. in Lond. Hort. Brit. Suppl. 11, 652, 1839 is quoted, so Lindley has priority. *O. aurantiaca extensa* Salm Dyck (Pfeiffer Enum. Cact. 147, 1837) is also quoted as a synonym(2).

In Britton and Rose (2) the following description of *O. aurantiaca* is given:-

"Low much branched spreading; stem terete or sub-terete 1 to 2 cm thick; joints very fragile, linear, 6 to 8 cm long 1·5 to 2·5 cm broad, almost terete at base, dark green shiny; areoles somewhat elevated, filled with white wool; spines 2 or 3 brownish 1 to 3 cm long; flowers yellow 2·5 cm broad fruit 2 to 2·5 cm long."

In comparison with this description the South African plants are much more robust. They have been known to attain a height of 7 or 8 feet. The joints vary a good deal in size but are usually thicker, and may be 10 to 20 cm or more in length. The spines in the areoles are more numerous, the flowers larger and the fruit very often bigger. In other respects it conforms to the description given by Britton and Rose.

The popular names for the plant are Jointed Cactus, "Litjies-kaktus and "suurtjies", the later being the name by which it is known in the Uitenhage district.

HISTORY

The plant is indigenous to Argentina and Uruguay (2) although Chili is a locality frequently quoted in error.

Apparently the first record of it in South Africa is from the Cape Province in 1878 (28) the general opinion being that it was imported as a garden plant. Garden enthusiasts are also attributed with its presence in the Eastern Province. Since then it has spread rapidly by vegetative propagation, being carried by stock and washed down by rivers to other parts of the country. It has been listed as a noxious weed in all four provinces. In the Eastern Cape it is particularly troublesome.

Schonland (45) in 1924 gave a rough estimate of the total infestation in the Eastern Province as :-

Commonages	14,000 morgen
Farms	35,000 morgen

He suggested that the approximate cost of clearing a farm of 500 morgen would be about £2 per morgen.

Opuntia aurantiaca has evidently been spread to Australia as well. There it is classed as one of the 9 species of Opuntia which are pests. Alexander (1) investigating the variations of acclimatised species of prickley pear, found no variation in *O.aurantiaca*, *O.vulgaris* and *O.streptacantha*. So far I have come across no literature refering to it as a pest in South America.

PREVIOUS WORK AND REFERENCES.

In the literature available I have found no mention of anatomical or stomatal work carried out on O. aurantiaca, with the single exception of Bukvic's (4) paper on the thylloid blocking of the stomata, where figures for O. aurantiaca are given.

I shall therefore endeavour to carry out the work on a comparative basis, refering to work done on other members of the Cactaceae, and Opuntia in particular, where possible.

For general anatomical references to the Cactaceae I have used the standard works of Solereder (47) and Engler Prantl(16), other incidental references to particular structures were found in Chodat (9), for which I have to thank Dr. A. C. Leemann, in Strasburger (51) and in the older text books of de Bary (12) and Sachs (44).

A list of references is included at the end of this thesis. Complete reference of the works of older authors whose papers it was impossible to obtain is not given, but reference is made in the text to the books in which extracts of their work is given.

W. F. Ganong(17) seems to have tried to arouse interest in the Cactaceae in the later part of the last century. His paper on "Present problems in the anatomy morphology and biology of the Cactaceae" gives a brief summary of the existing knowledge, and points out what is still to be done. He has endeavoured to fill some of these gaps himself in his work on the embryology and seedlings of Cact. (18,19). The works of d'Hubert 1896, Dabishire 1904, deFraine 1910 (13) supplement this to a small extent. Johnson's study of the fruit of O. fulgida 1918 (27) helps to clear up the morphology of certain parts. Present day references are not numerous and are mainly by German botanists in publications difficult to obtain in this country.

Cytological work is being done by Stockwell (51) and seems to point to the possibility of a generic distinction in the number of the chromosomes.

There has been no physiological work done on the stomata of O.aurantiaca. Direct reference to the work of Shreve (46), and Livingstone (30), on the movements of stomata and their influence on transpiration in the cacti, was not possible. Their chief results are however, briefly summarised by Maximov (37) and Loftfield (33). It is mainly on the stomatal work of Lloyd (32) and Loftfield (33) that the observations in this paper have been based.

OUTLINE OF WORK

Reference to the index will show the general scope of the work. Since in a subject of this nature illustrations are necessarily somewhat numerous, they are enclosed in a separate folder, which ~~enables~~ makes it easier to refer to them when reading the text.

The succulent nature of the Jointed Cactus must be emphasised. In this respect it is eminently suited to those parts of South Africa where the (sweeping!) natural vegetation is of the succulent type. It is in these areas that Jointed Cactus chiefly occurs.

The structure of the epidermis, stomata, hypoderm, ground tissue, and of the conducting system is dealt with in detail. They all differ to a greater or less extent from those of normal mesophytic plants. Attention is drawn to the numerous tracheids of the vascular system, they are apparently a common feature in other Cactaceae as well. The hydrocytes, which have been termed tracheids by most other investigators, are parenchymatons cells with peculiar thickening, and they act as water storage cells. They are dealt with in the section on the conducting system since they occur most frequently in conjunction with the vascular bundles. Their development is given here, since except for a passing reference in Chodat (9) no recent work has been done on them, and in the works of older writers there is some confusion as to their development and occurrence.

Mucilage is the outstanding feature of the plant. The occurrence and distribution of mucilage cells and canals is briefly mentioned here, since Dr. Leemann is making a thorough investigation of the subject.

The type of secondary thickening differs from that of most dicotyledonous stems and is well adapted to the production of succulent tissue.

The formation of underground storage organs by fallen stems and fruit is a feature of which I can find no previous mention. An account of these structures, which are here termed tubers, is given. Apparently the fruit of Jointed Cactus is unique in being able to produce underground storage structures. They are similar to those produced by the stem. These tubers contain enormous quantities of starch, and can retain their vitality during long periods of unfavourable conditions.

Following Johnson (27), the development of the flower is given, it is the same as in O.fulgida and leads to the same conclusion as to the morphology of the fruit. This is of interest in accounting for the areoles or axillary buds which occur on the ovary wall, and which enable the fruit to propagate vegetatively.

The development of the ~~fruit~~ ovule is especially noteworthy in connection with embryo formation, and in accounting for the dormancy of the seed. This has not been clearly illustrated before. (Recent work by Neumann, and Hubert, on the ovules of other members of Cactaceae, has not been procurable, but reference is made to the summaries of their work given in Biological Abstracts).

Polembryony in O.aurantiaca is here recorded for the first time to my knowledge. Proembryo stages and the causes of polyembryony have still to be investigated.

The percentage of germinable seed is very low and germination tests have necessarily been curtailed owing to the scarcity of seed. Germination in the field occurs only very rarely, the reason for this is given. Artificial germination of seed has not been recorded by other investigators.

Stomatal observations, on potted plants and on plants growing in the veld at Uitenhage are recorded. Readings from Uitenhage show that the stomata were apparently closed over considerable periods as far as microscopic investigation revealed.

The present work is not intended as a complete account. Several anatomical points require a closer investigation, and a further knowledge of the germination of pollen grains, fertilization and embryo development will probably show why germinable seed is so scarce. The question of abscission layers and the period, if any, of the formation of such layers has not yet been tackled. The presence or absence of wax requires a more exact investigation. Observations of the stomatal movements of young joints will probably be undertaken.

EXTERNAL APPEARANCE AND PLANT HABIT.

GENERAL APPEARANCE

As the accompanying photographs (fig 1,2,) show the plant is devoid of leaves except in the earliest stages of the young shoots (fig5a). The green colour of the stems, which are the assimilating organs, is often masked in the older stems by reddish anthrocyanin pigments (54). The stems are thick, (2 or 3 cm or more) and succulent, and are somewhat cylindrical in the young stage but when mature are rather flattened. They form an articulated system of "joints". Each shoot tapers toward the apex and rounds off, marking a period of growth, this constitutes a joint which may be 10- 20 cm or more in length. Further shoots are given off at the apex, slightly to one side, not as a continuation as in ordinary shoots, but as a separate unit as it were. Thus the plant gets its characteristic name of "Jointed Cactus" These joints do not mark the limit of a single year's growth, for a joint which has originated early in the growing season may give rise to other joints in the latter part of the season, while it itself continues to grow in length and thickness for several years.

The joints are covered with round markings, the areoles, (fig3a) which correspond to the nodes of an ordinary stem. In the young shoots the areoles appear in the axils of the small succulent cylindrical leaves (fig 5a,b). The base of the stem to which the leaf is attached protudes in a swelling or tubercle (fig 5a,b,). These tubercles become less and less prominent as the surrounding tissue fills out, and finally they loose their identity entirely, the leaves having fallen off, and the areoles, by a process of growth, appear in the old turgid joints to be almost flat with the surface (fig 3a). The morphology of these parts is commented upon in a later section.

The areoles give rise to trichomes, thorns and bristles appearing in the order named. The trichomes are soft white multicillular monosiphonic hairs, occuring in great numbers all over the areole. There may be 5 to 6 thorns in an areole, they are usually from 1 to 2·5 cm in length(Figs 3c, 3b) Bristles appear later forming a cushion on the base of the thorns, they are rather yellow and 2 - 3 mm in length. Further details of the anatomy and morphology will be given later. The question of nectaries has not yet been investigated. Although they are reported for a large number of <u>Opuntias</u> by Goebel (20) Johnson (27) and others, they were not noticeable in this case.

The flower buds are exactly similar in appearance to the vegetative buds in the early stages(fig3c) It is only later when the reddish sepals appear at the tip that they can be distinguished (fig7). The ovary is inferior and is enclosed by succulent tissue exactly similar in appearance in structure to a joint. The outer wall of this tissue is similar to that of a joint. It has areoles which bear leaves in the young stages and later thorns, bristles and trichomes, as do the areoles of a vegetative joint (fig7), and in this respect shows remarkabel difference when compared with the usual type of angiosperm ovary. This ovary wall may vary in length from 2 to 3 cm or more and is about 2 cms thick. The perianth members spring from a concave receptacle at the top. The petals (fig 4) which are fairly numerous are a pale but brilliant yellow Stamens are numerous and there is a thick central style all of uniform yellow colour. Thus when the perianth members fall the fruit may easily be mistaken for a small vegetative joint (fig 8a).

GROWING PERIODS.

The vegetative buds appear at the areoles with the first rains of August and September. The young bright green joints, from which the leaves soon start to fall off, grow rapidly during the spring and early summer months. Flower buds arise from the areoles of the older joints about November, and the flowering period continues until March or April. In the latter part of the summer the joints may appear wrinkled and shriveled, if they have been several weeks without rain, and had to endure high temperature and strong sun. On the second or third day after a good shower, however, they become round and turgid, and by the fourth day new vegetation and flower buds usually appear.

HABIT

In open ground the plant forms dense circular clumps (fig 1,2). It mingles with the natural bush however and becomes inextricablly entangled with it. Most commonly it reaches a height of 3 or 4 feet, but has been known to grow to 7 or 8 feet in height. The main stems become tough and woody and increase in size. (fig 3a.)

The thorns are provided with barbs (fig6) so that they cling to any body which brushes against them. The joints are only loosly attached to one another and are very easily pulled off and scattered by animal and human agency. Any joint which falls to the ground, given suitable moist conditions, is capable of sending forth roots from the lower areoles and shoots from the upper ones. It is by this mode of vegetative propagation that the plant has spread so rapidly (fig 3b). The fruit is also capable of root and shoot formation and vegetative propagation, a most unusual occurrence in comparison with the fruit of other plants.

Joints which fall to the ground and become buried, lose their photosynthetic powers, grow roots and shoots, store up large quantities of starch and increase in size considerably (fig 35). Since these are stems, which have become subterranean by force of circumstance, and have become adapted for storage purposes, whilst retaining their power to send further roots and shoots, it is proposed to call them "Tubers", since in all essentials they can be compared with tubers. In addition to vegetative joints, fruit form~~ing~~ structures similar in appearance, structure and function. Fruit that fall to the ground and become buried do not rot away, as with nearly all other types of fruit, but they send out roots and shoots from their areoles. Starch is stored in large quantities, and cork and secondary growth of vascular system, takes place in a manner exactly similar to that of the subterranean joint, since in all particulars of structure, the fruit is the same as the joint, except for the ovary cavity and seed within the upper portion. In the young stages of secondary growth it is possible to distinguish whether such a structure is formed from a joint or a fruit. The abrupt end to the top of the fruit is one distinguishing character, and if it be cut open, the ovary cavity gives a second distinguishing character. But in the older structure where considerable secondary growth has taken place, these distinguish marks become entirely obliterated, and one cannot tell whether the original structure was a fruit or a joint.

For reasons, which are given in the section on secondary growth and the formation of underground storage and perennating organs, I have decided to term such organs whether produced from joints of fruit, "tubers", distinguishing between them where necessary, as "joint tubers", and "fruit tubers".

xThe dangers of vegetative propagation are realised, when it is known, that very small joints, ranging in weight from ·08 gms to 1·5 gms, had taken root and were growing within 5 months after being placed on the ground.

THE AREOLES AND THEIR APPENDAGES.

As stated above the areoles of the joint correspond to the nodes of an ordinary stem. In the young joint they appear to be raised on tubersles (fig 5a,b), but in the older joints they are flat or almost flat with the epidermis. Fig 10 shows their origin from the growing point. The leaf is developed first. Some of the tissue in the axil of the leaf remains meristematic, and this soon gives rise to trichomes, and shortly afterwards the first thorns are formed from it. When the leaf is left behind by the rapid growing point, the meristematic tissue increases giving rise to a large number of monosiphonic multicellular trichomes (fig9) which mark off, on the epidermis, the circular area of the meristematic zone of the node and so give it the name of areole.

THE LEAF

The leaf is 2 to 3 mm in length and terete. The single vein from the leaf trace of the stem continues right up almost to the tip(fig10). At the base there are several phloem cells and annular or spirally thickened tracheids. The phloem elements do not continue far up into the leaf, but the tracheids continue almost to the tip. The remaining tissue of the leaf consists of small parenchymatous assimilating cells, and several large mucilage cells, which are specially conspicuous on the dorsal side of the leaf (fig10) A few crystals of calcium oxalate also occur. There is no conspicuous hypoderm and the epidermal cells are fairly regular with a thin layer of cuticle on their outer walls. The stomata are fairly large and numerous for so smalla leaf, and contrary to Solereder's (48) remark as to their abortive and anomalous development, they have been seen to function like ordinary stomata. The leaf fall takes place early in the life of a joint. The abscission layer (fig11) is responsible for this, the exposed portion being protected by cork formation.

THORNS

As already stated the soft thin walled hair-like trichomes appear first. Very soon afterwards the meristematic tissue gives rise to thorns. Dr. Leemanndrew my attention to the fact that the first two thorns to appear ~~are~~ to be always equally developed, and arise one on either side of the leaf. Shortly afterwards a third (~~[illegible]~~) thorn which is bigger than the other two, appears in the centre of the areole in the axil of the leaf (fig5a,b) Later, as the joint develops, other thorns appear irregularly placed in the areole, so that a well matured joint may have 5 to 6 thorns to an areole, each thorn from 2to3 cm long.

The thorns arise from the sub-epidermal tissues of the areoles. There is a central ~~foliar~~ conical mass of thick walled fibres filled with air. (see Johnson (27) and Solereder (47).) The outer cells haver rather thinner walls. The cells are elongated and arranged end to end in longitudinal rows, their walls are always straight. At the top of the thorn, the bases of the cells of the outer layer, project outwards and downwards, forming very effective barbs at the tip of the thorn (fig6). According to Englemann (48), these barbs are a characteristic which is peculiar to the Opuntias, and form a point of difference from other members of the order.

The morphology of these thorns has been commented on by many observers. Rudolph (48) thought that the thorns of O. missouriensis were simply trichomes; Delbronet (48) suggested that they were transitions between "leaf prickles" and trichome prickles; Caspari (48) and Schumann (48) that they were emergencies. While Zuccarini (48) Kaufmann (48) Goebel (20) and Gangong (17) stated that they were of a foliar nature. Darbishire (11) considers that in Mammillaria elongata at any rate, the tubercle is the highly developed leaf base. The spines together representing the leaf blade , the leaf stalk being absent. According to Strasburger the thorns of the Cactaceae represent leaf primordia . In *O.arborescens* Goebel (20) found all stages in transitions, from thorns to leaves. Such differences could also be brought about by artificial means.

In *O. aurantiaca* the position of the first two thorns is very suggestive of that of stipules, whilst the third thorn appears in the position of a branch and seems to be of more deep seated origin than the other two. There is no vascular supply to it however, nor could Caspani (48) find any vascular connection in the specimens he examined. The evidence in this case therefore favour the theory that the thorns are modified organs of foliar origin.

BRISTLES

The bristles are the last to appear in the areole. They are about 2 mm long, narrow straight and rather yellow. Like the thorn the outer cells at the tip of the bristles project outwards and downwards forming barbs. The bristles are of superficial origin, their weak connection making them very easily detachable. They appear first on the abaxial side of the areole, increasing in numbers with the age of the joint

The older areoles are further protected by cork forming at the base of the bristles and thorns (fig 12).

New shoots arise in the growing season from the meristimatic tissue which forms the areole. It is also this meristimatic tissue of the areole, both in fruit and joints, which is capable of sending further roots when the joint or fruit is lying on the ground. This process of root and shoot formation, and the manner and time of formation of the abscission layer at the base of the joints making them easily detachable, requires further investigation.

The presence of fungi on the thorns, and in the areoles, has been commented upon by Dabishire (11) for *Mammillaria elongata*. Dark brown hypal threads occasionally giving rise to spores of a type similar to *Fusarium*, have been seen frequently on the thorns and in the areoles of Jointed Cactus. The fungus apparently does no harm. The hyphal threads live on the surface of the thorn and do not appear to penetrate the cells. In the very old joints the fungus is apparently able to get between the cells of the thorn which can then easily be broken.

ANATOMY OF JOINT

STAINING METHODS

The chief difficulty in making sections of the joint is the enormous quantities of mucilage which are set free immediately a cut is made. It was found better to work with material which had been previously fixed in Carnoy's fluid (8). The stain which gave the most satisfactory results was Chodat's Geneva Red (50) which was recommended by Dr. Leemann. The sections were placed in Eau de Javel, immediately after cutting, and after an hour or less in this they were quickly passed through water and then stained and mounted in glycerine or glycerine jelly. It was impossible to cut microtome sections of any part of the joint, since the mucilage could not all be extracted, and with the heat of the embedding oven it lost water and the tissues contracted, beside the mucilage did not allow the wax to penetrate the tissue properly.

EPIDERMIS

In both the young and old joints (fig 15,21) the epidermis is somewhat undulating owing to the varying size of the epidermal cells. In surface view (fig13) the epidermal cells are irregular in shape with rather thick walls.

The cuticle of the young epidermis is only a very thin layer. It increases in thickness with the age of the joint as a comparison of figs 15-22 will show. The outer wall in fig 14-20 is not in sharp focus, so that in these figures the cuticle appears much thinner than it really is at this stage.

Wax has been reported to occur on various species of Opuntia by de Bary (12), Solereder (47), and Engler-Prantl(16), where it is said to occur as a thin continuous covering first, later it forms "very delicate, angular, homogenous scales, like the fragments of a delicate skin of wax, also found as a rudimentary form of the glaze on many smooth shining epidermal layers". (12)

No wax of this nature was noted in <u>O. aurantiaca</u>, nor does any sign of it show up in the photographs. It requires a more careful investigation with chemical tests before ~~this~~ it can be definitely stated whether wax is present or not, and if it is, in what form.

Anth~~r~~ocyanin pigment occurs in the epidermal cells of the joint and fruit (55) giving the parts where it occurs as a dark, dirty pink colour. Very often the pigment is more conspicuous round the areoles. Usually it is more abundant in the old joints, and was very much more noticeable in the plants growing in the veld at Uitenhage, than in those cultivated for the purpose of study. It was also noticed that in joints lying on the ground the colouring matter was so abundant as to hide the green appearance of the joint. But the lower unexposed portion, which was resting on the ground, was devoid of pigment.

HYPODERM

In a very young joint (fig 15) the hypoderm appears as a tissue, just below the epidermis, of 4 or 5 layers of irregularly shaped cells, whose walls appear to be slightly thicker than those of the epidermal or cuticle cells. The walls rapidly become thicker until finally the whole tissue becomes collenchymatous (fig 21, 22). The cell lumen is small, and very thick cell walls are perforated by large, simple pits. These cells are capable of increasing in size as the joints expands, as will be seen if Fig 17 -22 are compared, they are all of the same magnification. The cell walls turn a pale mauve when treated with Schultz's solution, the thickening being evidently a type of cellulose. The cells retain their nuclei and cytoplasm.

According to Engler-Prantl (16) the collenchymatous hypoderm is common to the majority of <u>Cactaceae</u>, except <u>Mammillaria</u>, where it may or may not occur. Ganong (17) has suggested that it may act as a water storage tissue, this has not been verified. Its chief function however appears to be mechanical, giving rigidity to the outer tissues of the joint while allowing for expansion. In fact it seems to be the only true mechanical tissue in the joint, until fibres appear, which is not until considerably afterwards.

The cells of the hypodermis adjacent to the epidermis contain large rosette crystals of calcium oxalate (crystals insoluble in acetic acid, but gradually soluble in sulphuric acid). They can be seen developing in the unthickened cells of the young hypoderm (fig) (17). They become so large as to distort the epidermal cells (fig 21) and occur at irregular intervals in a row below the epiderm (see fig 32, where cork formation is in progress). They show through the epidermis when seen in surface section (fig13) and greatly add to the difficulties of stomatal observations. They might also be said to act as mechanical elements, but what their further function is, beyond the storage of material, is rather obscure. Ganong (17) suggests they have a biological significance in preventing insects, or snails etc. from eating into the joint.

As in other Cactaceae (12) the hypoderm is only interrupted at the place of the respiration or substomatal cavities below the stomata. The schizogenous formation of this cavity or passage can be seen in fig 15. By the formation of the stomata and the growth of the cortical cells, it finally becomes a passage such as in fig 21. This passage has a lining layer of cuticle, a feature apparently common in the Cactaceae (47), but not known in any other plants as far as I can ascertain.

It is interesting to note the increase possible in the circumference and thickness of the joint, from increase in the size of the cells, as shown by the figs 17-22, which are all of the same magnification. The respiratory passage too, widens, with the increase circumfrence of the joint. The width of the passage shown in fig 21 was ? while that of fig 22 was

References to further changes in the hypoderm are made in the section on cork formation.

STOMATA

STRUCTURE AND DEVELOPMENT

According to Schleiden (47), the Cactaceae have a stomatal apparatus characteristic of the entire order.

In surface view the two rather large guard cells of the stoma of the stem of O.aurantiaca, ~~which~~ are about 48μ long and 20μ wide, + are surrounded by two, sometimes more, subsidiary cells, whose walls appear slightly thinner than those of the surrounding epidermal cells. (fig14). Only the guard cells contain chlorophyll and ~~form~~ starch.

In transverse section (fig 14, 21) the stoma shows itself to be a transition type, between the usual Dicotyledon type, and the Helleborus type, refered to by Strasburger (51). Rheforms (42) does not figure a similar type in his studies on stomata, in which the Cactaceae are not mentioned. The guard cells have on the whole rather thin walls (fig 21), the thickening is most pronounced on the top outer wall, and especially where it joins the walls of the subsidiary cells, both on the outer and inner sides. As with most dicotyledonous types it is the dorsal wall which is the thinnest. In transverse section the subsidiary cells are rather semi-circular in shape, their bulging ends projecting slightly both above and below the quard cells, which are thus sunk slightly below the surface of the epidermis. The CUTICLE outside cuticle of the epidermis forms a uniform layer over the subsidiary cells and guard cells.

Below the stoma is the long respiratory cavity formed by the interruption of the hypodermis, and sometimes a portion of the cortex (fig 21), so that it may be about 76μ to 146μ in depth, and in a normally functioning stoma is usually 17μ in diamter. With further growth of the joint and other changes mention below it may become even wider. ~~or more~~. As in other members of the Cactaceae, a layer of cuticle covers the slightly projecting lower ends of the subsidiary cells, and forms a lining to the whole of the respiratory cavity (fig 21). There is thus a remarkable difference between the stomatal ~~mechanism~~ structure just described and that of most mesophytic plants. It is more comparable with the stomata of many South African succulents and xerophytes, where sunken guard cells, large respiratory cavities, ~~and~~ are more common.

The development of the stoma, which has not, to my knowledge, been followed up for any species of the genus <u>Opuntia</u>, is essentially the same as in most other plants. An initial cell is divided by tworapid successive divisions into 3 cells, the middle one, the mother cell, being slightly larger than the other two, the subsidiary cells. The mother cell is soon halved by a third wall, the beginnings of which can be seen in fig 17. cf l. f.

At this stage also the cells of the hypoderm, immediately below the developing stoma, can be seen to separate schizogenously, as a result of the expansion caused by the stoma. Fig 17-20 show successively the further development of the guard cells, formed by the division of the mother cell. The dorsal walls become rounded, and project into the subsidiary cells, and thickening starts on the outer and inner walls. This increases, as the wall between the guard cells develops, and forms two separate walls, which split apart as the guard cells round off and push away from each other. Meanwhile the contents of the mother cell ~~has~~ have also divided, so that each guard cell contains chloroplasts etc. The respiratory cavity increases in depth and width with the formation of the stoma, though this is not shown ~~too~~ very clearly in figs 18-20, where it had to remain rather out of focus if the guard cells were to be shown up clearly. The stomata of the ovary wall are exactly similar to those of the joint.

The stomata of the leaves, which drop off quite early in the life of the joint, are somewhat similar to those of the joint in fig 20, i.e. the thickening is not so pronounced as in the fully developed stoma of the joint, the guard cells do not project below to such an extent, and the respiratory cavity is small and is more comparable to that of an ordinary mesophytic leaf, than to the exceptional elongated passage through the hypoderm in the joint. The leaf stomata are smaller too, being about 34μ long and 14μ wide.

According to Solereder (47), the stomata of the leaves of ~~*Optunia*~~ Opuntia are said to be abortive or anomalously developed. I have not found this to be the case in *O aurantiaca*, and observations showed, that the stomata of the leaves open and shut, and are influenced by external conditions, as are the joint stomata.

DISTRIBUTION OF STOMATA.

deBary (12) considers that 18 stomata per sq mm as found in *Cereus Speciosus* is a fairly high number, but only when compared with the stomatal frequency of stems. In *O.aurantiaca* the joint stomata occur about 16 to 23 per sq mm. The lower number being recorded from joints collected at Uitenhage. The younger joints have about 23 per sq.mm, and the leaves about 37 per sq mm, which is on the low side when compared with the numbers given for the majority of leaves, de Bary (12)

The stomata have their long axis parallel to the long axis of the joint, but some slight variations occur.

BLOCKING OF RESPIRATORY CAVITY

Bukvic (4) having investigated 14 species covering 6 genera of the *Cactaceae*, found that not infrequently the stomata were blocked in a peculiar manner. He found that this blocking might start from the subsidiary cells, from the mesophyll cells or from both at the same time. Often he observed all three instances in the same section, so that according to Bukvic there does not seem to be any one established mode of blocking for a particular species.

He gives figures to show how a cell of the mesophyll, may grow up from the mesophyll into the stomatal cavity, as in *Cereus bonplandii*. This thylloid cell may divide or it ma be sufficient in itself to completely block the cavity. In other cases the thylloid blocking originates from the subsidiary cells. The subsidiary cells grow out below the pore, come into contact, and one of them, or both, grow down into the respiratory cavity, divide and fill it completely. In *O.aurantiaca* for which he gives a figure, Bukvic says the stopping is of this last type. The subsidiary cells grow out towards each other, but they may or may not meet or fuse. In any case they make the opening below the pore considerably less, even if they do not actually block it.

As any interference with the free movement of the stomata, or blocking of the sub-stomatal cavity, might affect the penetration of a liquid, sprayed on the plant as a poison for eradicating, special attention was paid to the possibility of this occurrence in the Jointed Cactus of this country.

Sections of older joints showed that growth of the subsidiary cell had taken place as Bukvic described. If it is remembered, when comparing figures 21-22, that they are both equally magnified, it will be seen immediately what growth and increase takesplace in an old joint such as the one from which fig 22 was taken. Not only have the subsidiary cells grown out beneath the stoma, but they have also increased in width. The outer and ventral walls of the guard cells have become much thicker. The cuticle which has thickened on the epidermis is also much thicker on the top of the guard cells. The enlargement of the cells of the cortex and hypodermis, whose cellwalls are much thicker than in fig 21, has widened the respiratory cavity considerably, but in spite of this, the apperture below the stoma and between the subsidiary cells is very much less than that in fig 21.

This growth of the subsidiary cells, and thickening of the cuticle on the guard cells, was found to be very common in the old joints, and was first noted in the wrinkled joints collected at Uitenhage after a period of drought and heat. It is suggested that the increase ~~growth of~~ thickening interferes with the free movement of the guard cells, and accounts for the long periods during which the stomata are shut, as is shown in table. The same procedure seems to take place in the stomata of joints which have fallen to the ground, but which have been prevented by adverse condition from taking root. A similar blocking or narrowing of the respiratory cavity by the subsidiary cells immediately below the pore, has been figured by Rhefous for *Evanymus japonica* (42)

Further reference to the blocking of the respiratory cavity will be found in the section dealing with formation of cork.

CORTEX AND GROUND TISSUE

As fig 15 shows the cortex, which is the assimilating tissue, consists of somewhat elongated, regular cells, like pallisade tissue. The cells greatly increase in size with the age of the joint, and by cell division the appearance of regular arrangement disappears. The cells become round or oval in shape and the intercellular spaces, which are never large, become more numerous. Fig 15 shows that the pith cells are round even in the young joint. As with the cortical cells, these cells increase greatly in size as well as new cells being formed by cell division. According to Engler (16) all tissues of the Cactaceae stem are able to assist in the thickening of the stem, because they remain able to divide for quite a long time, besides greatly increasing in bulk themselves.

Except for the narrow margin of epidermis and hypoderm, and the small space occupied by the vascular system, the whole of the rest of the stem is occupied by this larged celled paranchyma. Their size largely depends on turgidity, as their walls are very thin and collapse as soon as water is withdrawn. As 91% of the fresh weight of a normal joint is water, it is not to be wondered at that the joint presents a very shrunken appearance when subject to drought conditions.

Conspicuous pit areas in which there are numerous simple pits, are a feature of adjacent walls of the parenchymatous cells of both pith and cortex.

The cells of the outer portion of the cortex, for a depth of about 7 or 8 cells, contain numerous ovoid chloroplasts, distributed along the walls of the cell. In these cells there are small quantities of starch. Larger starch grains, in much greater quantities, occur in the cells of the pith, and are especially noticeable in the older joints.

CALCIUM OXALATE CRYSTALS

According to Solereder (47) crystals of calcium oxalate are particularly characteristic of the genus Opuntia, where the form most commonly assumed is that of star shaped cluster crystals composed of monoclinic prisims (Lautenbach).

Fig 9, 51, of longitudinal sections through young vegetative and flower buds, show that such crystals occur at a very early stage below the growing point in the cells of both cortex and pith. While fig 17 shows their early formation in a row in the hypodem cells immediately below the epidermis. In the cortex of ~~the~~ old joints and tubers the crystals appear in large quantities around the phloem bundle (fig 23), and later, when lysigenous canals are formed outside the main bundles, numerous crystals are found in the mucilage in these canals (figs 30,44).

MUCILAGE CELLS AND CANALS.

In view of the fact that Dr. Leemann of the Division of Plant Industry, was making a chemical investigation of the mucilage, in connection with finding a suitable poison for eradicating ~~the~~ Jointed Cactus, the origin and nature of the mucilage is not being treated in this thesis. Since, however, the mucilage is the chief characteristis of both the cortex and medulla, a brief account of the occurence and distribution of the mucilage cells and canals is given here for the sake of completeness.

In *Opuntia susquehannensis*, according to Lloyd (31), the mucilage cells arise first in the medulla, and later in the cortex. This is also apparently the case in *O.aurantiacea*. The earliest may be found in the medulla, directly beneath the growing point in both the shoot and flower bud (fig 9, 51) Those first recognisable in the cortex are several mm. behind the growing point. They occur in greater abundance beyond the region of actively ~~expanding~~ growing tissue, and seem to arise in all regions during the whole period of growth of the joint, as is evinced by young mucilage cells being seen near the base of ~~the~~ a joint several inches long. When young, they can only be distinguished by staining, from the medullary cells, and also by their lack of chloroplasts, from the cotical cells. Geneva red was found to be a useful stain in this connection. The walls of young mucilage cells showed up as a rosy pink, the walls of the surrounding cells being brick red.

In older mucilage cells if the material has been fixed in alcohol and subsequently passed through water, the mucilage swells greatly, as it is capable of a high degree of hydration. In swelling it compresses the protoplast into an irregular star shaped mass in the centre of the cell, the protoplast being connected by strands of protoplasm to pits in the wall at points adjacent to neighbouring cells. The mucilage in such cells can be seen to be laminated owing to the varying degrees of hydration. If tissue containing young mucilage cells be stained with iodine and dilute sulphuric acid, the inner zone of the wall shows a much deeper blue, almost violet, than the outer portion of the wall. From this Lloyd (31) concludes that at this stage, the inner zone of the wall is in a much more hydrated condition. He quotes this fact in support of the theory that the mucilage arises by hydrolysis of the original cell wall, which shows striking or excessive secondary thickening, and not by deposition of additional new material on the walls by the plasma, or by alteration of a thick secondary wall, theories which have been put forward by various authors.

The size of the mucilage cell increases to a certain extent with the age of the joint. Crystals of calcium oxalate are sometimes found in the mucilage cells. Lloyd says starch is also present in amounts usually correlated with the amounts present in neighbouring cells. In view of these facts he assumes that the mucilage does not kill the protoplasm when compressing it into the centre of the cell, but that the protoplast remains alive, and the cell continues to grow.

Mucilage canals of lysigenous origin appear to be known in a number of species of *Opunttia*, de Bary (12) investigated them in *O.robusta*, and found that "they lie close to the outer limit of the phloem (not, as Schleiden states in it.) of the bundles of the trace, which are connected into a net, and follow than in their longitudinal course."

Mucilage canals of a similar nature were found in connection within the more strongly developed bundles of the older joints, and were especially conspicuous in the tubers.

In fig 15 several large cells adjacent to the phloem of the largest bundle, mark the probable origin of a canal. Fig 30, which is a section through an old joint, shows how the cavity enlarges and apparently destroys the older phloem cells as it does so. Further figs 40, 41, 44, 46, show still further increase in the size of the canal, and the quantity of mucilage in it. The increase in the numbers of calcium oxalate crystals in the surrounding tissue, and in the canal itself is also shown. Fig 41 shows how the neigbouring tissues are distorted as the canals increase in size.

VASCULAR SYSTEM.

The greater portion of the joint is occupied by the larged celled parenchyma of the cortex and pith. The vascular bundles are comparatively small, and occur in a ring well out in the periphery of the joint, each bundle being separated from its neighbours by a broad ray of large celled parenchyma.

Fig 23 is a diagram showing the conducting system of the joint. It agrees in general principle with that of most Dicotyledon stems. The leaf trace continues up into the small leaf (fig 9,53) which falls off early in the life of the joint.

Several small vascular bundles pass out from the main leaf traces, almost at right angles to the nodes or areoles. These strands thicken out and form the connection between the vascular system of the parent joint, and that of the young shoot.

The larger vascular bundles send off frequent small branches, which branch again and anastomise with similar branches from other main veins, so that there is a finely reticulate system between the bundles (fig23). de Bary (12) compares this system with the reticulations of a leaf, and points out that it is necessary for equalising the water supply in the various parts of the photosynthetic system According to de Bary (12), this reticulation in the vascular system of the stem, is quite common in succulents with small or rudimentary leaves. There are no cortical or medullary bundles, such as occur in some genera (16), the anatomising system does not seem to enter the cortex but keeps to the main circumference as main leaf trace bundles. At the base of the joint the vascular system is reduced to about 7 or 8 large bundles. These pass through the areole and join up with the vascular system of the parent joint.

DETAILS OF VASCULAR ELEMENTS.

In the vascular bundle of the young joint the phloem is well developed figs. 15, 25, 28. It consists of elongated narrow sieve tubes with small sieve plates, and equally narrow companion cells. The cell walls are thin. In the young joint the outside of the phloem is bordered by uniformerly large, isodiametric, parenchymatons cells of the cortex in which crystals of calcium oxalate are frequent. In the older joints these break down to form mucilage canals outside the phloem, ~~and~~ which follow the same course of the bundles.

The protoxylem elements are vessels with spiral or annular thickening. They are surrounded by narrow elongated wood parenchyma cells, fig 16, 17.

The metaxylem consists of tracheae* with lignified spiral or annular sometimes thickening, fig 27, 28.

TRACHEIDS

The tracheids of the Cactaceae are mentioned by nearly all the authorities on Cactaceae, as a feature more or less common to all members of the family.

It is necessary here to distinguish between two types of elements which have both been termed tracheids by the older writers. There are, in conjunction with the vascular bundles, large spindle shaped cells, with several rows of spiral or annular lignified thickning, which projects far into the cell lumen, figs 28, 42. In the young joints these occur on the inner side of the vascular bundle next to the protoxylem, and at first appear to be part of the vascular bundle.

Chodat (9) calls these cells hydrocytes or hydrocysts, and compares them with the hydrocytes that occur in the leaves of Sphagnum. This is the name by which I shall refer to them

Note:

* The term tracheae is used here as de Bary (p.155) defines it i.e. a collective term for tracheids and vessels, especially usefully in those cases where it is not certainly decided whether the elements in question belongs to one or the other type.

here, since they are not part of the vascular system but are formed from parenchymatous cells of the medulla and function as water storage elements as will be shown later.

The habit of using the same terminology for both these elements leads to some confusion in interpreting the accounts of former writers. Figures however help considerably towards a clear understanding. True tracheids are apparently common in all the genera. According to Darbishire (11) both the protoxylem and metaxylem of Mammillaria, consist of spirally or annularly thickened tracheids. de Bary (12) speaks of the reticulated vessels of species of Opuntia and also of tracheae with ridge shaped thickening projecting far into the lumen.

In Opuntia aurantiaca true vessels occur in proto- and metaxylem, but it is difficult to distinguish in many cases between vessels and tracheids, as it is not always possible to say whether the adjacent walls between tracheids of the metaxylem are perforated or not, fig 27, 28. In the old joints, and in tubers, where secondary growth has taken place, the tracheids are more easily distinguished, fig 45. Short spirally thickened tracheids, fig 24, 25, are the elements composing the reticulate network between the main bundles. De Bary compares these with the tracheids of the reticulations of many leaves, and points out that they perform similar functions. The tracheids of this reticulation do not appear to be accompanied by any phloem, or other xylem elements.

DETAILS OF

Tracheids are found in many other angiosperms, de Bary (pp. 481, 482) gives a list of some plants in which they occur and compares their length with the length of fibres. Brouland (3) has found them in the Ranunculaceae, in the stems and also in the vascular supply to the floral parts of Anemone, Clematis, Thalictrum, and Isopyrum. He suggests that their presence lends additional support to the theory of the polypetalous origin of angiospems. On Hutchinson's classification this would also be the case for the Cactaceae.

HYDROCYTES OR HYDROCYSTS.

Schleiden (47) was apparently one of the first to refer to these peculiar structures in the genus Opuntia. He termed them tracheids. They were later reported to occur in various other genera of the Cataceae e.g. Echinocactus, Melocactus, Cereus etc. In all cases they were described as 'Tracheids' with thickening projecting far into the cell lumen.

As indicated above and illustrated in figs 28, 45, they arelarge spindle shaped cells about 140μ or more long and about 60μ in diamter. They have thin walls like those of the adjacent parenchyma, but are distinguished from them by peculiar thickening of their cell walls. In stages such as shown in fig 28, they have no nucleus or protoplasmic contents.

van Teighem (47)was the first to question their nature. He demonstrated a nucleus and cytoplasm in them and held that they should be called parenchyma cells. Darbishire (11) 1904, investigating Mammillaria elongata, said that "the tracheids contain no protoplasm as soon as they become ling~~y~~ified. Frequently there can be seen, just inside the cambium, tracheids which already show a large spiral thickening which does not respond to ordinary wood stains. Such cells contain cytoplasm and a nucleus". He then suggested that van Teighem's observations were made on young tracheids which caused him to mistake them for possible parenchyma cells. Subsequent writers have continued to call them tracheids, at times distinguishing them as "barrel tracheids".

In O.aurantiaca they most commonly originate fromthe parenchyma cells adjacent to the protoxylem, but not in the bundle itself. As the thickening on the walls commences the cell contents disappears, so that when mature they do not seem to contain either nucleus or cytoplasm. They may elongate in the course of development, and very occasionally adjacent ones have been known to fuse by the breaking down of their intervening walls. The exact nature of the thickening is unknown, sometimes it reacts with phloroglucin, showing lignin, and in other cases no reaction takes place.

In applying the term hydrocyte to these cells I have followed Chodat (p164) who calls them hydrocytes. He uses this term for the water storage cells in the velamen of the aerial roots of Orchids, and compares them with the hyaline water storage cells of Sphagnum. The term is particularly appropriate since they are formed from parenchyma cells, and their function is evidently that of storing water. In material dehydrated with alcohol, the cell walls of these elements fall in where they are not supported by bands of thickening, which are apparently a mechanical device for keeping the walls extended, and preventing them from collapsing owing to the turgor pressure exerted by the neighbouring cells (Chodat). In O.aurantiaca these cells occur in the joint next to the ptotoxylem elements, and are more abundantly in the old joints than in the young. I have found them to be particularly abundant in the tubers, especially towards the end of the tuber. Fig 46 shows how numerous they are in this position, in the medullary ray parenchyma between the vascular bundles. Here they are intermingled with cells containing large quantities of starch, but they do not appear to contain starch themselves.

According to Solereder (47) these cells are absent in some species of Opuntia e.g. O.monacantha and other types with flat oval shoots. In other species they may occur sparingly in the articulations between the segments, or in the tissues of the medullary ray, or at the margin of the pith, but not in the bundles themselves. A further advance is found when they become an integral part of the vascular bundles e.g. O.tunicate, O.imbricata.

Chodat says that these hydrocytes may form a water system for young elongating organs which have no complicated system for transporting water for a great distance, and that they are associated with the primary strands which are the forerunner of the veins, and appear where growth and elongation is rapid. In Jointed Cactus this does not seem to be the case since the young joints have a very good reticulate vascular system, and hydrocytes are not at all so numerous as in older joints.

SECONDARY GROWTH

SECONDARY GROWTH OF ATTACHED JOINTS.

Interfascicular cambium formation takes place early. It produces only large celled parenchyma which forms the medullary ray tissue. The interfascicular cambium increases by radial division while the fascicular cambium only divides tangentially, so that the vascular bundles remain narrow, and do not join up to form a ring as in most secondarily thickened Dicotyledon stems. The increase in the thickness of the joint is chiefly due to the large celled parenchymatous tissue produced by the interfascicular cambium.

The secondary tissue of the vascular bundle is chiefly made up of narrow, elongated wood parenchyma cells, (fig29). The lignified elements, which are spirally or annularly thickened tracheae, occur in scattered groups of two or three. The phloem (fig 26, 28) is well developed, consisting of narrow sieve tubes and companion cells, there are never any bast fibres, however. The primary phloem is distorted and sometimes broken down by the lysigenous formation of mucilage canals just outside the bundle, and which follow the course of the bundle through the joint, (fig30). Crystals of calcium oxalate are particularly common around these canals.

Numerous large mucilage cells occur in the cortex, medullary rays, and pith.

Hydrocytes, near the protoxylem, are more numerous than in the young joints. The extent to which mechanical elements of fibrous nature occur is chiefly dependent on whether the joint persists on the plant or not. Joints, like that in fig 3a, which persist and form the main stems of the plant, bearing the burden of many lateral joints, have fibre patches in the vascular bundles, fig 46.

The form of secondary thickening occuring in these succulent stems may be contrasted with that of woody stems. In the former the vascular bundles remain isolated, and the chief tissue is large thinwalled parenchyma, while in the latter the vascular bundles usually join up to form a ring the chief tissue being formed of thick walled lignified elements.

CORK FORMATION.

Cork always occurs on parts exposed by abscission, such as the top of the ovary after the abscission of the perianth, and in the areoles.

The place of the epidermis is taken by cork in the older joints (fig3a), in joints which have fallen to the ground and in tubers. In such cases cork is formed by the tangential division of the epidermal cells, the cuticularised outer wall of the endiermis being carried up as new layers are formed. According to Bukvic such cork formation occurs in several Cactaceae. The cork cambium gives rise to a layer of typical brick shaped cork cells whose walls give a suberin reaction. After this a narrower layer of dark thick walled lignified cells are formed, then again a layer of thin walled cork cells (fig 32). Similar cork formation is recorded by Hemenway (22) for <u>Carnegia</u> <u>gigantea</u>.

Particular interest attaches to the method of cork formation over the stomata, and the later blocking of the substomatal cavity. This agrees to a large extent with the description and figures given by Bukvic for <u>O.aurantiaca</u> and other members of the Cactaceae. As previously stated and illustrated in fig 22, there is a partial blocking of the substomatal cavity, in the older joints, by the growing together of the subsidiary cells below the guard cells, fig 21, When the epidermal cells begin to divide, preparatory to cork formation, the subsidiary cells also divide tangentially and form part of the cork cambium. With cork formation the guard cells are pushed up with the cuticularised upper wall of the epdiermis, see fig 31. Subsequently one or both of the subsidiary cells grow down into the respiratory cavity forming thylloid cells which completely block the cavity (fig 32, 33). As the joint continues to increase in circumference with the addition of secondary tissue, the respiratory cavity becomes larger, as a comparison of figs 33, 34, which are of the same magnification, will show. It is adequately filled and blocked however, by the division of the first thylloid cell as fig 34 shows. In the tubers as a consequence of the great increase in girth this is particularly noticeable, and sometimes the thyloses forms quite a tissue almost indistinguishable from the cortex with which it comes in contact.

TUBER FORMATION

Vegetative propagation from fallen joints is the means by which the plant has spread so rapidly. Tuber formation, figs 35, apparently only takes place when a joint, which has roots and shoots, becomes buried. Conditions of tuber formation might be investigated. Absence of light is an important factor in tuber formation in some plants. (53).

There is no mention of the formation of underground storage organs in the two official publications (28, 40) of Jointed Cactus as a noxious weed, nor are such structures mentioned by Schonland (45), or by Britton and Rose (2), which are the only other records of Jointed Cactus available. There is no record of such structures occuring in other members of the Cactaceae. In describing the various species of Opuntia, Britton and Rose speak of tuberous root stocks. In the illustrations given for O.chaffey (fig276,voli) it seems as if this rootstock was really a swollen joint, whilst fig 175 suggests that in O.mackensensii the fruit may have taken part in forming the "tuberous root". Other types with large rootstocks are rare, Pterocactua tuberous and Wilcoxia poselgeri are the only two adequately illustrated in Britton and Rose.

These structures are being referred to as tubers since, they are stems which have become buried and transformed into storage organs, and are capable of vegetative propagation

A knowledge of the structure of these tubers is important from the point of view of eradication, since it is imperative that any poison used on the plant should circulate through the whole system and kill underground as well as aerial parts.

Tissues from which the tuber is chiefly formed are the xylem and medullary ray parenchyma. In most other tubers the medullary parenchyma contributes the major portion of the tissue. <u>In Helianthus tuberous</u> the medullary parenchyma, and the xylem and medullary ray parenchyma, are the most important, and the tubers of this plant show the same very long narrow vascular bundles, (as seen in transverse section) with lots of xylem parenchyma and a few isolated lignified elements, as occur in Jointed Cactus. Figs 39,40, show to what an extent the xylem parenchyma is developed. The bundles remain narrow since the fascicular cambium does not increase at all ? radially, but the interfascicular cambium does, and this gives rise to a very broad medullary ray of large thin walled parenchymatous cells and numerous mucilage cells. The medulla does not seem to increase at all and is often distorted by pressure from the surrounding tissue. The cortex does not contibute much to tuber formation.

The lignified elements of the vascular bundles seem to be chiefly spirally and annularly thickened tracheids (fig 42,43) though it is not always possible to say whether these are true tracheids or not.

Hydrocytes are more common in the tubers than in the aerial joints (fig 44, 45). In particular they seem to be very numerous towards the end of the tuber in the medullary ray tissue fig 47.

The phloem generally is not as well developed as in the aerial joints, and in many cases seems to be partially disorganised by the mucilage canal next to it which are much larger than in the joint. (see figs 40, 41, 44) Crystals of calcium oxalate also occur in much greater numbers particularly in association with the mucilage canals.

Starch grains in enormous quantities occur in the cells of the medulla, the medullary rays, and the cortex.

Cork formation which has already been described for the aerial joints continues to a greater extent in the tubers. It may be mentioned here that the collenchymatous thickening of the hypoderm apparently undergoes a change, it becomes much thinner so that the cell lumen is larger.

The very peculiar nature of the ovary wall bearing areoles or axillary buds, and being similar in every respect ~~with~~ to the structure of the vegetative joint, will be dealt with in a later section. Since the fruit does not rot away when it falls to the ground, but persists and sends forth roots and shoots from the axillary buds on its walls, and is capable of forming underground storage organs similar to the tubers of the stem when buried, it is necessary to mention it here.

Axillary buds on the ovary wall are fairly common in the genus Opuntia, and in one or two other genera of the Cactaceae, but I know of no similar case in any other family. The nearest approach Johnson (27) could find was in Calycanthus, where the outer wall of the cup of the fruit bears the scars of many fallen petaloid leaves, but there are no axillary buds above the leaves and thus no possibility of the development of secondary flowers or shoots from the wall of the flower or fruit.

Besides O.fulgida in which great masses of fruit occur attached to one another, O.rubescens, O.arbuscula and a few others are also capable of bearing fruit from the areoles or axillary buds of the ovary wall, but for the axillary buds to give roots and shoots seems rare even in the Cactaceae. Johnson (27) says that O.fulgida may proliferate vegetatively by means of its fruit. This also occurs in O.moniliformis, O.cholla and possibly one or two others. In no case however is there any clear evidence for the formation of underground storage organs or tubers from fruit, as so frequently occurs in O.aurantiaca, see fig36.

At first these may be distinguished from stem tubers by their shape, and by the ovary cavity within if they are cut open. Later however it is impossible to say whether the organ was originally a joint, or a fruit, for the tissues, their distribution and content, are exactly the same as in the joint tubers.

ROOT.

The primary root of the seedling does not persist long, its place is taken by adventitious roots developed from the base of the hypocotyl. Later adventitious roots arise from the stem. The adventitious roots of detached joints arise from the areoles closest to the ground, they soon acquire a thick protecting layer of cork.

According to de Fraine(13) the root of Opuntias is typically tetrarch. In the case of Opuntia Tuna however one series she examined showed, at first, a tetrarch root which changed to a pentarhh structure at a lower level. Her remarks apparently only apply to the primary root, since she deals with the seedling structure only and does not mention adventitious roots.

In O.aurantiaca the primary root is apparently tetrarch. In all the adventitious roots I have examined there is a hexarch structure, one root showing pentarch structure. Whether the primary root changes from tetrarch to pentarch or hexarch at a lower level as in O.tuna is not yet known. The whole matter of the transition from hypocotyle to root structure, and the formation of adventitious roots, requires further investigation. The adventitious roots in which secondary thickening takes place at an early stage show great development of fibrous tissue, see fig 46 . The xylem is composed of annular and spirally thickened tracheae and thick walled lignified fibres, with occassionaly pitted walls. The medullary rays, which are about 2 cell thick when seen in transverse section, contain large quantities of starch. The phloem which occupies a relatively small portion when compared with the xylem, is similar in structure to the phloem in the stem, there are apparently no bast fibres. The pericycle of the secondarily thickened adventitious root contains starch, and occasional crystals of calcium oxalate. In the root tip prior to secondary thickening, the cortex consists of large cells with numerous air spaces, and the commencement of cork formation can be seen in the region of the endodermis. The older portions of the adventitious root are covered with a thick layer of cork.

According to Cannon (6) the cacti are adapted to exist with infrequent rainy periods. Their superficial widely spread root system rapidly absorbs the water which penetrates the surface layers of the soil. In <u>O.aurantiaca</u> I have never found the roots to penetrate to any considerable depth. They branch considerably but generally keep to the upper layers of the soil.

DISCUSSION.

A review of the previous section on the anatomy of the joint shows several features of interest as applied to problems of eradication, whilst other parts have scientific value.

The method of eradication is to spray a poisonous liquid on to the plant. The ease and rapidity with which such a liquid spreads over the surface of the joint and moistens it, is largely dependent on the nature of the outermost covering of the joint, which, in cases where cork formation has not taken place, is the cuticle of the epidermis. A wax covering is mentioned for several types of Cactaceae but so far no wax has been detected on this plant. In the young joint the slight irregularities and undulations of the surface of the epidermis might account for the difficulty experienced in the rapid moistening of the surface of the joint. Since penetration of poisonous liquids into the inner tissues takes place through the epidermis and stomata, the blocking of the substomatal cavities, and the apparent absence of lenticels in cork tissue is of interest.

A diagram of the course of the vascular bundles shows that even in young joints the plant is well provided for circulation through the xylem elements, while the phloem is well developed in the main vascular bundles but does not seem to follow the tracheids in the reticulate system between the bundles.

Mucilage canals occuring just outside the vascular bundles, seem to partially disorganise the phloem when the canals increase in size as in the older joints and tubers. It is uncertain to what extent the hydrocytes take part in conduction.

Of more scientific interest is the structure of the stomata. The long narrow substomatal cavity, or in the older terminology, the respiratory cavity, should have, according to Brown and Escombe, a great effect in cutting down the rate of transpiration. The lining layer of cuticle to these substomatal cavities is of additional interest in considering transpiration and the methods of water preservation.

As is to be expected in a plant of succulent character, there are several points of difference from mesophytic plants, among these is the reticulate conducting system, the mode of secondary thickening, and the special function of cells for the storage of water, mucilage, and crystals of calcium oxalate.

The formation of underground storage organs by detached joints is interesting, and it is particularly remarkable that fruit are capable of forming similar storage organs. No other record of fruit behaving in a similar manner is found. The theory that the fruit is a stem, into the upper end of which the ovary is submerged, accounts for the possibility of vegetative propagation and tuber formation by the fruit.

The mode of tuber formation in most respects follows on the lines of secondary growth in the joints. Although joints which fall to the ground soon begin to grow roots and shoots they do not seem to form tubers until buried or cut off from light by decaying vegetation.

STRUCTURE AND DEVELOPMENT OF REPRODUCTIVE ORGANS.

In investigating the structure of the seed, and the reason why germination does not take place in the normal course of time, and also in view of the fact of the very low percentage of germinable seed per fruit, it became evident that, apart from the academic interest of the problem, a knowledge of the development of the ovule, and the process of fertilization, would help considerably in accounting for these peculiarities. The following account of the development of the ovule shows clearly the nature of the hindrance which prevents the embryo from germinating. While the structure of the ovule of various species of Opuntia has been described by several authors, as will be shown below, their accounts do not give a clear idea of its very peculiar nature, nor are there any illustrations of it in the papers to which I have had access. The illustrations given here, if somewhat numerous, at least show clearly how such a structure is formed. The question of fertilization and the reason for the paucity of germinable seed requires further investigation.

The morphology of the flower is included as a point of academic interest, but it also helps to give an adequate reason why the fruit is capable of vegetative propagation.

DEVELOPMENT OF THE FLOWER.

The flower bud arises from one of the areoles usually of the upper part of the joint. At first it is indistinguishable from the vegetative bud (fig 2c), later when the sepals are formed and assume a reddish colour, the difference is apparent.

The ovary wall possesses areoles, leaves, thorns etc., similar to the vegetative joint, (cf figs 5a, 7) and these are initiated in the same way as in the joint. The internal tissues of the ovary wall are exactly similar to those of the vegetative joint i.e. epidermis, hypoderm, assimilating tissue of cortex, vascular system, and cells containing mucilage and calcium oxalate.

When the bud is ·5 cm or slightly more in length, the growing point is still convex, although at the base of a cup-like depression formed by the more rapid growth of the outer parts of the receptacle. (figs 51, 52) This to be compared with the growing point of the vegetative bud. (figs 9, 10).

As Johnson (28) points out, the order of initiation of the floral parts is an acropetal one. In fig 51 the initials of the sepals and petals can be seen. They are formed in the same way as the leaves, only no axillary buds appear at their bases. A later stage in the bud development is shown in fig 53 where the growing point is now concave. Numerous stamen initials appear, and the carpels have grown out, projecting over the cavity of the growing point. [~~The carpels have grown out, projecting over the cavity of the growing point~~] The carpels which may be 5,6 or 7 in number grow together and meet in the centre forming a cavity over the arrested growing point. This becomes the ovary cavity. The lower portion of the carpels fuse and grow up to form a thick style (fig48) about 1·5 cm long, and 3 to 4 mm. in diameter. The tips of the carpels which do not fuse spread out to form the arms of the stigmatic surface (fig 49). They are thickly clothed with swollen papillae, which exude a sticky yellow substance when the flower opens, fig 49) The vascular system of the style ends in a mass of tracheids at the tip of the stigma arm (fig 49).

This development is similar to that of *O fulgida* as given by Johnson (28).

The general appearance of the flower can be seen from fig 4b , it is a pale yellow, with rather an irridescent dazzling quality. The specific name "aurantiaca" aludes to the colour of the flower.

The flowering period is from about November to April. The flowers open in the morning about 9 a.m. and close in the afternoon at about 4 or 5p.m. They last for 2 or 3 days.

ABSCISSION OF PERIANTH PARTS.

The shedding of perianth and other parts in the genus *Opuntia* is more complicated than in most flowers. In *O. Fulgida* Johnson (28) records that, in about 3 days after the flower has opened, the sepals and petals have withered to a cone of dry crisp remains. In *O.aurantiaca* it is a week or more after the flower has closed for the last time that the perianth parts have dried out to this extent.

The perianth parts do not fall off separately, but sepals, petals, stamens and style all wither together into a cone attached to a cup-like base and fall as a whole (fig 8c). In this respect it is similar to *O fulgida*. Johnson (28) describes an abscission layer which forms across the whole receptacle a little below the base of the perianth parts. He points out that this layer is not in a simple transverse plane, but is that of an inverted cone. In *O.fulgida* this cone is usually perforated at the apex since the abscission layer comes out again a little above the base of the style. Sometimes however, he says, it may extend across beneath the style which is then cut off, and with the other parts, is shed wholesale attached to the cone of the receptacle. Although I have not studied this abscission layer in *O.aurantiaca* it is apparently similar to this last case of *O.fulgida*, since the style is shed with the other perianth parts attached to the receptacle.

The withered remains of the perianth do not fall off mmediately the abscission layer is completed, but remain ticking to the top of the ovary for a week or more. By this me cork formation has covered the whole top portion of the ary exposed by the dehiscence of the perianth parts. This rk layer rapidly thickens as the fruit matures, and any entrance the ovary through the stylar passage is completely blocked g 8b).

MORPHOLOGY OF FLOWER.

(Based on the supposition that the flower of angiosperms is a branch bearing perianth members etc., instead of vegetative leaves.) The theory that the ovary of Opuntia consists of a shorter or longer vegetative joint into the depressed upper end of which the ovary is submerged, with stamens and perianth members inserted round the margin, was put forward by various authors working on the Cactaceae, Zuccarini 1844; Schumann 1894; Toumey 1905; Harris 1905 (for full references see Johnson(28)), did not adequately support this theory with developmental evidence, such as Johnson (28) has now given for O.fulgida. The facts will be briefly summarized with reference to O.aurantiaca.

As pointed out, the bud of the flower is indistinguishable in the early stages from the vegetative bud. The ovary wall is similar to a vegetative joint. It bears areoles, comparable with axillary buds, which possess each a leaf similar to those borne by the young joint. It also has thorn, bristles and trichomes similar to those of a vegetative joint, and originating in the same manner. In addition, the areoles of the fruit are capable of giving rise to new flowers while still on the plant, so that fruit bears fruit as in the case of O.fulgida, though not quite so prolifically. The structure of the epidermis, stomata and hypoderm is exactly similar to that of the joint. The vascular bundles follow the same course in supplying the areoles. The cortex is photosynthetic, mucilage cells and crystals of calcium oxalate, occur in the same tissues as in the joint.

Superficially the shortness of the fruit, and the crown of perianth members, are the sole features which distinguish it from the joint. As in O.fulgida a series of fruits can easily e collected to show how the stem like portion of the ovary all may vary in length, and how the ovary cavity may be restrictd to occupying a small portion at the top of the fruit.

As shown and illustrated in the section on tuber formation, when the fruit falls to the ground it is capable of sending out roots and shoots from any of its areoles. Once firmly rooted secondary thickening and cork formation, as was shown, takes place so that the ovary cavity is obliterated and the "fruit" grows in size and ultimately becomes indistinquishable from any tuber similarly formed from a vegetative joint.

Lastly in following the history of development of the perianth parts and carpels, it is seen that the growing point is at first convex, and like that of any ordinary stem gives rise to leaves and axillary buds. With the arresting of the growing point, and the formation of perianth parts, the rate of elongation is greatly reduced and the ovary cavity is formed by the more rapid growth of outer portions.

In all these cases the similarity to the stem is very striking. The case of vegetative propagation by the fruit, as shown in an earlier section, is apparently practically confined to the genius Opuntia.

Further, the formation from a fruit, of an organ similar in structure and function to the tuber from the vegetative joint, shows that the fruit can be compared not only structurally, but also functionally with a stem. This is additional evidence for Johnson's conclusion that, "the present type of fruit in Opuntia has arisen from an originally superior ovary which has progressively sunk more and more into the upper end of the joint bearing it."

THE OVULE.

According to Engler Prantl (16) the ovules of Opuntia and Nopolea show a remarkable structure. Externally they show nothing extraordinary, but in Longitudinal section they appear to be enclosed in a peculiar envelope, which is to be regarded as an extraordinary enlargement of the fold of the funicle. Payer (Engler and Prantl p.21 p 607) has indicated that the ovule describes in its most youthful stage, a complete spiral before it develops into an anatropus ovule.

In Peireskia sp (16) the ovules are turned and curved against the funicle, which broadens out in the neighbourhood of the micropyle and envelopes it as in a fold. Guighard (21) comments on the length of the funicle in Cercus, and describes the ovule as campylotropus "a son micropyle ramené presque au contact du funicle qui le porte et qui se recourbe sur lui comme une bouche pour le recouvrir".

In a summary in Biological Abstracts 4 (7-9) 1930 (22,497) of a paper by J.A.Huber (13) on the development of flower and seed in cacti, the ovule in Opuntia, Peireskia and Rhipsalis is said to be "campylotropus with a long funiculus", and the "third integument of Opuntia is interpreted as an enlargement of the funicle." Without seeing the original paper, which I have not yet been able to obtain, it is rather difficult to comment on the structures from this description.

Ganong's (18) account of the ovule of O.vulgaris agrees to a certain extent with that of Payer. He says "the ovule of O.vulgaris is at first amphitropous, but in its development it becomes elongated and bent, at the same time turning round in such a way that the funiculus makes a complete turn around it, so that finally it stimulates a compylotropous condition."

d'Hubert (18) has evidently described the development of the ovule for several species of Opuntia and ~~other species~~ of other genera. According to Ganong his account fits that of O.vulgaris fairly well.

The description of the seed of O.fulgida by Johnson (28) is not very clear, but agrees to a certain extent with that of Ganong. The seed has a protective jacket which, "derived from the funiculus, surrounds the seed completely having finally closed in above the micropyle".

The development of the ovule in O.aurantiaca has been followed from its intiation as a small swelling of tissue on the ovary wall see fig 54 . This grows up and increase in length as shown in fig 55 , the tip then bends over and small swellings arise showing the commencement of the nucellus and integuments, fig 56 . The tip then makes a complete turn so that it once more comes to lie in an anatropous position with reference to the ovary wall, and the funicle completely surroun the ovule as shown in fig, 57. By this time the embryo sac is in the fullc course of development. This description of the development of the ovule in O.auratiaca, confirms that of Payer (16) for other sp. of Opuntia. It also makes ther descriptions of Ganong clearer, and Johnson's more easily understood, for later stages, such as that shown in fig 64. illustrate how the ovule might be said to "simulate a campylotropous condition."

It seems probable then, that, in the genus Opuntia at least, the peculiarity of the funicle completely encircling the ovule, which is neither truly orthotropous, anatropous or campylotropous, is not uncommon.

In Opuntia aurantiaca two ovule initials may arise side by side. As the funicles lengthen they assume the appearance given in fig 55 . Later the base of the funicles fuse giving the impression that two ovules arise by branching of the funicle as in the fig . This fused portion is never very long however and often does not occur at all. In this connection it is interesting to recall the structure of the ? Cerus as given by Guignand (22). In C.tortuosus in particular the principle trunk a funicle may give rise to about 30 branches or secondary funicles, each terminated by an ovule.

A vascular bundle, a lateral bundle from the vascular bundle of the placenta, traverses the whole length of the funicle, and ~~tha~~ ends in the chalaza just below the nucellus.

At the time of the embryo sac formation the nucellus is extremely large, the tip between the embryo sac and the micropyle end being 5 or 6 cells deep. There are two integuments, the micropyle is formed by the inner integument only, the ends of which are thick and flared out, so that it projects above the outer integument which forms a collar round it (fig57-59) This agrees with the features mentioned as characteristic of the Cactaceae and Centrospermae by Neumann(39) (ex.Biol.Abstracts).

The inner and outer integuments are both made up of two distinct layers, agreeing in this with those observed in Opuntia, Piereskia and Ripsals species investigated by Huber (25). The two layers of the outer integument are each a single cell thick, the outer layer persists, and is found in the seed. The outer layer of the inner integument is a single cell thick, but the inner layer is two of three cells thick, this persists as a papery coat covering the embryo. ~~Whereas~~ Ganong (18) describes the integuments of O.vulgaris as made up of three distinct layers.

DEVELOPMENT OF EMBRYO SAC

Material collected during the flowering season for investigation of the development of the ovule, was only worked over some time later when it was impossible to get more material to supply the stages that were found to be missing. Thus it has not been possible to follow out the complete life history of the development of the embryo sac, and subsequent effects of fertilization. Of the material at my disposal I shall give a description of the most outstanding slides and quote the findings of other authors on the missing stages.

According to Neumann (39) there is one primary archesporial cell in Peireskia and one cover cell (Deckselle). The chalazal megaspore develops giving an embryo sac of the normal type.

Fig 60 shows the first two nuclei of the embryo sac, the result of the first division of the megaspore nucleus. At this stage the nucellus, which is comparatively large, is only slightly curved at the base. Its subsequent growth is shown in the illustrations of later stages. The two papillae.

like cells at the top of the nucellus fig 57, 58 are of interest, as are also the projecting hair-like cells on the inner side of the funicle.

Figs 61-63 are sections of ovules from an open flower. Guignard (22) says that starch grains in the embryo sac indicate the process of fertilaztion since they come from the pollen tube. Starch seems to be present in these sections and they are taken to represent a stage in fertilization, fig 62. In fig 62 it would seem that the pollen tube had already discharged its contents, and that the two nuclei mn. and en. represent the male and female nuclei about to fuse. The two polar nuclei pn are seen lower down on the right. According to Huber (23) these do not fuse until after fertilization.

The pollen tubes can be traced no farther back than the nucellus, and although many sections have been made it is not yet certain how they actually enter the ovule. Fig 63 shows what look like the ends of two pollen tubes. This is a section from a young fruit, and the nuclei of the egg have already disintegrated as occurs in all similar stages so far examined.

Later stages (fig 64) only show the increase growth of the nucellus and the obliteration of the embryo sac by pressure from the nucellus. At the top of the nucellus possible remains of the pollen tubes are always present.

Changes in the integuments are also illustrated. The single outer layer of the outer integument becomes more prominent, whilst the inner layer disintegrates. The inner layer of the inner integument perisits and the outer layer is obliterated. Fig 65 show diagrammatically the 3 chief stages encountered.

POSSIBLE FORM OF EMBRYO FORMATION

So far no proembryos have been found in the material examined, nor any stage, between the fertilization described and the fully formed embryo, which shows the possible form of embryo formation. In view of the fact of the very small percentage of germinable seed (about 1 germinable seed occurs in every 14 fruit) from fruit which contains on an average about 100 ovules per fruit, it seems that a fair amount of material will have to be examined in order to get such stages.

Reference to literature shows that cases of nucellar embryony have been reported for several Opuntia species. Ganong (18) gives a full account of embryo formation in Opuntia vulgaris. Here adventitious embryos arise from cells of the nucellus, near the pollen tube, which seem richer in protoplasmic contents than their neighbours. His figures show clearly how several embryos arise in one ovule by the budding out of cells of the nucellus. At the time of embryo formation he states that there is no sign of an egg cell, although before embryo formation he found ovules in all stages before and at the time of fertilization. In ovules showing the early stages of embryo formation from the nucellus there regularly lies, in the position of the egg cell, a crumpled mass of protoplasm which he concludes to be the remains of the disintegrated egg.

This disintegration of the egg corresponds to what I have seen in my own preparations but there is no sign ~~in my own preparations but there is no sign~~ of nucellar embryo formation.

Another case of embryoformation from the nucellus is givenby Hull (24) for O. Rafinesquii, whilst in younger stages nucellar buds were observed in O.ficus indica, and O.leucantha Ernst (15)

The fact that O.aurantiaca is quite commonly polyembryonic like O.vulgaris, might lead one to suppose that nucellar embryo formation occurs in this case too.

Further investigation of pollination, the germination of the pollen grain, and embryo formation is necessary. This will probably reveal the cause of the very low percentage of germinable seed.

POLYEMBRYONY

In *O.aurantiaca* there is clear evidence of polyembryony. About 24% of the seeds germinated showed double embryos. Polyembryony is quoted for *O.vulgaris* by Ganong (18), but he says he has seen no other case of it although he has worked over some 75 species of the family. According to Englemann (18), *O tortispina* showed an instance of two embryos in one seed, and Braun (18) suggests that the four cotyledons which he noticed in *O.glaucophylla* may indicate a fusion of two embryos. Other cases of polyembryony occur in *O.ficus indica* and *O.Rafinesquii* for both of which nucellar embryony has been recorded.

(References on polyembryony are from K.Schnarf's "Embryologie der Angiospermen" and I am indebted to Dr. Metcalfe of the Royal Botainic Gardens, Kew, for the extracts he made from this work at Dr. Smith's request.)

SEED STRUCTURE.

The germinable seed is about 4 mm. in length and 3 mm at its broader end (fig 68b,69 The outside is covered with a pulpy mass of long soft hairs (fig 68a) which arise from the outside of the funicle. Intermingled with these hairs are numerous large oval cells of varying size. Each cell has a large nucel~~l~~eus, and numerous plastids, and starch grains and mucilage. This pulpy mass is very firmly attached to the seed and is better developed in germinable seed than in sterile seed. (cf fig 68 and 70)

The funicle, which completely surrounds the embryo (fig 65iv) is composed of hard stone cells which make the seed very difficult to cut or crack. As shown in fig 65iii,67 the stony funicle forms a loop over the micropyle, which is not freely exposed as in other seeds. Germination could only be secured when this loop was cut away and the radicle exposed. Thus by its very peculiar structure, and because of its hard stony nature, the funicle prevents the embryo from germinating.

The embryo is surrounded by two coats, the outer one, which is formed from the outer layer of the outer integument adheres to the funicle (fig 66). The inner one, from the inner layer of the inner integument, forms a thin brown papery covering to the embryo.

The embryo is large and well developed and completely fills the seed except for a small portion occupied sometimes by the remains of the nucellus. The two cotyledons are large and bent round in the fashion of those of a campylotropus embryo (fig 66). The radicle is also well developed and has a conspicuous cap to the growing point. All the cells of the embryo are closely packed with small globules which give a protein reaction with Millon's Reagent, and are probably aleurome grains.

In the case of seed containing more than one embryo, the second embryo is squeezed within the curve of the other one.

Besides seeds containing embryos two other types of seed occur in the fruit. One is a thin flat papery seed with hardly any surrounding mass of hairs and mucilage cells (fig 70).

The second type has a hard stony funicle similar to that of germinable seed, but it is smaller and flatter and the mass of tissue surrounding it is not so plentiful and succulent as that of seed containing embryos. Inside this second type of seed is a flat papery object evidently the remains of the integuments and nucellus. With a little practise it soon becomes possible, on cutting open the fruit, to distinguish immediately between the germinable seed and the other two types. The pulpy mass surrounding the former is more abundant and has a translucent appearance.(cf fig 68-70)

Germinable seed occurs very rarely, and when it does there are usually only one or two in a fruit, which as an ovary, contains about a hundred ovules. For the most part the fruit contains sterile seed of the two types mentioned above, sometimes one type predominating and sometimes the other.
It is impossible to tell from external appearance whether the fruit contains germinable seed or not. The following table gives some idea of the proportion of germinable seed to fruit.

TABLE I.

Place of Collection	Time of Collection	No.of fruit	No.of germinable ~~fruit~~ seed
Uitenhage	Feb. and April	780	111
Uitenhage	July	1074	66
Uitenhage	September	1476	66
Uitenhage	October	81	3
Middle Drift (Alice)	August	1657	2
	Total	5068	248

As this table shows germinable seed was found with much more regularity in fruit from Uitenhage than in fruit from the Middle Drift area. The average amount of germinable seed in fruit from Uitenhage is about one seed to every 14 fruit, which is extremely low considering that each ovary contains from 80 to 200 ovules.

The seed takes some time to mature and the fruit of February flowers can only be collected about July. During this time the fruit first swells slightly and assumes a dark red colour,+ later it becomes wrinkled.

GERMINATION TESTS.

The second type of seed with the hard seed coat and papery inner tissue was at first mistaken for germinable seed. The first germination tests were made on this seed from fruit collected at Middle Drift. Of some 2,000 seed tested for germination, after various treatments, it is not necessary to make further mention, except to comment on the lack of good seed in fruit from this area. (see table I, only 2 good seed in 1657 fruit).

Later, when fruit collected at Uitenhage was examined, the true seed containing a well developed embryo was immediately noted. Owing to the fact that only a few hundred seed could be got after the labour of collecting and examing several thousand fruit, the germination tests were somewhat limited.

Test I. Four lots of 25 seeds each were treated as follows.

Lot 1. $H_2 SO_4$ and cut seed coat.

The seeds were treated with a 50% solution of sulphuric acid for 15 minutes, washed, and the pulpy outer coat removed. The micropyle end of the hard funicle was then cut so as to expose the radicle.

Lot 2. $H_2 SO_4$

Treated with 50% $H_2 SO_4$ for 15 minutes, the pulpy mass removed and the seeds well washed (Spaeth 48)

Lot 3. Boiling Water.

The pulpy mass removed and the seeds kept in 90°C water for 3 mins.

Lot 4. No treatment, pulpy mass not removed.

Seeds were placed on filter paper on small petri dishes with strips of filter paper dipping down into water in larger petri dishes. These were kept in an oven 24°C. Thus all seeds were equally supplied with water, and conditions of humidity, oxygen supply and heat in the oven remained fairly uniform (see Rules for Seed Testing 43).

The following table gives the results of Test I.

Table II Test I.

Lot.No.	No.Set.	TREATMENT	GERMINATION AFTER DAYS								%TOTAL GERM
			1	2	3	4	5	6	7	8	
1	21	H_2SO_4 and coat cut	-	2	1	-	6	-	-	-	43%
2	25	H_2SO_4	-	-	-	-	-	-	-	-	–
3	25	90·C water	-	-	-	-	-	-	-	-	–
4	25	No treatment	-	-	-	-	-	-	-	-	–

Of the 21 seed set in Lot I. 9 germinated, 3 of these had double embryos. The test was allowed to continue for 37 days but no further germination took place. After this period the seeds of 2,3,4, were used again in Test 2.

Table Test 2.

Lot.No.	No.Set	TREATMENT	GERMINATION AFTER DAYS						%TOTAL GERM
			1	2	3	4	5	6	
2	18	Seed coat cut	2	3	3	-	3	2	72%
3	18	Held in flame for 8secs	-	-	-	-	-	-	–
4		No treatment	-	-	-	-	-	-	–

Again only those with seed coat cut, so as to expose radicle, germinated. Out of the 13 which germinated 2 gave double embryos, both of them vigorous and strong.

Clearly dormancy in this case is secured by the third method mentioned by Crocker(10) "mechanical resistance to the expansion of the embryo and seed contents by enclosing structures". The embryo, which is well developed, is surrounded by the hard, stony funicle which forms a loop across the micropyle, which the embryo seems incapable of breaking through without the interference of some external influence.

Lansdell (28) carried out some germination tests on **the** jointed cactus. Seeds were sown, without previous treatment, **on** moist blotting paper in sawdust and in soil, in July and August 1920. 300 seeds were tested but no germination took place as recorded in results published in 1923. She does not say however whether the embryos were still alive after this period.

Evidently this mechanical obstruction by the funicle is the only cause of dormancy, for as the germination tests above indicate, within 24 hours of the funicle being removed, and on being supplied with moisture etc, the embryo showed signs of **germinating**.

GERMINATION.

As shown above the seeds could only be induced to germinate when the part of the funicle covering the micropyle was removed. How germination takes place in nature is difficult to imagine, as the funicle is so hard and stony that it would take a considerable time before it rotted away, and it is uncertain whether the embryo could retain its vitality during this time.

When artifically germinated the radicle emerges and is quickly followed by the hypoctyl. There is a tuft of hairs at the juncture of the radicle and hypocotyl (fig 71). **When** there are two embryos, one usually emerges before the other, so that there is usually one seedling further developed and stronger than the other (fig 73). Figures 72 -74 show various stages in the growth of the seedlings. The seedcoat is usually carried up on the tip of one of the cotyledons. This stage is reached within 3 or 4 days of the commencement of germination. Sometimes the cotyledons emerge quite freely, and at other times they do not seem able to emerge at all, so that the two cotyledons are held within the seed coat and the seedling eventually dies. This was noticed by Ganong (19) in other types of Cactaceae seedlings.

The hypocotyl. is long and fairly thick and at an early stage mucilage cells make their appearance. The two cotyledons are large, flat and succulent and in this respect correspond to Ganong's (19) Platopuntiae group as typified by <u>O.vulgaris</u>. The primary root does not persist long, its place is soon taken by adventitious roots from the hypocotyl.

The plumule or first shoot only makes its appearance a month or more after germination. Figure 74 shows that it is exactly like a small globular joint. It has areoles with rudimentary leaves, fine thorns and trichomes.

<u>DISCUSSION</u>.

Results of the investigation of the seed and fruit show that the possibility of the plant spreading rapidly from seeds is very remote since the percentage of germinable seed is very low, and the exit of the embryo is blocked by the hard stony funicle so that germination can only take place very rarely in nature. In eradicating the plant however, the fruit must not be neglected since vegetative propagation is very common from fallen fruit. The question of how long the embryo retains its vitality is not yet answerable . Seedlings should be easily recognisable in the veld by their large flat cotyledons which are usually green on top and reddish below.

The development of the flower and the morphology of its parts shows very clearly that the ovary wall can be considered as a transformed stem. The relatively primitive position assigned to the Cactaceae in a scheme of the evolution of Angiosperms such as Hutchinson's, is supported by this evidence.

The development and structure of the ovule is full of interest in comparison with the more normal types of ovule. Apparently the very long funicle completely surrounding the ovule is a feature common to other species of the genus Opuntia, but does not seem to occur in other genera of the Cactaceae, or in any other plant family. What the function of such a structure is, is obscure, since it complicates the passage of the pollen tubes to the ovule and ultimately prevents the embryo from germinating. It might indirectly account for the scarcity of germinable seed.

The absence of all proembryo stages makes it impossible to say whether the embryo originates from the fusion of the egg and pollen nucleus, as in the majority of angiosperms , or whether it is of nucellar origin, as is usually the case in species of Opuntia showing polyembryony. How far the pollen tube and its contents are responsible for the embryo formation, cannot be said until the course of the pollen tube has been followed up.

Seedling anatomy requires a closer investigation. de Fraine's study on the transition phenomena in the seedlings of Cactaceae (13), shows up some interesting possibilities.

GENERAL PHYSIOLOGY.

In connection with the stomatal observations which follow it is as well to give a few facts on the water content and water loss of joints, and to note the period and manner of growth.

WATER CONTENT AND WATER LOSS OF JOINTS.

The water content of a young but well developed joint is approximately 91% of the fresh weight. Joints dried in an oven at 40°C for 8 hours daily took roughly 21 days drying before constant weight was reached. Loss of water under these conditions is therefore very slow.

Loss of water under natural conditions is even slower. A set of joints, knocked off from the plant and allowed to lie on the ground, as occurs in nature in vegetative propagation, were weighed at intervals with results shown in Table III

Table III

Period elapsed after joints knocked off parent plant.		Young Joint		Old Joint.	
		Weight grms.	per cent	Weight grms	per cen
1st day	0	7·4524	100	45·4168	100
	2 hrs	7·4201	99·56	45·3000	99·74
	6hrs	7·3536	98·67	45·1208	99·34
2nd day	26 hrs	7·3368	98·49	45·1092	99·32
	30 hrs	7·2872	97·78	44·8300	98·71
3rd day	50 hrs	7·0800	95·00	44·5550	98.10
4th day		7.0214	94.21	44.5008	97.98
24th day		6.9290	92.87	43.5352	95.87
46th day		6.5216	87.90	41.4248	91.21

The loss is at first comparatively rapid, but it becomes proportionately less and less. On the 46th day after the commencement of the experiment the young joint had lost approximately 1/8 of its original weight, whilst the old joint had only lost 1/12th of its original weight.

Was this even in spite of ~~or because~~ of water-loss above described or was the plant damped before it produced the small shoots

On the 46th day 3 small shoots were observed at the areoles on the lower surface, and soon the new plant was in a flourishing condition. The young joint also sent out roots and shoots a little later. Similar results were obtained by Mac Dougal and Spalding for Opuntia.

From the above facts it is seen that separate joints are considerably resistant to water loss, and vegetative propagation is easily accomplished. The danger of very small joints, as a source of vegetative propagation, is evident from the fact that very small young joints, ranging in weight from .08 gms, ? dimensions which were left on undisturbed ground in April, **had** taken root and were growing vigorously by September.

GROWTH.

Vigorous growth starts with the first rains of August and September, when numerous young shoots appear in the areoles. These grow rapidly during the early spring and summer months.

A large tuber which had been stored for about a month or more was planted on the 26/6/35. By the 16/8/35, after heavy rain numerous vegetative buds had appeared. The following rough readings were made of the growth in length and thickness of some of the young joints.

Table I.

DATE	JOINT NO. I LENGTH	DIAM.	2 LENGTH	DIAM.	3 LENGTH	DIAM.	4 LENGTH	DIAM.
	m.m.	m.m.	m.m.	m.m.	m.m.	m.m.	m.m.	m.m.
26/8/35	35	10	18	9	8	6	5	5
27/8/35	39	10	21	9	10	7	6	5
28/8/35	40	10	21	10	10	7	6	5-8
29/8/35	40	10	21	10	11	7	6	5-8
18/9/35	77	15	55	14	44	8	25	7
10/10/35	139	19	94	20	101	15	58	12
TOTAL IN 45 DAYS	104	9	76	11	93	9	53	7

Thus in the specimens under sonsideration there was a maximum increase in length of 10.4 cm in 45 days

Omissions

Fig   is a photograph of the tuber from which the measurements were taken. It had put forth 19 new joints during the 2 months of during which it was under observation. Fig   is the same plant and shows the increase growth during a year.

During the summer months the joints may appear wrinkled and shriveled after several weeks without rain (see fig   )

spell

On the second or third day after a good shower however they become round and turgid, and by the fourth day new vegetative and flower buds have appeared. (The plant ~~thus~~ apparently draws on its water reserves during the drought, but as soon as rain falls it is able to make a rapid recovery, and even to put forth a new crop of shoots within a few days of recovery.)

Cut the bit in brackets & merely say "thus the speed of recovery is remarkable"

The Opuntias examined by MacDougal and Shreve(36) showed similar form alterations in response to rainfall. In the species mentioned however, bud formation seems to occur only month or more after the rains, and then during a second period of expansion. (As a result of experiments on hydration and growth MacDougall (34) comes to the conclusion that :-

? cut from here

"the flattened stems of Opuntia yield ample evidence that the fluctuations in growth show a direct relation to the hydration capacity of the growing cell masses, and that as a morphologically complex member or organ approaches maturity, the fully developed tissues show a varying water capacity different in many respects from that of the embryonic cell-masses."

In a later paper MacDougal and Shreve (   ) give a programme for the daily growth of Opuntia joints.)

What does "water capacity" mean?

? of use to insert paragraphs whose meaning is not clear

STOMATAL OBSERVATIONS.

APPARATUS FOR RECORDING PHYSICAL CONDITIONS OF ENVIRONMENT.

RAINFALL

Records of rainfall are those taken from the nearest meterological station which was never more than 2 miles distant from the place of observation. It is of interest to note the average yearly rainfall of the three areas in which stomatal observations were made, or from which fruit was collected.

? Government

	Mean Annual Rainfall	Mean Annual No. of rainy days.	Percent. Summer Rainfall	Percent Winter Rainfall
Alice (Middle Drift Area).	22 ins	73	66	34
Grahamstown	28 ins	89	61	39
Uitenhage	17 ins	60	54	46

LIGHT

Of the available instruments for recording light intensity the most reliable and best standardised results were given by the Eder-Hecht Dauerphotometer, this instrument is also compact and easy to handle when doing field work. Eder-Hecht Chloris Photometer Paper was used and an exposure of 2 minutes given. Hence it was possible to record the light intensity of practically the moment of stripping and fixing the stomata. By means of the table supplied with the instrument readings were translated in terms of relative light intensity add something here which can easily be transformed into absolute readings in Bunsen Roscoe units. Owing to an oversight short exposures to record early morning and evening light were not made which accounts for the absence of such readings in the tables given .

TEMPERATURE

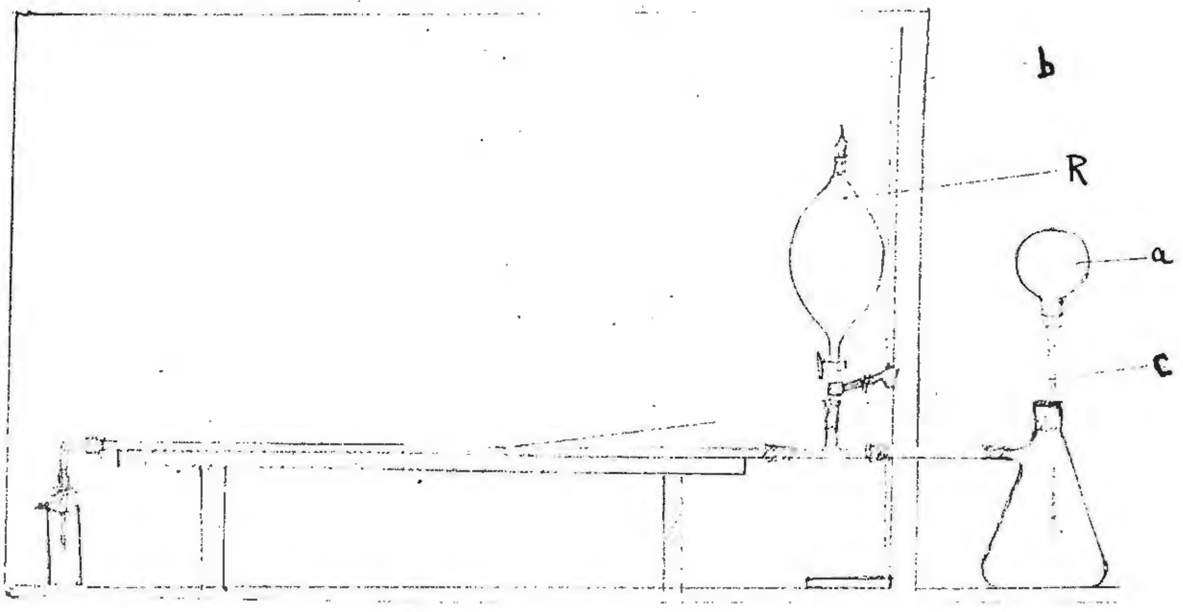
For the first observations and results given in tables V and VI temperature was recorded by swinging a thermometer in the air. For later observations a Stevenson Screen was procured on loan and temperature readings were made from a max and min. thermometer kept in the screen.

HUMIDITY -

The humidity readings of table VII were made from a wet and dry bulb~~s~~ thermometer kept in the Stevenson Screen. Readings are given as Degrees of Relative Humidity.

EVAPORATION

Several types of atmometer were tried including the volumetric type ~~of~~ figured by Maximov (37) and the weighing type suggested by Livingston. None of these were sensitive enough~~t~~ to give the small readings necessary if evaporation of the moment was to be recorded. On Dr. Léemann's advice the apparatus shown in figure below was constructed.



b box
R resevoir

a atmometer
c capillary tube

A white bulb Livingston atmometer with a reservoir was attached to a horizontally placed capillary tube supported by a wooden platform. Between the atmomter and the tube a second reservoir with a tap was inserted. The tube and second reservoir were protected in a wooden box with air vents in the side to regulate the temperature. This apparatus was easily portable and could be left outside at the place of observation. When not in use the tube was filled with water and the end stopped with rubber tube and holder. The tap of the resevoir R_2 was left open so that evaporation from the atmometer was provided for from this source.

In order to take a reading the resevoir tap was turned off and the stop removed from the end of the tube. The water was then drawn through the tube by evaporation from the atmometer, and the rate of its passage measured along a centimetre rule placed alongisde the tube could be recorded with a stop watch. The rule was placed alongside a portion of 20 cm. marked off on the tube so that the rate of evaporation would always be measured over the same volume. The tube was carefully chosen for its eveness of bore. In order to ascertain the volume of the 20 cm. through which readings were taken, this part of the tube was filled as accurately as possible with clean mercury. The mercury column was measured then the mercury ran out and weighed and hence the volume of the tube calculated.

column of mercury 20.4 cm long weighed 2.6062 gms at 21° C

spefic gravity of mercury at 21°C 13.5439

Volume occupied by column of mercury 20.0cm long =

$$\frac{2.6062}{13.5439} \times \frac{20.0}{20.4}$$

$$.1886 \text{ cm}^3 \text{ at } 21^\circ\text{C}.$$

Since the volume of water alters with the change in temperature, the temperature of the capillary tube was taken for each reading, and all readings refered to 21°C according to the formula.

~~$V_x = V_1 (1 x x t)$~~ $V_x = V_1(1 + \alpha t)$

where V_x is the required volume

V_1 the known volume at 21° C

α the coefficient of expansion of water in $\text{cm}^3/1^\circ$

t the difference in temperature.

This volume reduced to milligrams, multiplied by the atmometer coeffecient and divided by the time it took to evaporate, gave the rate of evaporation in mg/minute. The readings given may be considered accurate for the first decimal place.

METHODS.

Observation of stomatal movements is particularly difficult on account of the nature of the epidermal tissues of the plant, a full account of which is given in the section on anatomy. The crystals of calcium oxalate in the hypoderm, which cannot be separated from the epiderm in stripping, show through and greatly mar the clear definition of the cell walls. The stomata are slightly sunk and the subsidiary cells project over them, so that it is very difficult to distinguish the walls of the guard cells clearly. In order to overcome these difficulties the following methods were considered or tried.

(i) Microscopic observation *in situ* is impossible on account of the thickness of the joints.

(ii) The xylol, benzol, alcohol injection method (Molisch), did not give any satisfactory results as it was impossible to ascertain whether the liquid disappeared through evaporation or because of penetration. Nor was it possible to get any results if there was any moisture on the epidermis.

(iii) Darwin's porometer method was tried but found impracticable on account of the rounded surface of the joints, which did not permit the glass chamber to be fixed in an air tight manner.

(iv) Stripping the epidermis and fixing, by plunging it immediately into absolute alcohol, Lloyd(32) has been one of the most successful methods for stomatal observations on this plant. This method has been extensively tested by Lloyd, Loftfield and others, and has proved very reliable. There are many disadvantages however. The cuticle and hypodermal tissue become very brittle in alcohol and the strip is apt to curl, so that the cover glass does not lie flat and it is difficult to focus on such an object.

(v). Paraffin Method. Miss L. Britten drew my attention to the fact that liquid paraffin is used for a mounting medium for tissues used in experiments dealing with the osmotic pressure of cells, where it is absolutly necessary that the cells should retain their natural turgor. She suggested that I might find it useful in connection with stomatal observations. Ernest (14) gives the results of a series of tests using the paraffin method for studying suction pressure in plant cells. According to her results the cells retained their original turgor for several hours, when in paraffin. In the present instance the epidermis was stripped and plunged into liquid paraffin, a process occupying a few seconds, and then examined under the microscope while mounted in liquid paraffin. Tests showed that the stomata in this medium were the same 6 hours after stripping as at the moment of stripping. Extensive comparisons were made with strips of epidermis from the same plant, and taken at the same time, but fixed with alcohol. In every case the results agreed closely. Most of the results given in tables VI and table VII were done in duplicate with these two methods and the results agreed perfectly. Moreover paraffin as a mounting medium has several advantages over alcohol. In the first place the epidermis strips remain soft and pliable when mounted in paraffin, so that when a cover glass is placed on the top it lies absolutely flat, also there is no necessity to worry about the preparation drying out, as is often the case with alcohol. A third advantage, which was of great importance in this case, is that paraffin is an excellently clear mounting medium for microscopic examination. It was found that measurements of the pore and width of the guard cells, by which the state of the stomata is recorded, could be made much more easily and precisely than was the case with alcohol.

[illegible] dried strips need to be mounted in alcohol (see 5)

(vi) Collodium Method (38). A thin layer of collodium painted over the epiderm and afterwards removed, as a strip, when dry, was quite satisfactory for showing the distribution of the stomata and the number per sq. mm., but it did not give a clear enough impression, or show distinctly, whether the stomata were open or closed, nor could any accurate measurements be made.

Of the above methods the paraffin method was chiefly used, the alcohol method being usually used as well as a check, since this is apparently the first time liquid paraffin has been used as a medium for observing stomatal apertures. The advantages of the strip method may be briefly enumerated.

(i) microscopic observation reveals the actual degree of opening, which can then be measured with a micrometer eye piece scale.

(ii) The stomata are never all of the same degree of opening at the same moment, as is pointed out by Loftfield (33). By microscopic examination of a strip of epidermis, approximately 400 stomata can be viewed in quick succession, and measurements made of the maximum and minimum, as well as the average degree of opening. As most results were done in duplicate with the paraffin and alcohol methods, about 800 stomata were reviewed for each reading. The measurement given is that of the width of the stoma from the outer wall of one guard cell to the outer wall of the other, ~~as well as~~ with comments on the general state of the stomata.

OBSERVATIONS AND RESULTS

I. OBSERVATIONS AT GRAHAMSTOWN, JUNE.

Refering to the rainfall table given previously, it will be seen that Grahamstown has the highest average rainfall of the three areas mentioned. Mists are prevalent during the spring and winter months. The potted plants from which observations were made were kept in a spot sheltered from winds and early morning sun.

Table V gives the stomatal readings taken hourly over a period of 24 hours, June 9th-10th. All readings were done in duplicate the alcohol method only being used. Fig α is a graph of the same readings in which the maximum and minimum dimensions of the stomata are plotted, whereas in Table V average dimensions are given. The graph illustrates the fact that the stomata are never all of the same degree of opening at any one period. The dimension given is the width of the stoma from the outer wall of one guard cell to the outer wall of the other guard cell. This measurement was easier to take than that of the pore, and gives a good idea of the variation of the stoma as well as the degree of opening of the pore.

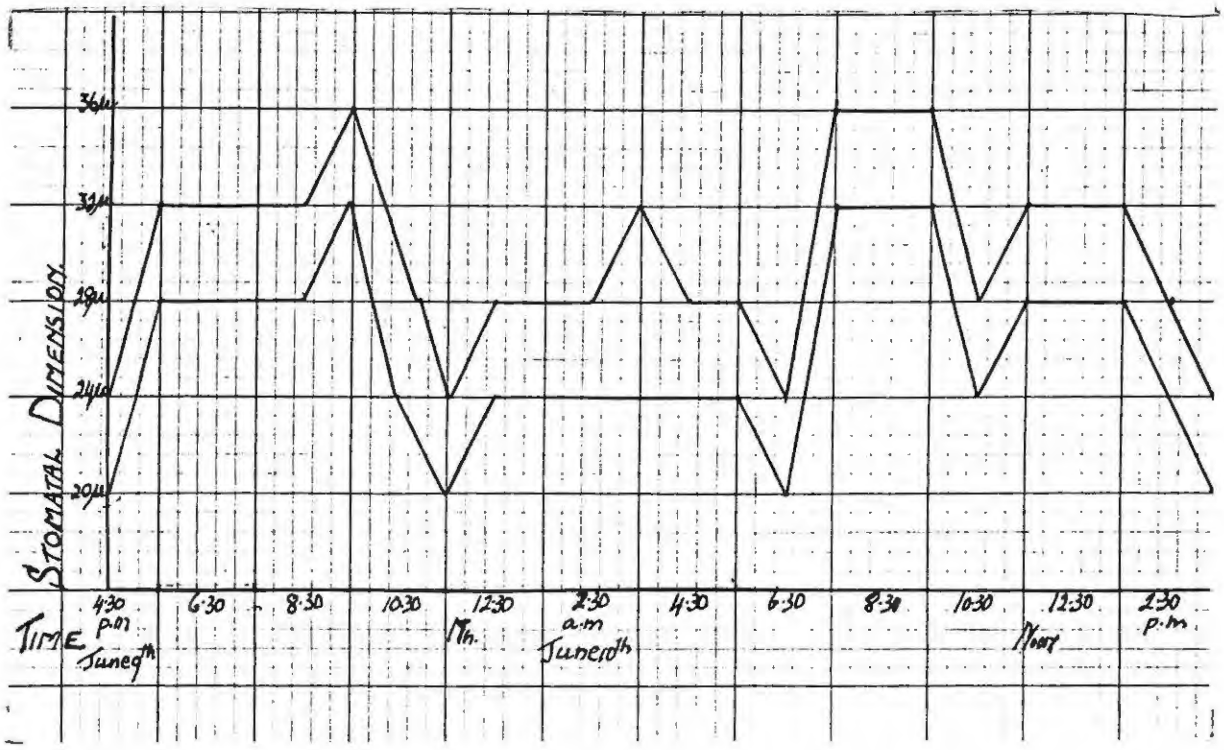
Temperature and humidity figures quoted in the table were obtained from the government meterological station situated about ½ mile away.

? Sound

A discussion of these and other results will be given at the end of the section.

II.OBSERVATIONS AT GRAHAMSTOWN OCTOBER 1935

In October when weather was warmer a number of readings were made. Typical results are given in Table VI. For the sake of comparison, simultaneous readings were made of the conditions of the stomata in Spekboom (Portulacaria afra) and Lucerne species.



Graph of Maximum and Minimum Width of Stomata, June 9th - 10th.

TABLE V HOURLY STOMATAL OBSERVATIONS 4.30 p.m. June9th-3.30 p.m. June 10th.

Date	Time	State of Stomata	Average Width of Stomata	Temperature Max F°	Temperature Min F°	Rel. Humidity	Remarks
June 9th	4.30p.m	Some shut others open	.22				Cloudy slight breeze
	5.30p.m.	Open	32				
	6.30p.m	Open	30				
	7.30p.m	Open	30				Calm dewy
	8.30p.m	Open	30				
	9.30p.m	Wide open	34	68.6	46.2	77.8	
	10.30p.m	Open	30				
	11.30p.m	Some shut others open	22				Cold, calm
	Midnight 12.30a.m	Open	26				
June 10th	1.30a.m	Open	26				
	2.30	Open	26				Slight breeze
	3.30	Open	28				
	4.30	Open	26				Slight breeze

Date	Time	State of Stomata	Average Width of Stomata	Temperature Max F°	Min F°	Rel. Humidity	Remarks
June 10th	5.30a.m	Open	26				
	6.30	Some shut others open	22				
	7.30	Wide open	34				
	8.30	Wide open	34				Slight breeze
	9.30	Wide open	34				
	10.30	Open	26				
	11.30	Open	30	58.5	45.4	84.8	Bright cool Windy close
	Midday 12.30	Open	30				
	1.30p.m	Open	30				
	2.30	Open	26				Over-Cast
	3.30	Some shut others open	22				

TABLE V Hourly Stomatal Observations, 4.30 June 9th -3.30 June 10th.

The above graph and figures show that at no time during the 24 hours period were the stomata all closed. Periods of greatest opening occurred reoughly from 8.30 to 10.30 p.m. and from 7.30 to 9.30. a.m.

ABLE VI

			JOINTED CACTUS		PORTULACARIA AFRA		LUCERNE		
Date	Time	Temperature	Average State of Stomata	Dimensions.	Average State of Stomata	Dimensions.	Average State of Stomata	Dimensions	REMARKS.
0th October	9 a.m.	80° F.	Shut	22μ	Shut	16μ	Open	15μ	Cloudy, close
	10 a.m.	75°	Shut	22μ	Shut	16μ	Open	15μ	Windy, getting cooler
	12 noon	78°	Shut	22μ	Shut	16μ	Open	15μ	
	2 p.m.	75°	Shut	22μ	Shut	17μ	Slightly shut	14μ	
3th October	9 a.m.	87°	Shut	23μ	Shut	17μ	Almost shut	13μ	Hot wind
	10 a.m.	90°	Shut	22μ	Shut	17μ	Almost shut	13μ	Hot wind
	3 p.m.	80°	Shut	22μ	Slightly open	20μ	Almost shut	13μ	Breeze cooler
2nd October	11 a.m.	62°	Shut	21μ	Open	15μ	Open	20μ	Cloudy, sudden rain,
	3 p.m.	63°	Slightly open	25μ	Wide open	19μ	Open	20μ	foll. by sunshine.
	5 p.m.	60°	Slightly open	24μ	Wide open	21μ	Slight closure	15μ	Cool, windy, cloudy
5th October	7 a.m.	50°	Slightly open	24μ	Open	15μ	Open	15μ	Cool, sunny.
	9 a.m.	60°	Shut	21μ	Open	15μ	Open	15μ	Windy, cloudy.
	5 p.m.	60°	Slightly open	24μ	Open	15μ ~~15μ~~	Open	15μ	Windy, cloudy.
8th October	11 a.m.	62°	Shut	21μ	Slightly open	14μ	Open	15μ	Rain.
	3 p.m.	68°	Shut	21μ	Shut	15μ	Open	15μ	Clear after rain
	6 p.m.	55°	Wide open	34μ	Shut	15μ	Slightly open	14μ	Sun set
	10 p.m.	50°	Shut	21μ	Shut	15μ	Shut	12μ	

29th October - continued

ABLE XI **Continued**

			JOINTED CACTUS		PORTULACARIA AFRA		LUCERNE		
ate.	Time.	Temperature.	Average State of Stomata.	Dimensions.	Average state of Stomata.	Dimensions.	Average state of Stomata.	Dimensions.	REMARKS
th October	7 a.m.	50°F.	Open	29μ	Open	19μ	Open	15μ	Dew still on leaves.
	9 a.m.	75°	Shut	21μ	Slightly open	19μ	Open	15μ	Dew gone.
	12 noon	80°	Shut	22μ	Shut	15μ	Nearly shut	13μ	Hot wind.
	2 p.m.	77°	Shut	21μ	Shut	15μ	Nearly shut	13μ	Windy.
	4 p.m.	60°	Wide open	32μ	Open	21μ	Nearly shut	13μ	Thick clouds coming up.
	6 p.m.	51°							Heavy shower rain made i impossible to take readings.

Analysis of these and other readings made during October show that the stomata of the Jointed Cactus are usually open in the early hours of the morning, but closed soon after sunrise, and remain closed 'till 4 or 5 o'clock in the afternoon. Low temperatures and high humidity would appear to cause them to open earlier in the afternoon, about 3 o'clock, but this is very variable and depends to a large extent on the climatic conditions. Under such circumstances closure appears to take place again between 9 and 10 at night.

III Observations at Uitenhage, February 4th to 21st, 1936

A Stevenson Screen with meterological instruments, and an atmometer, were set up about 100 yds from the plants under observation.

The first set of stomatal observations at Uitenhage were made on the day following an extremely hot day when tem perature was 103°F. Later observations, as records of temperature and humidity in Table XII show, were made under milder conditions and over a period of 5 days of intermittent rain, a total of .87 inches having fallen. Hence a fairly wide range of summer conditions were experienced by the plants during the time of observation. The effect of this weather was very noticeable in the condition of the plants. During the hot period, prior to the rain, the joints were very shrunken and wrinkled, but after two days of intermittent showers these joints had become turgid so that the epidermis was no longer cast into folds. On the fourth day of damp weather numerous vegetative and fruit buds had appeared. A remarkable feature showed up by the records obtained, is that for long periods the stomata are shut as far as can be ascertained by microscopic examination, though there must be a slight diffusion of gases through the presumably very narrow aperture.

ABLE VII.

A SERIES OF OBSERVATIONS, MADE ON PLANTS GROWING IN VELD AT UITENHAGE, CORRELATING STOMATAL MOVEMENT WITH CHANGES IN PHYSICAL CONDITIONS.

Date	Time	Relative Light Intensity	State of the Stomata	Dimensions of Stomata in Microns	Temperature		Relative Humidity	Rate of Evaporation mg/minute	REMARKS.	
					Max. °F.	Min. °F.			WEATHER.	STATE OF PLANTS.
/2/36.	11 p.m.	-	Shut	24	53.5	54	80	-	Clear, calm	Joints shrivelled.
idnight.	12	-	Shut	27	51	51	86	-		
/2/36.	1 a.m.	-	Shut	24-27	49	50	86	-		
	2 a.m.	-	Shut	24-27	47	47.5	93	-		
	3 a.m.	-	Shut	24-27	46	47	91.2	-		
	4 a.m.	-	Shut	24-27	45	46	92	-		
	5 a.m.	-	Slightly open	24-29	44	44.5	90	-		
	6 a.m.	3.07	Slightly open	24-29	44	44.5	92	-	Sun up at 6.5 a.m.	
	9 a.m.	77.70	Shut	22	70	71	50.6	18.24	Hot, sultry.	
	10 a.m.	77.70	Shut	22	75	76	46	40.74		
	5 p.m.	50.81	Shut	24	72.5	73.5	51	52.84	Windy	
	6 p.m.		Shut	27	71	71.5	50.7			
	7 p.m.	-	Shut	22-24	66.5	66	59.7	32.99		
	8 p.m.	-	Shut	22-24	65	66	63	17.56		
	9 p.m.	-	Shut	24	63	65.5	72			

↑
dashes mean no observations made

ABLE XII. continued.

A SERIES OF OBSERVATIONS, MADE ON PLANTS GROWING IN VELD AT UITENHAGE, CORRELATING STOMATAL MOVEMENT WITH CHANGES IN PHYSICAL CONDITIONS.

ate	Time	Relative Light Intensity	State of the Stomata	Dimensions of Stomata in Microns	TEMPERATURE Max. °F.	TEMPERATURE Min. °F.	Relative Humidity	Rate of Evaporation mg/minute	REMARKS. WEATHER.	REMARKS. STATE OF PLANTS.
0/2/36	7 p.m.	-	Shut	24	69	69.5	78	19.58	Cool breeze after	Joints shrivelled.
1/2/36	9 a.m.	77.70	Shut	22	80	81	56	40.35	Hot day. Max.93°F	
	2 p.m.	102.8	Shut	22	79	80	75	39.01		
	5 p.m.	29.04	Shut	24	75	76.5	94	24.70		
	6 p.m.	-	Shut	24	72	73.5	100	17.08	Rain started 6.35p.m.	
	8 p.m.	-	Shut	24	67	68	100	-	.06" fallen by 8.30	
	10 p.m.	-	Shut	24	67	68	88	-	a.m. 12/2/36	
idnight	12	-	Shut	24	67	68	83	-		
2/2/36	8 a.m.	33.42	Shut	24	66	67.5	85.5	6.44	Cloudy, slight breeze	
idday	12	136.2	Shut	24	76	77	89	25.22	Cloudy, sultry, no wind	
	3 p.m.	118.4	Shut	24	80	81.5	63	33.80	Very sultry.	
	8 p.m.	-	Shut	24	71	72.5	83	-		
idnight	12	-	Shut	24	65	66	94	-	Quite heavy dew,	
3/2/36	2 a.m.	-	Shut	24	63	64	97	-	No breeze.	
	4 a.m.	-	Shut	24	62	62	94	-	Cool, damp.	
	6 a.m.	-	Shut	24	60.5	60.5	97	-	Cool, sun up 6.00a.m.	
	8 a.m.	102.8	Slightly open	24-29	67	67	85.5	Not taken	Hot, cloudy	
	2 p.m.		Shut	24	80	80	56	34.92	Hot, few clouds.	
	8 p.m.	-	Shut	24	70	70	71	17.27	Warm night.	

14th February, 1936. - continued.

ABLE XII. continued.

A SERIES OF OBSERVATIONS, MADE ON PLANTS GROWING IN VELD AT UITENHAGE, CORRELATING STOMATAL MOVEMENT WITH CHANGES IN PHYSICAL CONDITIONS.

ate	Time	Relative Light Intensity	State of the Stomata	Dimensions of Stomata in Microns	TEMPERATURE		Relative Humidity	Rate of Evaporation mg/minute	REMARKS	
					Max. °F.	Min. °F.			WEATHER.	STATE OF PLANTS.
4/2/36	10 a.m.	157.0	Shut	24	80	80	67	40.68	Very hot	
	3 p.m.	157.0	Shut	24	82	83	42	66.54	Hot, slight breeze	Max. 89°F
	8 p.m.		Shut	24	70	71.5	73	8.72	Hot	
									.78" Rain from 15th - 18th.	
8/2/36	3 p.m.	136.2	Shut	24	72	72	69	42.33	Windy, few clouds	Joints fat and Turgid
	5 p.m.	29.04	Slightly open	24.29	67.5	68	65.5	20.11	Cool breeze	
	6 p.m.	4.07	Slightly open	27-29	66.5	67	73.5	16.87	Cool breeze	
	8 p.m.	-	Slightly open	29	66	66.5	73	6.80	Cool - close	
									.08" rain overnight	
9/2/36	8 a.m.	12.05	Some Slight open	24-29	64.75	64.5	99.4	-	Mist over everything	
	10 a.m.	89.33	Few slightly open	24-27	70.5	71	88.5	5.97	Mist lifted, slight breeze	
idday	12	102.8	Shut	24	74.75	75	75.3	19.39		
	2 p.m.	118.4	Shut	24	78.5	79	60.5	34.19	Windy, close, cloudy	
	4 p.m.	33.42	Shut	24	74.5	75	78	22.74	Cloudy, windy	
	6 p.m.	4.07	Slightly open	24-29	71.5	72	80.5	9.15	Cloudy, calm	
	8 p.m.	-	Slightly open	24-29	70	70	88	4.16	Cloudy.	
									.01" rain overnight.	

ABLE XII. continued.

A SERIES OF OBSERVATIONS, MADE ON PLANTS GROWING IN VELD AT UITENHAGE, CORRELATING STOMATAL MOVEMENT WITH CHANGES IN PHYSICAL CONDITIONS.

ate	Time	Relative Light Intensity	State of the Stomata	Dimensions of Stomata in Microns	TEMPERATURE Max. °F.	Min. °F.	Relative Humidity	Rate of Evaporation mg/minute	REMARKS. WEATHER.	STATE OF PLANTS.
0/2/36	8 a.m.	33.42	Some slightly open	24-29	69.5	70	97	-	Misty	Many new vegetative and fruit bud appearing.
	10 a.m.	118.4	Shut	24	78	79	75	17.30	Cloudy, hot	
idday	12	238.3	Shut	24	88	88.5	52	44.78	Hot, slight wind	
	2 p.m.	207.0	Shut	24	87.5	88	56.5	67.38		
	4 p.m.	67.46	Shut	24	84.5	85	60.5	45.01		
	8 p.m.	3 54	Some slightly open	24-29	78	78.5	74	15.90	Windy. Max 91°F.	
/2/36	8 a.m.	89.33	Shut	24	74	74.5	61	43.62	Windy	
	10 a.m.	180.0	Shut	24	76	76.5	52	61.07	Hot, windy	
dday	12	207.0	Shut	22-24	75.5	76	56.5	72.09	Windy	
	2 p.m.	136.2	Shut	22-24	72	73	57	53.04	Windy	
	4 p.m.	136.2	Shut	22-24	69.75	70	65	40.24	Cooler, windy.	
	6 p.m.	33.42	Shut	22-24	68	68.5	66			

A study of Table VII shows that of the hourly readings made from February 4th, 11 p.m. to February 6th, 6.a.m., the stomata were only seen open, very slightly, twice, i.e. at 5 a.m. and 6 a.m., while by 9a.m. there was no sign of opening. Maximum temperature the previous day had been 103°F, minimum during the time of observation was 44°F., ~~But~~ but the degree of humidity and rate of evaporation fairly high. The condition of the plants has also to be taken into consideration, as these observations were made towards the end of Summer, when the plants had been subject to fairly severe conditions. The rainfall for January was only .85 inches.

Another period from 9 a.m. 11/2/36 to 8 p.m. 13/2/36 passed without the stomata being seen open once. During this period a maximum of 88°F, minimum 60°F and .06" rain with high degree of humidity was recorded.

During the 16th and 17th., .77" of rain fell. On the 18th the joints were much improved in condition, all of them being quite turgid. From the 18th to 20th., .10" of rain fell, making .87" in all since the 16th February, which is greater by .02" than the rainfall for the whole of January.

It is interesting to note that with these more favourable conditions the stomata were seen open from 5 p.m. to 8 p.m. on the 18th; from 8-10 a.m. and 6-8 p.m. on the 19th; and at 8 a.m. and 8 p.m. on the 20th. At all other times they appeared to be shut.

It may be noted that these periods of opening correspond roughly to the periods of greatest opening as observed at Grahamstown during June and October.

DISCUSSION

The peculiar anomaly in the respiration of cacti, first noted by Livingston (30) was the subject of further investigation by Shreve (46) who made some observations on the movements of *Opuntia versicolor* in connection with it.

In *O.versicolor* she found that "under typical conditions the stomata begin to close soon after sunrise and they appear to be completely closed by noon. They begin to open at 5 to 6 o'clock in the evening and continue to open throughout the night, the maximum size occuring between 3 and 6 a.m. " Loftfield (33) commenting upon this statement says that it applies almost word for word to the behaviour of the alfalfa stomata under conditions of low water content, high temperature continuous brilliant sunshine, and very dry air. These being the habitual conditions under which the species of *Opuntia* under consideration lives, Loftfield suggests that it has adapted itself, and such movements have become typical of its stomata.

In the case of *Opuntia aurantiaca* table V shows that at no period during the 24 hours of observation were all the stomata of any particular portion of the epidermis seen to be shut, but that the period of greatest opening occurred in the morning from 8 to 10 a.m., and in the evening from 7 to 9 p.m., this during the winter month of July, the maximum temperature during the period of observation being 68°F and relative humidity 77.8.

Observations in October during the growing season show that the stomata are usually open in the early hours of the morning but close soon after sunrise, to open again at about 4 or 5 in the afternoon. Further information is necessary on the question of night opening. The period of opening in the morning corresponds with the period of maximum opening for *O.versicolor*.

Comparing these results with those of the fleshy leaved *Portulacaria afra*, and the thin leaved mesophytic type such as Lucerne,it will be seen that Partuclacaria afra shows results between the Jointed Cactus and Lucerne, agreeing sometimes more closely with the Jointed Cactus than with the Lucerne. The results given for Lucerne correspond in general with those given by Loftfield for alfalfa, as it is known in America, that is in common with most mesophytes the stomata remain open during the day under normal conditions. As conditions become less favourable midday closure appears, see table VI Oct. 10th , Oct 18th. According to Loftfield, with increasingly unfavourable conditions complete closure occurs,and becomes prolonged until stomata are closed all day. With the appearance of day closure,night opening occurs and increases with increase of day closure, showing that the stomata of these plants are capable of changing their normal movements and adapting themselves to the rigors of external conditions.

In some respects the movements of the Jointed Cactus stomata may be compared with Loftfield's third group which contains a large number of fleshy-leaved plants. In particular it may be compared with the cowbeet where the stomata, under favourable conditions,are wide open during the day and close very little at night , this agreeing in some respects with results in table V. As conditions grow less favourable the stomata of the cow beet only open widely an hour after sunrise,and close gradually and completely during the forenoon or even before morning is over. In the Jointed Cactus opening occurs in the early hours of the morning, closure starts soon after sunrise and continues until late afternoon when they start to open again. Table VII shows that,under conditions of high temperature and light intensity,and after a prolonged period without rain,but under condition of high humidity, see table III Feb. 11th-13th, the stomata are to all appearance shut during this period, only opening slightly at 8 a.m at the end of the period. While on the 18th and 19th Feb, after .78 inch rain and,under more favourable condition,the periods of opening approach more nearly those recorded in table VI.

These stomatal readings give some idea of the adaptability of the plant to external condition and the methods used in resisting drought. Attention is once more drawn to the nature of the stomata in older joints, the thickened cuticle of the guard cells, and the growth of the subsidiary cells, which causes them to project below the guard cells into the respiratory cavity and cause partial if not complete blockage of the air pore. How far this is affected by drought condition and how far by age of the joint is not yet certain. Its interference with the opening and closing of the stomata and the checking of transpiration, is of obvious importance to the plant. Further observations of such joints, and of the movements of stomata of very young joints during the growing season, are necessary.

SUMMARY.

1. An illustrated account of Jointed Cactus (Opuntia aurantiaca, Lindley) is given. Points which have direct bearing on problems of eradication are discussed at the end of each section, and are summarised below.

2. The terminology and morphology of the external parts are briefly dealt with.

3. Anatomical details of the epidermis, hypodermis stomata, ground tissue and vascular system are given. Of particular interest are the mucilage canals and cells, the numerous calcium oxalate crystals and the hydrocyts.

4. A brief account of secondary thickening in the joints shows how secondary growth in the fallen joints takes place, and how these are capable, when buried, of forming large storage organs or tubers.

5. Vegetative propagation is dealt with, it is the meristematic tissue in the areoles which is responsible for new growth. It is important to note that the fruit are capable of vegetative propagation, and also form underground storage organs.

6. The root system and root anatomy is briefly commented upon.

7. Development and structure of the floral parts is given and the homology between flower and vegetative shoots discussed.

8. Development and structure of the ovule are described. The exceptionally long funicle which completely surrounds the ovule, and which later forms a hard stony coat round the embryo, is of particular importance in accounting for the delay in germination.

9. Seeds containing embryos are very rare, roughly about 1 in every 1,400 ovules develops an embryo. Polyembryony is fairly frequent.

10. Germination tests are given. Seeds would only germinate when the hard, stony funicle covering the micropyle had been removed.

11. A few facts dealing with the physiology of the plants are given.

12. The results of stomatal observations on cultivated and wild plants are tabulated and discussed. Methods of observation including a new use of liquid paraffin are given.

Points of Interest in Problems of Eradication.

1. Vegetative propagation from very small joints, from portions (with areoles) of joints, from fruit, tubers and bits of tubers is possible. Such parts can retain their vitality for a long time, therefore all parts must be destroyed in order to prevent fresh infection.

2. Infection from seed is rare but possible. Germinable seed occurs very rarely, about 1 seed in every 14 fruit. The structure of the seed is such that germination can be delayed indefinitely. Seedlings can be recognised by the two large fleshy cotyledons which are reddish underneath.

3. Blocking of the stomata and cork formation must be reckoned with when considering the penetration of the surface by poisonous liquids.

4. The conducting system is illustrated, the reticulate system of tracheids is noteworthy. In older joints and in tubers the phloem seems to be seriously interfered with by the lysigenous formation of mucilage canals.

REFERENCES.

1. Alexander, W.B. Variations of acclimatised species of prickley pear. (Roy. Soc. Queensland, 38 (3) 1926).

2. Britton & Rose. The Cactaceae (Car. Inst. Wash. vol.1919

3. Brouland. Comp. Rend. Acad. Sci. (Paris) 98, 1934.

4. Bukvic. Die thylloiden Verstopfungen der Spaltöffnungen und ihre Beziehungen zur Korkbildung bei den Cactaceen, (Osterr. Bot. Zeitschr. LXII (1912) 401).

5. Cannon, W.A. Water Conducting Systems of Desert Plants (Bot. Gaz. 1905).

6. ———— Physiological Features of Roots, with Special Reference to the Relations of Roots to Aeration of Soil. (Car. Inst. Wash. 368, 1925).

7. ———— Features of the Vegetation of the More Arid Portions of South Africa (Car. Inst. Wash. 354).

8. Chamberlain. Methods in Plant Histology.

9. Chodat. Principes de Botanique 1920.

10. Crocker, W. Mechanics of Dormancy in Seeds (Amer. Jour. Bot. vol iii 1916).

11. Darbishire, O. Observations on Mammillaria elongata (Ann. Bot. XVIII p. 375, 1904).

12. de Bary. Comparative Anatomy of the Phanerogams and Ferns 1884.

13. de Fraine. The Seedling Structure of Certain Cactaceae (Ann. Bot. XXIV 1910).

14. Ernest, E. Paraffin Method of Studying Suction Pressure in Plant Cells. (Ann. Bot. XLVII 1934).

15. Ernst, A. Bastardierung als Ursache der Apogamie im Pflanzenreich. Jena 1918.

16. Engler-Prantl. Die Natürliche Pflanzenfamelien vol. 21.

17. Ganong, W.F. Present Problems in Anatomy, Morphology and Biology of Cactaceae (Bot. Gaz. 1895).

18. ———— Upon Polyembryony and Its Morphology in the Cactaceae (Bot. Gaz. 1898).

19. ———— Comparative Morphology of Embryos of Seedlings in Cactaceae (Ann. Bot. XII, 1898).

20. Goebel. Organography of Plants 1905.

21. Guignard, L. Observations sur les ovules et la fécondati des Cactees. (Bull. Soc. Bot. France XXXIII p. 276, 1886).

22. Hemenway, A.F. An Anatomical Study of Traumatic and other Abnormal Tissues in Carnegia gigantea. (Amer. Jour. Bot. 21,9,1934).

23. Huber, J.A. Bluten und Samenentwicklung der Kakteen und ihre Bedeutung für deren systematische Slettung. (Monatsschrift Deutschen Kakteen Ges 1 (9) 1929).

24. Hull, E.D. Polyembryony in *Opuntia Rafinesquii* (Am. Bot., 21, 56-57).

25. Index Kewensis. Fasc I and II 1893, 1894.

26. Jackson,B.D. Glossary of Botanical Terms.

27. Johnson, D.S. Fruit of Opuntia fulgida (Car. Inst. Wash.1

28. Lansdell, K. The Weeds of South Africa, X Jointed Cactus, (*Opuntia aurantiaca*) Gilles. (Journ. Dept. Agric., South Africa, XVII, 2. 1923).

29. Lindley. Edwards Botanical Register 19, 1833.

30. Livingston,B.E. Relative Transpiration in the Cacti. (Plant World. 10:110).

31. LLoyd, F.E. Origin and Nature of Mucilage in the Cacti and Other Succulents (Amer. Jour. Bot.VI,19

32. ——— The Physiology of Stomata (Car. Inst. Wash. 1908).

33. Loftfield, T.V.G. The Behavior of Stomata (Car. Inst. Wash. 314, 1921).

34. MacDougal, D.T. Hydration and Growth (Car. Inst. Wash. 297, 1920).

35. ——— and Spalding E.S. The Water Balance of Succulent Plants (Car. Inst. Wash. 141, 1910).

36. ——— and Shreve. Growth in Trees and Massive Organs of Plants (Car. Inst. Wash. 350, 1924).

37. Maximov. The Plant in Relation to Water.

38. Mes and Amyer Ainslie. Studies on the Water Relations of Grasses I. (South African Ass. Adv. Sci. vol. XXXII, p. 280, 1935).

39. Neumann, M. Die Eintwicklung des Pollens, der Samenenlag und des Embryosackes von *Preseskia amapola* var. *argentia*. (Oesterreich. Bot. Zeitschr. 84 (1) 1935).

40.	Pole Evans, I.B. and Lansdell, K.	The weeds of South Africa VIII. The jointed cactus, <u>Opuntia aurantiaca</u> Gilles. (Union of South Africa, Dept. Agric. Bull. Local Ser. 75, 1919).
41.	Reed, T.	On the Anatomy of Some Tubers (Ann. Bot. XXIV, 1910).
42.	Rhefous.	Etude sur les Stomates (Inst. Bot. Univ. Geneve, [illegible] Serie, VI Fascicule).
43.	Rules for Seed Testing ~~U.S.~~.	U.S. Dept. Agric. Circ. 406, 1928.
44.	Sachs.	On the Physiology of Plants. 1882.
X45.	Schonland, S.	The Jointed Cactus (South African Journ. Dept. Agric. Reprint no 38, 1924).
46.	Shreve, E.B.	An analysis of causes of variations of the transpiring power of cacti. (Physiol. Res. 2: 73).
47.	Solereder.	Systematic Anatomy of Dicotyledons. 1908.
48.	Spaeth, J.M.	A Physiological Study of Dormancy of Tilia Seeds. (Cornell. Univ. Agric. Exp. Stat. Memoir 169, 1934).
49.	Strasburger.	Prakticium.
50.	————	Text Book of Botany 1930 ed.
51.	Stockwell, P.	Chromosome Numbers of some Cactaceae (Bot. Gaz. 96 (3) p. 565).
52.	Tincker & Darbishire.	Studies on the Formation of Tubers and othe Storage Organs (Ann. Bot. XLVII, 1933).
53.	Toumey, J.W.	Vegetal dissemination in the genus Opuntia (Bot. Gaz. p. 356 1895).
54.	Wheldale Onslow.	The Anthocyanin Pigments of Plants.