VIVIPARY IN NYMPHAEA

x DAUBENIANA

by

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Vivipary is a rare occurence outside the animal kingdom; and vivipary by means of foliar embryos is an even rarer occurence. The production of foliar embryos is limited to about 10 genera of four widely divergent families. Even though the genera which exhibit this characteristic are evolutionarily as widely separated as Bryophyllum (Crassulaceae) and Camptosorus (Polypodiaceae), the ontogeny of the individual plantlets is remarkably similar (Yarbrough 1932, 1934, 1936a, and 1936b). The differences lie in the location of the embryos on the lamina, their number per leaf, and the length of time it takes for each of the developmental steps to be completed (Heide 1965; Yarbrough 1932, 1934, 1936a, and 1936b). The species Nymphaea micrantha (Guillemin and Perrottet) and Tolmeia menziesii (Torrey and Gray) are the only plants which produce their foliar embryos at the point of the insertion of the petiole into the lamina (Yarbrough 1934 and 1936b).

The only developmental difference between the plantlets of N. micrantha and T. menziesii is that the plantlets of Tolmeia generally have no dormant period (Yarbrough 1936a) as do those of N. micrantha (Planchon 1852).

The first record of the viviparous habit of N. micrantha was in the description of the species by Guillemin and Perrottet in 1830. They used the viviparous habit to separate N. micrantha from N. stellata to which it was otherwise very similar morphologically. J. E. Planchon (1852) was the first person to describe the development of the foliar embryo; and he was also the one who first applied the

term "tubercle" to the embryonic structure. Planchon's description was limited only to what could be seen with the naked eye. He attributed this development to an overabundance of life force, "d' un foyer où la vie surabonde."

Conard (1905) in his monograph of the genus Nymphaea repeated Planchon's description of the tubercle's development and records the names of various other writers who had noted this viviparous characteristic before the year 1900. From 1905 until the present there is no record of any scientific study of the foliar embryos of this waterlily. Although in 1922 G. H. Pring, the noted breeder of tropical waterlilies, wrote about his attempts to spread this viviparous habit through a larger number of cultivars because it would make propagation much simpler.

In the late 1920's and the early 1930's there were a number of papers published examining in detail the ontogeny of the foliar embryos of various species that display this characteristic, but Nymphaea micrantha was neglected. It was not until 1965 that part of the biochemical control mechanism for foliar embryo production of one of these species, Bryophyllum daigremontianum, was discovered by O. M. Heide. He found that naphthaleneacetic acid inhibited the development of the foliar embryos and that the cytokinin, 6-benzyl-aminopurine, had a stimulatory effect on their growth. He also noted that several other researchers (Götz, 1953, Meyer, 1953, and Kröner, 1953) had found that development of the foliar embryos was also influenced by the photoperiod.

Of all the waterlilies, only Nymphaea micrantha and some of its hybrid progeny are viviparous, Nymphaea x Daubeniana is one of these. Nymphaea micrantha is native to the coastal freshwater swamps

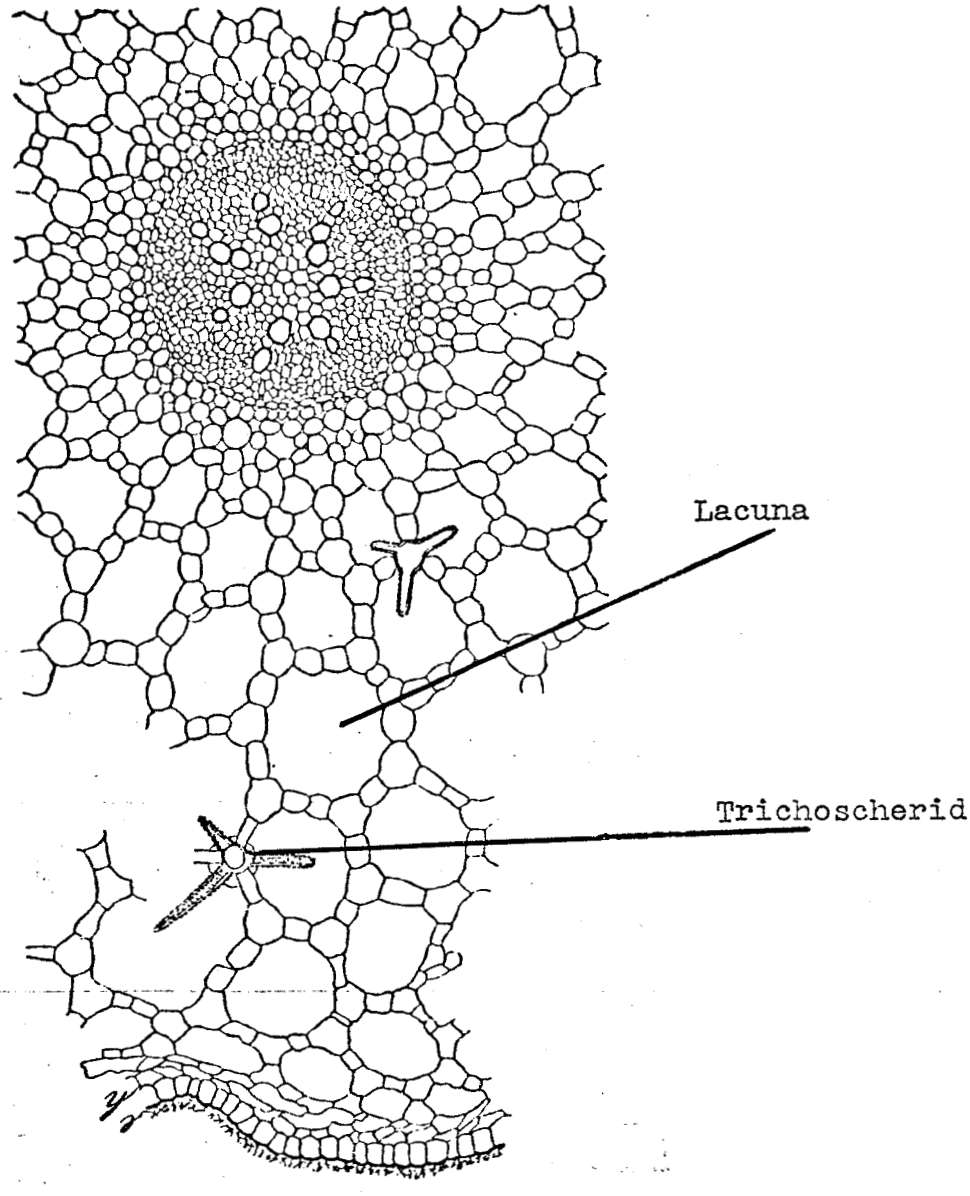

Figure 1. The structure of the air canals in the underwater sections of the waterlily (taken from Conard- The Waterlilies).

of Nigeria. These lie in an area about seven degrees north of the equator where the rainfall is 70- 170 inches per year. Most of it comes during the May to October rainy season. The air around the leaves of these plants, according to Kendrew (1961), has a mean annual temperature of 80° F. The monthly mean temperature variation around this annual mean is only plus or minus 4° F. It is coolest during the rainy season.

The water in which these plants are found varies in depth between twelve and thirty-six inches. The main axis of the plant is a short upright tuberous stem. The leaves grow upwards from this stem so that the lamina floats upon the surface. Air is conducted from the surface of the water to the rest of the plant by numerous

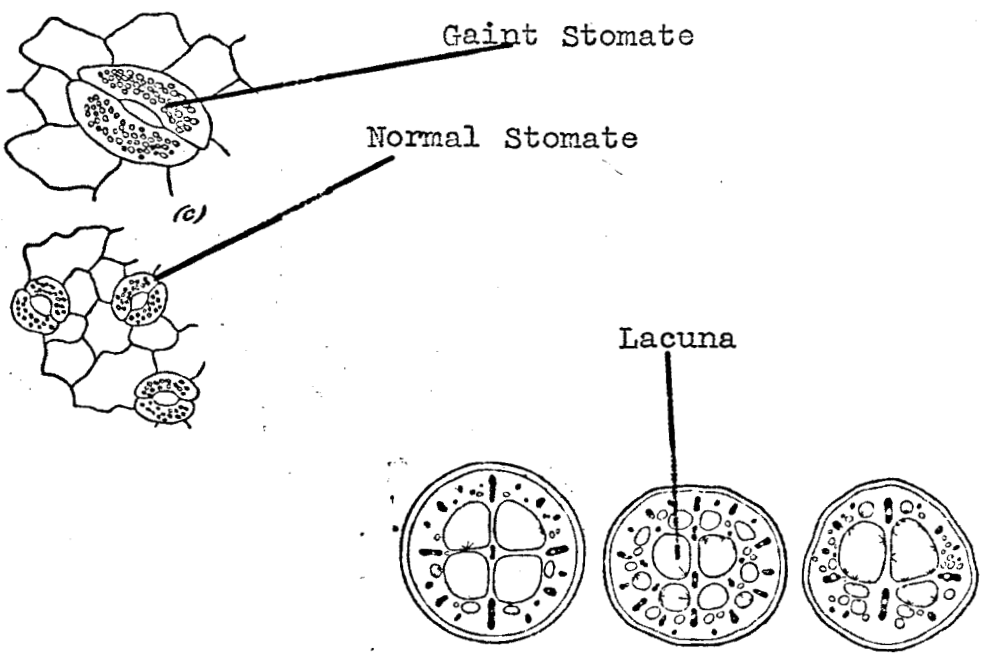

Figure 2. Structure of air conducting system of waterlilies in the leaf. (Taken from Conard - The Waterlilies).

lysigenous air canals which traverse longitudinally the length of the petiole and are parallel to the vascular bundles (Conard 1905). In the stem these canals empty into a central pithy area that consists largely of various sized, thinwalled cells, with large intercellular spaces and many trichoscherids which permit air diffusion to the rest of the underwater parts of the plant (refer to figure 1). The canals open to the atmosphere through several giant stomates (refer to figure 2).

The vascular bundles, in joining the leaf, are all connected at the uppermost end of the petiole by "transverse commissures,

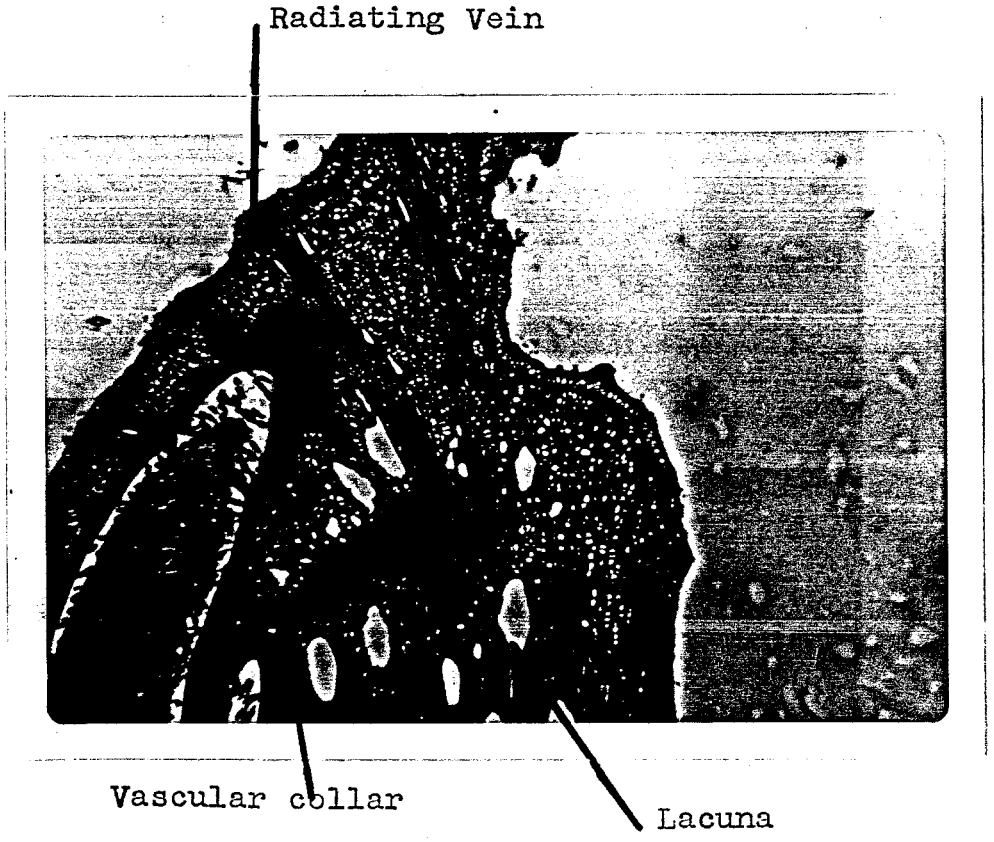

Figure 3. The vascular system of the leaf showing the vascular ring and radiating veins.

forming a ring of vascular tissues parallel to the surface of the leaf. From this ring the primary veins radiate out into the leaf" (Conard 1905). It is from cells within this vascular ring and around the air canals that the plantlets form.

There are no vascular elements entering this area. Thus, it will be assumed that the cellular nutrients and hormones diffuse inward evenly from the surrounding vascular ring and there is a plentiful and even supply of oxygen from the lacunar system (figures 3 and 4). This physical arrangement is similar to the environment to which a piece of callus tissue is exposed when grown in culture.

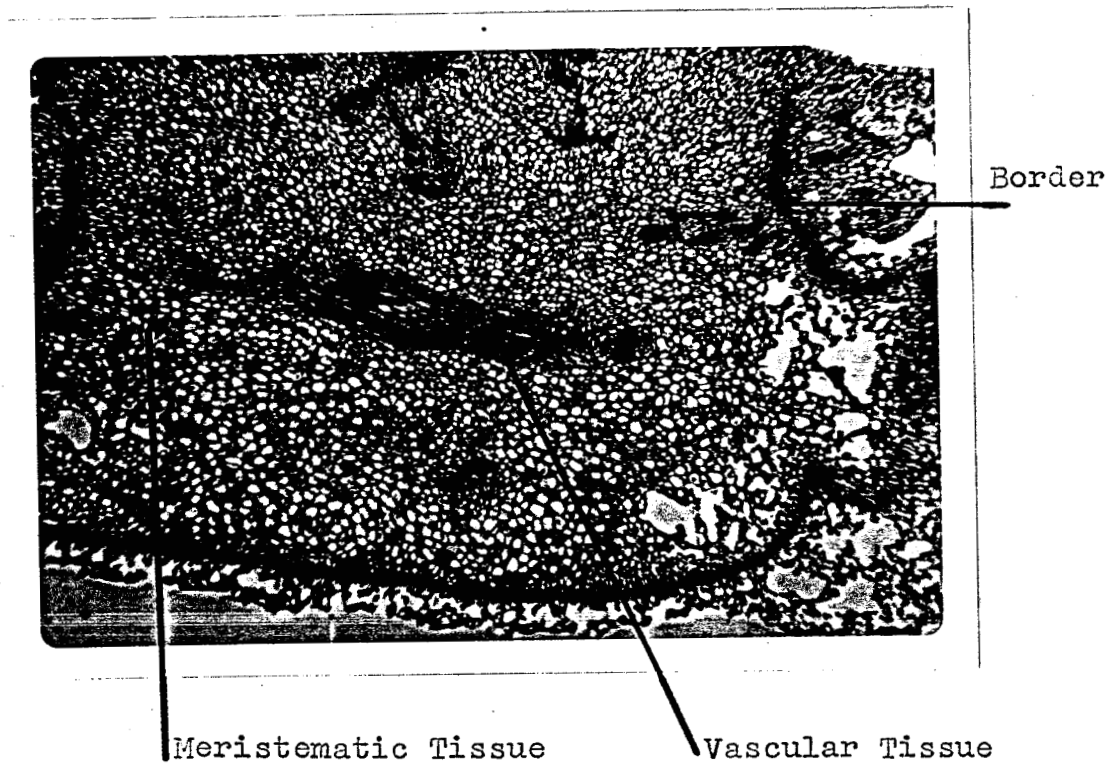

Figure 4. The border area of the tubercle showing the lack of vascular connection.

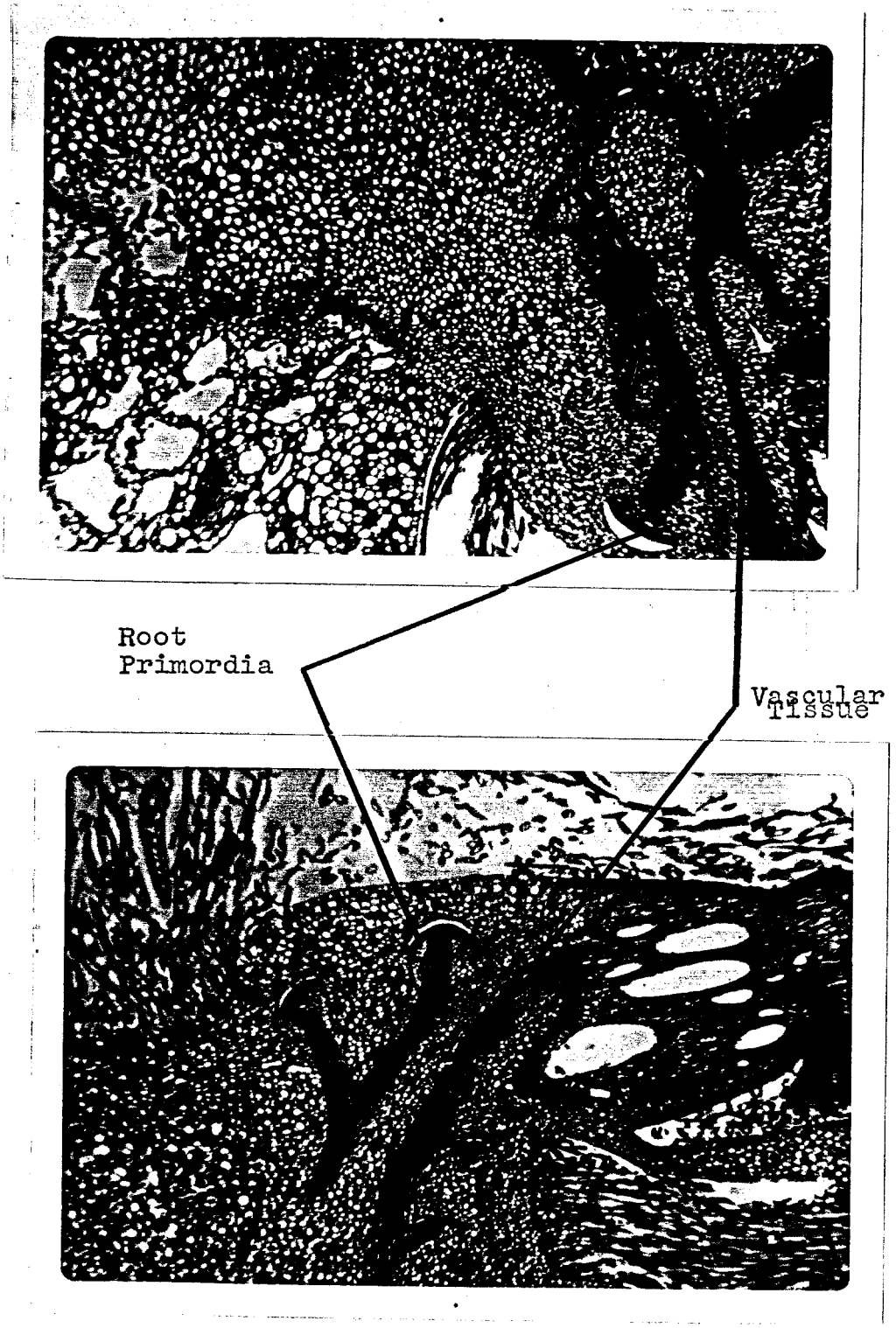

Figure 5. The similarity of root development within the stem (on top) and the tubercle (on bottom).

The plantlet develops from a mass of meristematic cells "left behind" by the rest of the "parent leaf's" cells as they differentiate into the various tissues that make up a mature leaf. The mass of cells which soon cause a humping under the upper epidermis readily noticeable in a transverse section of the insertion area. The hump increases in size until a typical tunica and corpus meristematic arrangement forms. Leaf primordia are initiated as the plantlet goes into a dormant period as the parent leaf matures on the water's surface. By this time the meristem has differentiated a short (2-5mm) stem axis, but no roots have formed. It is after this dormant period that roots form in the same manner that roots form from tissues in the stem (refer to photographs in figure 5).

As the parent leaf begins to senesce, the dormancy of the plantlet is broken and growth resumes. The leaves rapidly increase to a larger size, and the roots grow out across the parent leaf's surface. The lamina of the parent leaf rots and disentegrates within a few days; the petiole persists longer. The final result is a small floating plant which currents may carry away to a new location, before the roots find the bottom and anchor the plant to a new home. This rapid and easy means of reproduction is probably the evolutionary advantage which favored its development.

There are several concepts which must be accepted axiomatically, for these concepts to form the basis of this paper. These are: the concept of totipotency, the concept of the unit genetic switch, the concept of developmental pathways, and the concept of switching networks. Also helpful in understanding how small amounts of plant hormones work is Monod's concept of the mode of hormonal action.

Totipotency is defined by Bonner and Varner (1965) as the theory that each living cell of an adult organism contains all the genetic information necessary to regenerate the adult organism. "An example of this is shown by Steward, Mapes, and Kent (1963) who have shown that whole carrot plants can be readily grown from clumps of carrot phloem cells and that whole carrot plants can also be caused to arise from single differentiated cells of very diverse types." The secret lies in placing the single cells in the proper

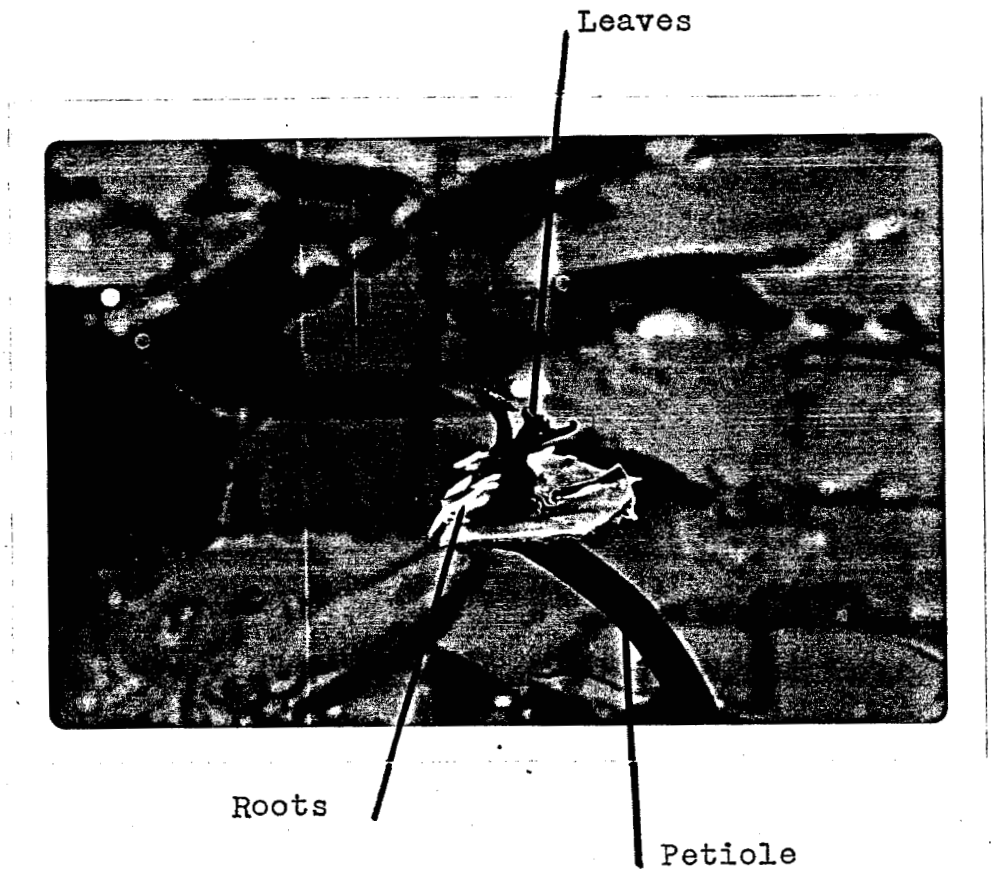

Figure 6. The final stage of tubercle development. The tubercle is now equipped for independent life; the lamina is now senescing (note yellow). Lamina in this photograph has been trimmed for the picture.

type of nutrient medium, one which contains the materials necessary to bring about renewed division in the isolated cell. The same is also true of callus cultures. Under appropriate conditions these cells can be caused to give rise to buds and thence to generate a new plant which passes normally through the whole plant life cycle.

All this information is contained in the cell's DNA. The information stored in the DNA molecule must be interpreted in some organized manner. Bonner (1963) and others have proposed that the group of chemical compounds known as histones are the controllers of which sections of the DNA is read by repressing and derepressing sections of the genetic code. Monod et. al. (1963) proposed the idea that the histones could be controlled by particular small molecules which bind to the protein repressor (histone) substance: such binding changes the configuration and thus some of the other properties of the repressor. The plant hormones are believed to be one such group of small molecules.

"Excellent proof of this concept is given in the case of potato tuber bud dormancy (Tuan and Bonner 1964)." These buds do not grow, even when favorable conditions are supplied. They lack the ability to synthesize any substantial amount of RNA in vivo (Bonner and Varner 1965). When the dormancy has ended, the buds again posses the ability to synthesize RNA rapidly. In addition to those genes which are derepressed by the presence of appropriate hormones, there are also those other genes which are repressed or derepressed by the proper metabolite (Bonner and Varner 1965).

> It is characteristic of development that a cell, or cells, once set upon a particular route or pathway of development, continues inexorably along this pathway. Thus, the cells produced by the apical meristem differentiate step by step into the

> specialized cells of stem and leaves. The meristematic cells of the apical bud continue to produce cells which follow the stem-leaf pathway until dormancy, flowering, or death intervene. The developmental pathway, once turned on, is followed until fresh signals set it upon a new course.
>
> Switching of the developmental course is dramatically evident in the case of bud dormancy considered above. Dormancy is a pathway along which no development takes place, a sort of ground state. When the bud is awakened from dormancy it passes at once to the appropriate path by which buds make leaves and stems. The agent which awakens dormant buds does so by derepressing genes previously repressed (Bonner and Varner 1965).

The triggers of the developmental pathways are physical and chemical. Among the physical triggers are the presence of cellular neighbors and the type of neighbor. This information could be transmitted by chemicals excreted by one cell and absorbed by its neighbor(s). These chemical signals could be hormones or metabolites.

> We know, then, of many individual instances in which a particular kind of genetic activity is called into play by one or another agent. The cell is set on a pathway of development. Let us now inquire in more detail exactly what we mean by a pathway of development. In the case of the flowering pathway, for example, the flowering hormone sets things into motion. Once the initial act of floral induction has occurred, the cells of the bud proceed inexorably toward flower formation. It is to be expected that further changes in the posture of the genome will occur as flower development proceeds. The meristematic apex swells, protuberences are cut off, the individual organs of the flower: sepals, petals, stamens, ovaries appear, meiosis takes place, and so on. All this complicated sequence flows, as it were, automatically from the initial act of induction. It is as though induction calls into

> action a preprogrammed plan of all steps subsequent to the first which must be executed if flowers are to be formed (Bonner and Varner 1965).

Bonner and Varner (1965) further state that "the same may be said for the cells of the vegetative meristem, the root meristem, and the cambium. It is convenient to think of the genetic information of the plant as subdivided into a series of such programs," one for the making of each of the kinds of plant organs as well as for the conduct of basic cellular biochemistry (Bonner and Varner 1965). According to this theory, the proper developmental program is called into action by the presence of the required signal or inducer substance, the flowering hormone in the case of flower induction.

This theory uses the idea that the cell periodically samples its environment to find out the number of surrounding cells. This test might consist, for example, of sensing the concentration of some substance given off by the cells, which therefore builds up to high concentrations in masses of cells but which quickly diffuses away from single cells.

> With the concept of a developmental test, it becomes possible to specify model programs which, if executed, would cause a cell to develop step by step with strict sequential programing into an organ or organism. Such a program for the generation of stem from a single cell is outlined (by the following). The apical cell first divides into two cells. Each cell then tests itself to find out whether it is apical or not. The one that is apical then divides again. The cell that discovers from the outcome of the test that it is not apical proceeds to the next instruction in the developmental program. This instruction is to divide and to continue dividing and producing more cells until a specified number of cells have been accumulated in the growing bud. The subapical cells, divide, after each division testing themselves

for the size of the cell mass in which they are embedded. When the required ultimate size has been achieved, as shown by the outcome of the appropriate size test, the cells of the bud are required to conduct further tests to discover what kind of specialized cells they should turn into...The cells are first required to test themselves for whether they are on the outside of the tissue mass or not. If they are on the outside, they then become epidermal cells. Cells which are not on the extreme outside then test themselves to find out whether

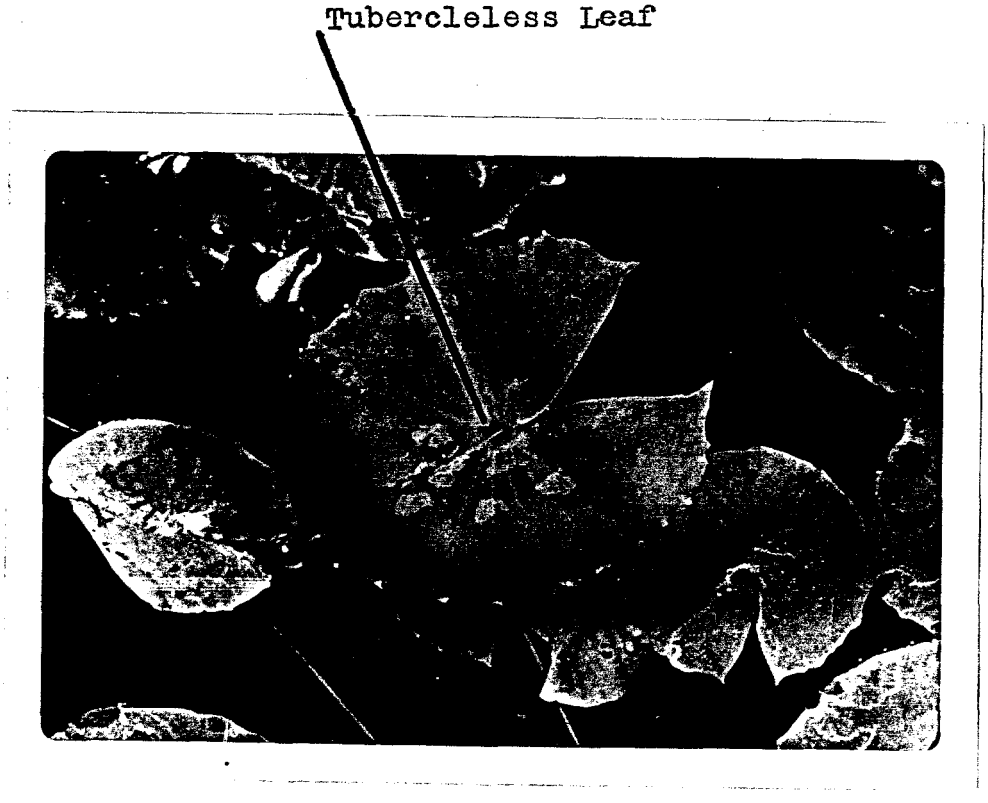

Figure 7. Photograph of a leaf of Nymphaea x Duabeniana without a tubercle.

> they are on the extreme inside. If they are, they turn into xylem. Cells that are in neither category test themselves again to find out whether they are next to the epidermis; if they are, they turn into phloem, if they are neither, they are told to divide again, that is, to behave as cambium (Bonner and Varner 1965).

It has been intended that this section, and especially the outline of stem formation, to be suggestive of the processes necessary for the development of the waterlily tubercle.

In the switching network which leads to tubercle development, the first step causes the cells within the vascular ring to remain meristematic instead of differentiating into parenchyma. This first step must exist because some leaves never have tubercles. If the leaf does not have a tubercle as it matures on the water's surface, it will not subsequently produce a tubercle and thus will not produce plantlets (figure 7). This triggering may simply be a high level of vigor since Table One shows that all the factors which favor the accumulation of photosynthates, i.e., length of photosynthetic period, and temperatures are correlated with leaf and tubercle formation. The data in Table One were computed by a multiple stepwise regression analysis from the data listed in the Appendix. If the level of vigor is above an unknown threshold, a tubercle is produced; below this threshold there is no tubercle produced. Bonner's assertion that a metabolite may act as a trigger to set a cell along the path of a switching network is consistent with this model. Once the mechanism is triggered, growth is regulated by the plant's hormones. The two hormones involved are indoleacetic acid (IAA) and the cytokinin, zeatin. Table Two shows that naphthalene acetic acid (NAA) and 6-benzylaminopurine, structural

Table One. Factors affecting plantlet and tubercle production. The figures in the columns give the percentage of the total variance the various independent variables have on the dependent variables as calculated by a multiple stepwise regression analysis.

Independent Variables	Dependent Variables					
	Number of Tubercles	Number of Leaves	Number of Plantlets	Number of Leaves Removed	Milligrams of CH_2O/gram	Milligrams of N/gram
Daylength	4%	4%	12%	.9%	14%	.2%
Water Temp.	17	13	23	.9	.4	33
High Air Temp.	12	12	15	.1	.3	3
Low Air Temp.	58	63	36	53	10	.7
Fertilization	1	1	11	6	2.2	9.3
Total Explained Variation	92%	93%	97%	61%	27%	46%

analogs to zeatin and indoleacetic acid are active in the regulation of tubercle growth. The native chemicals, thus, are probably IAA, because it is found in plants while NAA is synthetic; and zeatin, because Heide and Skoog found zeatin readily produced in the leaves of Begonia rex-culturum and Bryophyllum (S. C. I. Monograph No. 31). That both an auxin and a cytokinin are involved is indicated by Jublonski and Skoog (1954), who showed that for cells of tobacco pith to divide, it was necessary to supply them with both an auxin and a cytokinin. Romberger (1963) states that "when used alone, neither substance can elicit cell division. The high levels of these hormones would be conducive to rapid disorganized cell proliferation."

Table Two. The effects of kinetin and NAA on plantlet production.

TEST	PLANTLET GROWTH	
Cut Leaves	Number yes[1]	Number no[2]
NAA in Lanolin	30%	70%
Kinetin in Lanolin	90%	10%
Lanolin	60%	40%
No Lanolin	75%	25%
Uncut Leaves [3]	21%	--

[1] The number of plantlets produced.

[2] The number of leaves that died before producing plantlets.

[3] Population mean for plantlet production on undamaged leaves; standard deviation from mean ± 16%.

The cells that become the tubercle remain meristematic while the cells of the rest of the leaf differentiate. Microscopic examination indicated that the cells of the tubercle remained small

and isodiametric. The nuclei are small and dense, and the vacuoles are poorly developed and few. At the same time the leaf cells outside the tubercle are taking on the shape and cell wall structure of epidermal, palisade, spongey mesophyll and vascular cells (figure 4). Romberger (1963) states that the ratio between indoleacetic acid and kinetin is very important in determining whether or not tobacco tissue cultures remain undifferentiated.

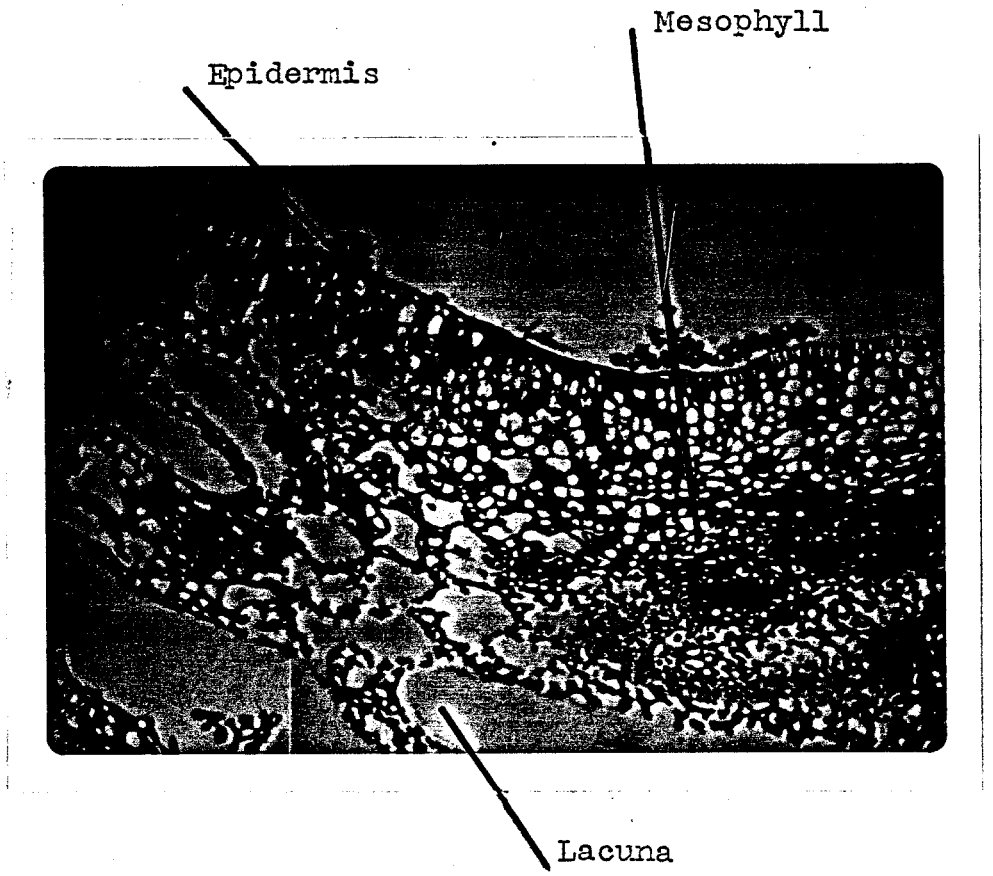

Figure 8. The tissue within the vascular ring of a tubercleless leaf. This is the same area where a tubercle could have formed.

While the tubercle is accumulating a cell mass before differentiating a tunica and corpus, the factor controlling tubercle development is an even balance of the two hormones which permits only disorganized cell growth in the tubercle.

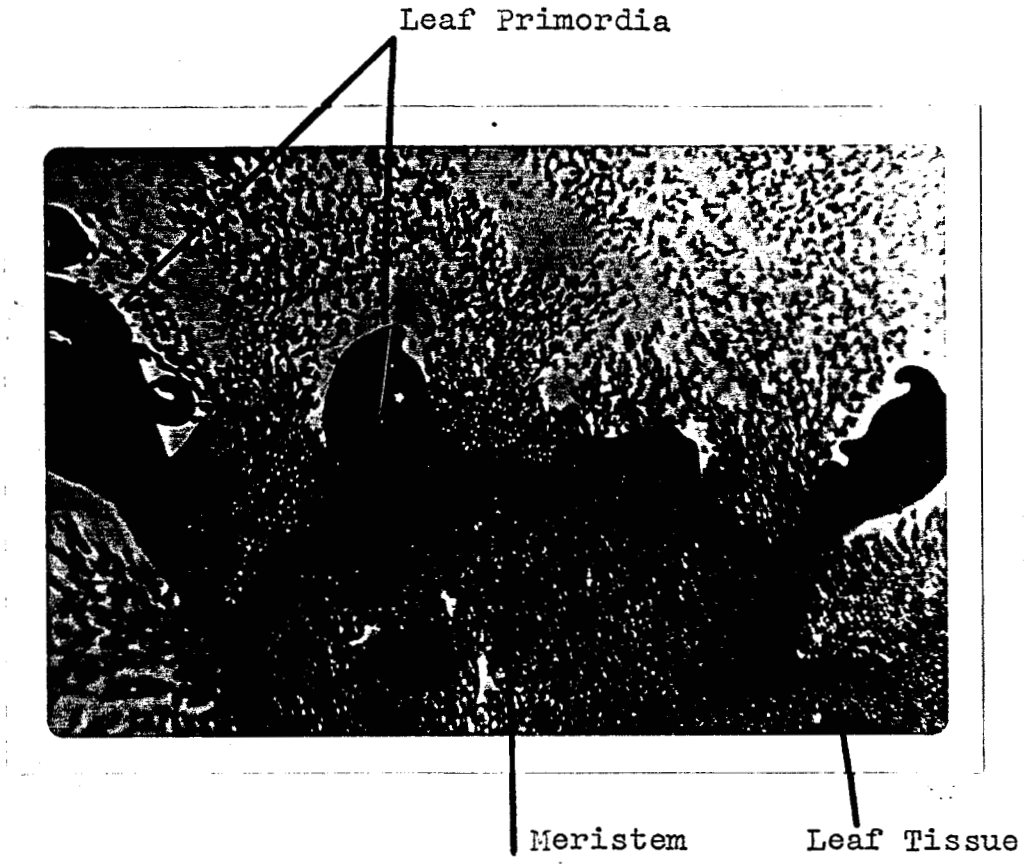

Figure 9. Transverse section of a tubercle showing leaf primordia.

While the parent leaf is still underwater and growing, the tubercle mass apparently reaches a size that triggers the formation of a meristem that soon differentiates several leaf primordia. The

tubercle's meristem and the fully differentiated parent leaf at this stage reach the surface, and the tubercle becomes dormant. Hartman and Kester (1968) noted that stems and leaves are produced by callus tissue in a medium low in indoleacetic acid and high in kinetin. Perhaps, it is a similar balance which triggers the meristem development.

As the parent leaf unfolds on the water's surface, the tubercle appears to be dormant. Many observations over a two year period showed that there is no growth of the tubercle meristem for a period as short as a few days or as long as three weeks. The mechanism controlling this dormancy was studied with leaves where the laminas were removed and kinetin, 6-benzylaminopurine, and the auxin, naphthalene acetic acid, were applied to the cut edges of the remaining lamina. Corks were attached to the petioles to maintain the leaf's normal orientation to the surface of the water. The experiment began one week before the spring equinox and lasted for two weeks. The results of this experiment are summarized in Table Two. These data show, even though the number of replications is small, that the tubercles treated with naphthalene acetic acid react very much like untreated and undamaged leaves. The leaves treated with lanolin on the cut laminas behave very much as leaves with their dormancies broken by kinetin. Romberger (1963) defines a type of dormancy which he calls "correlative inhibition," after Dorrembos. This dormancy is maintained by agents within the plant but not within the organ effected. When we consider that the auxin, indoleacetic acid, is synthesized in the leaves, and is translocated in a basipetal direction (Wightman and Setterfield 1967), we fill the definition for correlative inhibition. "Chvojka (1961), and Engelbrecht and Mothes (1962) have shown that cytokinins can

be used to break correlative types of dormancy" (Romberger 1963). As shown in Table Two, kinetin breaks the dormancy of the tubercles. Thus, this dormancy can be properly called correlative inhibition. The mechanism described previously is very similar to that described for the inhibition of axillary buds by leaves at the same node (Romberger 1963).

The roots of the tubercle form in the same manner that new roots form in the stem of the plant, i.e., in association with the vascular traces near the base of the petioles (refer to figure 5).

After the plantlet's development is complete, the parent leaf begins to senesce, the dormancy is broken, the tubercle now plantlet resumes growth. This resumption of growth by the tubercle and the parent leaf's senescence always go together. Bonner and Varner (1965) suggest that senescence is the result of a hormone balance which will not allow the transcription and translation of the information needed to keep the cellular machinery in good repair. Whether the senescence is controlled by mechanisms within the lamina or within the tubercle was examined in an experiment where groups of four leaves were put into three categories. The first group (A) had the tubercles removed with a sterilized scalpel and the wound sealed with lanolin paste; the second group (B) had tubercles which were not disturbed; the third group (C) was tubercleless leaves. Group (A) had an additional four leaves added to the group every four days until there was a total of sixteen leaves. All the leaves in this experiment were from the same plant. Observations were made daily and records kept as to whether the leaves were healthy (all green to ¼ yellow), senescing (¼ to entirely yellow), or dead. The results, Table Three, are not conclusive. A Chi square test of the means revealed that all the observed variation can be

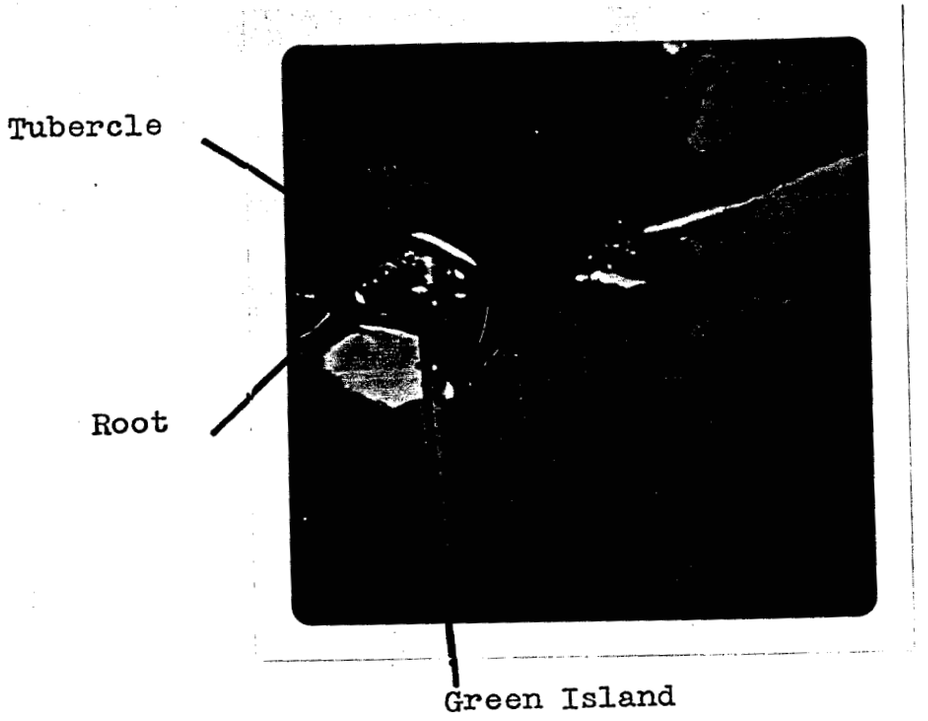

Figure 10. Senescing leaf with rapidly growing tubercle now plantlet. Note the "green island" effect around tubercle.

explained by chance. A standard deviation calculation showed that two of these means were outside the limits of one standard deviation.

Senescence is thought to be associated with a decrease in a cell's ability to reproduce its protein because of a breakdown of its DNA-RNA translation system. A decrease in protein synthesis would infer a decrease in enzyme synthesis also. Thus the pathways which produce plant hormone would also decrease, leaving little indole-acetic acid to be translocated and the dormancy of the tubercle released.

Table Three. The effect of the tubercle on the longevity of waterlily leaves.[1]

Condition	Tubercles Removed	Tubercles	Tubercleless
Healthy	10.8	10.7	10.7
Senescing	9.28	12.0	10.0
Dead	14.6	22.0	20.2

[1] The numbers in this table are arithmetic means; 9.28 and 14.6 are the only numbers outside one standard deviation (the above numbers are numbers of days in each of the three categories).

After the dormant period, the now rapidly growing plantlet exerts its own influence on the lamina. There is a pronounced "green island effect" (figure 10) around the base of the tubercle, suggesting at this point a flow of hormones out of the tubercle into the lamina. According to Mothes (1961) "kinetin increases the ability of cells to accumulate solutes." Perhaps the plantlet can thus drain the parent lamina of nutrients to facilitate plantlet growth. The plantlet shortly thereafter is able to start an independent life.

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APPENDIX

Daylength (min.)	Water temp. (^{o}F)	High air temp.	Low air temp.	Number of leaves	Number of tubercles	Number of plantlets	Number of leaves removed	Milligrams CH_2O/gm	Milligrams N/gm	Fertilized?
901	80	90	66	245	226	32	00	239	370	0*
901	80	90	66	245	226	32	00	198	360	0
901	80	90	66	245	226	32	00	148	340	0
898	80	94	66	252	240	21	78	148	310	0
898	80	94	66	252	240	21	78	140	440	0
898	80	94	66	252	240	21	78	115	300	0
893	78	94	78	187	172	11	29	181	170	1
893	78	94	78	187	172	11	29	239	180	1
893	78	94	78	187	172	11	29	49	170	1
887	78	80	78	281	275	32	34	53	0	0
887	78	80	78	281	275	32	34	42	0	0
887	78	80	78	281	275	32	34	49	0	0
870	78	80	78	252	243	34	0	173	300	0
870	78	80	78	252	243	34	0	247	350	0
870	78	80	78	252	243	34	0	157	380	0
863	78	100	62	299	292	28	48	152	0	0
863	78	100	62	299	292	28	48	150	0	0
863	78	100	62	299	292	28	48	143	0	0
849	78	95	62	317	310	47	71	239	330	1
849	78	95	62	317	310	47	71	8	340	1
849	78	95	62	317	310	47	71	49	260	1
800	80	96	50	376	367	53	127	74	330	1
800	80	96	50	376	367	53	127	115	300	1
800	80	96	50	376	367	53	127	239	270	1
783	79	85	65	277	268	22	53	16	370	0
783	79	85	65	277	268	22	53	8	333	0
783	79	85	65	277	268	22	53	16	260	0
860	79	90	67	276	265	31	45	122	237	.3 = x

* One means fertilized at this time, and zero means unfertilized.

The high and low air temperatures are recorded from a thermometer which recorded the maximum high and low temperature. The thermometer was read once a week so the temperatures in these columns represent weekly highs and lows.

Also, Nymphaea micrantha is one of the parents of N. x Daubeniana.