THE DEVELOPMENT AND STRUCTURE OF THE JUVENILE LEAVES IN *Marsilea quadrifolia* WITH NOTES ON THE ANATOMY OF THE STEM AND ADULT PETIOLE

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Historical. Most of the literature on Marsilea is concerned with the development and structure of the spore fruit and on the gametophytes, but comparatively little has been written on the juvenile leaves.

Engler and Prantl (5) state that in the development of the sporophyte of Marsilea from the spore, there are four stages in the leaf development. First is the cotyledon; second, submerged primordial leaves which develop but a single blade having stomata on the upper side; third, floating leaves whose blades spread out upon the surface and develop stomata on the upper side; and fourth, foliage leaves borne in the air having stomata on both sides.

Bower (3) calls attention to six forms of juvenile leaves as figured by A. Braun. The sixth leaf in the figure is shown as having four lobes but the lobes are not disposed as the four lobes in the adult form. He states that the first juvenile leaf is subulate, those that follow are spatulate, then two lobed, and finally the four lobed leaf. Bower points out the form of venation in these leaves and especially the fact that the marginal loops by which the separate veins are linked, form a closed system.

Campbell (4) points out that the cotyledon grows for a time from the regular divisions of the primary octant cells and the subsequent growth is purely basal. The cotyledon is a slender, cylindrical leaf tapering to a fine point, where the cells are much elongated and almost colorless. Its growth is at first slow, but at a later period it begins to grow with great rapidity and soon reaches its full size. This is largely due to a simple elongation and expansion of cells.

Material and Methods. The plants upon which this work is based were grown in the laboratory from the spores. The sporocarps were sown in water in a glass jar and in a glazed earthen-ware vessel on June 18, 1931. An opening was made in the spore fruit to facilitate the penetration of water in order to cause, in a shorter time, the escape of the sori which are attached to the well-known gelatinous cord.

Both vessels contained one inch of rich soil, one inch of sand and about two inches of water. The glass jar was covered with a piece of glass during the experiment to prevent too rapid evaporation of the water. Both vessels remained outside on the ledge of a north window

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of the laboratory. The temperature of the water in the glass jar varied from 2—5° C. higher than that in the earthen vessel.

Observations. The following data were obtained from plants growing in the glass jar because they grew much more rapidly than those in the earthen vessel.

There were seven juvenile leaves produced by the plants. The first juvenile leaf or cotyledon appeared June 21, 3 days after sowing. At first, the growth of the leaf was apical and later basal. The tip of the leaf which terminated in three, elongated, almost colorless cells, was slightly twisted. The leaf was mature June 26, 8 days after sowing, with a length of 14 mm. and a width of 1 mm. across the middle which is the widest part (Fig. 1, A).

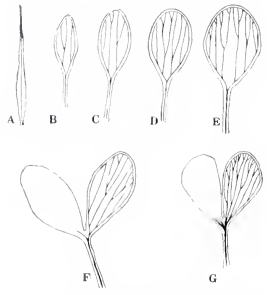

Fig. 1. *Marsilea quadrifolia.* The seven juvenile leaves in order from A to G.

This leaf produces one to three celled trichomes which are scattered over the under surface. Water and air stomata occur on both sides but more abundantly on the upper side. The water stomata are large and round due to the open pore, while the air stomata are slightly smaller and are usually closed. In the case of this first leaf, the water stomata occurs at the apex and when grown in air, a small drop of water is formed at the tip. A single vein extends from the base to near the extreme tip. The extremity of this vein consists of a row of single spiral tracheids.

The second juvenile leaf made its first visible appearance on June 24, 6 days after sowing. On June 26, this leaf was 4.5 mm. long and 1.33 mm. wide. The leaf was full grown on the 9th day, June 27, with a length of 7 mm. and a width of 2 mm. (Fig. 1, B).

The leaf blade is much shorter and broader than the cotyledon, and the petiole is almost as long as the blade which has 5-6 veins. These veins are closed or joined together along the margin which is the region where the water stomata occur.

The following juvenile leaves resemble the above leaf very closely. The difference consists in their increase in size and change in shape of the leaf blade. They all have trichomes and air and water stomata. The trichomes appear on the under side of the leaf, while the air stomata are found on the upper surface and are very few or lacking on the under surface.

The third juvenile leaf appeared June 26, 8 days after sowing. On June 27, this leaf was 8 mm. long and 2 mm. wide. The leaf was full grown June 28, when the plant was 10 days old, having a length of 10 mm. and a width of 4 mm. (Fig. 1, C). The leaf had 7-8 closed veins.

The fourth leaf appeared June 27, 9 days after sowing. On the 11th day the leaf was 7 mm. long and the leaf blade was 3 mm. wide. The leaf was full grown July 2, when the plant was 14 days old, having a length of 12 mm. and a width of 3 mm. The leaf blade and petiole are about the same length and the leaf has 10-12 veins (Fig. 1, D).

The fifth leaf appeared on June 30, 12 days after sowing. On the 14th day, the leaf was 10 mm. long and 4 mm. wide. It reached its full size July 3, 15 days after sowing, having a length of 15 mm. and a width of 5 mm. There were 15-17 veins in the leaf blade which was about as long as the petiole (Fig. 1, E).

The sixth leaf made its first visible appearance on July 2, 14 days after sowing. This leaf consisted of two blades. On the 16th day, the leaf was 11 mm. long and each blade was 3 mm. wide. The full size was reached on July 9, when the plant was 21 days old. It had a length of 16 mm. and a width of 3 mm. per blade (Fig. 1, F). The petiole is about one and one-half times as long as the blades. One blade had 14 veins and another 13, while on another plant each of the two blades had 13 veins.

The seventh and last juvenile leaf appeared July 6, when the plant was 18 days old. On the 23rd day, this leaf was 19 mm. long and each of the two blades were 4 mm. wide. The leaf was full grown July 15, 27 days after sowing, with a length of 22 mm. and each blade had a width of 5 mm. (Fig. 1, G). The petiole is almost twice as long as the blades. The leaf is circinate in the bud which is a characteristic of the adult leaves. In the case of one plant there were 21 veins in one blade and 25 in the other, and in another case there were 24 veins in each blade.

The first adult leaf (the eighth) appeared on July 7, 19 days after sowing. On July 11, the leaf was 41 mm. long and the blades were not unfolded. On July 21, when the plant was 33 days old, the leaf was 10.5 mm. long and each of the four blades was 9 mm. wide and 9 mm. long. During the development of the juvenile leaves, the stem remained very short, but began to elongate as the adult leaves were produced. The stem between the seventh and first adult leaf was 1 mm. long, between the first and second adult leaf 2 mm., and between the second and third 3 mm. as shown in table 2.

Adult Leaf. Engler and Prantl (5) point out that the adult floating leaves differ from the juvenile leaves by having long slender petioles and by being circinate in the bud. In shallow water, the young petioles grow from 8-10 cm. above the surface of the water, but sink back when mature. On the under side of the blades of the floating leaves are golden brown interstitial strands, which originate from the epidermis due to the intense golden brown color of the thick epidermal cells, and the neighboring colorless cells which do not have stomata.

Hildebrand (7), whose paper was the earliest accessible literature, states that he could not find any literature concerning the floating leaves of Marsilea and as a result he studied them. He found that adult petioles will not elongate when submerged but that the young petioles will grow above the water producing floating leaves. Some of the petioles grew to be three feet long which is an adaptation of the plant to the depth of the water. There are slightly sunken stomata on the upper and lower sides of the air leaves. On the upper side of the floating leaves there are twice as many stomata as on the upper side of the aerial leaves, but on the under side of the floating leaves the stomata are entirely lacking. The upper guard cells are, however, on a level with the epidermal cells.

Agnes Arber (1) makes the statement, based upon the findings of Karsten, that if tubes of oxygen-free air were inverted over the leaves of Marsilea, the growth of the petiole would continue after the lamina had come in contact with air. The conclusion is then drawn that the oxygen of the atmosphere checks further growth of the petiole.

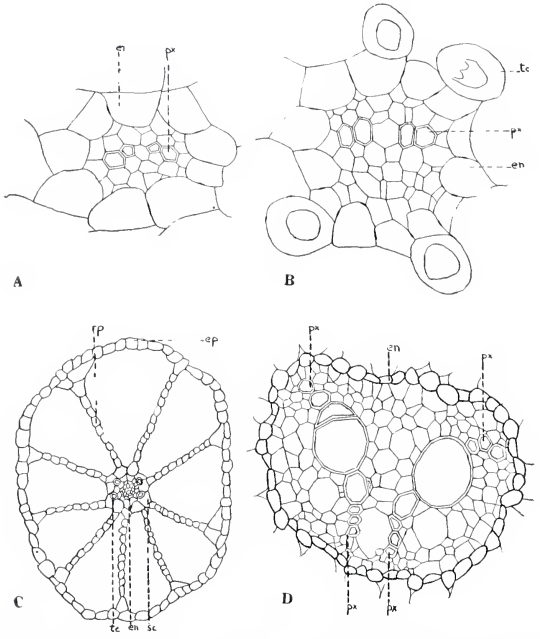

Fig. 2. *Marsilea quadrifolia.* A, cross section of the petiole of the first juvenile leaf, showing vascular strand; B, cross section of vascular strand of petiole of the third juvenile leaf; C, cross section of the petiole of fourth leaf; C, cross section of adult petiole, showing vascular bundle. *en*, endodermis; *tc*, tannin cell; *px*, protoxylem; *rp*, radiating partitions; *sc*, storage cortex; *ep*, epidermis.

Observations. Both the aerial and floating leaves have stomata on both surfaces but the stomata of the floating leaves are more abundant on the upper surface. The number of stomata on the upper surface of the floating leaves is greater than that on the upper surface of the aerial leaves. There are water stomata along the margin and when the plant is placed under a bell jar over night, small drops of water are found. It was seen that when the plants are grown in deep water, the young petioles did not grow above the surface of the water and then sink back as stated in Engler and Prantl. Brown interstitial strands are found on the under side of the older floating leaves.

Anatomy. Campbell (4) states that during the development of the first juvenile leaf, the cells are separated in places and form a series of longitudinal air chambers which are separated by radiating plates of tissue. The simple vascular strand traversing the axis is concentric and has a definite endodermis, but the tracheary tissue is very slightly developed.

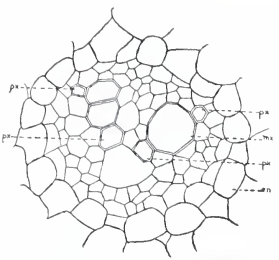

Fig. 3. *Marsilea quadrifolia.* Cross section of the vascular strand of the seventh juvenile leaf. *en*, endodermis; *px*, protoxylem; *mx*, metaxylem.

In regard to the structure, my observations are in accord with the account given by Campbell. In a cross section near the base of the first juvenile leaf, the vascular strand is in the center of the section and is surrounded by an endodermis and one layer of storage cells. Between this central strand and the epidermis, there is a series of six longitudinal air chambers which are separated by radiating plates of tissue one cell in thickness. These radiating plates or partitions extend from the storage cells to the epidermis where they may be one or more cells thick. The structure of the entire leaf is similar to that of the petiole of the fourth juvenile leaf which is shown in Fig. 2 C. At intervals in the older juvenile leaves, perforated diaphragm-like partitions, one cell in thickness, extend transversely across the intercellular chambers. This type of structure is common in the tissues of aquatic plants.

The vascular bundle is composed of two masses of protoxylem which are surrounded by the phloem. The cells of the endodermis are relatively large and show slight Casparian thickenings (Fig. 2, A). The xylem is composed of four annular or spiral tracheids. Tannin cells, which are characteristic of the other juvenile and adult leaves, were not found in the first leaf.

In all of the juvenile leaves, except the seventh, the vascular strand which is surrounded by a more evident endodermis, has two masses of protoxylem. They all have in the petioles a cortex which is made up of two regions, the storage tissue and the radiating partitions.

In a cross section of the petiole of the second juvenile leaf, there are 7-8 large intercellular spaces formed by radiating partitions. The storage cortex which is one cell thick, surrounds the endodermis. There are 2-3 tannin cells joining the endodermis and cortex. The vascular bundle is slightly larger than those of the cotyledon and the xylem is similar.

The cross section of the petioles of the third, fourth, and fifth leaf shows 7-8 intercellular spaces and 2-4 tannin cells. With each successive leaf, the vascular strand increases in size but the number of tracheids remains the same.

In the sixth juvenile leaf, there are 7-10 intercellular spaces and 4-5 tannin cells. The entire vascular bundle is larger than the preceding ones, and one of the xylem masses is composed of three tracheids.

In a cross section of the petiole of the seventh juvenile leaf, there are 9-10 intercellular chambers. One layer of cortical cells lies just beneath the epidermis. This is a characteristic of the adult leaves and stem. The bundle is composed of four masses of protoxylem which is a characteristic of the adult petioles. The entire bundle is composed of 7-8 tracheids (Fig. 3).

In the petiole of the adult leaf, there are 15 large air chambers formed by the radiating plates (Fig. 4, A).

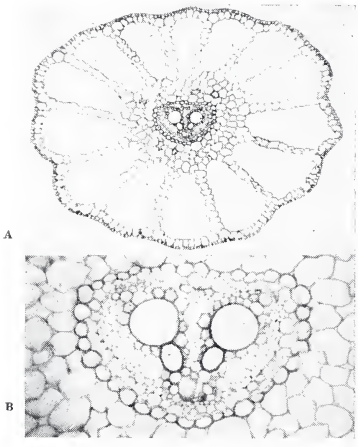

Fig. 4. *Marsilea quadrifolia.* Photomicrographs of cross sections of adult petiole. A, entire petiole; B, vascular bundle with surrounding cells more highly magnified.

The storage cortex is much thicker than in the juvenile leaves and it contains five or more tannin cells. The vascular bundle is composed of four masses of protoxylem which are surrounded by phloem (Figs. 2, D and 4, B).

A cross section of the leaf blade of the adult leaf shows that the palisade layer is not entirely compact but contains air spaces which are more abundant between the veins (Fig. 7, A). The spongy parenchyma is composed of rows of cells that extend from the palisade layer to the lower epidermis forming large intercellular spaces. The upper epidermis has thicker cell walls than those of the lower epidermis. Each vein which is surrounded by border parenchyma is composed of 3-6 tracheids. Just outside of the border parenchyma are 3-6 tannin cells.

Stem. Luerssen (8) states that in Marsilea the stems are frequently creeping for a long distance and are supplied with numerous branched roots which develop near the vegetative tip. In comparison with the size of the plants, the stems are thin, cylindrical, more or less well branched and the branches are extra axillary, developing on the sides below the leaves. The growing point is not enveloped by young leaves but is densely hairy, while the older parts are frequently bare. The central vascular cylinder, closed siphonostele, shows a narrow gap, only beneath the insertion of the leaves above the branching where the vascular strand enters the leaf petiole, through which the pith and cortex are united. The cortex is perforated by air chambers which are longitudinally placed, separated by radial plates of one layer of cells.

Campbell (4) makes the statement that the solid vascular cylinder of the young stem is later usually replaced by a tubular one. Its structure is concentric with phloem completely surrounding the xylem, and it has both an inner and an outer endodermis. When the plants are completely submerged, the ground tissue is mainly parenchyma, but in the terrestrial forms, sclerenchyma may be developed in the cortex of the stem and petiole.

Bower (3) points out that in Marsilea, the stem is traversed by a solenostele with inner phloem and endodermis. The undivided leaf trace separates a sector of the ring. The cortex is differentiated into an inner storage tissue, separated by a thin sclerotic band from an outer tissue containing large radiating lacunae with thin parenchyma septa. This is a peculiarity seen in aquatic plants. The vascular supply to the stalk of the sporocarp comes off by marginal abstriction. The petiolar trace is marked off by the endodermis from the parenchyma which is differentiated into storage tissue having tannin sacs, and an outer porous sys-

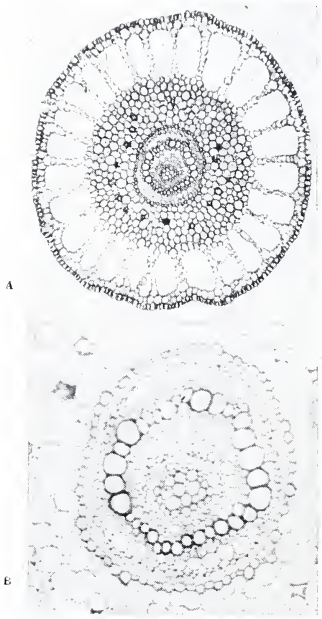

Fig. 5. *Marsilea quadrifolia*. A, photomicrograph of young rhizome; B, enlargement of the siphonostele. *oen*, outer endodermis; *ien*, inner endodermis; *p*, pith.

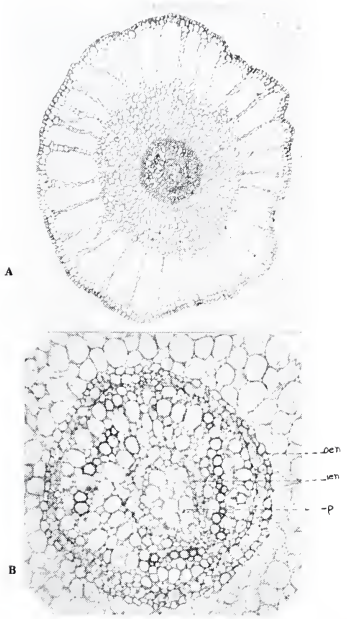

Fig. 6. *Marsilea quadrifolia*. A, cross section of the adult rhizome; B, stele enlarged showing vascular tissue.

tem with peripheral epidermis and sclerotic tissue between. The roots are diarch with a conical apical cell, and a regular lacunar cortex.

De Bary (2) states that the sieve tubes are fitted one on another with pointed or horizontal ends and have sieve plates on the latter and also on the surface which touches other sieve plates. The pores are very numerous.

According to Sinnott (11) there are three main types of leaf traces in the ferns which may be characterized as monarch, diarch, and triarch, possessing one, two, or three masses of protoxylem respectively. The first type is found in Osmundaceae and Ophiglossaceae, the second in Maratticeae, and the third in all other families of the Filicales. He states that in Marsilea the leaf trace as it goes from the stele, is an arc with three endarch protoxylem. The median one soon forks dividing the trace into two bundles each of which has a mass of protoxylem at either end.

In another paper (10) Sinnott states that in certain ferns, the leaf trace is small as it leaves the stele and increases in size as it goes through the base of the petiole. In those types of ferns where the rhizome is superficial and the petiole arises directly from it, there is no difference in either the petiole or the vascular bundle.

Observations. In a cross section of the young stem there are about 30 intercellular spaces due to rows of radiating partitions (Fig. 5). The storage cortex is about 10 cells thick. There are 5-7 masses of proto-

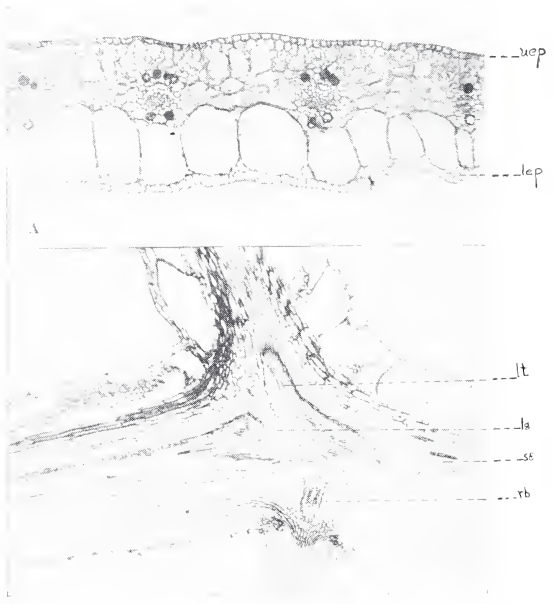

Fig. 7. *Marsilea quadrifolia.* A, cross section of the adult leaf blade. *ucp,* upper epidermis; *lep,* lower epidermis. B, longitudinal section of stem, showing the origin of petiole and root; *lg,* leaf gap; *rb,* root branch; *lt,* leaf trace; *sc,* storage cortex.

xylem in the young stem. As the stem matures these masses of protoxylem are connected by metaxylem to form a circle. This is surrounded on the inside and outside by phloem and an endodermis. The cells of the endodermis and pith have thickened walls (Fig. 6, A and B).

A suitable cross section was made which showed the connection between the siphonostele and leaf and root. Here it is observed that the petiole has four masses of protoxylem which are separated at the median arc by one large parenchyma cell (Fig. 2, D). The vascular strand of the petiole is of the same size while in the cortex of the stem as it is after leaving the cortex.

The manner in which the leaf trace leaves the stele is shown in Fig. 7. The pith and cortex are united by means of the gap. The root arises from the base of the petiole. Roots and petioles originate at the same node but there may be roots produced without the petioles. Branches are also produced at the nodes but there are no gaps formed. The xylem of the root is diarch.

There may be several variations in the number of blades produced on different plants. The fourth, fifth, sixth, and seventh juvenile leaves of one plant had two blades and the eighth which was the adult had four blades. In another instance an adult leaf had five blades instead of four. The fifth blade was similar in size and shape to those of the second juvenile leaf. The adult leaf may also consist of three blades as is found in plants grown in air. It was also found that the sixth leaf may be cleft instead of two lobed.

Plants were grown in air on soil that was continually moist. The growth was slower than that in the earthen-ware vessel, and the entire plant was smaller. More stomata were produced on the upper and lower surfaces of these leaves than on those grown under water. In the older juvenile leaves, the stomata on the upper surface had chloroplasts in the guard cells while those on the under side were colorless. The adult leaf was about 30-40 mm. in length (Table 2).

Neither the plants grown in the air nor in the earthen vessel produced a long creeping stem. The stem remained very short as it did while the juvenile leaves were produced. Hildebrand (7) calls attention to the fact that during the growing period of one season, the stem attained a length of ten feet.

Plants were grown in water 14 inches deep and all of the adult leaves were floating, but the length of the petiole was greater than the depth of the water. In one case a petiole attained a length of 19 inches.

SUMMARY

1. *Marsilea quadrifolia* has seven juvenile leaves. The first five have one blade and the next two have two blades.

2. The juvenile and adult floating leaves have stomata more abundantly on the upper surface while the aerial adult leaves have the same number of stomata on both surfaces. Both the juvenile and adult leaves have water stomata along the margin near the closed veins.

3. The petioles of the first six juvenile leaves have two masses of protoxylem and the seventh juvenile leaf has four masses which is a characteristic of the adult petiole. The seventh juvenile leaf is circinate in the bud which is a character also found in the adult leaf.

4. The vascular system of the rhizome is an amphiphloic siphonostele. A gap is present on the adaxial side where the leaf trace leaves the stem. The root arises from the base of the petiole.

5. The stem and petioles of the adult and juvenile leaves are composed of storage and radial cortex. In all leaves except the first, tannin vessels are found in the cortex.

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TABLE 1. Juvenile Leaves

	Number of veins	Number of tannin vessels	Number of air spaces	Number of xylem vessels
1st leaf	1	none	6	3-4
2nd leaf	5-6	2-3	7-8	3-4
3rd leaf	7-10	2-4	7-8	3-4
4th leaf	10-12	3-4	7-8	3-4
5th leaf	13-17	3-4	7-8	4-5
6th leaf	13-14	4-5	7-10	4-5
7th leaf	21-24	4-5	7-10	7-8

TABLE 2. Difference in Size due to Environment
(Measurements in mm.)

Where Grown	Size of Cotyledon	Second leaf	Third leaf	Fourth leaf	Fifth leaf	Sixth leaf	Seventh leaf	1st adult leaf	2nd adult leaf	3rd adult leaf
Glass Jar:										
14 days.....	11 long 1 wide	6 1.5	9 2.5	11 3.5	9 4					
23 days.....	11 long 1 wide	8 2	10 3	11.5 3	12 4.5	16 3.5 2 blades	19 4.5 2 blades	41 1 blades not unfolded		
27 days.....	11 long 1 wide	7 1.5	10 2.5	11 3	14 4	18 3.5 2 blades	22 4 2 blades	9cm 4 4 blades		
33 days.....	10 long 1 wide	7 1.5	9 2.5	11 3	14 5	16 7 partly lobed	22 5 2 blades	10.5cm 9 2 blades	11.3cm 7 4 blades	14cm 12 4 blades
							Rhizome	1	2	3
Grown in Crock:										
14 days.....	9 long 1 wide	8 1.5	9 2.5	6 2						
23 days.....	10 long 1 wide	8 1.5	9 2	9.5 3	10 3.5	18 2 2 blades				
27 days.....	11 long 1 wide	8.5 1.5	9 2.5	10 3.5	14 4	40 3 2 blades	3.5 1			
33 days.....	10 long 1 wide	8.5 1.5	9 2.5	10.5 3	12 4	53 3.5 2 blades	63 5.5 2 blades	57 3 4 blades		

Table 2. Continued

Grown in Air:										
15 days.....	8 long .75 wide	6 1	7 1.5	2 .75						
22 days.....	7 long .75 wide	6 1.5	7 2	7.5 3	13 4					
28 days.....	7 long .75 wide	5 1.5	6 2	9 3.5	14 5	16 4 2 blades	7 1			
37 days.....	7 long .75 wide	6 1	8 1.5	9 2	15 5	18 3 2 blades	30 6 2 blades	42 4 4 blades		

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