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Fat Deposits in Certain Ericacere

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FAT DEPOSITS IN CERTAIN ERICACEÆ[1]

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The purpose of this work was to investigate the structures of certain available plants of the family Ericaceæ with special interest in fat deposits in the tissues.

HISTORICAL. Priestly and Hinchliffe (8) have called attention to the characteristic appearance of plants growing in peat moors of England. These plants are of a stunted nature, dark green in color, leathery in texture, and with very little leaf surface. These characteristics, along with certain others, have led to these plants being grouped with xerophytes. This seems strange, as xerophytism is associated, normally, with scarcity of water.

The cuticle of a plant consists largely of cutin, a substance derived from various organic acids, mainly oxyfatty acids. The rapid deposit of cutin on a young vascular plant would therefore suggest the presence of a large amount of fatty acid. Priestly has confirmed this assumption by anatomical investigation of several of the peat plants from the Yorkshire moors.

An explanation is made on the basis that peat plants are growing in a soil that is deficient in oxygen. If a root grows under conditions of deficient oxygen, one of the best sources of energy for its constructive metabolism would be found in the converting of carbohydrates into fatty acids, with the elimination of CO_2. Such changes as these have been shown to exist in the living plant. Behind the growing region, these fatty acids seem either to be deposited on the walls as insoluble calcium soaps, or to be carried with the sap up in the vascular cylinder as soluble potassium or sodium soaps, or as free fatty acids. Peat soils are deficient in calcium, so it may be expected that the fatty acids would be carried away from the roots, up into leaf and stem.

Pearsall (7) states also that sour soils are notoriously deficient in calcium, and comparatively rich in sodium and potassium. If Ca is scarce, the fatty acids will be carried upward in the plant as soluble soaps of Na and K. If Ca is abundant, the fatty acids will be deposited in roots as insoluble calcium soaps

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METHODS. Collection of the following material was done by Dr. Stanley A. Cain, of the Department of Botany at Butler University in Indianapolis. Leaf and stem material of *Cassandra calyculata* D. Don, was collected from a peat substratum in Mineral Springs bog, in Northern Indiana. Leaf and stem material of *Arctostaphylos uva-ursi* (L.) Spreng. was collected from sand habitat of Wilson dunes, Northern Indiana.

Roots of *Kalmia latifolia* L., *Dendrium prostratum* (Loud.) Small and *Rhododendron catawbiense* Michx., from both upland peat, Cain (1), and sand-clay habitats, were collected from the Great Smoky mountains in Eastern Tennessee.

The following were collected by the writer: *Vaccinium macrocarpon* Ait., roots, stems and leaves, from the Gaston peat bog north of Muncie, Indiana, and *Gaylussacia baccata* K. Koch, *Vaccinium stamineum* L., and *Vaccinium vacillans* Kalm. from sand-clay habitats in the Sycamore Creek region of Morgan county, Indiana. Part of this material was preserved in 4-60 formalin alcohol, part in a weak formalin solution, and some was dried or sectioned fresh.

Comparisons of the same material treated in different ways showed that the treatment did not alter the amount of fat in the tissue, as determined by the staining method described below.

Some difficulty was experienced in sectioning the smaller roots and stems, as well as leaves, because of the necessity of avoiding imbedding methods. The fats, had the material been cut in paraffin, would have been removed by the action of the higher grades of alcohol and chloroform, as Gatenby (4) and McClung (6) point out in their texts on microscopical technique

Freehand sectioning was employed with a fair degree of success, but a method of cutting the sections on a sliding microtome (the material being held between pith), proved most satisfactory. Even then, some of the more delicate tissues were sometimes torn and rendered useless.

As a stain, Oil Red O proved to be the most desirable of several fat stains tried. Sudan III and Sudan IV were both tried in solutions of 70 per cent. alcohol and in acetone alcohol solutions like that used by Miss Haynes (5). Oil Red O gives a much brighter stain than either of the two Sudans. It was first used in a 70 per cent. watery solution of pyridine, as employed by Proescher (9). The one objection to pyridine is its extreme volatility and stifling odor. The Oil Red O was then tried in a solution of one part acetone to one part of 50 per cent. ethyl alcohol, and this stain was used throughout the work. A saturated solution of the stain was prepared, allowed to stand over night, with occasional shaking, and then filtered before using. The sections were cut in water, transferred to 70 per cent. alcohol for five minutes, then to the stain for about one minute, then washed in 70 per cent. alcohol and mounted in the glycerine jelly suggested by Chamberlin (2). The mount was then sealed with balsam.

A counterstain of Delafield's hæmatoxylin or Unna's polychrome methylene blue, as described by Conn (3), may be used with this stain, but it seemed more advisable to omit this counterstain, since microphotographs of the tissue were to be made and they would not differentiate between a red and a blue staining tissue.

RESULTS AND DISCUSSION

I. Plants Grown in Sand-Clay Substrata. In *Gaylussacia baccata* K. Koch., the old root had a thin cuticle, a fatty periderm internal to the pericyclic fibers, and a few fat globules scattered through the phloëm (Figure 1). The young stem had a cuticle, fatty deposits in the epidermis, and epidermal hairs taking a fat stain. Scattered fat globules were present in the cortex and pith (Figures 2, 3). The old stem had a cuticle partly surrounding the section, and fat deposits as globules and as linings of the cell walls in the epidermal cells. A fatty periderm was present, internal to the pericycle. Fat globules were present in the cortex, phloëm, phloëm rays, xylem rays and pith (Figures 4, 5).

In *Vaccinium vacillans* Kalm. the young stem was covered with a heavy cuticle, and fairly large numbers of fat globules were deposited in the epidermis, especially in the guard cells of the stomata (Figure 6). The old root had a rather thin cuticle. Fat was present in the epidermis as deposits on the walls of the cells and as globules in the cells. A fatty periderm was present internal to the pericycle (Figure 7).

In *Vaccinium stamineum* L. only the young stem was available for examination. Fat globules were found in the epidermal cells, and a cuticle was present.

The young leaf of *Arctostaphylos uva-ursi* (L.) Spreng. had a cuticle, epidermal hairs and some fatty deposits in the walls of the epidermal cells. The mesophyll had practically no fat globules. The old leaf had a heavy cuticle, also fat deposited as globules in the epidermis. The

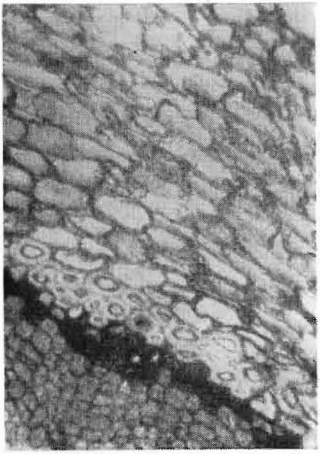

FIGURE 1

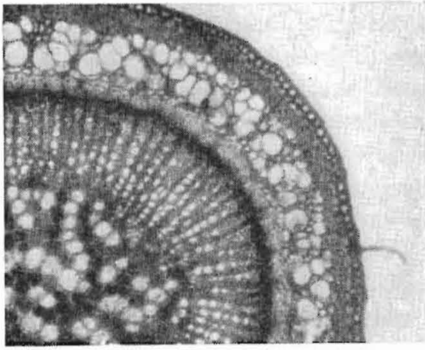

FIGURE 2

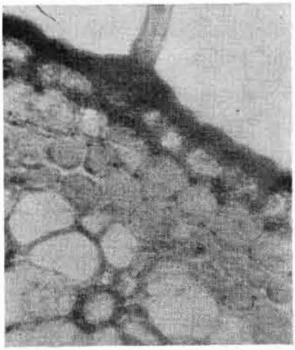

FIGURE 3

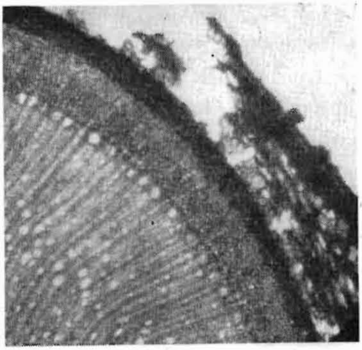

FIGURE 4

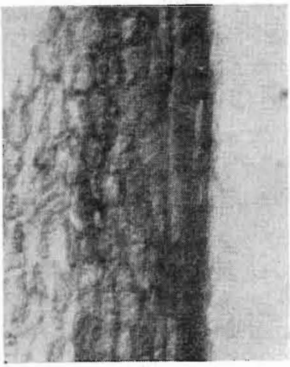

FIGURE 5

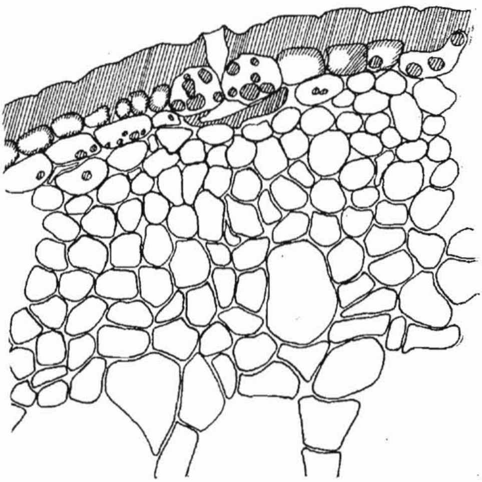

FIGURE 6

FIGURE 1. Section of old root of *Gaylussacia baccata*. Note fatty periderm just beneath the pericyclic fibers.

FIGURE 2. Section of young stem of *Gaylussacia baccata*, showing cuticle, epidermal hairs and fatty deposits in epidermal region.

FIGURE 3. Portion of young stem in Figure 2 in detail, showing heavy cuticle and fat deposits in epidermal cells.

FIGURE 4. Section of old stem of *Gaylussacia baccata*. Note heavy periderm which is replacing the outer tissue, this outer tissue being lost.

FIGURE 5. Portion of periderm from Figure 4, showing details.

FIGURE 6. Camera lucida drawing of a portion of the stem of *Vaccinium vacillans*, showing heavy cuticle and fat deposits in guard cells of stoma. Shaded portions represent deposits taking a fat stain.

FIGURE 7. Section of old root of *Vaccinium vacillans*. Note heavy periderm internal to pericyclic fibers.

FIGURE 8. Camera lucida drawing of a portion of a cross section of the leaf of *Arctostaphylos uva-ursi*, showing upper and lower cuticles and fat globules deposited in the palisade and mesophyll regions of the leaf. Shaded portions represent deposits taking a fat stain.

FIGURE 9. Section of old stem of *Cassandra calyculata*, showing disintegration of tissue from periderm outward.

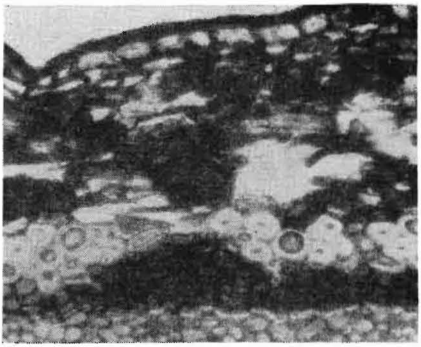

FIGURE 7

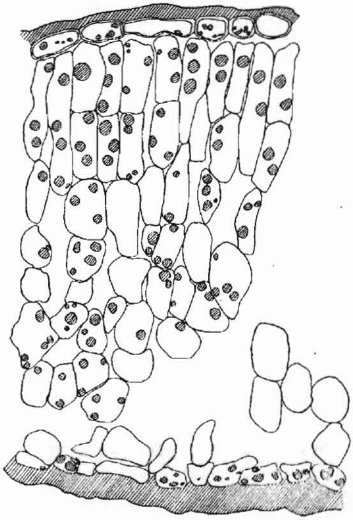

FIGURE 8

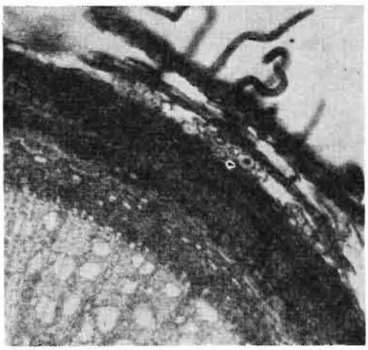

FIGURE 9

mesophyll was densely filled with fat globules (Figure 8). A cuticle and the presence of fat globules in the epidermis marked the young stem. The old stem also had a cuticle present. Fat was found in the epidermis both as globules and as deposits on walls of the cells. Fat globules were also sparsely scattered through parts of the cortex and xylem rays.

The young root of *Dendrium prostratum* (Loud.) Small was lacking cuticle and epidermis. Fat deposits were found in the walls of the cortex. Fatty deposits were also noted in the periderm, which was internal to the pericyclic fibers. Fat globules were noticeable in phloëm, phloëm rays and xylem rays.

Kalmia latifolia L. had the young root lacking cuticle and epidermis. Fat deposits were abundant in the walls of the cortex and periderm.

In *Rhododendron catawbiense* Michx. the young root likewise was lacking cuticle and epidermis. The cortex, too, was mostly gone. Fat deposits were noted in the walls of the periderm and as globules in the heart of the xylem.

II. Plants Grown in Peat Substrata. The studies of *Vaccinium macrocarpon* Ait. showed the old root to be lacking cuticle and epidermis. Fat deposits (not very heavy) were found in the walls of the periderm. The young stem had a cuticle present, and fat deposits as globules in the epidermal cells were common. A fatty periderm was present, internal to the pericycle, and fat globules in the xylem rays and pith were also noted. The old stem was lacking cuticle, epidermis and cortex. This tissue was lost, as the fatty periderm formed internal to the pericycle. The leaf was surrounded by a cuticle and the mesophyll contained numerous fat globules.

The old stem of *Cassandra calyculata* D. Don. had a cuticle and numerous epidermal hairs taking the fat stain. A fatty periderm was present internal to the pericycle. Fat globules in the xylem rays and pith were also noted. The tissue from the periderm outward was disintegrated and rapidly falling off (Figure 9). A cuticle and epidermal hairs were present in the young stem. There was, however, no trace of a periderm.

The young root of *Dendrium prostratum* (Loud.) Small was lacking cuticle and epidermis. The cortex was seen to have fatty deposits on the walls, and a periderm containing fat globules and fat deposits on the walls was very evident. Some fat globules in the xylem rays were also noted.

Rhododendron catawbiense Michx. had the young root lacking

TABLE I. PLANTS GROWN IN SAND-CLAY SUBSTRATA

Species	Organ	Cuticle	Epidermis	Periderm	Cortex	Phloëm	Phloëm Rays	Xylem Heart	Xylem Rays	Pith
Gaylussacia baccata K. Koch	Old root	w		W		G				
	Young stem	W	g-hairs		g					g
	Old stem	W	g-w	W	g	g	g		g	g
Vaccinium stamineum L.	Young stem	W	g							
Vaccinium vacillans Kalm	Young stem	W	G		g					
	Old root	w	w-g	W						
Arctostapbylos uva-ursi (L.) Spreng.	Young leaf	W	w-hairs		Mesophyll with practically no fat globules					
	Young stem	w	w-hairs							
	Old leaf	W	g		Mesophyll densely filled with fat globules					
	Old stem	W	w-g		g				g	
Dendrium prostratum (Loud.) Small	Young root			W	w	g	g		g	
Rhododendron catawbiense Michx.	Young root			W				g		
Kalmia latifolia L.	Young root			W	w					

Explanation: W, large deposit of fat in cell walls; w, small deposit of fat in cell walls; G, large deposit of fat as globules in cells; g, small deposit of fat as globules in cells.

TABLE II. PLANTS GROWN IN PEAT SUBSTRATA

Species	Organ	Cuticle	Epidermis	Periderm	Cortex	Phloëm	Phloëm Rays	Xylem Heart	Xylem Rays	Pith
Vaccinium macrocarpon Ait.	Old root			W						
	Stem	W		W					g	g
	Leaf	W			Mesophyll with fat globules					
Cassandra calyculata D. Don.	Old stem	W	w-hairs	W					g	g
	Young stem	W	w-hairs							
Dendrium prostratum	Young root			wg	w				g	
Rhododendron catawbiense	Young root			w				g		
	Old root			w				g	g	
Kalmia latifolia	Young root			w						

Explanation: W, large deposit of fat in cell walls; w, small deposit of fat in cell walls; G, large deposit of fat as globules in cells; g, small deposit of fat as globules in cells.

cuticle, epidermis and cortex. A periderm with fat deposits in the walls of the cells and some globules in the heart region of the xylem constituted the fat deposits in this specimen. The old root was like the young root above, but with some fat globules in the xylem rays, as well as in the heart region of the xylem.

The young root of *Kalmia latifolia* L. was lacking cuticle, epidermis and practically all of the cortex. A heavy periderm with fat in the walls of the cells was the extent of fat deposits in this species.

Relation of Fat Deposits in Root and Stem. Table I furnishes the following calculations: (1) Fat occurred in fifteen places out of a possible forty in the roots studied. (2) There were twenty-three deposits out of a total of forty-eight possibilities in the stem. Reducing these fractions to a common denominator, a total of forty-one fat deposits are found for root and stem. Taking this total number of deposits as 100 per cent., it is found that 43.9 per cent. of the total number of deposits occurs in the root, while 56.1 per cent. occurs in the stem.

Table II, similarly, will show that fat occurs in roots to the extent of 35.3 per cent. of the total occurrence, while 64.7 per cent. of the fat deposited occurs in the stem.

This seems to bear out some of the theory of work previously mentioned. The deposits in plants from the peat substrata would be found to a greater extent in the stem on account of the lack of calcium and the comparative abundance of sodium and potassium in the peat substrata.

Occurrence of Fatty Periderm. Following the same procedure as above, it is found that the periderm occurs as follows:

Sand-Clay Substrata—Roots, 85.7 per cent.; stems, 14.3 per cent.
Peat Substrata—Roots, 60 per cent.; stems, 40 per cent.

Again a decrease in the occurrence of periderm in the roots of plants from peat substrata is shown, and there is a corresponding increase in occurrence of periderm in the stems of those plants.

This problem was suggested and directed by Dr. Stanley A. Cain.

SUMMARY

1. Nine species of Ericaceæ, representing seven genera, were studied with the purpose of investigating the extent and localization of fat deposits.

2. Cuticle, taking a fat stain, was present in all species where the outer tissues had not been lost.

3. Epidermal hairs, taking a fat stain, were present in three species, *Gaylussacia baccata, Arctostaphylos uva-ursi* and *Cassandra calyculata.*

4. A periderm was present in 85.7 per cent. of the roots and 14.3 per cent. of the stems of plants grown in sand-clay substrata, and in 60 per cent. of the roots and 40 per cent. of the stems of the plants grown in peat substrata. Periderm was always formed internal to pericyclic fibers

5. Fat globules were present in the pith of three species.

6. Fat globules were present in the heart or rays of the xylem in five species.

7. In *Arctostaphylos uva-ursi,* the young leaf showed practically no fat globules, while the old leaf was densely filled with such bodies.

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