

u m.

UNIVERSITY OF
MINNESOTA
LIBRARY

REPORT
of
COMMITTEE ON EXAMINATION

This is to certify that we the undersigned, as a Committee of the Graduate School, have given Louise Terese Dosdall final oral examination for the degree of Master of Arts. We recommend that the degree of Master of Arts be conferred upon the candidate.

Minneapolis, Minnesota

June 1 1917

Frederic E. Clements
Chairman

E. A. Brown

William S. Cooper
A. Foster

MOM
D74

REPORT
of
COMMITTEE ON THESIS

The undersigned, acting as a Committee of the Graduate School, have read the accompanying thesis submitted by Louise Terese Dossall for the degree of Master of Arts. They approve it as a thesis meeting the requirements of the Graduate School of the University of Minnesota, and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Arts.

Minneapolis, Minnesota
May 1917

Frederic B. Crooks
Edwin Furman
William S. Cooper

6-7-17 6885

WATER REQUIREMENT AND ADAPTATION IN
EQUISETUM.

BY

Louise Terese Dosdall

A THESIS
SUBMITTED TO THE GRADUATE SCHOOL OF
THE UNIVERSITY OF MINNESOTA IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
OF
MASTER OF ARTS.

MINNEAPOLIS, MINNESOTA.

MAY 1917.

24

WATER REQUIREMENT AND ADAPTATION IN EQUISETUM.

I. INTRODUCTION.

A study of the water relations of Equisetum was undertaken to throw light upon the nature of bog xerophytes and the successional relations of Equisetum in the hydrosere and paleosere.

The functional responses of a plant to the water content of its habitat determine the xerophily of the plant. Usually the impress of an extreme habitat is recognizable at once in the structure of the plant. This, however, is not always the case. In the group of plants known as bog xerophytes many of the members, while superficially exhibiting xerophytic structures, also show characteristics of hydrophytes, namely, large air-spaces and diaphragms. Moreover, these plants grow in the same habitat with true hydrophytes such as *Sagittaria*, *Ranunculus*, and *Caltha* (Clements 1907: 168). While the nature of the habitat has been subjected to much investigation, very little inquiry has been made as to the nature of the plants themselves. The purpose of this experimental study of the water requirements and adaptations of Equisetum was to determine whether this plant is a xerophyte as has been supposed, or whether it is truly a hydrophyte as its habitat suggests.

The problem was suggested by Dr. F. E. Clements and the work was carried on under his direction. The writer gratefully acknowledges her indebtedness for the many helpful suggestions in carrying out the work. Acknowledgments are also offered Miss Frances Long for the determinations of photosynthate and to Mr. Gorn Loftfield for the night readings on the position of the stomata.

1. SUMMARY OF VIEWS AS TO BOG XEROPHYTES.

(1). Physiological aridity.

Much emphasis has been placed upon the physiological aridity of bog habitats and its causes, but as yet an adequate explanation has not been found. It is generally believed that the water available for plant use is limited in certain zones (Burns 1911:106) characterized by xeromorphic plants. Clements (1916:90), however, questions the theory of physiological drought, while Gates (1914:455) has shown that winter is the deciding factor in the xerophily of the most typical of the bog plants, namely, the ever-green heaths.

Schimper (1899: 6) pointed out the difference between physical and physiological dryness. The latter is attributed to high concentrations of salt and humous acids in the water (1898: 691). This suggested to Livingston "that if the physiological dryness of the bog be due to humous acids or humous salts, these substances may check the absorption of water by plants either physically by high osmotic pressure or chemically by toxic or stimulation effects", (1904:383). He therefore made a study of the osmotic pressure of a series of bog waters. He found practically no difference in the osmotic pressures, and concluded that bog waters do not have an appreciably higher concentration of dissolved substances than do the streams and lakes of the same region.

Livingston (1905:348) later made an investigation of the physiological properties of bog waters by growing an alga (Stigeoclonium) in them and found that "many bog waters act upon the plant like poisoned solutions." He says, "The stimulating substances are

most markedly present in water from those swamps whose vegetation is most definitely of the bog type. They are absent from river swamps and large lakes; in water from swamps whose vegetation is of a character intermediate between those of the river swamp and the bog, they are present to some degree, their amount being roughly proportional to the extent of the xerophilous character of the vegetation."

Dachnowski (1910:339) has extended the toxic theory. From a study of physiological aridity, he concludes that "the real determining factor in the bog habitat is the ratio of the possible rate of water absorption to the rate of transpiration, and that the toxicity of the bog habitat has a primary role in bringing about bog conditions." From his study of cultures of isolated bog bacteria he concludes that bacterial toxins are the leading factor to be considered in the physiological aridity of bogs.

Riggs (1913:310) from his investigation of the effect of bog waters on the root-hairs of Tradescantia, suggests that "the toxins act through their stunting effect on root-hairs." In other words, plants which grow in bogs can have only a low absorptive power.

The conclusion of these writers is that the xerophily of bog plants is due to the toxic effect of the habitat.

- (2) Xerophytic nature due to persistence of stable structures.

Clements (1905:126) holds the view that many of the so-called swamp xerophytes are not xerophytes at all, but are plants which have developed their xerophytic features while growing on dry land. They have subsequently been able to adjust themselves to present conditions without undergoing a corresponding change in

structure. His view is based upon the presence of hydrophytic structures in the plants and the association of the plants with typical hydrophytes.

(3) Winter xerophily among plants.

Gates (1914:445) has made a very thorough study of the nature of the evergreen heaths growing in bogs, principally *Chamaedaphne*, and concludes that "in view of the fact that exposure to the very extreme summer conditions in 1911 and 1912 did not affect the vitality of the evergreen ericads, that neither did the average winter of 1910-1911, with its scanty snow covering during the coldest weather, while the extreme winter of 1911-1912 killed the parts of the evergreen ericads which projected above the snow; and in view of the fact that the evergreen habit is hereditary, that the position of the leaves in winter is different from that in summer, and that the transpiration is decidedly less than that of deciduous shrubs and of herbaceous plants in summer, but greater in winter, the xeromorphy of these plants is real xerophyty, occasioned fundamentally by the necessity of protection when exposed to winter conditions and used advantageously by these plants during the summer."

2. SUCCESSIONAL RELATIONS OF EQUISETUM.

(1) In the Hydrosere.

Equisetum is found in such a wide range of places that its natural position in the development of vegetation is somewhat puzzling. From Clements (1916:184-278) summary of climax formations we find that Shaffner, Jennings, and Tyler in their descrip-

tion of the concentric zones about Brush Lake in Ohio found Equisetum fluviatile in the swamp zone along with Carex and Scirpus. Cowles in sketching the general course of succession on rock hills, sand hills, clay hills, and lake bluffs found the secondary succession on the lake bluff beginning with xerophytic herbs such as Melilotus, Aster, Equisetum, and various grasses. Resoll found a pool which in 1898 contained little but Sparganium minimum a dense mass of Equisetum fluviatile in 1912. Burns (1911:106) in his description of the distribution and position of zones in the bogs of southern Michigan gives Equisetum fluviatile as a member of the floating sedge zone. This together with the two succeeding zones, bog and tamarack, is called the xerophytic region of the habitat. Bergman and Stallard (1916:339) reported Equisetum fluviatile often occurring as a species in the Scirpus-Zizania associates. "In places it forms rather extensive areas of pure growth, notably on Leech Lake near the mouth of Boy river and at the west end of Long Lake near Ely. Usually, however, Equisetum occurs mixed with other species of the associates." It is also reported among the plants often occurring in small numbers in the Carex associates.

(2) In the Paleosere.

Equisetums reached their most luxuriant growth during the Pennsylvanian Period of the Carboniferous Age. The Calamites, great tree-like equisetums one to two feet in diameter and often sixty to ninety feet in height, are reported by Grand Eury to have formed great forests in Central France. According to Chamberlin they probably frequented swamps and lowlands. The evidence of the present day water relations of Equisetum substantiates the view of Clements (1916:421) that the reed swamp associates of today was pro-

bably represented by the smaller Calamites in the paleosere.

(3) The climate of the Carboniferous age.

The following, from Chamberlin and Salisbury, is a summary of the plant life of the Pennsylvania Period and the inference as to climate. The Coal Flora was made up of the following groups of plants: Filicales, Cycadofilices, Equisetales, Sphenophyllales and Lycopodiales. Equisetales were represented by Calamites, whose branches and leaves were arranged in whorls. The leaves were much reduced in size but not so much so as in the present day forms. "The structure was of the type adapted to dry weather as in the pine and many desert plants, and also, strangely enough, in undrained swamp plants. They (Calamites) probably frequented swamps and lowlands." The opinion of some geologists is that the climate of this period was more or less dry and cold. Their judgment is based on the xerophytic nature of the dominant plants, the lepidodendrous, sigillarias, calamites, and cordaites.

"The force of the inference from the xerophytic aspect of the overgrowth is much weakened, however, by the fact that the vegetation of undrained swamps and bogs assumes many of these xerophytic features, which in such cases, obviously become pseudoxerophytic. A satisfactory explanation of this phenomenon has not yet been found, nor has its extent and its limitations, either in respect to the nature and degree of swampiness necessary to produce it, or in respect to the kind of plants or plant parts affected, been so thoroughly worked out as to permit a close application to the problem in hand. It is obvious that to fit the case, marsh or bog conditions must have produced pseudoxerophytic

adaptations in the great mass of the tree growth, but not in ferns and sphenophyllous plants. The effects must apparently also be extended to all plants brought into the depositing basins by drainage, whether these were true swamp plants or not, unless a closer study of the coal flora shall show that the xerophytic aspect was not assumed by all species of the trees involved. It is clear that a more critical study of the problem on all sides is necessary before a final conclusion can be reached."

Dachnowski (1912:278) questions this theory on the ground that the plants encountered adversities of soil-water content rather than of climate.

II. METHODS.

The general plan followed was to make a quantitative comparison of the water used by Equisetum and that used by certain other plants of well known habits. The later include Bryophyllum calycinum, a xerophyte, Helianthus annuus and Phaseolus vulgaris, mesophytes, and Ranunculus scleratus, an amphibious hydrophyte. Under the term water requirements the water used in various ways by the plant is included, the purpose being to determine whether in its physiological processes Equisetum uses more or less water than the other plants.

(1) Source of Plants.

In the spring of 1916 attempts were made to transplant the fertile and sterile stems of Equisetum arvense into glazed pots in order to compare their rates of transpiration. No matter how carefully the plants were taken up or how little the soil was disturbed, they wilted almost as soon as they were put in the green-

house. This may be accounted for by the fact that the rootstocks of this species grow very deep into the ground and it was necessary to cut the underground stems. In a few cases dormant buds on the plants transferred developed quite respectable plants. This suggested that it would be necessary to dig up the stems before the spring growth started but by this time it was too late to find any more buds.

Early in June similar attempts were made to transplant Equisetum hyemale with similar results. In one case a block of soil twelve inches in diameter and eighteen inches deep was cut out and carefully lifted into a large jar. This was done on a rainy day when conditions were ideal for transplanting, but by the next day the plants were wilted. A trench was then dug in order to trace the root systems. Underground stems were frequent at a depth of two feet, although most of them were in the first twelve to fifteen inches of soil. Roots were traced to a depth of three feet and one inch from which point they were submerged in the water of an underground spring.

Potometers with cut stems of Equisetum hyemale were then tried but, although there were no signs of wilting, the rate of transpiration decreased so rapidly and steadily that this method was discarded as unreliable. The only possible solution seemed to be to get the buds on the underground stems during their resting period.

During October and November large clumps of earth matted together with the root stocks of Equisetum fluviatile were dug from the edge of a tamarack swamp near Glenwood Park, Minneapolis and brought to the greenhouse. Profiting by past experiences, pieces of soil were cut to just fit into their pots but it was soon



PLATE 1

General view of the work-bench showing some of the plants used in the investigation.

found that too much competition between the many Equisetum stems and other plants in the soil was hindering good growth. Other Equisetum buds were then carefully separated from the soil in which they had grown and planted in porous pots containing ordinary garden loam. The stems began coming up at once and very good plants developed in the course of six weeks. Some of the material brought in from the swamp was covered with a sack and left out in the yard all winter. In March, when the frost was beginning to leave the ground, these were brought in and planted in loam just as the others had been. In some cases these spring plants grew almost to the full size attained in the field.

During the latter part of October some Equisetum hyemale was found growing in the glen along the Mississippi river at Fort Snelling, Minnesota. These plants were growing on the side of a hill in deep shade. The root systems proved to be rather shallow and when the plants were transferred to the greenhouse only a few of the stems turned yellow. Throughout the winter abundant new shoots developed while most of the old stems died down towards January. Equisetum arvense was dug early in April. Plate 1, shows some of the plants used in spring and the methods of growing them. Ranunculus sceleratus was grown from seedlings, which Mr. Folsom of the Botanical Department of the University had grown in mud for a number of generations. The plants of Bryophyllum calycinum were cuttings from plants which had grown in the greenhouse for a number of years. Helianthus annuus and Phaseolus vulgaris were grown from seeds.

(2) Water Content Methods.

In determining the wilting coefficient of Equisetum fluviatile, and Ranunculus sceleratus essentially the same wax-seal method recommended by Briggs and Shantz (1912:10) for the direct determination of the wilting point was used. The plants, however, were grown approximately six weeks in soil sifted through a two millimeter screen before being sealed for wilting. The exact time elapsing before the seal was applied differed with the season because the plants started in spring grew much more rapidly than those started in fall. Through an inadvertence Equisetum hyemale growing in sandy loam was used for the wilting coefficient and consequently a direct comparison with the others cannot be made.

In most cases Equisetum wilts very suddenly, the collapse of the upper part of the plant indicating that the critical point in the absorption of water has been reached. Very similar wilting effects result from other causes. If the wax is too warm when put around the stem the latter is injured and the whole plant collapses. This can be distinguished from true wilting by the collapse of the tissue at the point of sealing. A damping-off fungus also produces a sudden wilting, but in this case the stem always shows decay at the surface of the soil. In wilting due to lack of water in the soil, the top of the plant collapses first and the stem is not injured at the surface of the soil or at the point of sealing.

Ranunculus sceleratus on the other hand has no definite wilting point. The time of the yellowing of the first leaves of the rosette varies from four to eight days. By the time the younger parts wilt the older parts are completely dead. As these parts continue to draw water from the soil and evaporate it from their



A. *Phaseolus vulgaris* in the same pots with *Equisetum hyemale*, *Equisetum arvense*, and *Equisetum fluviatile*, to determine the time of wilting.



B. *Helianthus annuus* in the same pots with *Equisetum hyemale*, *Equisetum arvense* and *Equisetum fluviatile*, to determine the time of wilting.

leaves (Briggs and Shantz 1912:47), the coefficients are too low.

The relative time of wilting was compared with that of mesophytes such as Helianthus annuus and Phaseolus vulgaris by growing them in the same pots (Plate 2) and allowing them to wilt. In some cases Equisetum and the beans and sunflowers were started in the same pots, in others, plants from ten to twelve days old were transplanted into the same pots, care being taken to injure the roots as little as possible. Two weeks were allowed for the plants to establish themselves and develop good root systems before sealing.

(3) Transpiration Methods.

Water loss by the plant was determined by weighing potted plants as this is the most convenient and reliable of the several methods for measuring transpiration. The plants were either sealed in glazed pots or in Ganong aluminum shells. The wax used as a seal was made of twenty parts of vaseline to eight parts of paraffin, the formula recommended by Briggs and Shantz (1912:13). In cases where the wax was to be put directly on the soil, a piece of paper was cut to fit neatly over the top of the pot, dipped into the melted wax, and fitted in place. The edges were sealed by pouring more wax over the surface and slightly tipping the pot so that it would run up the sides. The object of the paper was to prevent the wax from creeping into the soil when heated by the sun's rays. The plants themselves are very sensitive to heat so that the wax cannot be applied near the stems until it has cooled sufficiently to form a scum over the surface. The waxed paper also proved a satisfactory substitute for rubber on the Ganong shells.

The plants were grown in loam which had been sifted through a two-millimeter screen in order that absorption by the roots would be as nearly uniform as possible. Records of humidity and temperature during each experiment were made by a Draper self-recording hygrometer and thermometer.

The transpiration of two or more plants was compared on the basis of the number of grams of water lost per unit area. The stem and branches of Equisetum are almost cylindrical so that the determination of the area of the surface was comparatively simple. The diameter of a stem was measured with a vernier micrometer at the middle of the lowest and topmost internodes. These represented the extremes in size, and from the average, the average circumference of the stem was calculated. This multiplied by the length of the stem gave the area of the surface.

Leaf areas were found in two ways, by the planimeter and by weighing paper prints. Leaves with comparatively simple outlines were traced on paper and these retraced with the planimeter. After practice this could be done readily and the average reading of three tracings was recorded. This method has the advantage of giving the area directly but also has disadvantages in that the square inches must be converted into square decimeters, and that it is difficult to trace leaves with very toothed margins. In the paper weighing method either tracings or blue prints of the leaves were made and cut out with a scissors. The weight of these prints divided by the weight of one square decimeter of the same paper, multiplied by two gave the transpiring area of the leaves. The following table shows how the two methods check with one another.

COMPARISON OF PLANIMETER AND WEIGHING
METHODS OF DETERMINING LEAF AREAS.

Paper weighing method			∴	∴	∴	∴	∴
			∴	∴	∴	∴	∴
			∴	∴	∴	∴	∴
Wt of 1 sq.d.m. paper (grams)	Wt of leaf prints (gms)	Area sq.d.m.	∴	Area sq.in.	∴	Area sq.d.m.	Difference Sq.d.m.
0.500	0.18	0.72	∴	10.72	∴	0.69	.03
0.533	0.26	1.04	∴	16.60	∴	1.07	.03
0.538	0.44	1.65	∴	26.04	∴	1.65	.03
						Average area	1.14 sq.d.m.
						Average difference	.03 sq.d.m.
						Error	3%

This comparison of the two methods shows an average difference of only three hundredths of a square decimeter. This was considered so small that the two methods might be used interchangeably. Whichever method was the more convenient under the circumstances was used to determine the areas.

(4) Photosynthesis Methods.

To determine the efficiency of Equisetum as compared with that of other plants as to the activity of the chlorenchyma in the manufacture of carbohydrates, determinations of the amounts of glucose present in the stem in the morning and evening were made. During the same period a record was kept of the transpiration and the results correlated. The chemical analyses were made by Miss Long according to a modification of the Munson-Walker method of testing for glucose.

(5) Adjustments and Adaptations.

The adjustments of a plant to the physical factors of its habitat are most strikingly shown in the physiological responses of growth and transpiration to light and to the water content of the soil. To determine the effect of soil-water content upon growth, Equisetum fluviatile was grown with roots submerged in water, in mud, and in a soil-water content of thirty-five per cent. All other factors were kept uniform by using the same volume of the same type of soil sifted through a two-millimeter screen. The plants stood beside each other in the greenhouse bench so that they were subjected to the same conditions of temperature, humidity and light. The intensity of light in another group of plants was varied by placing plants in a muslin shade tent which cut down the light to .3 per cent as measured by a photometer. Plants in the bench receiving normal sunlight in the greenhouse, or 50 per cent full sunlight, served as checks.

A study of the position of the stomata was made according to the method worked out by Lloyd (1908:22). Thin slices of epiderm were cut out from the surface and plunged into absolute alcohol. Before using, the sections were transferred to a saturated solution of Congo red in absolute alcohol. After several days they were examined under the microscope. The stain deeply colored the cell walls so that the opening between the guard cells is easily observed.

Cross sections of the stems which had grown under varying soil-water contents for four months were made with the freezing machine and examined for structural modifications. Strips of epidermis were also studied for the number of stomata.

III EXPERIMENTAL RESULTS.

General Outline of Experiments.

- (1) Water content of the soil.
 - (a) Maximum and Minimum.
 - (b) Wilting coefficient of
 - Equisetum fluviatile
 - Equisetum hyemale
 - Ranunculus sceleratus
 - (c) Time of wilting
 - (1) Equisetum fluviatile and Helianthus annuus.
 - Equisetum fluviatile and Phaseolus vulgaris.
 - (2) Equisetum hyemale and Helianthus annuus.
 - Equisetum hyemale and Phaseolus vulgaris.
 - (3) Equisetum arvense and Helianthus annuus.
 - Equisetum arvense and Phaseolus vulgaris.
- (2) Water loss by the plant.
 - (a) Relation of transpiration to plant functions
 - (b) Comparative transpiration
 - Equisetum fluviatile
 - Equisetum hyemale
 - Xerophyte : Bryophyllum calycinum
 - Mesophyte : Helianthus, Phaseolus vulgaris
 - Hydrophyte: Ranunculus sceleratus
 - (c) Guttation.
- (3) Transpiration and Photosynthesis
- (4) Physiological responses
 - (a) Growth and soil-water content
 - (b) Growth and light intensity

- (c) Transpiration of soil-water content
- (d) Stomata behavior
- (5) Adaptations.

1. Water content of soil.

(1) Determination of maximum and minimum soil water content.

Two series of *Equisetum fluviatile* were run from November 4, 1916 to December 21, 1916 in soil-water contents varying from roots submerged in water to a holard of 9% based upon the dry weight of the soil. In series A the soil was sifted through a five-millimeter screen to give uniformity while in series B the soil was not sifted. In all other respects the series were duplicates. Plants numbered 1 grew in an inch layer of soil covered with three inches of water. Plants numbered 2 grew in porous pots standing in water. The others grew in a four inch layer of soil (2500 cc.) in eight inch glazed jars. Buds of approximately the same size and with the same amount of rootstock were used to plant in each pot so that none would have the advantage of a larger stored food supply. The amount of water added weekly was measured and recorded. A set of soil samples was taken every two weeks.

Average Soil-water Content.

Series A (Soil sifted)		Series B (Not sifted)	
No. 1 Submerged		No. 1 Submerged	
No. 2	60%	No. 2	58%
No. 3	33%	No. 3	37%
No. 4	23%	No. 4	30%
No. 5	17%	No. 5	18%
No. 6	12%	No. 6	9%

The plants in No. 5 and No. 6 withered when about two inches above the ground while those in No. 4 grew to be eight inches high. Very few branches were developed and these dried at the tips. The best plants were obtained in No. 1 and No. 2, those growing in mud having slightly more branches than those with the roots submerged. Thus, Equisetum fluviatile demands a high amount of moisture in the soil, the range being from 23% to saturation with the optimum at the highest concentrations.

2. Wilting Coefficient.

(a) Separate cultures.

Equisetum fluviatile and Ranunculus sceleratus were grown in loam with a water content from 35-45 per cent maintained by keeping the pots in deep saucers filled with water. The wilting point was determined by the wax seal method. At the time of sealing, the Equisetum had grown six weeks and the Ranunculus two months under the conditions mentioned. A series of Equisetum hyemale growing in sandy loam was also sealed for wilting. Because of the difference in the soil the latter were checked against the wilting of two sunflowers also growing in sandy loam.

Results:

Soil-water content at time of wilting.

Equisetum fluviatile

Plant (1)	23.1)
" (2)	24.4)
" (3)	23.0)
" (4)	30.0)
Average	- 25.1

LOAM

Ranunculus sceleratus

Plant (1)	13.5)	
" (2)	11.0)	
" (3)	14.1)	LOAM
" (4)	14.7)	
Average	<u>13.3</u>	

Equisetum hyemale

Plant (1)	5.3)	
" (2)	5.3)	SANDY
" (3)	4.2)	LOAM
" (4)	4.7)	
Average	<u>4.9</u>	

Helianthus annuus

Plant (1)	5.8)	SANDY
" (2)	5.4)	LOAM
Average	<u>5.6</u>	

In order to check the soil water requirements of Equisetum against those of typical mesophytes the following series of experiments were undertaken.

(b) Cultures with mesophytes.

(The methods of growing the plants in the same pots is shown in (Plate 2).

RESULTS:

Relative time of wilting of Equisetum and mesophytes grown simultaneously in the same pots.

Plants	:Wilting of :Equisetum	:wilting of :Mesophyte	: Remarks on : Root Systems.
<u>Equisetum fluviatile</u>	:	:	:
<u>Helianthus annuus</u>	: 2 days	: 10 days	: Both plants had : good root symstems : well distributed : in pot.
<u>Helianthus annuus</u>	: 7 "	: 12 "	: " " " "
<u>Phaseolus vulgaris</u>	: 2 "	: 17 "	: good root systems
<u>Phaseolus vulgaris</u>	: 12 "	: 19 "	:
<u>Equisetum hyemale</u>	:	:	:
<u>Helianthus annuus</u>	: 15 "	: 15 "	: good root systems
<u>Helianthus annuus</u>	: 13 "	: 17 "	: Fair root systems
<u>Phaseolus vulgaris</u>	: 13 "	: later	: Good root systems : but not very well : distributed
<u>Phaseolus vulgaris</u>	: 10 "	: Bean : injured	: Root system of bean : very poor. Equisetum : signs of wilting : first
<u>Equisetum Arvense</u>	:	:	:
<u>Helianthus annuus</u>	: 5 "	: 12 "	: Good roots
<u>Phaseolus vulgaris</u>	: 7 "	: yellowing : on 7th day	: Root system of bean : poorly developed : Equisetum roots : good.

The table shows, with the exception of Equisetum hyemale, that Equisetum wilted before the mesophytes when the root systems of both were in good condition and well distributed in the pots.

PLATE 3



Equisetum fluviatile wilting. *Phaseolus vulgaris* and *Helianthus annuus* still turgid.

In some cases the exact number of days for the wilting of the mesophytes was not obtained, but the fact that the Equisetum wilted first is indicated. The discrepancies in the cases of Phaseolus are attributed to the poorly developed root systems, but the results with Helianthus show beyond a doubt that it is able to draw water from the soil after Equisetum fluviatile and Equisetum arvense are dead. Plate 3 shows Equisetum fluviatile wilting while both Helianthus and Phaseolus are in good condition. The conclusion to be drawn from this evidence are that the latter are less hydrophytic than either Equisetum arvense or Equisetum fluviatile. Equisetum hyemale differs from the other two in that it seems to be mesophytic in its relations.

These results on the wilting of Equisetum, Helianthus, and Phaseolus agree with the work of Hedgecock (1902:65) who states, in his conclusion that "the ability of plants to take water from the soil varies in an ascending scale from hydrophytes, through mesophytes, to xerophytes" and the view of Clements (1905:31) that "a plant of xerophytic tendency is naturally able to remove more water from the same soil than one of mesophytic or hydrophytic character." Briggs and Shantz (1912:75) found the wilting coefficient of a great number of plants with which they worked to vary but little, or from 92 for Japan rice to 106 for a variety of corn, with 100 as the standard. From their results of simultaneous wilting of plants grown together in the same pots, the writers conclude that one plant is not able to draw more moisture from the soil than another. The results of the present investigation indicate that certain mesophytes are able to take more moisture from the soil than the hydrophytic species of Equisetum can.

2. Water loss by the plant.

(1) Comparative transpiration.

Series 1.

Factors:

Average temperature 80° F.

Average humidity 57%

Plants	:Total loss :(grams)	:Area :sq.dm.	:Loss per :sq.dm. :grams.	:Average :loss	:Ratio
<u>Equisetum fluviatile</u>	: 7.3	: .38	: 19.2	:	:
" "	: 7.4	: .43	: 17.2	: 18.8	: 1.0
" "	: 6.0	: .30	: 20.0	:	:
Bryophyllum calycinum:	5.5	2.18	2.5	1.9	0.1
" "	5.8	4.54	1.3	:	:
Ranunculus sceleratus:	27.4	2.08	13.2	:	:
" "	27.0	2.50	10.4	14.5	0.8
" "	50.0	3.02	19.8	:	:

Series 2.

Factors:

Average temperature 67° F.

Average humidity 60%

Plants	:Total loss :(grams)	:Area :sq.dm.	:Loss per :sq.d.m.	:Ratio
Equisetum fluviatile	: 10.9	: .37	: 29.5	: 1.0
Ranunculus sceleratus:	77.4	3.02	25.6	0.9
Equisetum hyemale	: 68.5	: 3.83	: 17.9	: 0.6
Helianthus annuus	: 62.5	: 4.24	: 14.7	: 0.5
Phaseolus vulgaris	: 28.4	: 5.42	: 5.2	: 0.2
Bryophyllum calycinum:	9.9	4.54	2.2	0.1

Series 3.

Factors:

Average temperature 65°F.

Average humidity 60%

Plants	:Total loss :(grams)	:Area :sq.dm.	:Loss per :sq.d.m.	: Ratio
Equisetum fluviatile	: 9.9	: 0.84	: 11.8	: 1.0
Equisetum fluviatile	: 7.8	: 0.75	: 10.4	:
	:	:	:	:
Equisetum arvense	: 4.0	: 0.84	: 4.8	::
Equisetum arvense	: 4.2	: 0.89	: 4.7	: 0.4

Series 4.

Factors:

Average temperature 44°F.

Average humidity 39%

Plants	:Loss :Grams H ₂ O	:Area :sq.d.m	:Grams H ₂ O: :lost per :sq.d.m.	:Average :loss per :sq.d.m.	:Ratio
Equisetum fluviatile	:	:	:	:	:
Plant 1	: 5.3	: .38	: 13.9	:	:
2	: 10.0	: .68	: 14.7	: 15.2	: 1.0
3	: 5.1	: .30	: 17.0	:	:
Helianthus annuus	:	:	:	:	:
Plant 7	: 21.3	: 2.20	: 9.7	:	:
8	: 29.0	: 3.15	: 9.2	: 9.8	: 0.6
9	: 40.8	: 3.91	: 10.4	:	:
Phaseolus vulgaris	:	:	:	:	:
Plant 4	: 48.7	: 6.51	: 7.5	:	:
5	: 53.5	: 8.82	: 6.1	: 6.5	: 0.4
6	: 20.0	: 3.47	: 5.8	:	:

A comparison of the tables shows that in the amount of water lost by transpiration Equisetum fluviatile exceeds both the mesophytes and the xerophytes. Ranunculus sceleratus which is a typical hydrophyte loses but from eight to nine tenths the amount

of water that Equisetum fluviatile does. The mesophytes Helianthus annuus and Phaseolus vulgaris lose from two to six tenths as much, while the xerophyte, Bryophyllum calycinum, loses only one tenth as much. In other words, Equisetum fluviatile transpires from two to five times as much water from its surface as do two typical mesophytes, and ten times as much as a typical land xerophyte. Ranunculus is an exceedingly plastic species which accounts for its ranking below Equisetum, for during the experiment the roots of the former were not in water. Helianthus is an exceedingly wasteful plant as far as water loss is concerned which explains the higher transpiration than that of Phaseolus. The conclusion to be drawn from this evidence is that Equisetum fluviatile is unquestionably a hydrophyte.

These results agree very closely with those of Sampson and Allen (1909:55) who found that Scirpus lacustris also a "bog xerophyte" loses twice as much water as Helianthus annuus.

Comparing the transpiration of the different species of Equisetum, it is found that Equisetum hyemale loses but six tenths as much water as does Equisetum fluviatile and that Equisetum arvense but four tenths as much, showing that these two are more mesophytic species.

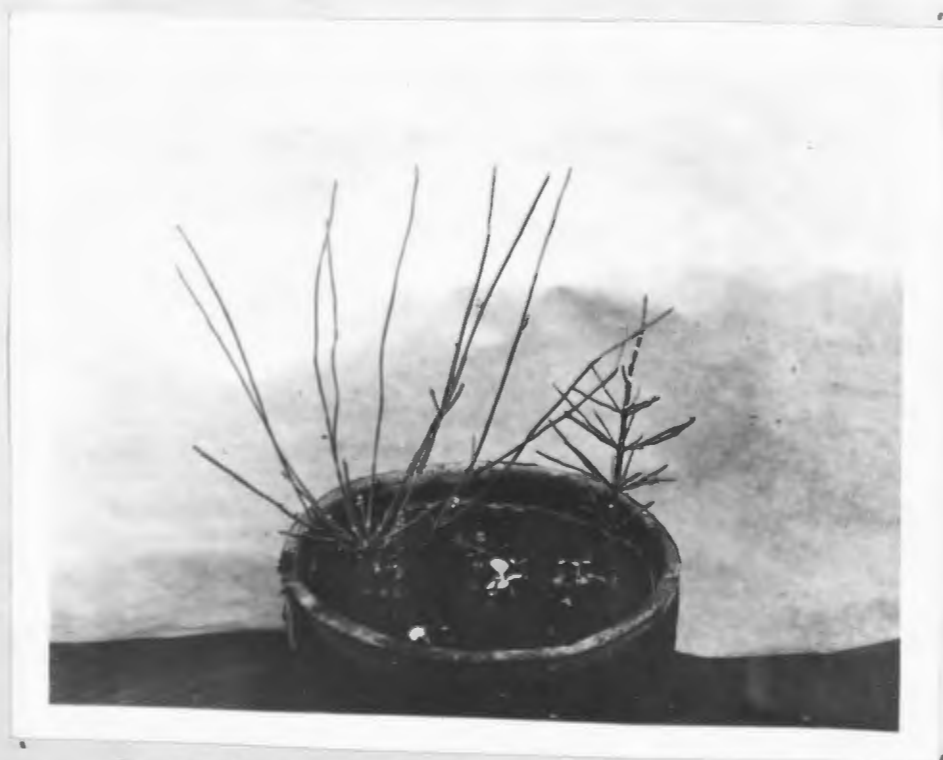
(2) Guttation.

Leaves of many species of plants, among which the best known are aroids, fuchsia, cabbage, and nasturtium, exude drops of water from their surface when transpiration is checked by external factors. (Sachs 1887:278). Usually the water is forced out of permanently open stomata or water pores at the end of the veins. Although a common phenomenon, it is scarcely to be expected in a

PLATE 4



A. Stem of *Equisetum hyemale* showing drops of water exuded by guttation.



B. *Equisetum arvense* showing drops of water exuded by guttation.

xerophyte. During the week of May 18-23 on four successive cloudy mornings, all three species of Equisetum were found to be guttating vigorously. In some cases the drops of water were four millimeters in diameter, and 25-50 occurred on single stems. Old stems as well as young were exuding water, (Plate 4). On these mornings the relative humidity in the greenhouse was 72%. The plants were then placed under bell jars and the following morning large drops of water were found at the nodes between the points of the leaf scales and much smaller ones in the grooves of the internodes at positions corresponding to those of the stomata.

This furnishes additional evidence that Equisetum is not a xerophyte and particularly a "bog xerophyte" whose roots are supposed to have low absorption powers.

(3) Transpiration and photosynthesis.

Relative transpiration and photosynthesis efficiency of Equisetum fluviatile, Helianthus annuus and Phaseolus vulgaris.

This experiment was done in cooperation with Miss Frances Long who determined the photosynthate.

The plants for this experiment were grown in soil kept saturated with water. The morning of the experiment the pots were placed in Ganong aluminum shells filled with water. Evaporation from the soil was avoided by covering it with a layer of waxed paper held in place by the aluminum ring. The space between the plant and paper was sealed with the Shantz mixture of paraffin and vaseline.

For the photosynthate readings similar plants were used for the morning and noon readings, since those used for transpiration could not be destroyed until evening.

Leaf prints were made of the leaves as soon as cut and the areas found by a planimeter. The leaves were killed in chloroform and dried in a steam oven at 90°. The glucose determination was made by the modified Munson-Walker method used in Miss Long's photosynthate experiments.

The experiment was carried on May 4, 1917. The temperature and humidity conditions were as follows:

	: Humidity	: Temperature:
7.30 a.m.:	70%	70°
12 noon	: 28%	: 78°
5.30 p.m.	: 20%	: 85°

TRANSPIRATION - 7.30 a.m. - 5.30 p.m. May 4, 1917.

Plant	: Loss : Grams H ₂ O	: Area : sq.d.m.	: Grams H ₂ O : lost per : sq.d.m.	: Average : loss per : sq.d.m.
<i>Equisetum fluviatile</i>				
Plant 1	: 5.3	: .38	: 13.9	: 15.2
2	: 10.0	: .68	: 14.7	
3	: 5.1	: .30	: 17.0	
<i>Helianthus annuus</i>				
Plant 7	: 21.3	: 2.20	: 9.7	: 9.8
8	: 29.0	: 3.15	: 9.2	
9	: 40.8	: 3.91	: 10.4	
<i>Phaseolus vulgaris</i>				
Plant 4	: 48.7	: 6.51	: 7.5	: 6.5
5	: 53.5	: 8.82	: 6.1	
6	: 20.0	: 3.47	: 5.8	



Submerged Mud 35%
Equisetum fluviatile showing effect of
varying soil-water contents on growth.

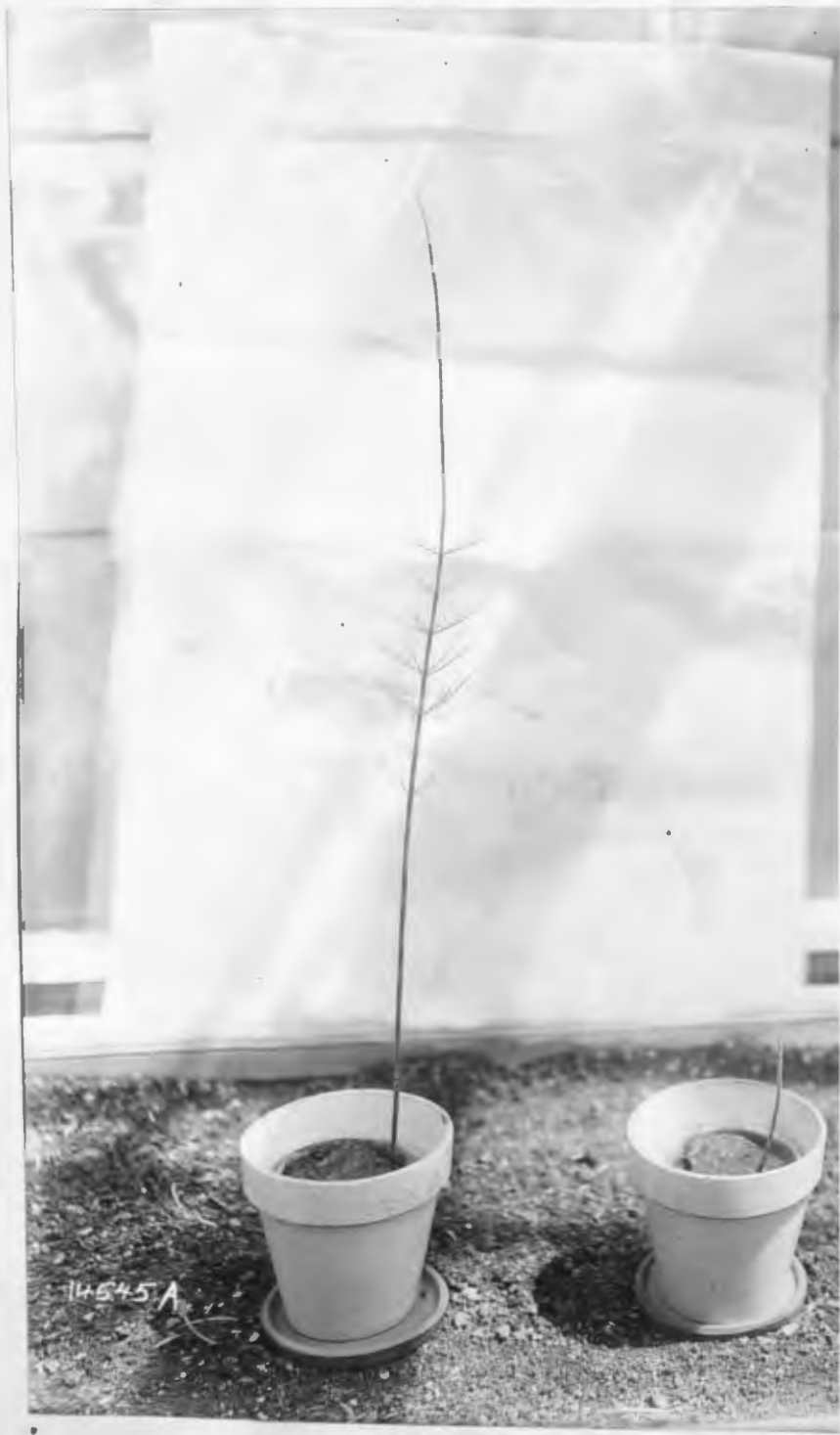
Photosynthate.

	:gms.dried: :leaf used:	:Area :sq.d.m.:	:Glucose: :total :Mgs	:Glucose: :Mgs.per: :sq.d.m.:	:Mgs. :per :during day	:Glucose :per sq.d.m.
Equisetum fluviatile	:	:	:	:	:	:
7.30 a.m.	: .2100	: .6	: 17.4	: 29.0	:	: .
5.30 p.m.	: .2884	: .68	: 30.9	: 44.0	:	: 15.0
Helianthus annuus	:	:	:	:	:	:
7.30 a.m.	: 1.0446	: 3.785	: 2.0	: .52	:	:
12 noon	: .9354	: 2.907	: 33.0	: 11.35	:	: 10.83
5.30 p.m.	: .6674	: 2.68	: 76.5	: 28.54	:	: 28.02
Phaseolus vulgaris	:	:	:	:	:	:
7.30 a.m.	: 1.509	: 7.54	: 126.7	: 16.81	:	:
12 noon	: 1.015	: 5.17935	: 272.4	: 52.59	:	: 35.78
5.30 p.m.	: 2.4540	: 7.67	: 472.4	: 61.72	:	: 44.91

A glance at the table shows that Equisetum transpires 1.5 as much as Helianthus and 2.3 times as rapidly as Phaseolus. Helianthus loses 1.5 times the amount of water lost by Phaseolus. Helianthus is regarded as a very wasteful plant so far as water efficiency is concerned.

Helianthus shows 1.8 the ability to make carbohydrates shown by Equisetum, while Phaseolus is three times as efficient as Equisetum. Phaseolus makes 1.6 times as much photosynthate as Helianthus. The low efficiency of Equisetum may be accounted for by the fact that the stem is the photosynthetic organ rather than the leaves. The position of the stem prevents the rays of the sun from falling directly on the surface so that a given amount of light is spread over a greater area. These slanting rays which reach it are of much less intensity than the direct rays and thus the source of energy for the photosynthetic work is much reduced.

Helianthus has an epidermis covered with hairs which serve as a light screen and cut out 70 per cent of the light which strikes the epidermis. Phaseolus epidermis screens out 45 per cent



50%

3%

Equisetum fluviatile showing effect of
varying light intensities on growth.

of the light incident upon it. The chloroplasts are then functioning not in bright light, even though the leaves are in full sunlight, but in 30 per cent and 55 per cent relative light intensity.

It is interesting to note that the photosynthetic efficiency in these three species is in inverse proportion to the amount of transpiration.

4 Physiological responses.

(1) Growth and water content.

A series of Equisetum fluviatile are grown with roots submerged, roots in mud, and roots in a water content of thirty-five per cent, from April 2, to May 18. The effect of the varying water contents on the size of the plants is shown in Plate 5. The following is the height of each:

Submerged	Mud	35%
80.1 c.m.	80.5 c.m.	68.2 c.m.

As shown on the plate almost no branches developed on the plant in thirty-five per cent water content. With all the plants used in the greenhouse growth was best in mud, although in many cases the difference between the plants growing in mud and those with submerged roots was very slight.

(2) Growth and light intensity.

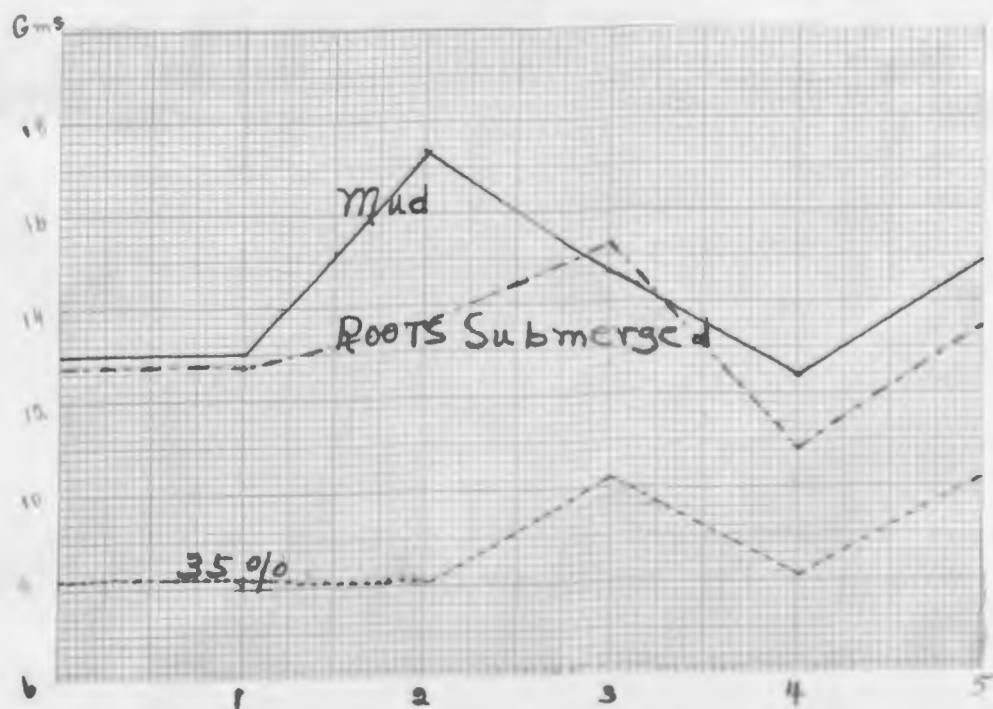
Plants of Equisetum fluviatile were grown in a muslin shade tent which cut down the light to 3 per cent and in the normal light of the greenhouse, or 50 per cent full sunlight. The effect is shown in Plate 6. At the end of six weeks the plant from the shade tent was 7.4 centimeters high and the other 80.0 centimeters high. These plants grew in mud.

The results show that both water content and light intensity have a marked effect on growth in Equisetum fluviatile.

(3) Transpiration and water content.

The effect of the three water contents on the amount of transpiration was determined for Equisetum fluviatile. To check the results obtained the experiment was repeated three times with twenty plants. The difference in the amounts of water lost are shown in the following graph.

Effect of soil-water content on transpiration.



The plants growing in mud lost the most water while those in soil with thirty-five per cent water content lost the least.

(4) Stomatal behavior.

Equisetum hyemale has thirty-six stomata per square millimeter of surface while Equisetum fluviatile has one hundred and eighteen. In both cases the stomata are sunk in grooves resembling the modifications of a xerophyte. A set of readings on the position of the guard cells in the pidermis of Equisetum hyemale at 8:00 a.m., 12:00 noon, and 4:00 p.m. and an hourly series from 10:00 p.m. to 3:00 a.m. showed no difference in the size of the opening between the cells. During the night epidermis from both old and young stems were taken. This fixed position of the stomata explains why Equisetum can loose so much water per unit area.

(5) Adaptations.

That Equisetum fluviatile is a very stable form is proved by the fact that no structural differences could be discovered in cross sections of the stems growing in the different water contents. The epidermis was also studied for differences in the number of stomata but the average number per square millimeter did not vary.

The results of adaptation experiments were entirely negative. No structural differences could be discovered in the epidermis chlorenchyma, vascular tissue, or air space from the cross section of Equisetum fluviatile which had grown in the varying water contents, from November 1916 to April 1917 in the greenhouse. Sections of epidermis were also studied for the number of

stomata but the average per square millimeter did not vary. These results correspond with those of E. S. Clements (1905:87) who found no variation in certain xerophytic forms grown under varying conditions of water content.

IV. SUMMARY AND CONCLUSIONS.

1. The results of the present investigation of the water requirements of Equisetum fluviatile show that this species requires a higher concentration of water in the soil in order to maintain its normal functions. The amount of water remaining in the soil at the death of the plant is greater than the amount at the death of Ranunculus sceleratus. When growing in the same soil mass with Helianthus annuus and Phaseolus vulgaris, Equisetum wilts first. A comparison of the transpiration shows that the water loss is much greater than from the mesophytes and the xerophyte with which it was compared. Equisetum fluviatile is therefore to be considered a true hydrophyte.

2. The high transpiration is correlated with low photosynthetic efficiency as compared with Helianthus annuus and Phaseolus vulgaris.

3. The difference in the time of wilting between Equisetum and the mesophytes with which it was compared shows that the range in the amount of water that plants can take from the soil is greater than indicated by the work of Briggs and Shantz.

4. The fact that Equisetum can adjust itself to physical changes in the habitat by marked physiological responses but gives negative results as to adaptation indicates that the xeromorphic features are extremely stable structures and that adaptation to hydrophytic conditions has come about through the development of air-spaces, diaphragms, and permanently open stomata.

n 5. The great amount of water lost through transpiration and guttation indicates that this species does not grow in the xerophytic regions of bogs because of its low absorptive powers as Dachnowski maintains, and that it cannot be considered an actual xerophyte at all. Its xerophily is only superficial and is probably due to the persistence of ancestral structures, now compensated by adjustment and by adaptation in the aerenchym.

6. Since the structure and habitat of the Paleozoic Calamites and Mesozoic Equisetum were essentially the same as those of the present day form, the apparent xerophily cannot be considered as an indication of a dry climate, or of a physiologically arid habitat because the water lost by the plants is great and means of checking it are absent. It is probable that the superficial xeromorphy of Equisetum was acquired in a cold arid period, such as the Permian.

7. The hydrophytic nature of Equisetum fluviatile places it in the hydrarch succession. It usually plays only a secondary part in the various kinds of swamps where it probably holds its place as a relic of former eoseres. Successionally it is a member of the reed-swamp or sedge-swamp associates, usually forming a societies.

8. Equisetum hyemale and Equisetum arvense are found in much dryer areas than Equisetum fluviatile. A comparison of their transpiration per unit area shows that the former are more mesophytic in their tendencies. This accounts for the fact that these two species are capable of invading grass land.

B I B L I O G R A P H Y.

- Bergman, H. F. and Stallard, H. 1916. The development of climaxformations in Northern Minnesota. Minn. Bot. Studies 4:333-378.
- Briggs, L. J. and Schantz, H.L. 1912. The wilting coefficient of plants and its indirect determination. Bur. Plant Ind. Bull. 230.
1913. The water requirements of plants, II. A review of the literature.
- Burns, G. P. 1911. A botanical survey of the Huron River Valley. VIII. Edaphic conditions in peat bogs of southern Michigan. Bot. Gaz. 52:105-125.
- Chamberlain, T. C. and R. D. Salisbury; Geology. 1907.
- Clements, E. S. 1905. The relation of leaf structure to physical factors. Trans. Am. Mic. Soc. 26:19.
- Clements, E. F. 1905. Research methods in ecology.
1907. Plant physiology and ecology.
1916. Plant succession. An analysis of the development of vegetation.
- Dachnowski, A., 1910. Physiologically arid habitats and drought resistance in plants. Bot. Gaz. 49:325-339.
- Dachnowski, A., 1912. The succession of vegetation in Ohio lakes and peat deposits. Plant World 15:25-29.
- Dachnowski, A., 1912. Peat deposits of Ohio, their origin, formation and uses. Geological Survey of Ohio 4th ser. Bull. 16.
- Gates, F. C., 1914. Winter as a factor in the xerophily of certain evergreen plants. Bot. Gaz. 57:445-489.
- Hedgecock, G. G., 1902. The relation of the water content of the soil to certain plants, principally mesophytes. Rept. Bot. Surv. Nebr. 6.
- Kiesselbach, T. A., 1916. Transpiration as a factor in crop production. Nebr. Agric. Exp. Stat. Bull. 6.

- Livingston, B. C., 1904. Physical properties of bog water.
Bot. Gaz. 37:383-385.
- Livingston, B. C., 1905. Physiological properties of bog water.
Bot. Gaz. 39:348-355.
- Lloyd, F. E., 1908. The physiology of stomata.
- Pfeffer, W., 1897. Pflanzenphysiologie. Leipzig.
- Renner, O., 1910. Beitrage zur Physik der Transpiration.
Flora 100:451.
- Riggs, G. B., 1913. The effect of some Puget Sound bog waters
on the root hairs of Tradescantia.
Bot. Gaz. 55:314-326
- Riggs, G. B., 1916. A summary of bog theories.
Plant World 19:310-325.
- Sachs, J. von, 1887. Physiology of plants. Oxford.
- Sampson, A. W., and Louise Marie Allen. 1909. Influence of
physical factors on transpiration.
Minn. Not. Studies 4:33-59.
- Schimper, A.T.W., 1898. Pflanzen-Geographie auf physiologischer
Grundlage Jena.