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UNIVERSITY OF IOWA STUDIES IN NATURAL HISTORY

HENRY FREDERICK WICKHAM, Editor

VOLUME XI NUMBER 10

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CONTENTS

THE IOWA SPECIES OF RUSSULA

GRACE WINTERS

INTRODUCTION

The name *Russula* was first used by Persoon in 1796 to designate a section of the genus *Agaricus*. In his Synopsis of 1801 (5) he includes twenty-four species in the section. *R. emetica*, described by Schaeffer in 1774 as *Agaricus emeticus*, is usually regarded as the type. Among European botanists Persoon, Schaeffer, Fries, Quelet, and Romell have studied the genus extensively. In America most of the work on *Russula* has been contributed by Peck, Kauffman, (3) Beardslee, (1) and Burlingham (2).

Practically no work has been done on the local occurrence of the genus in Iowa, and in view of this fact a taxonomic study of Russulas was begun in the summer of 1924. The work has been carried on in the mycological laboratory of the State University of Iowa, under the direction of Professor G. W. Martin. The mycological herbarium contains thirty-nine determined species of *Russula* collected within the state. The only previous mention of Russulas in Iowa seems to be the three reported by Shimek (6) from the Lake Okoboji region. The thirty-nine species at Iowa City include specimens from Johnson, Dubuque, and Clayton counties, which were gathered over a period of three years from 1923 to 1926.

In this state, Russulas form a conspicuous part of the mycological undergrowth in open oak woods. Usually they make their appearance in the middle of the summer and last through early fall.

The present study is only a beginning, as much work remains to be done on this genus. It is hoped that in the future collections can be secured from various parts of the state which will permit a more comprehensive knowledge of the Russulas of Iowa. In compiling the descriptions of species constant reference has been made to the works of Beardslee, Burlingham, and Kauffman, especially the last-named. The order of species is, in general, that of Kauffman.

TECHNIQUE

For collecting Russulas no elaborate equipment is necessary; a large market-basket and plenty of newspaper are all that is needed.

A heavy knife or narrow trowel is handy to loosen the soil around the base of the stipe, for while no volva is present, the complete stipe should always be secured.

Each individual collection can readily be kept separate by wrapping in newspaper and packing loosely in a basket. This procedure is important, since many of the species are much alike and without a great deal of experience it is difficult to distinguish between them. The spores are not as liable to become mixed when each collection is kept separate, and any spore-prints deposited on a wrapper will be with the specimens that produced them.

In the study of Russulas it is essential to secure a good spore-print. Only mature pilei should be used. Cut the stipe near the pileus and place the pileus with gills down on a white paper. Cover with a beaker or bell-jar and leave for several hours. Black paper may be used where the gills look white. With mature specimens a mass of spores will be deposited whose color is distinct. Often small worms or insects are bothersome. If so, a few crystals of paradichlorbenzene under the bell-jar will kill the pests without injuring the specimens.

The value of adequate collection data can not be over-stressed. They should include date, locality, habitat, color of spore-print, taste, odor, and name of collector. These data can be effectively listed on the slip on which the spore-print is deposited. As the specimen is unwrapped, in preparation for the work in the laboratory, a small piece of the pileus may be tasted. It is not at all difficult to distinguish between the mild and acrid forms, as the difference is quite pronounced.

The taste is an extremely important taxonomic character and must be obtained when the specimen is fresh. There are several forms in the *Russula* collection at Iowa City where the spore-print is lacking and the taste not recorded, hence it has been impossible to identify them with certainty.

A record of the locality in which collections are made is important, especially when one is working over a given area such as a state. Material may be abundant in one part of the area and scanty in another. Some species of *Russula* are found only in open oak woods, others among conifers. In this state Russulas have been collected only in open oak woods. A description or mention of habitat often aids in identification.

The summer of 1924 was hot and rainy, an ideal season for fungi.

Russulas are summer and early fall forms, August being ordinarily the best month for their collection. Though the latter part of the summer of 1925 was dry, the early fall was rainy, which resulted in an excellent production of Russulas in many forms. Abundant collections were made until the middle of October, which is rather unusual for this region.

Drying of the specimens is simple. When the sun is bright enough and the atmosphere not too humid, air drying is effective. If this method can not be used, the specimens may be dried in a slightly heated oven. In the laboratory at Iowa City the specimens are placed on a wire net over a radiator and dried. In this process the heat kills the parasites. After the specimens are thoroughly dry, they are ready to be put in the herbarium, the spore-print and collection data being filed with each specimen. It is well to add a small amount of naphthalene to guard against further attack by insects.

In identification work, microscopic cross-sections of the gills are often of service. A small piece of pileus with gills attached may be placed between pieces of pith and sectioned with a razor. These sections must be sufficiently thin for the study of the structure of the hymenium and trama. Dried specimens lend themselves very nicely to sectioning. A small portion of the pileus may be soaked in water for a short time, then sectioned in the same manner as the fresh material. When the section is placed on the slide a drop of 7 per cent potassium hydroxide may be added. This causes the cells to distend, giving them a more natural appearance. An ordinary compound microscope with low and high power objectives may be employed to examine the slide. The low power gives the general contour of the section, while the higher power serves to show the details of the hymenium. An oil immersion objective is not necessary in the study of cross-sections, but is very helpful in studying the spores. It is often necessary to resort to the use of microscopic sections when identifying closely related species.

DISCUSSION OF THE GENUS

The genus *Russula* may be characterized as follows: Trama vesiculose, without milky juice; pileus fleshy, continuous with stipe, variable in color, with or without separable pellicle, dry or viscid; margin even or striate; gills attached, rigid but fragile; stem central, rigid; veil absent, spores white, creamy, or yellow; taste acrid or mild; odor none or characteristic in a few species only.

Russula is a very distinct genus, most closely related to *Lactarius*, from which it differs by its lack of a milky juice. *Hygraphorus* differs in the thicker and more waxy nature of the gills, although there are evident signs of relationship between this genus and certain species of *Russula*.

The most distinctive feature of the genus is the character of the trama, which with that of the Lactarii is most unique among the Agaricaceæ. Hyphæ of the usual slender, filamentous type, as found in the other genera, are rather scanty and interweave among clusters of thin-walled, parenchyma-like, isodiametric cells, forming the so-called vesiculose trama; this accounts for the more or less brittle consistency of the pileus.

The hymenium and subhymenium are, in some species, quite characteristic. Cystidia may or may not be present; if present, they may be few, scattered, abundant, short, blunt, clavate or long and pointed. This character is found to be constant for each species. The subhymenium may be distinct or may merge gradually into the tissue of the trama.

While the trama is the most outstanding generic character, spore-prints and spores are the most essential means of settling the identity of closely related species. The color of the spore-print is constant for each species, but may fade with age, hence all herbarium spore-prints should be accompanied by careful notes of the print when fresh. The color varies from a pure chalk-white to a rather pronounced ochre and is one of the most valuable diagnostic characters.

By staining the spores with Melzer's[1] reagent and using an oil immersion objective, the spore-markings may be determined. This reagent stains the markings on the spore wall without staining the wall itself. If this reagent is not used, the markings may be made out only by means of an oil immersion objective and even then with difficulty and uncertainty.

The spores may be divided into two groups, according to the markings; the reticulate and echinulate types. The echinulate group is further subdivided into those with long spines and those with blunt spines; the reticulate group also consists of two types, those with crests or ridges and those with fine connections. When the diagnos-

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[1] Melzer's reagent (4)

Potassium Iodide	1.5 grams
Iodine	.5 grams
Water	20.0 grams

Add to this solution an equal amount of chloral hydrate.

tic characters are almost identical, the spore markings may be the determining factor. In fact, in closely related species, the presence or absence of cystidia and the spore markings are the only differentiating factors.

The pileus may be convex, plane, or depressed in the center, and exhibits a great variety of colors: red, purple, yellow, green, and white. The specimens of the same collection may vary in color, according to light exposure or age.

A differentiated pellicle composed of more or less specialized hyphæ is present on the surface of the pileus. It may become viscid in wet weather, or may remain dry and become pruinose or velvety. The pellicle is somewhat separable along the margin and in some species may be easily peeled from nearly the whole surface.

The flesh of the pileus, when fresh, is white or whitish, or it may be tinged grayish or purplish. In many of the red forms, the flesh under the pellicle is tinged red or reddish; on exposure to the air, after bruising or in age, it may turn ochraceous or blackish.

The margin may be even or striate, or even at first, becoming striate in age. In species with thin pilei, the lines of attachment of the gills to the pileus show through as raised ridges and these striations may extend toward the center of the pileus. In the species with firm thick pilei the striations are not as clearly marked or are obscurely developed on the margin. They may appear when the plant becomes old. This character is somewhat variable and must be used with caution.

The gills are brittle, thin, with acute edges, simple or forked, of equal or unequal length. The colors for the different species are of all shades between white and deep ochre-yellow. This fact alone separates them from any spore-color groups of the Agaricaceæ. The gills may become darker with age or may stain where bruised. The shape and width are constant and are of value in identification.

The stem is central or nearly so; solid, firm, spongy or stuffed, becoming hollow, but never fibrous, usually white, sometimes red or slightly ochraceous, in some species changing to ashy or brownish where bruised.

The taste is sharply acrid in some species, slowly or slightly acrid in others, and entirely mild in a considerable number. This is an important character for the identification of the species and is fairly constant. In all species it is necessary to have fresh specimens in order to determine whether or not acridity is present. Almost all

the species are edible after careful cooking, since even the peppery forms then lose their sharp taste; in any case the mild species are said to be perfectly safe when fresh, young, and clean.

The odor of some species is quite characteristic and should always be considered. One must not confuse this test by applying it to plants already in the first stages of decay.

KEY TO THE IOWA SPECIES OF RUSSULA

Pileus some shade of red or purple 1
Pileus not some shade of red or purple 27
1– Spores pure white in mass—not creamy white 2
1– Spores not white in mass including creamy white 10
2– Taste promptly acrid 3
2– Taste mild or tardily acrid 6
3– Flesh white under pellicle 4
3– Flesh red under pellicle 5
4– Gills thin, crowded, moderately broad; margin striate; very fragile ..... *R. fragilis*
4– Gills subdistant, attached by a point; edge even ..... *R. fallax*
5– Pileus rosy to blood red, color even; pellicle separable; margin strongly tuberculate striate ..... *R. emetica*
5– Pileus darker, often purplish; pellicle adnate, scarcely separable; often yellow spotted; margin slightly striatulate in age ..... *R. atropurpurea*
6– Edge of gills flocculose-crenulate; blood-red; viscid, when dry as if with bloom; striate only when fully expanded ..... *R. purpurina*
6– Not as above 7
7– Pileus purplish or deep rose pink, later variegated with olive or greenish; flesh grayish, under pellicle; often tardily acrid ..... *R. variata*
7– Not as above 8
8– Pileus rigid, unpolished; margin obtuse; not striate; sometimes slightly bitterish or subacrid ..... *R. lepida*
8– Pileus thin; fragile 9
9– Pileus 2-5 cm. broad; pink or bright flesh, unicolorous ..... *R. uncialis*
9– Pileus 5-14 cm. broad; bright rosy red, shading into yellowish blotches ..... *R. subdepallens*
10– Taste acrid 11
10– Taste mild 15
11– Taste tardily but truly acrid 12
11– Taste promptly acrid 14
12– Pileus 6-12 cm. broad; uniform red or spotted; gills crowded, narrow, fragile, white to yellow ochraceous ..... *R. tenuiceps*
12– Not as above 13
13– Pileus rosy red; 3-6 cm. broad; spores and gills creamy white ..... *R. sanguinea*
13– Pileus Corinthian red, fading; up to 9 cm. broad; spores ochraceous in mass; gills yellowish ..... *R. corinthiirubra*
14– Pileus 2-5 cm. broad; pale dull red to rosy red; soon dry; stem white or rosy tinged ..... *R. subpunctata*
14– Pileus 5-7 cm. broad; deep rosy red; viscid when moist; stem white, never red ..... *R. veternosa*
15– Flesh white, unchanging 16
15– Flesh changing with age or where wounded, or tinged under pellicle 22
16– Pileus salmon or salmon tinged to dull red in center; margin drooping; spores maize yellow ..... *R. humidicola*
16– Pileus without salmon color or tinge; spores not maize yellow 17
17– Pileus 2.5-8 cm. broad; red to paler, yellowish on disk; margin even, slightly striate when old; stem white to yellowish at base ..... *R. luteobasis*

17– Not as above....18
18– Pileus 2-5 cm. broad; some shade of red or purple, fading to yellowish on disk; stem 2-5 cm. long, 4-6 mm. thick, slender, white....*R. chamælcontina*
18– Not as above....19
19– Pileus 2.5-5 cm. broad; rosy red to flesh red fading to yellowish on disk, stem white or rose tinged, 2.5-5 cm. long, 5-12 mm. thick, tapering upwards....*R. roseipes*
19– Not as above....20
20– Pileus rosy-flesh to peach color; fragile; gills white at first to bright ochraceous yellow....*R. amygdaloides*
20– Not as above....21
21– Pileus 8-12 cm. broad; dull colors, reddish purple, sordid red, reddish predominating; gills ochraceous becoming darker with age....*R. alutacea*
21– Pileus 5-10 cm. broad; color from buff to reddish-brown to dark dull red, fading; gills white to cream or buff....*R. integra*
22– Flesh staining slowly red then black where wounded....*R. rubescens*
22– Not staining slowly red then black where wounded....23
23– Flesh red under cuticle; firm; blood red....*R. borealis*
23– Flesh not red....24
24– Odor disagreeable in age; pileus purplish red, olivaceous, variegated; stem changing to ochraceous brown where handled....*R. xerampelina*
24– Odor none; pileus not variegated with olive tints....25
25– Purple or dark purplish red; cuticle adnate; gills yellowish to bright ochraceous buff....*R. ochrophylla*
25– Not as above....26
26– Pileus 4-7 cm. broad; rather pliant; dark dull red, sometimes blackish on disk; stem becoming ashy or blackish....*R. obscura*
26– Pileus 5-12 cm. broad; firm; orange-red to ochre on disk, darker red on margin; stem becoming cinereous....*R. decolorans*
27– Spores white in mass, not creamy white....28
27– Spores not white in mass, including creamy white....39
28– Pileus white....29
28– Pileus not white....31
29– Pileus 8-15 cm. broad; gills short and long alternating; mild to weakly acrid....*R. delica*
29– Pileus 2.5-5 cm. broad; gills not short and long alternating; acrid....30
30– Margin even....*R. albidula*
30– Margin striate....*R. fragilis*
31– Flesh white, unchanging, not tinged under cuticle....32
31– Flesh white, changing or tinged under cuticle....37
32– Stem stained at base by cinnabar red stains....*R. fœtentula*
32– Base not stained at base by cinnabar red stains....33
33– Taste tardily and slightly bitterish....34
33– Taste mild....35
34– Pileus 6-12 cm. broad; soon dry; stem 4-5 cm. long....*R. ochraleucoides*
34– Pileus 3-6 cm. broad; viscid; stem 3-4 cm. long....*R. raoultii*
35– Pileus 5-10 cm. broad; pale grayish green paler or sub-ochraceous in center; margin even; spores white tinged yellow....*R. viridella*
35– Not as above....36
36– Margin striate when mature; cystidia present; pileus with crust-like areas; viscid when young or moist....*R. crustosa*
36– Margin not striate; no cystidia; pileus with floccose pulverulent areas; dry....*R. virescens*
37– Pileus whitish, clouded with umber; flesh changing to reddish where bruised then blackish....*R. nigricans*
37– Not as above....38
38– Pileus 3-7 cm. broad; straw color to brown; striate....*R. pectinatoides*
38– Pileus 5-12 cm. broad; variegated; not striate....*R. variata*
39– Flesh white, not changing, tinged under pellicle....40
39– Flesh changing with age or tinged under pellicle....41

40– Pileus purple-brown on disk; margin dull garnet; mild to somewhat nauseous ..... *R. nauseosa*
40– Not as above 42
41– Pileus buff to reddish brown to dull red; spore-print cream yellow to pale ochraceous ..... *R. integra*
41– Pileus umber-yellow to golden yellow; spore-print maize yellow ..... *R. flaviceps*
42– Flesh thick on disk, thin elsewhere; grayish or grayish purple under pellicle; odor unpleasant when fresh ..... *R. xerampelina*
42– Flesh not grayish nor grayish purple under pellicle 43
43– Pileus 3-7 cm. broad; from dingy straw color through to umber brown; margin striate ..... *R. pectinatoides*
43– Pileus 5-8 cm. broad; dull yellow; even or slightly striate in age ..... *R. flava*

DESCRIPTION OF SPECIES

1. *Russula delica* Fr.

Pileus 8-15 cm. broad, firm, convex—umbilicate then depressed to infundibuliform, *dull white*, sometimes with rusty-brown stains, *unpolished*, glabrous, pubescent or obscurely tomentose, even, dry, margin at first involute, not striate.

Flesh compact, white or whitish, not changing where bruised.

Gills subdecurrent, narrowed behind, broader in middle, distant or subdistant, thickish, short and long alternating, few forked, *white or whitish*, edge often distinctly greenish.

Stem 2-5 cm. long, 1.5-2 cm. thick, *short*, stout, *solid*, equal or subequal or tapering down, white becoming dingy, not turning blackish when bruised, glabrous or subtomentose above, often with a narrow pale-green zone at apex.

Taste mild to tardily but weakly acrid.

Odor none.

Spores globose, 9-10 microns, tubereulate, white in mass. This species is reported by Shimek from the Okoboji region. I have not collected it.

2. *Russula nigricans* Fr.

Pileus 7-15 cm. broad, subrigid, convex then depressed to subinfundibuliform; margin at first incurved then spreading and elevated, often irregularly wavy, at first whitish and clouded with umber, *soon smoky-umber*, subviscid at first, glabrous, even on margin.

Flesh, compact, white, *changing to reddish* where bruised, *then blackish*.

Gills narrowed or rounded behind, adnexed, thick and firm, *subdistant to distant*, short and long alternating, white becoming grayish, reddish at first when bruised.

Stem 2-6 cm. long, 1-3 cm. thick, *solid,* hard, stout, white at first, at length *smoky-umber, reddish then blackish where bruised.*

Taste mild, sometimes tardily but slightly acrid.

Odor none.

Spores subglobose, 8-10 microns, reticulate, white in mass.

Cystidia short and blunt.

3. *Russula virescens* Fr.

Pileus 5-12 cm. broad, at first globose, soon convex and expanded, often somewhat depressed, firm, *dry* or velvety, surface of disk broken into many floccose or pulverulent areas or patches, green or grayish green, the margin not striate or rarely so, cuticle scarcely distinguishable or separable.

Flesh white.

Gills white, rather close, narrowed towards stem, almost or entirely free, few shorter and forked.

Stem 3-7 cm. long, 1-2 cm. thick, white, firm, equal or subequal, solid or spongy.

Spores subglobose, 6-8 microns, echinulate, with few very fine reticulations, spines scattered and blunt, white in mass.

Cystidia none, no differentiated subhymenium.

4. *Russula crustosa* Pk.

Pileus 5-12 cm. broad, firm, convex then expanded and depressed in the center, surface cracked except on disk, the *areas crustlike,* sordid cream-color, dirty brownish or ochraceous, usually tinged with olive or green, viscid when young or moist, especially on the disk, *striate on margin* when mature.

Flesh white.

Gills dull white, dingy cream color in age, rather broad in front, narrowed toward stem, adnexed or free, *thick, distinct,* not crowded, rather brittle, few forked, few short.

Stem 3-6 cm. long. 1-2.5 cm. thick, short, stout, spongy-stuffed, subequal, ventricose or white.

Taste mild.

Odor none.

Spores broadly ovate, 7-8 x 8-10 microns, reticulate, white in mass.

Cystidia rather numerous extending through subhymenium.

5. *Russula viridella* Pk.

Pileus subglobose or very convex, becoming nearly plane or centrally depressed, 5-10 cm. broad, surface pale grayish-green, paler or subochraceous in the center, dry, soon minutely squamulose or furfuraceous, except in the center, margin even.

Flesh white.

Gills white, few short ones present, some forked, thin, narrow, close.

Stem white, equal or nearly so, even, solid or spongy within, 5-7.5 cm. long, 1-1.6 cm. thick.

Taste mild.

Odor none.

Spores subglobose, 7-8 microns in diameter, reticulations very fine, white tinged with yellow in mass.

Cystidia subfusiform 80 x 16 microns.

6. *Russula ochraleucoides* Kauff.

Pileus 6-12 cm. broad, large, rigid, convex, soon expanded-plane, varying *straw yellow to pale ochraceous*, usually dull ochre to reddish ochre toward center, pellicle adnate, soon dry, pulverulent or subrimose, even on the obtuse margin.

Flesh, *thick*, compact, white, unchanging or slightly sordid in age.

Gills adnexed or free, rather narrow, rounded or slightly broader in front, *white* or whitish, close to subdistant, shorter ones intermingled, often forked in posterior part, intervenose.

Stem 4-6 cm. long, 1.5-2 cm. thick, short, rigid, equal or tapering slightly downward, white, glabrous or subpruinose, spongy-solid, even or obscurely wrinkled.

Taste tardily and slightly bitterish, acrid or disagreeably bitter.

Odor faintly aromatic or none.

Spores globose, 7-9 microns, reticulate, white in mass.

Cystidia few to moderately abundant.

7. *Russula raoultii* Quel.

Pileus broadly convex, then plane or slightly depressed in center; 3-6 cm. broad, surface straw-yellow or massicot yellow, viscid, glabrous, margin even or at length very faintly striate.

Flesh pure white, unchanging.

Gills white, some short ones intermingled, rarely forking next to the stipe, interspaces slightly venose, narrow, 2-6 mm. broad, acute at the inner ends, close.

Stem white, not changing color, somewhat pruinose, tapering downward, stuffed, 3-4 cm. long, 1-2 cm. thick.

Taste tardily peppery.

Odor none.

Spores globose, 6-9 microns in diameter, reticulate, white in mass.

Cystidia rather abundant.

8. *Russula lepida* Fr.

Pileus 4-10 cm. broad, *rigid,* convex, then expanded-depressed, cuticle adnate, and disappearing on disk, *unpolished, soon dry,* rose-red to pale blood-red, fading, disk soon pallid or variegated with paler yellowish-reddish hues, sometimes rimulose-cracked or rugulose on disk, margin obtuse, not striate.

Flesh compact, white or reddish under the cuticle, thick, abruptly thin on margin.

Gills narrowed behind and narrowly adnate or almost free, close, *rather narrow,* broader and rounded in front, *white then whitish,* few shorter, occasionally forked.

Stem 4-7 cm. long, 1-2 cm. thick, equal or slightly tapering downward, *white or tinged rosy-pink,* spongy-stuffed, rather rigid, obscurely wrinkled.

Taste *mild,* sometimes slightly bitterish, subacrid.

Odor none or very slightly disagreeable.

Spores subglobose, 7-8 x 9-10 microns, reticulate, white in mass.

Cystidia moderately abundant, subcylindrical, 70-75 x 10-12 microns.

9. *Russula fœtentula* Pk.

Pileus 3-7 cm. broad, soon fragile, at first subhemispherical then convex to plane and depressed, *viscid,* livid-ochraceous, russet-tinged, disk darker and innately granular, long tuberculate-striate, margin at first incurved.

Flesh thin, whitish.

Gills adnexed or nearly free, close, rather narrow, broader in front, thin, whitish, *often spotted or stained reddish.*

Stem 2.5-5 cm. long, 6-12 mm. thick, subequal, somewhat firm, spongy-stuffed, soon cavernous, whitish or sordid-white, *stained at base by cinnabar-red stains.*

Taste very slightly acrid.

Odor none or somewhat like oil of bitter almonds, varying in intensity.

Spores 6-7 x 7-9 microns, echinulate, creamy-white in mass.
Cystidia moderately abundant.

10. *Russula pectinatoides* Pk.

Pileus 3-7 cm. broad, rather firm, becoming fragile, thin, convex, then plano-depressed, viscid when moist, covered by a thin separable pellicle, radiately rugose-striate on the margin, often half way to the center, or *strongly tubercular-striate*, dingy straw color, brownish, yellowish-brown or umber-brown.

Flesh white, thin, becoming fragile, slightly *ashy under the cuticle*, not changing.

Gills whitish, close to subdistant, thin, distinct, equal, moderately broad, broadest in front, narrowed behind, often stained or broken half way from stem, some forked at base.

Stem 2-5 cm. long, 5-1 cm. thick, white or dingy, subequal glabrous, *spongy-stuffed, then hollow*, even.

Taste mild or slightly and tardily acrid.

Odor not noticeable.

Spores subglobose, 6-8 microns, echinulate, white in mass.

Cystidia few.

11. *Russula subpunctata* Kauff.

Pileus 2-5 cm. broad, *rigid*, convex then expanded-plane to depressed, cuticle adnate and scarcely separable on margin, subviscid, soon dry, *pale dull red to rosy red*, often white-spotted where cuticle disappears, minutely rivulose or subgranular, margin even, acute.

Flesh compact, firm, rather thick on disk, abruptly thin on margin.

Gills adnate to subdecurrent, thin, slightly alternate at both ends, not broad, close to subdistant, whitish, then *pale, cream-colored*, few short or forked at base, pruinose, intervenose.

Stem 2-4 cm. long, 4-10 mm. thick, subequal or tapering down, spongy-stuffed, becoming cavernous, white or rosy-tinged, unchanging, attached at times to roots and forming mycorrhiza.

Taste quickly and very acrid.

Odor none.

Spores subglobose, 7-9 x 9-11 microns, reticulate, *creamy white in mass.*

Cystidia abundant, subcylindrical, 90-110 x 8-12 microns.

12. *Russula variata* Banning and Pk.

Pileus 5-12 cm. broad, fleshy, firm, convex then depressed to subinfundibuliform, viscid, *not striate*, purplish or *deep rose pink when*

young, later variegated with olive or dark umber, or sometimes *greenish* with only a trace of *purple*, opaque and reticulate-wrinkled under lens, the thin pellicle slightly separable on the thin margin, with a subsilky or dull lustre when dry.

Flesh white, firm, cheesy, tinged grayish under pellicle.

Gills shining and persistently white, adnate-decurrent, thin, *rather crowded*, narrowed at both ends, not broad, *subdichotomously forked*, interspaces venose.

Stem 4-7 cm. long, 1-3 cm. thick, white, firm, solid, equal or subequal, sometimes tapering downward, even.

Taste mild to tardily acrid or slightly astringent.

Odor none.

Spores subglobose, 6-7 x 9-10 microns, echinulate with very fine reticulations, white in mass.

Cystidia few and short.

13. *Russula atropurpurea* Maire

Pileus 5-14 cm. broad, rigid, medium to large size, convex then plane, soon depressed, rather firm, viscid, pellicle adnate and scarcely separable on the margin only, scarlet to dark crimson when fresh and young, *becoming darker to purplish when mature or on dying*, pruinose, disk often darker, sometimes blackish, red to livid olivaceous purple, sometimes yellow spotted; margin even or only slightly striatulate in age.

Flesh dark red under the pellicle, white elsewhere, not changing to ashy.

Gills white, dingy in age, rather narrow, close behind, subdistant in front, adnexed, few short, interspaces venose.

Stem 4-7 cm. long, 1-3 cm. thick, subequal, medium stout, white with a dull lustre, *pruinose*, even, spongy-stuffed, apex floccose-punctate.

Taste acrid.

Odor none.

Spores oval, 8-10 microns, reticulate, white in mass.

Cystidia numerous.

This is the form described under this name by Kauffman. *R. atropurpurea* as used by Burlingham and others seems to be quite distinct. See note under *R. xerampelina*.

14. *Russula xerampelina* Fr.

Pileus 5-10 cm. broad, firm, convex then plano-depressed, dry or very slightly viscid in wet weather; pellicle hardly separable; not

striate on margin; surface glabrous or subpruinose, purplish-red to purplish-olive, disk olivaceous variegated.

Flesh compact, whitish then dingy.

Gills creamy, *creamy-white to creamy-yellowish*, then sordid, rather close, adnexed, moderately broad throughout, thickish, often forked, shorter ones usually intermingled, interspaces venose.

Stem white or rosy-tinged, soon dingy olivaceous-yellowish where handled, 5-7 cm. long, 1.5-2.5 cm. thick, firm, subventricose or equal, spongy-stuffed, even or obscurely wrinkled, changing where bruised to dirty *ochraceous-brown*.

Taste mild.

Odor disagreeable with age or when dying.

Spores subglobose, 7-8 x 9-10 microns, echinulate with a few reticulations, creamy-yellowish in mass.

Cystidia present, not extending much beyond basidia.

I include in this species forms that grade into *R. squalida* Pk. and *R. atropurpurea* Pk. since I fail to find in our specimens any sharp distinctions which seem to justify separation.

15. *Russula ochrophylla* Pk.

Pileus fleshy, firm, convex, becoming nearly plane or slightly depressed in the center, 5-10 cm. broad, surface purple or dark-purplish-red, dry, cuticle not easily separable, unpolished, glabrous, margin even, rarely very slightly striate when old.

Flesh white, purplish under the adnate cuticle.

Gills yellowish at first, becoming bright ochraceous-buff when mature, pruinose, equal, few forked at the stipe, venose-connected, subdistant, adnate.

Stem reddish or rose-tinted, paler than the pileus, white in one variety, solid, spongy within, equal or nearly so, 3.5-6.5 cm. long, 1-2 cm. thick.

Taste mild, edible.

Odor none.

Spores globose, 10 microns, echinulate with a few reticulations, bright ochraceous in mass.

Cystidia moderately abundant, rather short.

16. *Russula decolorans* Fr.

Pileus 5-12 cm. broad, *often large*, firm, globose at first then convex and plano-depressed, *orange-red* usually ochre on disk and dark

red on margin, pellicle separable, subviscid, margin even, slightly striate in age.

Flesh white, *becoming cinereous with age*, or where broken, becoming fragile.

Gills pale yellowish-ochraceous at maturity, white at first, thin, fragile, moderately broad, close, adnexed, forked at base, few short.

Stem 5-12 cm. long, 1-2.5 cm. thick, *stout*, long spongy or solid, wrinkled-rivulose, white, *the flesh becoming cinereous with age or where bruised.*

Taste mild.

Odor none.

Spores subglobose, 7-9 microns, reticulate, pale ochraceous-yellow in mass.

Cystidia very numerous.

17. *Russula flava* Romell

Pileus 5-8 cm. broad, *rather fragile*, convex, then plano-depressed, even or *slightly striate in age*, dry in dry weather, somewhat viscid when moist, pellicle separable, *dull yellow*, color hardly fading, but sometimes ashy, discolored in age.

Flesh white becoming cinereous with age.

Gills white at first, becoming yellowish, broadest towards front, narrowly adnate, close, distinct, becoming slowly gray in age.

Stem chalk-white at first, the flesh becoming ashy, equal or subequal, spongy-stuffed, obscurely reticulate-rivulose, rather fragile, 6-8 cm. long, 1-2 cm. thick.

Taste mild.

Odor none.

Spores globose, 8-9 microns, echinulate with a few reticulations, yellowish in mass.

Cystidia present, rather short.

18. *Russula obscura* Romell

Pileus 4-7 cm. broad, rather pliant, convex then plano-depressed, dull, *dark blood-red,* pileus sometimes blackish on disk, thin, the pellicle continuous and separable, hardly viscid when moist, *subpruinose* when dry, even or slightly striate in age.

Flesh whitish, becoming ashy.

Gills white at first, then dingy straw-color, moderately broad, narrowly adnate, close, mostly forked at base, equal, interspaces sometimes venose.

Stem white, becoming ashy or blackish, rarely tinged red, subequal, 4-6 cm. long, 10-15 mm. thick, spongy-stuffed, rigid, soon soft, obscurely wrinkled.

Taste mild.

Odor none.

Spores subglobose to ovate, 7-8 x 8-10 microns, echinulate, pale ochraceous in mass.

Cystidia rather numerous, subulate.

19. *Russula rubescens* Beards.

Pileus 4-10 cm. broad, firm, becoming fragile, convex-plane, *dull red, variegated* with yellowish, ochraceous or olivaceous-purplish hues, at first darker, fading, pellicle adnate, dry, scarcely separable and substriate on the margin, subglabrous, margin acute at first.

Flesh whitish, *staining slowly red then black where wounded*, becoming cinereous with age.

Gills narrowly adnate, broader in front, close to subdistant, medium broad, *equal*, rarely forked, white at first then pale creamy-ochraceous, intervenose.

Stem 3-7 cm. long, 1-2.5 cm. thick, subequal or tapering down, spongy-stuffed, glabrous, even, white, becoming cinereous in age, *changing slowly* to red then blackish where bruised.

Taste mild.

Odor none.

Spores globose, 7-10 microns, echinulate, pale ochraceous in mass.

Cystidia few and short, subhymenium not differentiated.

This species is very abundant in the vicinity of Iowa City.

20. *Russula borealis* Kauff.

Pileus 5-9 cm. broad, *firm and rather compact*, convex then plano-depressed, outline broadly elliptical, often with a sinus on one side, blood-red, disk darker or color uniform and not fading, pellicle somewhat separable, hardly viscid, margin even or obscurely striate.

Flesh white, red under the cuticle, not very thick.

Gills ochraceous, subdistant or moderately close, medium, broad, broader in front, narrowly adnate, rather distinct, edge often reddish anteriorly, equal, a few forked toward base, interspaces venose.

Stem mostly white, tinged red in places; *firm*, spongy-stuffed, thickened below, 5-7 cm. long, 1.5-2 cm. thick.

Taste mild, sometimes slightly and tardily acrid.

Odor none.

Spores subglobose, 7 x 9.5 microns, echinulate with few reticulations, deep ochraceous-yellow in mass.

Cystidia few and pointed.

21. *Russula alutacea* Fr.

Pileus 8-15 cm. broad, large, firm, convex then depressed, with dull colors, dark reddish-purple, sordid red, sometimes mixed with other shades, the reddish color predominating, with somewhat separable pellicle, glabrous, somewhat viscid in wet weather, soon dry, *pruinose and subgranulose*, margin even or somewhat short-striate in age.

Flesh white thick.

Gills *ochraceous from the beginning*, deeper ochraceous to tan-colored when mature, *rather broad*, thick, *subdistant*, broader in front, rounded adnexed, of *equal* length.

Stem 7-10 cm. long, 3-4 cm. thick, very *firm*, *stout*, solid, *tinged red* or entirely white, subequal or ventricose, almost even.

Taste mild.

Odor none or pleasant.

Spores subglobose, 9-11 microns, echinulate, ochraceous-yellow to alutaceous in mass.

Cystidia present.

22. *Russula nauseosa* Fr.

Pileus broadly convex, becoming plane to depressed, up to 5 cm. broad, surface purple-brown at the center, shading to garnet-brown or dull garnet toward the margin, sometimes becoming pale, viscid when wet, with the pellicle separable, glabrous, margin becoming tuberculate-striate or furrowed.

Flesh white, fragile.

Gills light yellow, then dingy ochraceous, with a few shorter ones intermingled here and there, adnexed, ventricose, somewhat distant.

Stem white, up to 2.5 cm. long, and about .8 cm. thick.

Taste mild, but somewhat nauseous.

Odor disagreeable with age.

Spores 8-9 microns in diameter, echinulate, yellow in mass.

Cystidia present.

23. *Russula emetica* Fr.

Pileus 5-10 cm. broad, fleshy, *soon fragile*, convex to plano-depressed, *rosy to blood-red*, sometimes faded to white, pellicle separ-

able, *margin strongly tubercular-striate* or even sulcate, viscid and shining.

Flesh white, *red under the cuticle.*

Gills pure white, subdistant to close, distinct, rather broad, equal, broadest toward front, narrowly adnexed or free, interspaces venose.

Stem 4-7 cm. long, 1-2 cm. thick, white or tinged red, subequal, spongy-stuffed, even.

Taste very acrid.

Odor none.

Spores subglobose to globose, 7.5-10 microns, reticulate, white in mass.

Cystidia numerous.

Our specimens of this species are usually small. The pilei, rarely, if ever, reach a diameter of 10 cm.

24. *Russula fragile* Fr.

Pileus 2.5-5 cm. broad, *very thin* and fragile, convex then plano-depressed with a thin viscid pellicle, tubercular-striate on the thin margin, glabrous, rather uniform rosy or pale red, sometimes faded or bleached to white.

Flesh *white under the pellicle*, thin.

Gills white, thin, crowded, adnexed, ventricose, moderately broad.

Stem 2.3-5 cm. long, .5-1 cm. thick, white, spongy then hollow, equal, fragile.

Taste promptly and very acrid.

Order none.

Spores subglobose, 8-9 microns, echinulate with a few reticulations, white in mass.

Cystidia very numerous and rounded.

25. *Russula fallax* Cke.

Pileus 3-7 cm. broad, *thin, fragile*, color incarnate or pale rose, the *disk* pale *olivaceous or livid*, sometimes darker or purplish, soon plane or slightly depressed on the disk, quite viscid, margin striate and becoming elevated, surface faintly rugulose under lens.

Flesh white.

Gills white, unchanged, subdistant, attached by a point, *narrow*, edge even.

Stem 3-4 cm. long, 6-10 mm. thick, pure white, cylindrical or compressed, equal, spongy-stuffed, soon hollow.

Taste promptly and very acrid.

Odor none.

Spores subglobose, 7-9 x 9-10 microns, reticulate, white in mass.

Cystidia present, short.

26. *Russula albidula* Pk.

Pileus 2-5 cm. broad, *white*, broadly convex, glabrous, pellicle *viscid* and separable when fresh, margin even.

Flesh white, subfragile.

Gills white, moderately crowded, adnexed, not broad, of equal length, some forking at base, interspaces venose.

Stem 2.5-4 cm. long, 8-12 mm. thick, white, equal, spongy-stuffed, even.

Taste acrid.

Odor none.

Spores subglobose, 7-10 microns, reticulate, white in mass.

Cystidia present, rather few.

27. *Russula sanguinea* Fr.

Pileus 3-6 cm. broad, rather firm at first, subfragile, convex-plane or depressed, *rosy-red*, viscid, margin acute and thin, pellicle subadnate, easily separable on margin and tubercular-striate.

Flesh rather thin, white, red under pellicle.

Gills slightly adnate, close to subdistant, *equal*, not broad, *creamy-white*.

Stem 4-6 cm. long, subequal or tapering downward, often eccentric, *white or tinged rosy-red*, spongy-stuffed then cavernous, rather fragile, glabrous, even.

Taste tardily but truly acrid.

Odor none.

Spores globose, 8-10 microns, echinulate, creamy-white in mass.

Cystidia present, few.

28. *Russula corinthiürubra* Burl.

Pileus becoming plane or slightly depressed in the center, up to 9 cm. broad, surface Corinthian-red, fading, the center becoming tinged with maize-yellow, viscid, with the pellicle separable half way to the center, glabrous, margin becoming slightly striate-tuberculate.

Flesh white, tinged red next to pellicle.

Gills becoming yellow, equal, some forked next to the stipe, venose-connected, narrow at the inner ends, broad and ventricose toward the outer; rather thick, close.

Stem white, firm, nearly equal, 4 cm. long, 1.7 cm. thick.

Taste slowly acrid.

Odor none.

Spores subglobose, 7-8 x 9-10 microns, reticulate, ochraceous in mass.

Cystidia very numerous, rounded at tip.

29. *Russula tenuiceps* Kauff.

Pileus 7-12 cm. broad, thin, fragile, convex to expanded, the somewhat viscid pellicle easily separable, margin at first connivent, *striate*, deep rosy or blood red, sometimes white, spotted or tinged with orange blotches, sometimes uniform red, with or without minute rugæ.

Flesh white, red under cuticle, *very fragile at maturity.*

Gills white, then yellow-ochraceous, crowded, narrow, fragile, narrowly adnate to free, few forked, interspaces venose, equal.

Stem fragile, white or rosy-tinged, spongy-stuffed, subequal or ventricose, obscurely rivulose, white within and unchanged, 5-9 cm. long, 2-2.5 cm. thick.

Taste acrid, sometimes tardily but very acrid.

Odor not marked.

Spores subglobose, 6-9 x 8-11 microns, echinulate, yellow-ochraceous in mass.

Cystidia present.

30. *Russula veternosa* Fr.

Pileus 5-7.5 cm. broad, convex then expanded, with a somewhat separable pellicle, *indistinctly striate* on the margin, deep rose-red, viscid when moist.

Flesh white, red under the cuticle.

Gills white at first, *then straw-color or pale ochraceous*, narrow, adnate, close, broader in front, equal or few shorter, few forked, interspaces venose.

Stem white, *never red*, equal or subequal, spongy-stuffed, somewhat slender, fragile, hollow, even, 4-5 cm. long, 1-1.5 cm. thick.

Taste very acrid.

Odor none.

Spores subglobose, 8-10 microns, echinulate, yellowish-ochraceous in mass.

Cystidia numerous.

31. *Russula subdepallens* Pk.

Pileus 5-14 cm. broad, *fragile,* convex then plane and depressed, margin elevated in age, *bright rosy-red,* shading into yellowish blotches as if the red color were put over the yellow, disk paler in old specimens, disk dark red in very young plants, with a thin separable, viscid pellicle, *tubercular-striate* on margin, obscurely wrinkled elsewhere.

Flesh white-rosy under cuticle, becoming slightly cinereous, very fragile, thin.

Gills white, broad in front, narrowed behind, adnate, subdistant, few forked, interspaces venose.

Stem white, spongy-stuffed, rather stout, 4-10 cm. long, 1-3 cm. thick, subequal.

Taste mild.

Odor none.

Spores globose, 7.5-8 microns, echinulate with fine reticulations, white in mass.

Cystidia none.

32. *Russula purpurina* Quel. and Schultz

Pileus 3-7 cm. broad, fragile, *viscid,* usually very viscid, subglobose then expanded and slightly depressed at the disk, *brilliant rosy-red to blood-red* or even darker, pellicle somewhat separable, margin thin but *not striate* except when fully expanded, surface when dry as if with a bloom.

Flesh white, red under the cuticle, thin, fragile, unchangeable.

Gills white, later dingy-white or yellowish; medium close to subdistant, adnexed, not broad, broadest in front, mostly equal, few or none forked, interspaces sometimes venose, edge floccose-crenulate.

Stem rather long, 5-8 cm. long, 8-12 mm. thick, sprinkled rosy-pink, equal or subequal, spongy-stuffed, fragile but rather soft.

Taste mild.

Odor none.

Spores subglobose to globose, 6-8 x 8-10 microns, reticulate, white in mass.

Cystidia present.

33. *Russula uncialis* Pk.

Pileus 2-5 cm. *broad,* thin, rather fragile, convex then expanded-depressed, *pink or bright flesh-color,* unicolorous, the rather adnate pellicle slightly separable, slightly viscid when moist, pruinose and

pulverulent when dry, margin not striate till old.

Flesh white, pink under pellicle, unchanged.

Gills pure white, scarcely changing with age, rather broad, broadest in front, narrowed behind and adnate; subdistant or moderately close, distinct, entire on edge, few forked, interspaces venose.

Stem white, rarely tinged pink, rather short, 1-3.5 cm. long, 4-10 mm. thick, spongy-stuffed, equal, glabrous.

Taste mild.

Odor none.

Spores subglobose, 7-8 microns, echinulate with a few reticulations, white in mass.

Cystidia few.

This species with us is generally fully 5 cm. broad but in other respects agrees with the descriptions.

34. *Russula integra* Fr.

Pileus 5-10 cm. broad, firm, *soon fragile*, discoid, convex or campanulate then plano-depressed, covered with a *viscid separable pellicle*, thin on the margin, at length *coarsely tubercular-striate*, variable as to color in different plants, *colors dingy or sordid*, from buff through to reddish-brown and dark dull red, *fading*.

Flesh white not changing.

Gills white at first, then creamy-yellow to buff-ochraceous, not strongly ochre, *broad*, *distant*, equal, nearly free.

Stem white, unchanged, never red, soon quite fragile, conic or short-clavate at first, then subequal or ventricose, spongy-stuffed, even.

Taste mild.

Odor none.

Spores subglobose, 8-9 x 9-10 microns, echinulate, creamy-yellow to pale ochraceous in mass.

Cystidia none.

35. *Russula amygdaloides* Kauff.

Pileus 4-8 cm. broad, thin, medium size, ovate at first with a straight margin, then convex-plane or depressed, very viscid, *fragile*, pale rosy-flesh color tinged with yellow, sometimes *peach color*, sometimes dull citron-yellow, varying in color from young to old, pellicle continuous and entirely separable, margin becoming strongly tuberculate-striate.

Flesh thin, white, not changing color, soft.

Gills bright ochraceous-yellow, white at first, rather narrow, broadest in front, narrowed and adnexed behind, subdistant at maturity, dusted by spores.

Stem 4-8 cm. long, 1-2 cm. thick, subequal to ventricose, soft and fragile, loosely stuffed then cavernous (but not from grubs), white, rarely tinged with delicate pink, slightly wrinkled, subglabrous.

Taste mild.

Odor none.

Spores subglobose, 7-9 microns, echinulate with very few reticulations, bright ochre-yellow in mass.

Cystidia very few.

36. *Russula flaviceps* Pk.

Pileus convex, then expanding and slightly depressed in the center, 5-10 cm. broad, surface amber-yellow to golden-yellow, viscid, with a separable pellicle, glabrous, margin even, when young, faintly striate when old.

Flesh white.

Gills white, soon becoming Naples-yellow and dusted with spores, equal, simple, adnate or slightly rounded next to the stipe, broader at the outer ends, rather narrow, close.

Stem white, equal or nearly so, stuffed or spongy within, 4-6.5 cm. long, 8-12 mm. thick.

Taste mild or slightly acrid.

Odor none.

Spores subglobose, 8-9 x 9-10 microns, echinulate, maize-yellow in mass.

Cystidia very few.

37. *Russula chamæleontina* Fr.

Pileus 2-5 cm. broad, *rather small*, fragile, thin, plano-depressed, with a viscid separable pellicle, margin even at first then striatulate, *color varying for different pilei*, mostly same shade of red, purple, etc., fading to yellowish especially on disk.

Flesh white, thin.

Gills thin, crowded or close, adnexed or almost free, equal, rather broad, sometimes almost narrow, few forked, interspaces venose, ochraceous or ochraceous yellow.

Stem 2-5 cm. long, 4-6 mm. thick, white, spongy-stuffed then hollow, *slender*, equal or subequal to subventricose, sometimes subclavate, even or obscurely rivulose.

Taste mild.

Odor none.

Spore subglobose to globose, 6-8 x 8-10 microns, echinulate with a few reticulations, ochraceous in mass.

Cystidia present.

38. *Russula humidicola* Burl.

Pileus broadly convex, soon becoming depressed in the center, up to 6 cm. broad, surface varying in color from salmon, reddish-salmon, and yellowish salmon to Morocco-red in the center, sometimes fading, viscid, with pellicle separable except on disk, glabrous, margin drooping, soon tuberculate-striate.

Flesh thin, white fragile.

Gills white, becoming cream-colored, equal, rarely forking next to the stipe, interveined, acute, narrow and nearly free at the inner ends, broad and rounded at the outer, close, thin, pruinose.

Stem white, nearly equal, spongy, then hollow, 3-5 cm. long, 5-10 mm. thick.

Taste mild.

Odor none.

Spores globose to elliptic, 5-6 x 7 microns, echinulate with few reticulations, maize-yellow in mass.

Cystidia present, short and blunt.

39. *Russula luteobasis* Pk.

Pileus convex, then nearly plane, 2.5-8 cm. broad, surface at first rosy or red, then paler; yellowish in center, viscid when wet, cuticle separable, glabrous, margin even, indistinctly striate when old.

Flesh white or whitish.

Gills white to cream-yellow or pale-ochraceous when old or in dying, equal, not forking, except near the stipe, adnexed to adnate, rather close, broad.

Stem white, yellow to orange-yellow at base, subequal, stuffed, 2.5-7 cm. long, .4-2 cm. thick.

Taste mild.

Odor none.

Spores subglobose, 7-8 x 8-9 microns in diameter, reticulate, yellow-ochraceous in mass.

Cystidia none.

40. *Russula roseipes* (Secr.) Bres.

Pileus 2.5-5 cm. broad, *thin, fragile,* convex then plano-depressed, with a viscid, separable pellicle, margin tubercular-striate when mature, soon dry, *rosy-red* or *flesh-red,* disk tending to ochre-yellowish.

Flesh white, thin, unchanged.

Gills soon truly ochraceous, subdistant, mostly equal, broadest in front, ventricose, narrowly adnate or almost free, few forked, interspaces venose.

Stem white and rosy-sprinkled, stuffed then cavernous, equal or tapering upward, even, 2.5-5 cm. long, 5-12 mm. thick.

Taste mild.

Odor none or pleasant.

Spores subglobose to globose, 7-8 x 9-10, microns, echinulate with few reticulations, ochraceous in mass.

Cystidia none.

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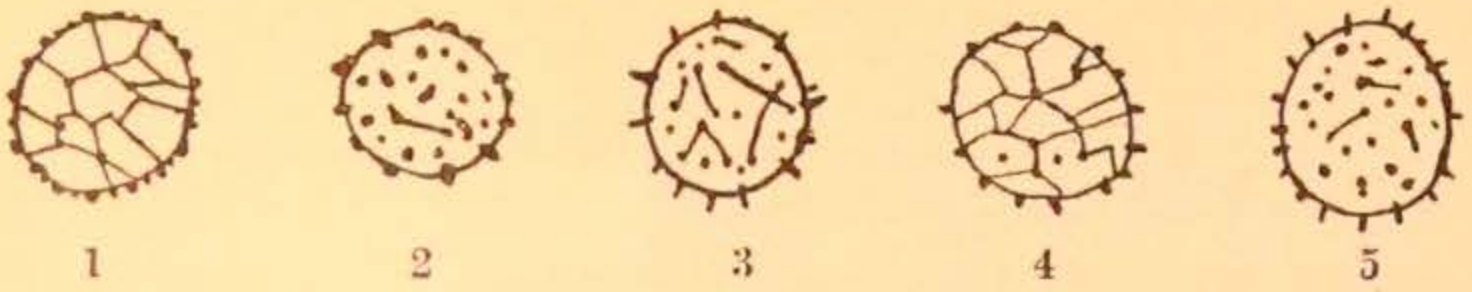
EXPLANATION OF PLATE

Spores of Russula

1. R. albidula
2. R. alutacea
3. R. amygdaloides
4. R. atropurpurea
5. R. borealis
6. R. chamæleontina
7. R. corinthiirubra
8. R. crustosa
9. R. decolorans
10. R. emetica
11. R. fallax
12. R. flava
13. R. flaviceps
14. R. fœtentula
15. R. fragilis
16. R. humidicola
17. R. integra
18. R. lepida
19. R. luteobasis
20. R. nauseosa
21. R. nigricans
22. R. obscura
23. R. ochraleucoides
24. R. ochrophylla
25. R. pectinatoides
26. R. purpurina
27. R. raoultii
28. R. roseipes
29. R. rubescens
30. R. sanguinea
31. R. subdepallens
32. R. subpunctata
33. R. tenuiceps
34. R. uncialis
35. R. variata
36. R. veternosa
37. R. virescens
38. R. viridella
39. R. xerampelina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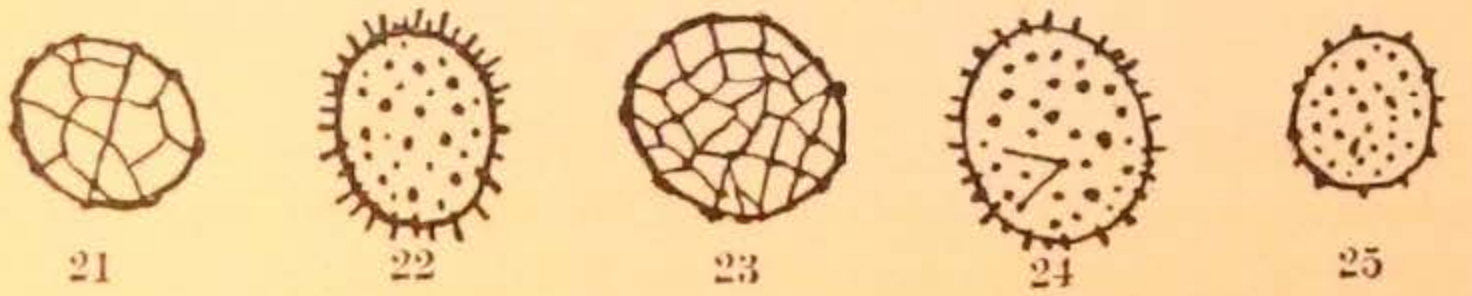
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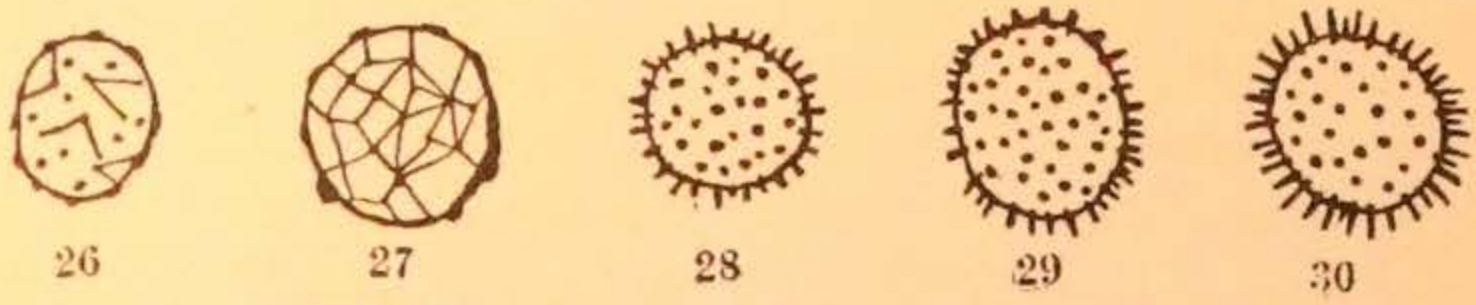
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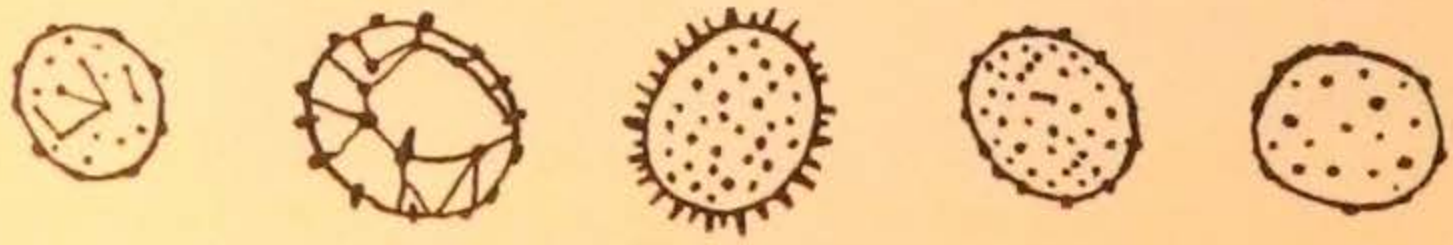
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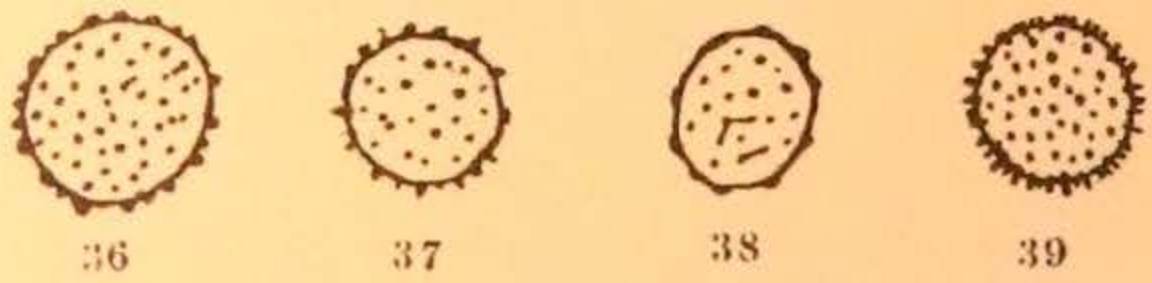
36 37 38 39

OCCURRENCE OF MYCORRHIZA IN IOWA FOREST PLANTS

M. L. Lohman

INTRODUCTION

The root-fungus association in the higher plants has had attention in botanical literature since the middle of the nineteenth century. The classical investigations of Kamienski (1881), Frank (1885), and Stahl (1900) gave an impetus to this phase of mycological and physiological research which has resulted in a continuous series of papers up to the present time. Most investigations have been made by European workers, and the most recent studies are those of Demeter (2), Melin (9, 10, 11, 12, 13), and Peyronel (17) on the continent, and Rayner (18, 19, 20, 21) in England.

The term "mycorrhiza" was first used by Frank (3) to designate that condition of a fungus-root system in which the fungus mycelium forms a weft of interwoven hyphæ about the root-tips of the higher plants. This term was adopted by later workers and it is the accepted term to-day, although it has been extended to include those conditions in which the fungus hyphæ are present in parts other than the root, or throughout the entire plant. The term "mycotrophic" has been suggested as more accurate, especially when applied to such plants as *Corallorrhiza*, in which there are no roots (Skene, 22). "Mycorrhiza" has also been used quite loosely by some writers to imply in one instance the fungus-root system, and in others the fungus alone, which, of course, is misleading and incorrect.

The technique involved is complicated, and it is exceedingly difficult to identify the fungus found in mycorrhizal relationship with the root of a particular plant. Considerable care must be exercised to succeed in getting the fungus in culture.[1] Whether in culture or not, not all mycorrhizal fungi produce fruit bodies, but the mycelium may remain sterile in the soil for years, continuing to form mycorrhiza. The method of tracing the mycelium from the fruiting body, through the soil, to the root of the higher plant has been employed as a means of identification, but it is

[1] See Rayner (18) for culture methods.

believed that such methods allow considerable opportunity for error. Paulson (16) states that the bulk of mycorrhiza is found in decaying leaves near the surface layers where decomposition is not far advanced. Thus the soil is permeated with the mycelium of soil fungi, many of which form mycorrhizal connnections with the higher plants.

Types of Mycorrhiza

In most literature the types of mycorrhizal development are given as ectotrophic and endotrophic, signifying respectively that the fungus is growing on the outside or on the inside of the root of the higher plant. A third quite peculiar type of development has been reported, i.e., those endotrophic forms developing the characteristic vesicles and arbuscles. Rayner (20) believes the distinction between ectotrophic and endotrophic forms is in the degree of infection of the root by the fungus. This suggests a possible transition on the part of a mycorrhiza-forming fungus from the endotrophic character to the ectotrophic, and that a given fungus may be endotrophic with one plant, and ectotrophic with another. It is possible that a mycorrhiza-forming fungus of a general endotrophic habit may form vesicles or arbuscles in one plant, and not in another (Demeter, 2).

The ectotrophic form (fig. 46, pl. 8) is readily noted since it occurs commonly in connection with the roots of many common forest trees such as beech and oak, also pine, larch and other conifers, the root having a coral-like appearance, being short, thickened, profusely branched, and with root hairs usually lacking or few. This was the type noticed by Frank which led to his further investigations. He regarded the outer part of the root simply as a fungus sclerotium, and described it as a pseudoparenchymous-like layer, formed by the massed hyphæ, resulting in a thick mantle or weft of fungus mycelium about the root of the higher plant. The fungus sends haustoria in between the epidermal cells, and these penetrate the root between the outer cortical cells. Melin (1921) has shown for pine and spruce that infection in this ectotrophic form takes place through the root hairs or epidermal layer of cells, and that the fungus first exists inside the cortical cells, but after digestion commences, the hyphæ pass between the cells of the epidermis and form the typical mantle.

In the endotrophic mycorrhiza (15) the hyphæ penetrate the

root and develop within the cortical cells, obtaining nourishment there. In such cases the mycelium may be intercellular, intracellular, or both, as observed by West (24) in mycorrhiza of the Marattiaceæ. The fungus in many forms of endotrophic mycorrhiza is thought to be a Phycomycete or a closely allied form. Peyronel is of the opinion that the mycelium of these phycomycetoid endotrophs forms in humus soil a continuous network which involves the root-system of the higher plants, passing from one to another, and also that these phycomycetoid endotrophs live a saprophytic existence, continuing their existence and development in the cortical tissues of the root after the death of the latter. In most endotrophic mycorrhiza the fungus forms haustorium-like arbuscles and spore-like vesicles (17), both of which will be discussed later. This type seems to differ sharply from the endotrophic mycorrhizal fungus of the orchids which has been isolated and cultivated, and is regarded as belonging to the genus *Rhizoctonia*.

Rayner (18, 19, 20) has worked with an endotrophic fungus in *Calluna vulgaris* which affects all parts of the plant; root, stem, leaf, flower, and fruit. The distinction made by her between ectotrophic and endotrophic forms is one of degree of infection only. The hyphæ on entering the root cell seem to be attracted towards the nucleus. Branching then occurs and the cells are filled with large hyphæ which are capable of absorbing material from the cell as well as from the external mycelium.

In many endotrophic mycorrhiza, especially when the mycorrhizal fungus suggests a Phycomycete, the peculiar vesicles and arbuscles (figs. 44 and 45, pl. 8) first described by Gallaud (4) are found. Peyronel (17) has observed these structures in endotrophic fungi, not, however, in orchids, and he believes the fungus concerned to be a Phycomycete, close to the genus *Endogone*. West (24) reports their presence in the mycorrhiza of the Marattiaceæ. A complete discussion of these forms is given by Demeter (2) in connection with his studies on the mycorrhizal fungus of *Vinca minor*, *Vincetoxicum officinale*, and *Asclepias cornuti*. He believes the fungus in these species to be the same as that found in the orchids, but in these species it develops the vesicles and arbuscles. Apparently these peculiar endotrophic structures have nowhere been reported for the orchids. Terminal and intercalary vesicles may form on either intercellular or intracellular hyphæ, and appear to be storage organs. But more important than the vesicles, according to Deme-

ter are the tree-like structures which Gallaud termed "arbuscles." These arbuscles are formed by a cloud of fine granules about the tips of the fungus branches. These are regarded by Demeter as apparently a protein precipitate formed by the mixing of the protoplasm of the cell of the higher plant and that of the fungus when the tips of the fungus branches burst. The granules later diffuse throughout the cell, and finally unite to form organized bodies—the so-called "sporangioles." He suggests the name "Plasmoptysiemycorrhiza" for this type of fungus.

Magrou (8) found vesicles in the endotroph of the wild potato and arbuscles in the fungus of *Mercurialis annua*. Upon inoculating *Solanum tuberosum* with *Mucor solani* he obtained terminal and intercalary vesicles resembling very much those of the endotroph found in nature.

Symbiosis in Mycorrhizal Connection

Several theories have been advanced concerning the nutritive relation existing in mycorrhiza, and in connection with each a considerable amount of experimental evidence has accumulated. It is still a question as to whether a mycorrhizal fungus is in any way parasitic on the higher plant, or whether there is established a state of true symbiosis, and if so, in what way the higher plant benefits by the presence of the fungus. It is undoubtedly true that no one rule can be set down for mycorrhiza in general, and that this physiological relationship varies between different plants and the same fungus; and between different fungi with the same plant. In any case there is probably a time in mycorrhizal development when the relationship is parasitic, and when it is symbiotic. In other words, such a physiological relationship is highly variable.

Magrou (8) defines symbiosis as that condition where both participants (higher plant and fungus) are in an equal state of reciprocity. The plants resist the attack of the fungus by some mechanical means of rendering themselves immune, such as the suffocation of the fungus by the development of cell structure. Miehe (14) believes that the fungus on *Casuarina equisetifolia* is a typical symbiont, changing nitrogenous materials already derived from the soil into such forms as can be used by the plant.

Molisch (15) in discussing the general physiological conditions states that perhaps the fungus simply assimilates free nitrogen as do the nodule-bacteria in the Leguminosæ, or that it aids in the

nutrition of the higher plant to some extent by making undigestible humus materials digestible, the plant thus obtaining nourishment through the fungus. In such cases the fungus also obtains organic material from the plant. He also suggests the idea that the fungus digests itself, and that the higher plant may in this way assimilate organic and inorganic materials. Whether or not soil fungi definitely assimilate free nitrogen is still a problem for research, although some positive and a considerable amount of negative evidence has been obtained (5, 22).

Rayner (19) in discussing the nutrition of mycorrhizal plants in connection with the endotroph of *Calluna vulgaris* states emphatically that the fungus has the general appearance of a parasite, and can act as such under certain conditions. She concludes that the reciprocity involved in the formation of active mycorrhiza as represented in *Calluna vulgaris* marks a relatively advanced stage of relationship, implying an extremely intimate association, and resulting in a "balance-of-profit" for the higher plant. The view put forward by the same author (18) in discussing the obligate symbiosis in *Calluna vulgaris*, is that the first step towards the formation of mycorrhiza involves a temporary modification of the parasitic habit on the part of the fungus concerned.

Melin (11, 13) upholds the fungus-symbiont theory for the plants with which he has worked. He believes that various symbionts of forest trees may enable their partners to assimilate the different organic nitrogenous compounds of the soil with a varying degree of ease, and that certain species of fungi can utilize the complex albumin bodies of the humus layer better than their disintegration products; while others more readily assimilate the latter. Melin produced ectotrophic mycorrhiza in culture on the birch with *Boletus scaber*, and on the ash with *B. rufus*. The relationship ascribed is pure symbiosis, the higher partner deriving much food from the digestion of the hyphæ of the mycorrhizal fungus.

The establishment of a state of true symbiosis is referred to by Thomas (23) in his paper on the genus *Corallorrhiza*, and recent work by Melin (10) has led the latter to believe that in many cases the higher plant and the fungus do live in a true state of symbiosis, both the fungus and the higher plant being benefited by this close morphological and physiological association. He bases his conclusion, first, on results of synthetic culture experiments; second,

on observations made under natural conditions; and third, on anatomical structure of the mycorrhiza.

It was long believed that the fungus relationship in the orchids was one of true mycosymbiosis, and that the fungus was absolutely essential to germination and growth of the orchid. Knudson (7) has demonstrated recently that the germinating orchid seed is benefited by the presence of the fungus in that the fungus increases the hydrogen-ion concentration, and changes complex carbohydrates into forms more available. Uninfected seeds, in solutions with an acidity equivalent to that produced by the fungus, germinated just as well as infected seeds in control solutions which were less acid, and in which uninoculated seeds would not germinate. His uninoculated control cultures had a pH value of 5.7, and on inoculation of such cultures with the fungus the acidity was increased so that the pH value ranged from 4.2 to 4.4.

Thus, in general, it may be said that the early workers believed that there was a state of true reciprocity existing between the higher plant and the mycorrhizal fungus. There was then a period in which the theory of parasitism on the part of the fungus was advanced, followed by more recent work in which the relationship was again thought to be one of true symbiosis as defined by Melin.

METHODS USED IN COLLECTION AND PREPARATION FOR STUDY

The purpose of this research was to make as extensive a survey as possible, in the time at my disposal, of the occurrence and nature of mycorrhiza in Iowa forest plants. Thus the methods used in collecting, preliminary study of roots and soil, fixing, and staining, were those most convenient for use in such a survey. The intention was to examine and study the roots of as many plants belonging to representative families as possible, without intensive study of any one particular plant.

All but one of the collections were made from April to October, 1925. The exception was an abundant supply of *Corallorrhiza*, collected in September, 1924. Collections were made in the typical Iowa forests of the river valleys of Johnson and Iowa counties, and in the forests of the lake borderlands of Dickinson County, with a few collections from open plant associations about West Okoboji Lake. Plants collected in the field were taken into the laboratory with a considerable quantity of soil attached, the pH reading of the

soil solution was made, and the plants were then placed in the refrigerator until the preliminary examination could be made.

The pH values of the soil solutions were determined as soon as the material was brought from the field. Experiment showed that a reading of the hydrogen-ion concentration of the soil solution in the field was not necessary, and that soils kept in the refrigerator for from twenty-four to thirty-six hours exhibited surprisingly little variation in the pH reading from that taken in the field, or immediately upon arriving at the laboratory, which was rarely more than four hours from the time of collection. In obtaining the pH value, about 5 cc. of soil was well mixed by shaking in a test tube with 20 cc. of neutral distilled water. This was allowed to stand until the turbidity of the soil solution was considerably reduced, ten to fifteen minutes being sufficient. The determination of the hydrogen-ion concentration was made with the standard indicators to one decimal place. Such a method, of course, is not free from error, but in general the error involved was compensating, and the relative acidity or alkalinity of the various soils tested was sufficiently accurate for the purposes of such a survey. In general, the soil taken for the pH reading was that about those roots exhibiting the usual external characteristics of mycorrhiza, or, in plants which did not disclose mycorrhiza in external appearance of the roots, the soil sample was usually taken at a depth of several inches. In several collections, as a matter of experiment, pH readings of the soil solutions were made from the surface layer, and also from various depths down to a depth of six and eight inches, and little variation in the pH values was revealed.

A preliminary examination was made on all plants collected, which involved a careful study of the external characters of the roots, and a microscopic examination of the internal structure by the study of free-hand sections. Note was taken of the general appearance, form, root-hair formation, and of any traces of fungus hyphæ found externally or internally. Free-hand sections were made across the root, beginning at the root-tip and then at intervals in the older root parts up to 3-4 cm. from the tip until no trace of fungus hyphæ was found. The staining of free-hand sections with Gram's iodine solution, or by an aqueous solution of eosin, or both, was found very useful. Roots of collections which disclosed ectotrophic or endotrophic fungus hyphæ in this examination were fixed and preserved for further study.

Several attempts were made, with the usual precautions and methods of sterilization, to obtain cultures of the ectotrophic fungus present on the roots of *Ostrya virginiana* and *Quercus macrocarpa*, but these were unsuccessful.

Chromo-acetic acid (1 per cent) was used in general as a fixing agent, although several collections were fixed and preserved in formalin-alcohol. Material fixed in chromo-acetic acid was washed, and then preserved in 5 per cent formalin solution until further study. Sections of different parts of roots of the same collection were kept in separate vials. Preserved material for microscopic study was run through the complete series of alcohols, at 10 per cent intervals, for dehydration, with four steps from absolute alcohol to pure xylol, and was imbedded in 48° paraffin. Longitudinal sections were made of roots of all plants which disclosed mycorrhiza in the preliminary examination, as a check on such findings, and cross sections were made of roots of those collections which disclosed outstanding mycorrhizal types in the longitudinal sections. The microtome sections were cut from 10 to 15 microns, the 15 micron sections being more satisfactory for this particular study. Good paraffin ribbons were obtained showing no great amount of tearing of vascular and cortical tissue in either longitudinal or cross sections of such woody roots as those of oak, ash, hornbeam, cottonwood, and the older roots of ferns, without special treatment. Land's albumin fixative as given by Chamberlain was the only material used in fixing the paraffin sections to the slide. This proved very successful in general, but surprisingly unsuccessful in the case of two ferns. In those sections of *Adiantum pedatum* which possessed an ectotrophic fungus layer of considerable thickness, the weft of fungus hyphæ did not adhere to the slide well; and in the material of *Osmunda claytoniana* which possessed an endotrophic fungus, the cells containing the disorganizing fungus hyphæ were mostly lost.

No attempt was made to experiment with various stains, but since roots of many different species of plants, and different collections of the same species were being investigated, Flemming's triple stain was used as the general stain. In this the safranin was made from equal parts of a saturated solution in 95 per cent alcohol and a saturated aqueous solution, with 1 per cent aqueous solutions of gentian violet and orange G. In cases where more careful study was desired, iron-alum hæmatoxylin counterstained with 1 per cent

aqueous solutions of orange G or erythrosin was used as a check. The hæmatoxylin proved the better stain in the case of most ectotrophic fungi, and also for those orchids which exhibited endotrophic fungus mycelium.

RESULTS

Seventy plant collections were made, forty-three of which disclosed fungi in mycorrhiza-like relationship, the other twenty-seven collections being negative. Of the collections all but one (the stock collections of *Corallorrhiza*) were made between April 13 and October 10, 1925, in Dickinson and Johnson counties, with one collection from Iowa County. The localities worked in these three counties are covered with typical Iowa forests. Most of the Johnson County collections, and the collection from Iowa County were made in the months of April and May. The temperature was practically normal for southeastern Iowa, but the rainfall until June was above the average. Conditions were favorable for an abundant fungus flora and especially for mycelial growth in the soil. Eight collections were made in Johnson County in September and October, following an extremely warm summer with a normal amount and distribution of rainfall. Warm weather continued until about October 15, when heavy frosts, early snows, and frozen ground prevented any more collecting. The ground was slightly frozen when the last collections were made.

The collections in Dickinson County were made in June and July. Cool, wet weather prevailed the latter part of June, but the month of July was very hot and dry. Dickinson County had an extremely dry spring. Conspicuous fungi were very scarce in the Okoboji region in the summer of 1925, only a few specimens of the very common genera being found during the entire summer. It is extremely probable that this was reflected in the growth and fewer numbers of soil fungi.

The total of seventy plant collections made represented twenty-one families, thirty-four genera, and forty species. The results of these collections, including field notes, the most important notes from preliminary examinations, and examinations of prepared slides, can be best correlated in tabular form. In Table I, which concerns those species in which mycorrhiza was present in all examinations, the arrangement is in phylogenetic order[2] according

[2] The nomenclature and phylogenetic order used is that of Gray's Manual of Botany; Ed. 7.

TABLE I

PLANTS IN WHICH MYCORRHIZA WERE FOUND ON EACH EXAMINATION

Species	pH of soil solution	Habitat of higher plant	Important characters of higher plant or fungus	Place of collection (county)	Date of collection
Pteris aquilina	6.4	oak-hickory forest; dense under-growth	ectotroph of very fine mycelium; endotroph of larger, septate hyphæ,	Johnson	Oct. 10, 1925
,, ,,	7.2	oak-hickory forest	ectotrophic weft, septate hpyhæ plus endotroph of same character	,,	,, ,, ,,
Onoclea sensibilis	4.9	open edge of oak-hickory forest	ectotroph about lateral roots	,,	May 20, 1925
Botrychium virginianum	5.0	oak-hickory forest	large phycomycetoid endotroph; numberous arbuscles	,,	May 20, 1925
Equisetum arvense	5.1	sandy soil along road by east edge of woods	phycomycetoid endotroph; abundant throughout cortical cells	,,	,, ,, ,,
Uvularia perfoliata	7.2	oak-hickory forest; north ravine	no root hairs; disorganizing endotroph in root tips	,,	Apr. 19, 1925
Erythronium albidum	6.2	oak-hickory forest; north ravine	large phycomycetoid endotroph	,,	,, ,, ,,
Smilacina racemosa	6.6	oak-hickory forest; dense	few root hairs; disorganizing endotroph; with arbuscles	,,	Oct. 10, 1925
,, ,,	7.0	oak-hickory forest; dense	root hairs present; disorganizing hyphæ in cortical and epidermal cells	,,	,, ,, ,,
Orchis spectabilis	6.8	oak-hickory forest; ravine	few root hairs; typical orchid fungus in few cells 5 mm. from tip	,,	Apr. 19, 1925
,, ,,	5.3	coppice growth; N.E. exposure	typical orchid endotroph; more abundant; 1 to 2 mm. from root tip	,,	May 20, 1925
Corallorrhiza odontorhiza	.....	hornbeam thicket in oak woods	typical orchid endotroph; very abundant in subterranean parts	,,	Sept. 13, 1924
Populus deltoides	6.6	sandy beach 15' from water line of lake	thick ectotroph as in *Ostrya*	Dickinson	July 21, 1925
,, ,,	6.5	,, ,, ,, ,,	usual ectotroph plus large phycomycetoid endotroph	,,	,, ,, ,,
Ostrya virginiana	7.6	hornbeam grove, east exposure along lake shore	ectotroph forming thick mantle; endotroph present; apparently the same fungus; seedling of 1st yr.	,,	July 3, 1925

Ostrya virginiana (cont'd)	7.6	" " " "	ectotroph only;	" " 2nd "	" " " "	
" "	7.9	bur-oak-hornbeam grove, E. exposure along lake shore	" " " "	1st "	" " 10, "	
" "	8.0	" " " "	" " " "	1st "	" " " "	
" "	7.9	" " " "	" " " "	2nd "	" " " "	
" "	7.2	" " " "	" " " "	1st "	" " 28, "	
" "	7.3	" " " "	" " " "	2nd "	" " " "	
Hepatica acutiloba	7.2	north exposure; lime-stone cliff	no root hairs; endotroph several mm. from tip of root		Johnson Apr. 13, 1925	
" "	6.5	north ravine; oak-hickory forest	root hairs abundant; large disorganizing endotroph in root tips		" " 19, "	
Podophyllum peltatum	6.8	" " " " "	phycomycetoid endotroph to 1.5 cm. from tip; in cortical and epidermal cells disorganization of fungus		" " " "	
" "	6.2	" " " " "	same as above; root hairs present		" May 20, 1925	
Caulophyllum thalictroides	6.5	" " " " "	no root hairs; long spiralled roots; large phycomycetoid endotroph		" Apr. 19, 1925	
Sanguinaria canadensis	5.2	N.E. exposure; coppice growth	no root hairs; ectotrophic fungus found in preliminary examination		" May 20, 1925	
Melilotus alba	6.5	sandy beach, 20' from water line of lake	few root hairs; endotroph in thickened root tips		Dickinson July 20, 1925	
" "	6.5	" " " "	endotroph in roots above nodules; forming vesicles		" " 21, "	
Viola sororia	6.6	oak-hickory forest	few root hairs; tips of roots darkened		Johnson Apr. 19, 1925	
Fraxinus pennsylvanica var. lanceolata	6.8	" " "	root hairs sparse; distorted; tips of roots swollen; ectotroph and abundant endotroph of apparently the same fungus		" " " "	
Galium aparine	6.2	" " "	root hairs present but scarce; digestion of phycomycetoid endotroph; arbuscles present		" May 20, 1925	
Galium triflorum	6.3	" " "	same as above; more arbuscle formation		" May 20, 1925	

TABLE II

PLANTS IN WHICH MYCORRHIZA WERE FOUND BUT NOT ON EVERY EXAMINATION

Species	pH of soil solution	Habitat of higher plant	Fungus Pres.	Fungus Abs.	Important characters of higher plant or fungus	Place of collection (county)	Date of Collection
Adiantum pedatum	6.9	oak-hickory forest	X		ectotroph; basidiomycete hyphæ	Johnson	Apr. 19, 1925
,, ,,	5.4	,, ,,	X		endotroph; vesicles; root hairs	,,	May 20, 1925
,, ,,	7.0	,, ,,		X	root hairs abundant	,,	Oct. 10, 1925
,, ,,	6.8	,, ,,		X	mycelium abundant in soil about root hairs, but no connection with root noticed	,,	,, ,, ,,
Cystopteris fragilis	6.7	,, ,,		X	roots well developed; root hairs abundant	,,	Apr. 19, 1925
,, ,,	5.2	,, ,, dense undergrowth	X		root hairs present; disorganizing endotroph in outer cortical cells of root tip	,,	May 20, 1925
Osmunda claytoniana	5.1	oak-hickory forest		X	root hairs sparse	,,	,, ,, ,,
,, ,,	6.2	,, ,,		X	roots normal, cells packed with starch grains	,,	Oct. 10, 1925
,, ,,	6.8	,, ,,	X		no root hairs; disorganizing endotroph in outer cortical cells of root tip	,,	,, ,, ,,
Arisæma triphyllum	6.7	,, ,,		X	root hairs sparse	,,	Apr. 19, 1925
,, ,,	5.4	,, ,,	X		root hairs few; large phycomycetoid endotroph with numerous arbuscles	,,	May 20, 1925
Quercus macrocarpa	7.6	open edge, bur-oak forest	X		ectotroph abundant; roots examined 8-10″ below surface; seedling of 3rd year	Dickinson	June 19, 1925
,, ,,	8.1	,, ,, ,, ,, ,,	X		same fungal cond. seedling, 2nd yr.	,,	,, 20, ,,
,, ,,	7.7	,, ,, ,, ,, ,,	X		,, ,, ,, ,, ,, ,,	,,	,, ,, ,,
,, ,,	7.5	,, ,, ,, ,, ,,	X		,, ,, ,, ,, ,, ,,	,,	,, ,, ,,
,, ,,	7.6	,, ,, ,, ,, ,,	X		,, ,, ,, ,, 1st ,,	,,	July 16, 1925
,, ,,	6.6	sandy beach; 15′ from water line of lake		X	roots normal and root hairs present	,,	,, ,, ,, July 21, 1925

TABLE III

PLANTS IN WHICH MYCORRHIZA WERE NOT FOUND

Species	pH of soil solution	Habitat of higher plant	Important characters of higher plant or fungus	Place of collection (county)	Date of Collection
Aspidium spinulosum	6.5	north ravine in oak-hickory forest	root hairs present	Johnson	Apr. 19, 1925
Equisetum kansanum	alk.	N.E. slope; open prairie	root hairs abundant	Dickinson	July 27, 1925
Smilacina stellata	7.9	east exposure; oak-hornbeam grove	roots normal	"	" 10, "
" "	8.0	east exposure; long lake shore	" "	"	" " "
Polygonatum commutatum	8.2	wooded east exp. long lake shore	root hairs present	"	" 9, "
" "	8.2	sandy slope east exp. lake shore	" " "	"	" " "
" "	7.7	rather open oak-ash peninsula	" " "	"	" 10, "
Trillium grandiflorum	5.4	coppice growth	few root hairs	Johnson	May 20, 1925
Cypripedium pubescens	6.2	dense woods; north of Homestead	root hairs present but much distorted	Iowa	" 10, "
" "	alk.	dense oak forest	roots long, thick, and tortuous; few root hairs	Dickinson	July 27, 1925
Asarum canadense	5.2	north exposure; coppice growth	no root hairs and no trace of fungus found	Johnson	May 20, 1925
Claytonia virginica	6.6	north ravine; oak-hickory forest	root hairs present	Johnson	Apr. 19, 1925
Anemonella thalictroides	6.8	north ravine; oak-hickory forest	root hairs numerous	"	" " "

(Continued on page 46)

TABLE III (Cont'd.)

Species	pH of soil solution	Habitat of higher plant	Important characters of higher plant or fungus	Place of collection (county)	Date of collection
Dicentra cucullaria	6.6	ravine; oak-hickory forest	root hairs well developed; no trace of fungus in roots or tubers	Johnson	Apr. 19, 1925
Psedera quinquefolia	8.2	sandy slope; east exposure; wooded shore line of lake	root hairs present	Dickinson	July 9, 1925
Viola pubescens	7.0	ravine; oak-hickory forest	no root hairs; not distorted	Johnson	Apr. 19, 1925
Viola sp.	6.4	sandy beach; 15′ from water line of lake	root hairs abundant; phycomycetoid mycelium about roots in soil	Dickinson	July 21, 1925
,, ,,	6.5	,, ,, ,, ,,	same condition as above; no connection with root in either case	,,	,, ,, ,,
Polemonium reptans	6.7	north ravine; oak-hickory forest	roots normal; root hairs present	,,	Apr. 19, 1925
Galium sp.	6.6	,, ,, ,, ,,	root hairs well developed	,,	,, ,, ,,

to families, and genera within each family. With each collection is given the hydrogen-ion concentration of the soil solution; the distinctive characters of the root-fungus association, and of the fungus involved; root characters of the higher plant; the habitat of the higher plant; and the place and date of collection.

Table II lists in phylogenetic order those species in which mycorrhiza were found in some but not all specimens examined, giving the number of collections of each; the pH of the soil solution; the habitat of the higher plant; and the place and date of collection. In those cases which revealed mycorrhiza the fungal condition is given.

Table III lists in like manner those species in which no mycorrhizal condition was found giving for each collection the hydrogen-ion concentration of the soil solution; the habitat of the higher plant, and its important characters; and the place and date of collection.

DISCUSSION

The endotroph found in the two examinations of *Melilotus* proved of interest, as mycorrhiza has been reported for the Leguminosæ only since 1923 (Jones, 6). Atkinson (1) reported on a microsymbiont in the root tubercle of *Vicia sativa* in 1893, but did not refer to it as a fungus. His figures of this microsymbiont clearly show it to be similar to the mycorrhizal, phycomycetoid, endotrophic fungi with the arbuscle formations. Jones has found mycorrhiza-like fungi in the roots of fifteen species of Leguminosæ. The fungus is reported as a Phycomycete, occasionally forming vesicles and arbuscles. The roots of *Melilotus* which I examined possessed bacterial nodules, and the fungus endotroph was found in the cortical and epidermal cells some distance above the nodules (figs. 27, 28; pl. 5). No trace of a fungus was found in the hypertrophied cells of the nodules.

The two collections of *Hepatica* were from widely separated localities, one being from a ravine in a dense woods, and the other from a north-exposed, limestone cliff. The branching, phycomycetoid, endotroph found in both was apparently the same fungus, found always in roots that were distorted and wanting in root hairs. No trace of a fungus was found more than 1 cm. from the root tip (figs. 24, 25; pl. 5). On the same plants no trace of a fungus was

found in those roots which possessed root hairs and appeared normal.

The fungus found in *Orchis* (fig. 18; pl. 4), and in *Corallorrhiza* (pl. 3) appeared to be the typical orchid fungus as described by Knudson (7). This was very abundant in the subterranean parts of *Corallorrhiza* in various stages of infection by the fungus, and of digestion of the fungus by the host. The two collections of *Orchis* were from different localities, one being from a group of plants in a rather dense woods, and the other being a lone specimen from an open coppice growth. Both specimens possessed good root hair development, and the fungus of the former was confined to but two cortical cells in one root (of the six roots examined), while the latter showed abundant endotrophic hyphæ about 2 cm. from the tips of the roots. The first was collected April 19, and the second May 20. In considering the data of Table I it is seen that more than one-third of the collections were made in April, but of those species collected in both April and May, and in collections made in May alone, the development of the root fungus was usually better in the later collections.

The two collections of *Podophyllum* made in April revealed a late state in the fungus infection (figs. 42, 43; pl. 7). Most of the roots possessed an endotroph in the shape of a small branched, phycomycetoid fungus, mostly in the inner cortical cells near the endodermis. The cells near the endodermis showed stages of disorganization and digestion of the fungus with the appearance of starch grains. Normal cells were filled with starch grains. The fungus occurred as far back as 2 cm. from the root tip. Root hairs were present in both collections.

It is also to be noticed in the first table that the mycorrhiza of the ferns and herbaceous plants are endotrophic in habit, with the exception of *Sanguinaria*, *Onoclea* and one collection of *Pteris*. In examining such plants in the field it is often difficult to determine whether or not there is a fungus association present, and it is usually necessary to examine the roots microscopically before one can be certain. In the endotrophic association there are in most cases a few root hairs present on the roots, although they may be greatly twisted and distorted, but in some, as in the case of *Podophyllum* discussed previously, the root hair development may appear to be quite normal. The roots of *Caulophyllum* were extremely interesting, being massed into a great tangle, all eight to ten inches long,

seldom branched, spirally twisted, and of the same diameter throughout, except for a slight swelling at the tip. There was no sign of root hair formation. On examination of freehand sections, the root tips were found to be filled with a large, phycomycetoid endotroph. The hyphæ were seen to enter between epidermal cells, the growth being both intercellular, and intracellular.

Of the ferns possessing mycorrhiza, all revealed an endotrophic fungus except *Onoclea*, and one collection of *Pteris*. The ectotroph of *Onoclea sensibilis* appeared to be the same as that of the hornbeam and oak. The ectotroph of *Pteris*, however, was composed of very small, branching hyphæ. The weft was of considerable thickness near the root tip, and at places on the older roots (fig. 11; pl. 2). One collection of *Pteris* possessed an enormous endotroph forming arbuscles (fig. 10; pl. 2), in addition to the ectotroph. These were apparently different fungi. The latter collection, as can be noticed in the table, came from a forest soil with a pH value of 7.2, whereas the former came from a soil which gave a reaction of pH 6.4. Such results are not in accordance with what some of the literature on this phase of mycorrhiza would lead one to expect. Melin (12) has stated that mycorrhizal fungi thrive best in a soil of pH 5.0 or lower, and that there is poor development in neutral soil. Knudson (7) in studying the germination of orchid seeds, found that best growth of seedlings was obtained in inoculated cultures where the pH value was 0.2 to 4.4. The controls, or uninoculated cultures, remained at pH 5.7. Knudson is of the opinion that in the orchid, at least, the increase in the hydrogen-ion concentration is due to the fungus. The two orchids in which mycorrhiza were found in this investigation were in acid soil, one being in a soil of pH 6.8. But of the thirty-three collections including twenty species of plants listed in Table I, all but nine were in a soil of pH 6.5 or more, ten of them being collected in soils with pH values ranging from 7.0 to 8.0.

Of those species collected in alkaline soils the mycorrhizal fungus was endotrophic in habit except in *Ostrya* and *Pteris*. The ectotrophic weft found in all plants of *Ostrya* that were examined was similar to the weft found in oak and cottonwood (pl. 6). The weft, or fungus mantle, was composed of septate hyphæ, and in several collections masses of loose, basidiomycete mycelium were found in the soil about the root. No clamp-connections were found in the hyphæ of the weft, nor in those collections where the same fungus

was endotrophic as well as ectotrophic. Melin (9) reports in this type of fungus the existence of a pseudoparenchymous mass of hyphæ within the cortical cells, and believes that it is here that infection sets in, the fungus mantle being formed later. In the hornbeam, under the oil immersion lens, I observed the mass of septate haustoria or intercellular hyphal tips about the epidermal cells and penetrating between the cortical cells, but I saw no instance in which they seemed to be intracellular. Five of the young hornbeam plants examined were seedlings of the first year, and two were in their second year of growth. It should be noted in this connection that not only in *Ostrya*, but in *Fraxinus*, and in one collection of *Pteris*, the well developed ectotroph was associated with an endotroph believed to be the same fungus.

The ectotroph of *Populus* seems to be the same fungus as that of *Ostrya*. The cottonwood plants were collected in the sand about fifteen feet from the water line of the lake, where the pH value for all collections was near 6.5. In one collection a phycomycetoid endotroph was present also (figs. 35, 36; pl. 6).

The data of Table II require more discussion. It should be noted that the collections of *Arisæma* and *Cystopteris* are the only collections definitely corroborating previous evidence on mycorrhizal relationship to hydrogen-ion concentration as given by other workers. In the first species the collection which revealed an abundant, phycomycetoid endotroph and the collection which proved to be negative, were made in the same woods, the former in late May, and the latter on April 19. The hydrogen-ion concentrations of the two soils were pH 5.4 and 6.7 respectively. The endotroph of *Arisæma* (fig. 22; pl. 4) exhibited numerous arbusele formations similar to those described by Demeter (2) and Gallaud (4). Likewise, in the case of the two collections of *Cystopteris*, one possessed a mycorrhizal endotroph, and was collected the same time and place as the positive collection of *Arisæma*. The other, which was negative, was collected with the negative collection of *Arisæma*. The hydrogen-ion concentrations of the two soils were pH 5.2 and 6.7, being practically the same as the values for the two collections of *Arisæma*. It should be noted that both negative collections were made in the middle of April, the positive late in May, and that in the discussion of Table I it was emphasized in connection with *Orchis* that although over one-third of the collections in that table were made

in April, there was in most cases a better mycorrhizal development in those collections made in May.

The third collection of *Osmunda,* and the second collection of *Adiantum,* possessed endotrophic mycorrhiza as did most of the ferns listed in the first table. In the first collection of *Adiantum* an ectotrophic basidiomycete fungus (figs. 1, 2; pl. 1) formed a mantle of considerable thickness. Thus of the eight collections of ferns in which mycorrhizal fungi were found, all possessed an endotrophic fungus except one collection of *Adiantum,* and the collection of *Onoclea.* The two examples cited are in agreement with the findings of others as to the occurrence of mycorrhiza, but it will be noted that in the case of *Adiantum* the positive collection possessing an ectotrophic fungus was from a neutral soil, and the positive collection possessing an endotrophic fungus was from an acid soil, while both negative collections were from nearly neutral soils. However, the two positive collections were made in the spring, and the two negative in October. Also, in the case of *Osmunda,* the negative collections were from soils of pH 5.1 and 6.2, one being made in April and one in October, while the positive collection was from a soil of pH 6.8, collected in October.

The vesicles in the endotroph of *Adiantum* (figs. 3, 5; pl. 1; and fig. 2; pl. 8), were similar to those described by West (24) for the mycorrhiza of the Marattiaceæ, and by Demeter (2), and Magrou (8). Terminal and intercalary vesicles were observed, both occurring intracellularly and intercellularly. There were no root hairs present, and the roots were much distorted, with short thick branches, the tips being black. In examination of prepared slides the extent of cellular disorganization near the endodermis, and the abundance of the large, branched, elongate hyphæ suggest a possible case of true parasitism on the part of the fungus. Since, in external appearance the roots exhibited the usual mycorrhizal characters for the ferns, it seems probable that the mycorrhizal endotroph had overcome the capacity of the roots to hold the fungus in check.

The ectotroph of the bur-oak (figs. 38, 39; pl. 6) appeared identical with that found in the cottonwood and hornbeam, forming a pseudoparenchymous layer about the short, thick, lateral roots. (See fig. 46; pl. 8). The external characters of the roots and the character of the fungus layer forming the mantle were typical of the ectotrophic mycorrhiza of common forest trees, as described by Frank (3) and others. On a lateral root, 0.12 mm. in diameter, the

thickness of the fungus weft averaged 20 microns, a condition found in general for all woodland collections of *Quercus*. Six of the collections were made at the edge of a bur-oak forest, some of the seedlings being quite in the open. One of the seedlings examined was in the first year of growth, three were in the second, and one in the third. All were rooted from three to ten inches below the surface of the soil, and none possessed roots with root hairs. The soil was alkaline, the hydrogen-ion concentrations for the soil solutions ranging from pH 7.5 to 8.1, as shown in the table.

One collection of *Quercus*, a seedling of the first year, growing in the sand about fifteen feet from the water of the lake, proved negative. This collection was made at the same time as that of *Populus* which possessed the ectotrophic fungus and the plants were only a few feet apart. The pH value of the soil solution of the sandy beach was 6.6 for both collections. In the oak the lateral roots were normal, root hairs were present in abundance, but distorted due to growth in the sand.

Table III requires little discussion, as negative results contribute no conclusive evidence, especially when so few collections of individual species are recorded. Of the fifteen species in which mycorrhiza were not found there was but one collection each of all but four. On comparing the notes in Tables I and II it will be found that of the thirteen species collected, with two or more collections of each, approximately all but one-third gave positive results for each collection. Thus it is highly probable that at least five of the fifteen species listed in Table III do ordinarily have mycorrhiza. With positive results in *Equisetum arvense*, *Smilacina racemosa*, *Viola sororia*, and two species of *Galium*, mycorrhiza would be expected to be present in the species of the same genera listed in Table III. With so much evidence in the literature regarding the presence of a fungus in the orchids, the negative results in the case of *Cypripedium* were surprising. Root hairs were present in both collections of *Cypripedium*. One collection was made in Iowa County, growing in a fairly acid soil in a dense woods, and flowering; while the other was a lone specimen in a bur-oak woods of Dickinson County, where the soil was alkaline. Likewise, the two collections of *Smilacina stellata* were from the alkaline soil of Dickinson County, whereas the two collections of *S. racemosa* giving positive results were from the more acid soils of Johnson County.

Correlating the data of the three tables in regard to the hydrogen-

ion concentration of the soil solution it is found that for the seventy collections made, fifty-one were from soils of pH 6.5, or more, the highest pH value recorded being 8.2. The value of pH 6.5 is taken as an arbitrary value below which conditions are in general more favorable for the development of soil and root fungi. Melin (12) has investigated the influence of hydrogen-ion concentration of soils and culture media on the vigor of the pine and fir root-fungi. He concludes that in general mycorrhizal fungi thrive best at pH 5.0 or slightly lower, with poor development at pH 7.0; and that *Rhizoctonia sylvestris* and *Mycelium r. atrovirens* thrive equally well in neutral and acid media, the latter thriving in all types of forest soil. In view of these observations it seems probable that the value of pH 6.5 chosen above is too high. The ectotrophic fungus found so commonly in this investigation on *Ostrya* and *Quercus* (and on one collection of *Populus* from the same region), is believed to be a *Rhizoctonia*, and all except the collection of *Populus* were taken from soils with pH values ranging from 7.2 to 8.1.

Of the fifty-one collections taken from soils of pH 6.5 or of a higher pH value, thirty (representing seventeen species) possessed mycorrhiza-like conditions. Seventeen (56 per cent) of the thirty were from soils ranging from 7.0 to 8.1 (representing eight species of plants). Of the remaining nineteen collections of pH value less than 6.5, thirteen (representing the same number of species) were positive. Eight of these (representing eight species) were from soils ranging from pH 4.9 to 5.5. These numbers substantiate to some extent the results of Melin and other workers, as discussed previously, in that approximately 70 per cent of the collections with a pH value below 6.5 were positive, indicating that an acid soil is the more favorable for mycorrhiza; also in that nearly half of the collections from soils of pH 6.5 or of a higher pH value were negative. It is evident that approximately 77 per cent of the negative collections, and 70 per cent of all positive collections were from soils of pH 6.5 or above, but these figures cannot be balanced against the preceding figures, for more than 70 per cent of all collections were made on alkaline, neutral, or slightly acid soils.

Thus, it seems evident that in the Iowa forest flora, mycorrhiza or mycorrhiza-like fungi are of common occurrence, and are widespread among species of higher plants, in acid, neutral, and alkaline soils, but are more common and better developed in soils of pH value below 6.5. Far-fetched generalizations should not be drawn

from too little evidence, but it is believed that the evidence cited in the above discussion concerning mycorrhizal development and hydrogen-ion concentration, is worthy of note in that (1) the pH values are fairly accurate; (2) the species of plants examined are quite representative of the Iowa forest flora; (3) the types of soils from which collections were made are also representative forest soils of Iowa; and (4) collections were made throughout the growing season.

SUMMARY OF RESULTS

(1) The seventy individual plants collected and examined from typical forested areas of Johnson, Iowa, and Dickinson counties represented twenty-one families, thirty-four genera, and forty species.

(2) Sixteen of the collections were of Pteridophytes, representing four families, eight genera, and nine species. All other collections were of higher plants.

(3) Of the total number of collections, forty-three, representing sixteen families, twenty-four genera, and twenty-five species, disclosed association with a mycorrhiza-like fungus.

(4) Of the forty species examined, twenty showed a root-fungus association in every plant examined, five in some cases but not in others, and fifteen showed no trace of mycorrhizal fungi.

(5) Of the forty-three positive collections twenty-four possessed mycorrhizal endotrophs, fifteen mycorrhizal ectotrophs, and four had both endotrophic and ectotrophic root-fungi.

(6) In the collection of *Fraxinus*, one collection of *Ostrya* and one collection of *Pteris*, the endotroph and ectotroph are in each case believed to be the same fungus.

(7) Two of the twenty-four collections with endotrophic fungi showed vesicle formations, one being of *Adiantum pedatum*, and the other of *Melilotus alba*.

(8) The following species with endotrophs showed arbusele formations: *Arisæma triphyllum*, *Botrychium virginianum*, *Erythronium albidum*, *Galium aparine*, *G. triflorum*, *Pteris aquilina*, *Smilacina racemosa*, and *Uvularia perfoliata*.

(9) The ectotroph of *Ostrya*, *Quercus*, *Populus*, and *Onoclea*, is believed to be the same fungus, probably a *Rhizoctonia*. *Ostrya virginiana* and *Quercus macrocarpa* are provided constantly with a fungus ectotrophic in character.

(10) In ectotrophic forms intercellular hyphæ were observed about the epidermal cells, and between cortical cells, but no intracellular, pseudo-parenchymous masses were found.

(11) In endotrophic forms both intercellular and intracellular hyphæ were observed, the hyphæ passing freely from one cell to another, but most growth being intracellular.

(12) In endotrophic forms hyphæ were observed entering between epidermal cells and penetrating the walls of the cortical cells.

(13) The usual stages of infection and disorganization as reported by other workers were observed in the endotrophic forms.

(14) Fungus hyphæ were found in epidermal cells in several cases but the means of infection could not be determined. They were observed to extend from epidermal cells into cortical cells in *Smilacina racemosa*. They were never found entering the epidermal cells directly as reported by Melin (9) and West (24).

(15) The endotroph of *Orchis*, and of *Corallorrhiza* is believed to be the typical orchid endotroph.

(16) Numerous roots of two collections of *Cypripedium* were examined and no trace of a fungus was found. Both plants were thriving, one being in flower.

(17) The pH value of the soil solutions of plants examined ranged from 4.9 to 8.2. The soils were typical forest soils of Johnson, Iowa, and Dickinson counties.

(18) Fifty-one of the seventy collections were from soils of pH 6.5 or more. Thus more than 70 per cent of all collections were from alkaline, neutral, or slightly acid soil.

(19) Of the forty-three positive collections and twenty-seven negative collections, approximately 70 per cent of the former and 77 per cent of the latter were from soils of pH 6.5 or more.

(20) Of the nineteen collections from soils below 6.5 in pH value, 70 per cent were positive.

CONCLUSIONS

(1) Mycorrhiza-like fungi are of common occurrence in the Iowa forest flora, and are associated with higher plants of wide family relationship.

(2) In general, the endotrophic forms are more common than the ectotrophic in ferns and herbaceous higher plants.

(3) In ectotrophic forms haustoria or intercellular hyphal

branches penetrate between the epidermal cells, and in some cases penetrate between the cortical cells of the root.

(4) A fungus may be either ectotrophic, or endotrophic, or both in habit, in its association with the root of a higher plant.

(5) Mycorrhiza-like fungi occur in Iowa, associated with higher plants in acid, neutral, and alkaline soils, but they are more common and develop better in soils with a pH value below 6.5.

These studies were carried out under the direction of Professor G. W. Martin, to whom I am indebted for helpful suggestions and criticisms, and for the abundant collections of *Corallorrhiza odontorhiza* which was the incentive for this particular study at the State University of Iowa.

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PLATES

All drawings were made with the aid of camera lucida, at a magnification of 630 diameters unless otherwise stated, and reduced one-half in reproduction.

Abbreviations used in labeling

ep—epidermis
en—endodermis
ec—epidermal cell
cc—cortical cell
st—stele
cn—cell nucleus
rc—root cap
fw—fungus weft
ctx—cortex

PLATE I

Fig. 1. Longitudinal section of lateral root of *Adiantum pedatum* showing pseudoparenchymous layer formed by ectotrophic hyphæ.

Fig. 2. Epidermal cells in longitudinal section near tip of root of same plant. The septate hyphæ of the fungus ectotroph show a clamp connection.

Fig. 3. Longitudinal section of root of another plant of same species showing intercalary, intracellular vesicle about 8 mm. from tip of root.

Fig. 4. Longitudinal section of same root showing hyphal branch entering the root between epidermal cells.

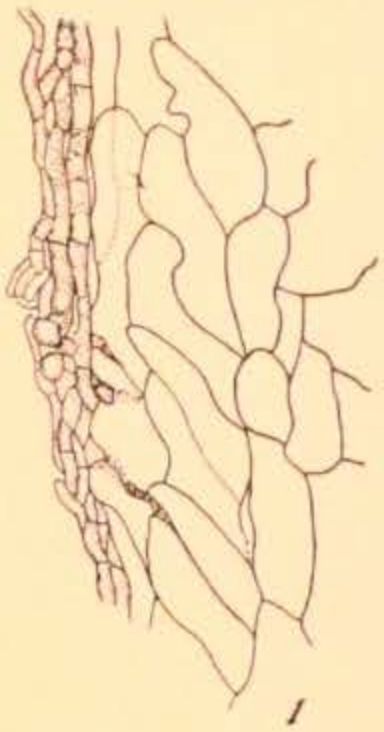
Fig. 5. Cortical cells of same root in longitudinal section showing hyphal growth from one cell to another, but most growth being intracellular. Also a terminal, intracellular vesicle in second layer of cortical cells beneath the epidermis, about 1 cm. from root tip.

Fig. 6. Longitudinal section of same root showing septate hyphæ of an endotrophic fungus associated with the one forming vesicles, but probably another fungus.

Fig. 7. Longitudinal section of root of *Botrychium virginianum* showing disorganizing hyphæ of fungus endotroph, and unorganized cell contents, in third and fourth layer of cortical cells beneath the epidermis, about 5 mm. from tip of root.

Fig. 8. Root of *Equisetum arvense* in longitudinal section showing hyphal characters of fungus in middle cortical cells.

PLATE I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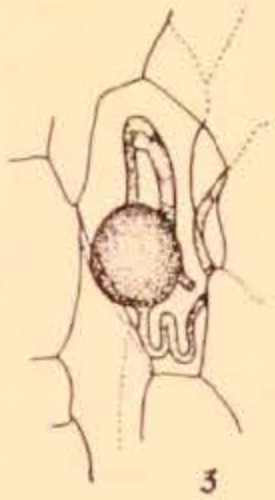
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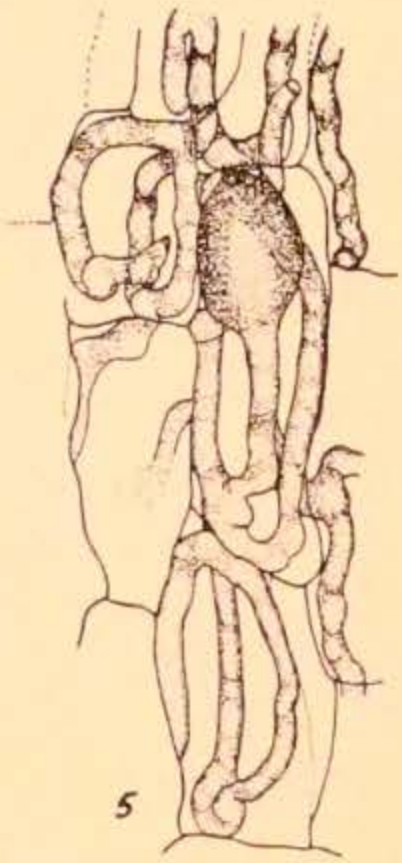
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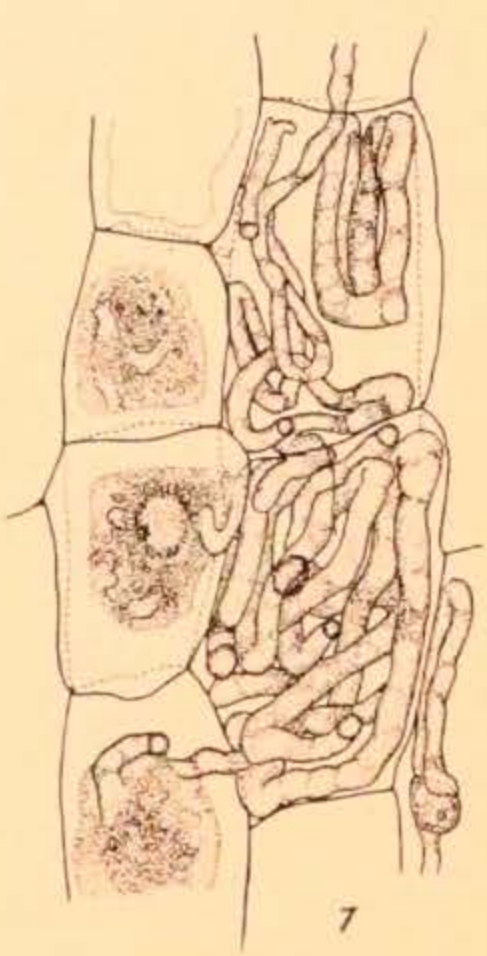
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PLATE II

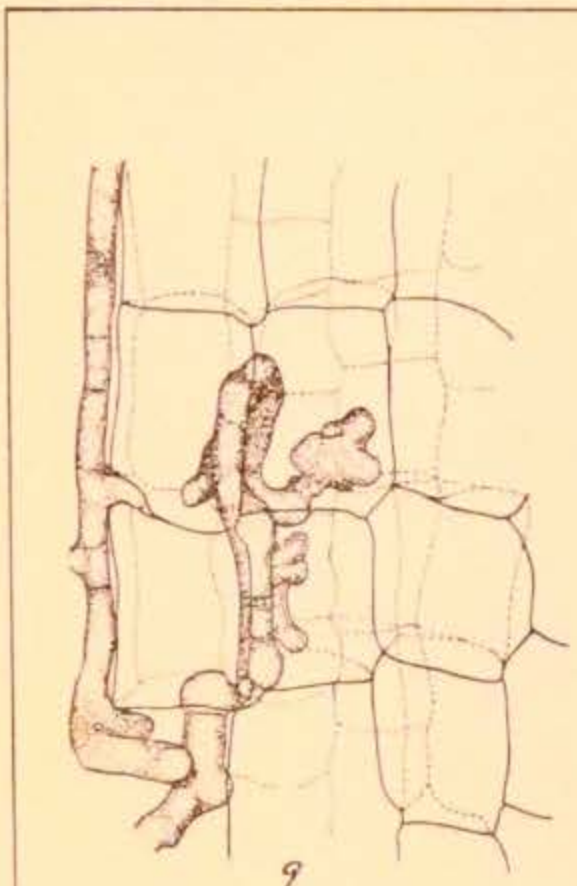
Fig. 9. Longitudinal section of same root of *Equisetum arvense* showing external epidermal surface and cortical cells beneath, with fungus hyphae entering between epidermal cells. The flattened tips of the intercellular hyphal branches are pressed against the walls of the first layer of cortical cells beneath.

Fig. 10. Root of *Pteris aquilina* in longitudinal section showing arbuscle formations in large cortical cells, and hyphae of an ectotrophic fungus of different character; *ep*, epidermis, and *en*, endodermis.

Fig. 11. Longitudinal section of another part of same root showing hyphae of fungus ectotroph of fig. 10 entering between two epidermal cells, *ec*, with their intercellular tips pressed against lower surface of large cortical cells, *cc*. Oil immersion; original magnification approximately x 1400.

Fig. 12. Longitudinal section of root of *Onoclea sensibilis* showing thick weft of ectotrophic hyphae forming pseudoparenchymous outer layer, and the haustoria penetrating between epidermal cells.

Fig. 13. Diagram of portion of median longitudinal section of same root tip showing relative thickness of fungus weft to other parts of root. Note the haustoria between the epidermal cells; *en*, endodermis, and *st*, stele.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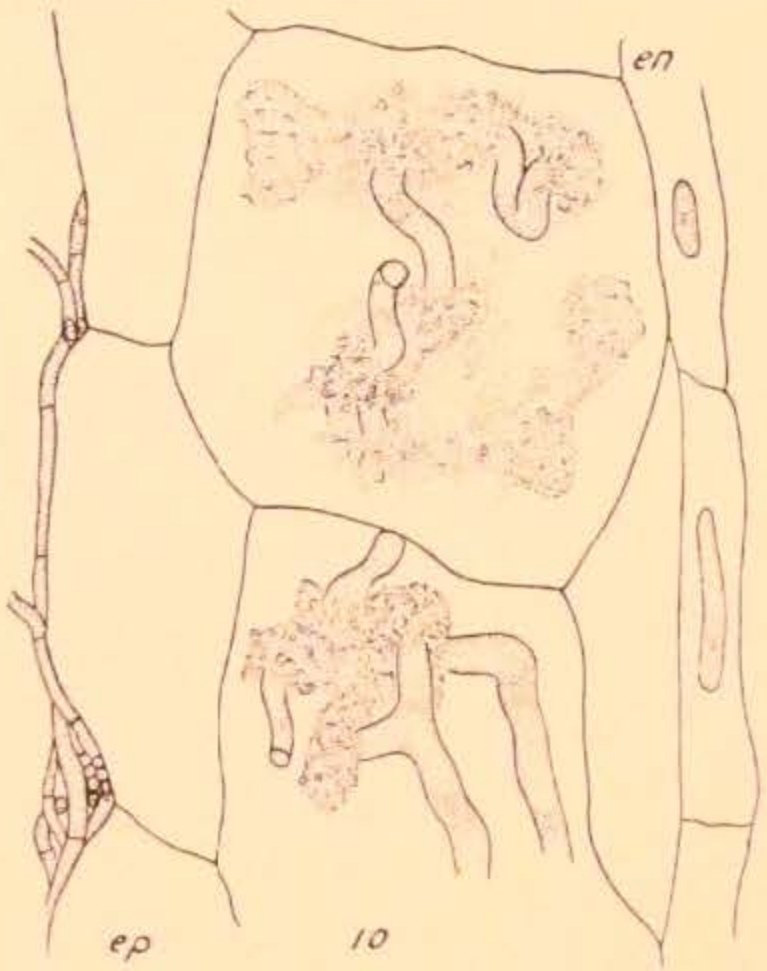
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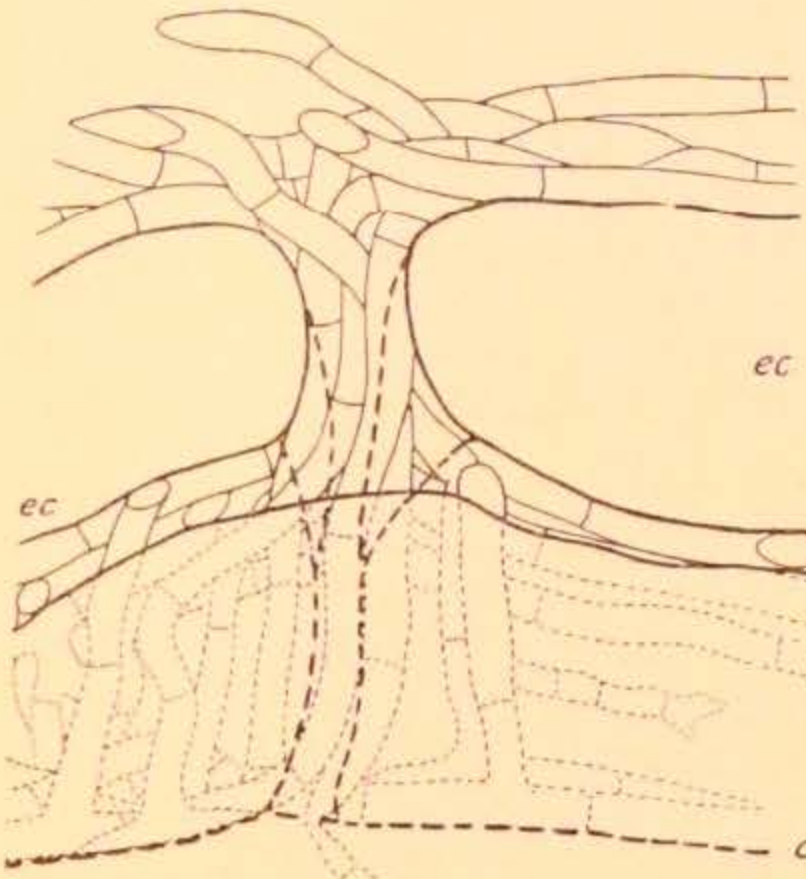
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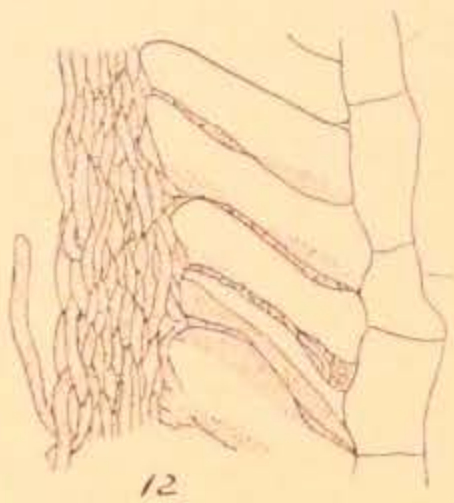
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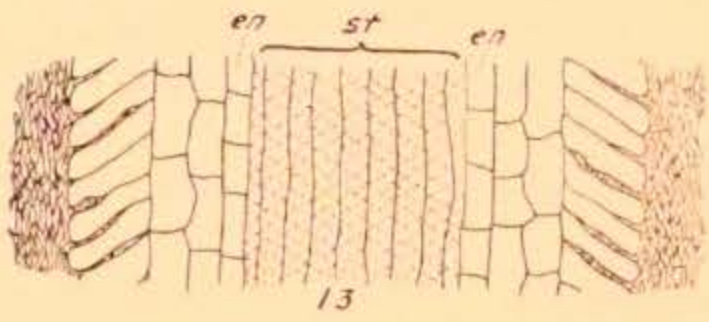
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PLATE III

Fig. 14. *Corallorrhiza odontorhiza.* Longitudinal section of slender, root-like, subterrancan part showing hyphæ of typical orchid endotroph in cortical cells immediately beneath the epidermis, *ep*, 1 mm. from tip of "root"; *cn*, cell nucleus.

Fig. 15. Cross section of similar part ("root") of same plant showing disorganization of fungus hyphæ, in inner cortical cells 4 mm. from the tip. Note enlargement of cell nucleus *cn*, on infection of cell by the fungus.

Fig. 16. Cross section of short, thick, tuberous, subterranean part of same plant showing fungus hyphæ in epidermal cells.

Fig. 17. Longitudinal section of similar part showing stages of mycelial disorganization in inner cortical cells.

PLATE III

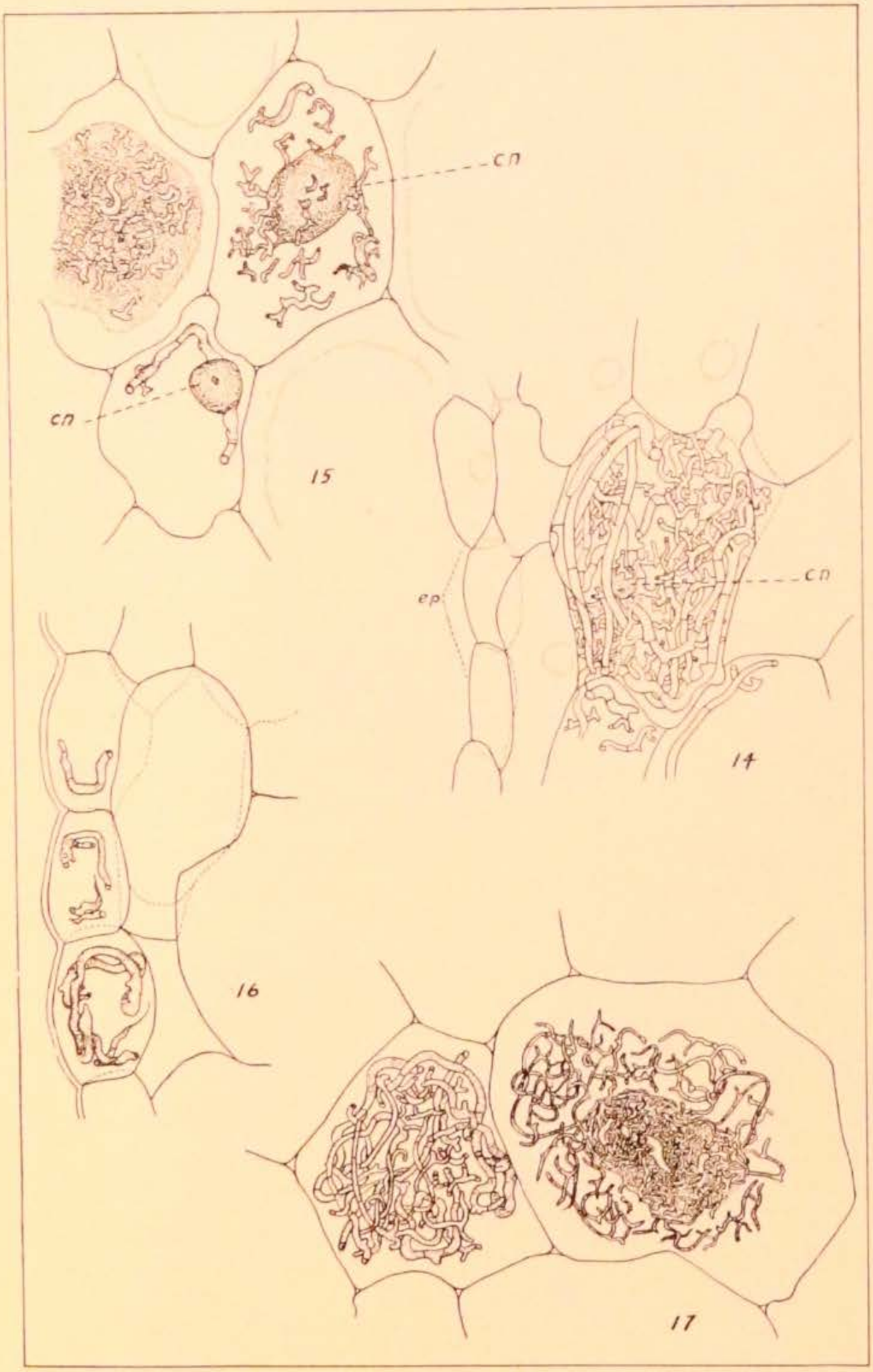

PLATE IV

Fig. 18. *Orchis spectabilis*. Cross section of root showing typical orchid fungus in cortical cells, and enlargement of cell nucleus *en*, on infection of cell by fungus.

Fig. 19. Longitudinal section of root of *Caulophyllum thalictroides* showing phycomycetoid endotroph with branching hyphæ, in inner cortical cells. The normal cell on left shows starch grains, and cell above shows disorganization of hyphæ; *en*, cell nucleus.

Fig. 20. Cross section of another root of same plant showing disorganization of hyphæ in an inner cortical cell. The cells are of the third and fourth row beneath the epidermis about 4 mm. from root tip; *en*, cell nucleus.

Fig. 21. *Erythronium albidum*. Cross section of root showing hyphæ of the endotroph in second and third layers of cortical cells beneath epidermis.

Fig. 22. *Arisæma triphyllum*. Longitudinal section of root showing endotroph forming arbuscles in third layer of cortical cells beneath epidermis about 2 cm. from root tip; *en*, cell nucleus.

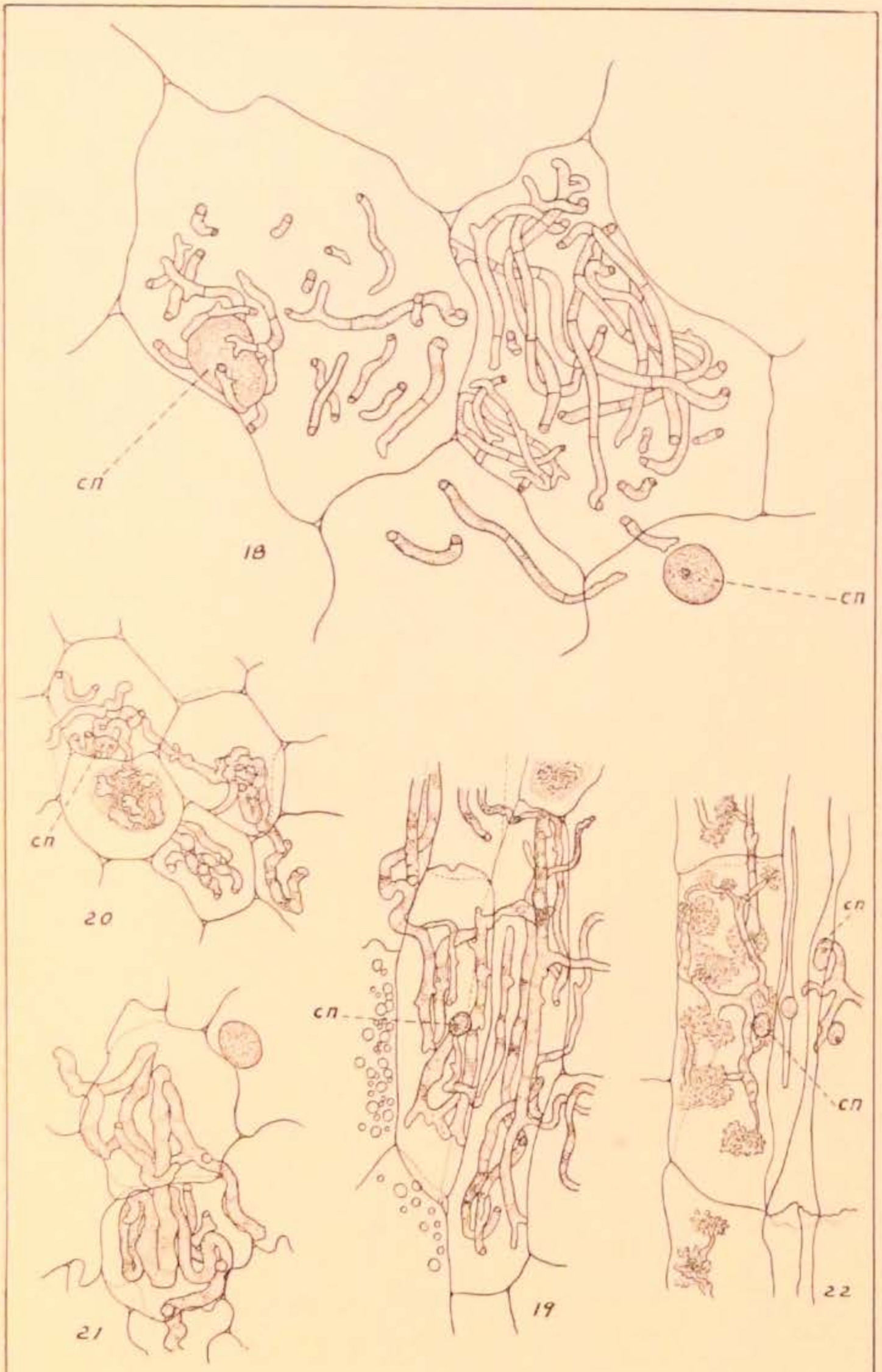
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PLATE V

Fig. 23. *Erythronium albidum.* Longitudinal section of root showing branching hyphæ of fungus endotroph and formation of arbuscles in second and third layers of cortical cells 3 mm. from tip of root.

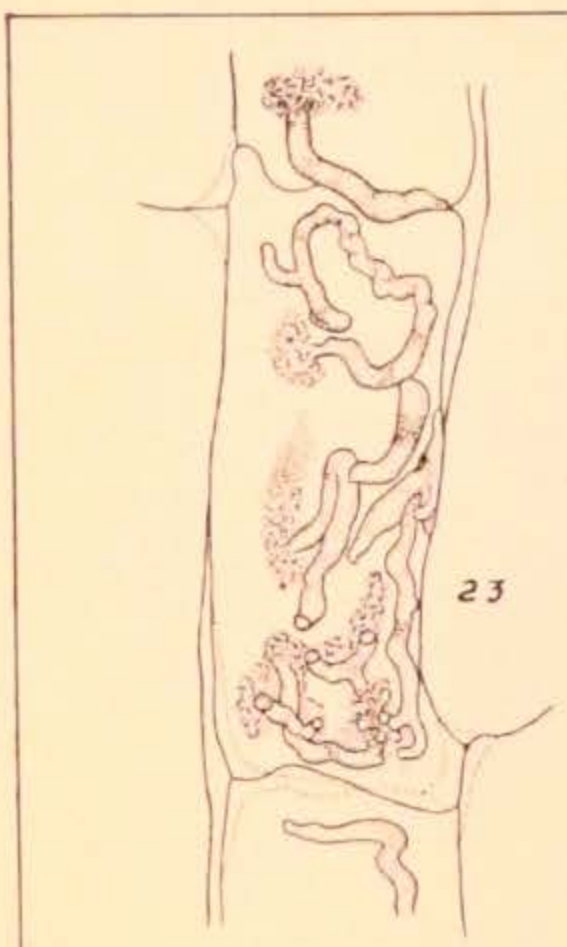
Fig. 24. Longitudinal section of root of *Hepatica acutiloba* showing in cortical cells the hyphæ of the endotroph 3 mm. from tip of root.

Fig. 25. Cross section of root of same plant 2 mm. from tip showing fungus hyphæ and hyphæ being digested; *en*, endodermis; *st*, stele; and *ep*, epidermis.

Fig. 26. *Melilotus alba.* Cross section of root showing disorganizing phycomycetoid hyphæ in cortical cells.

Fig. 27. Longitudinal section of root of another plant of same species, through older part of root above bacterial nodules, showing terminal intercellular vesicles between cortical cells.

Fig. 28. Longitudinal section of same root showing intracellular and intercellular hyphæ near endodermis *en*; *ep*, epidermis.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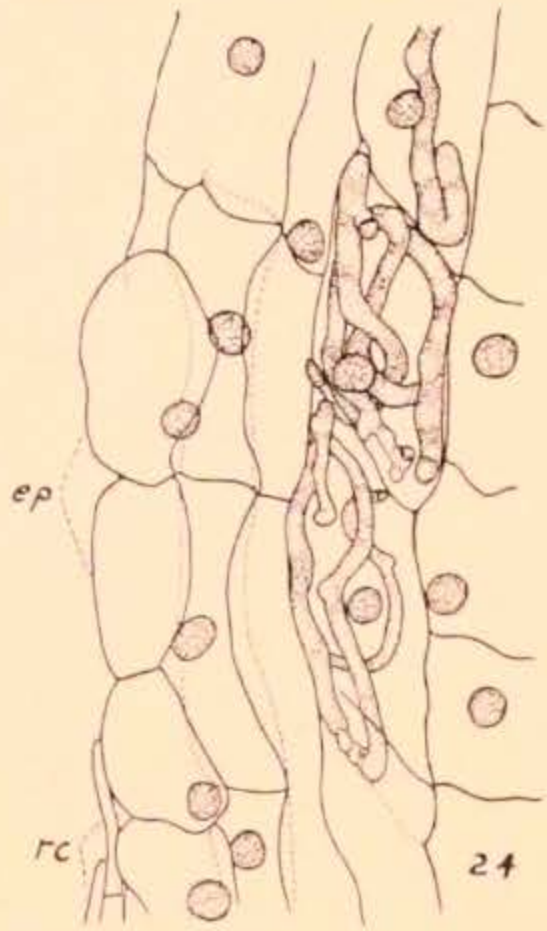
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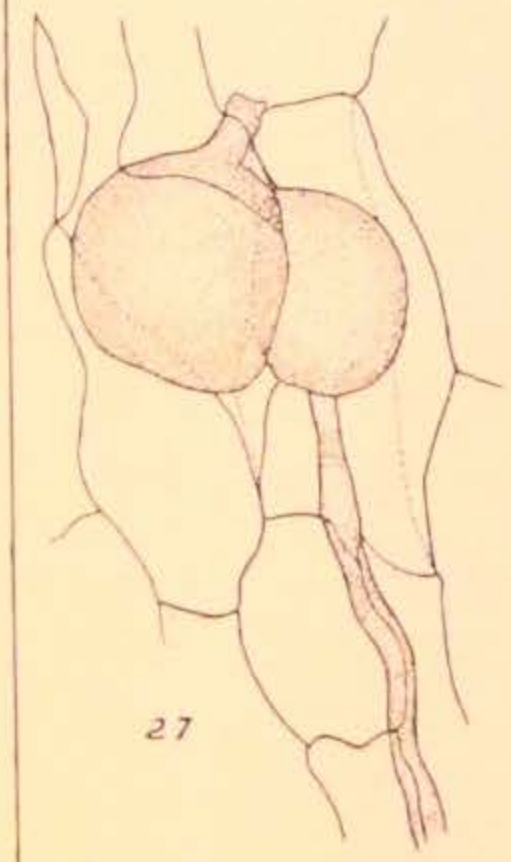
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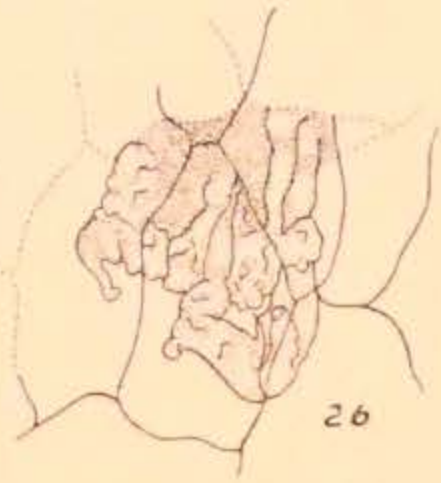
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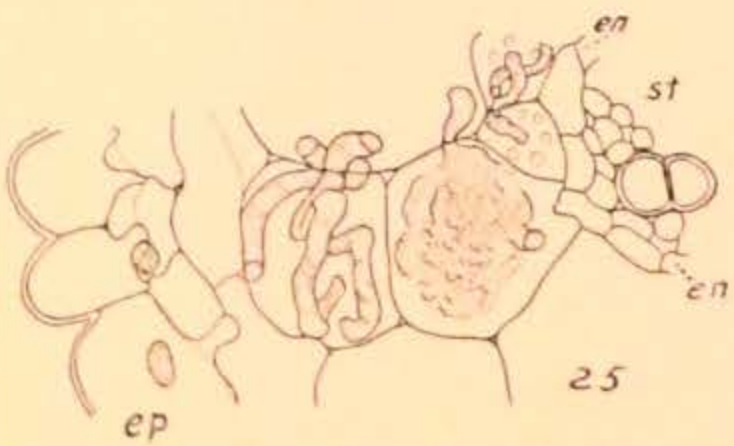
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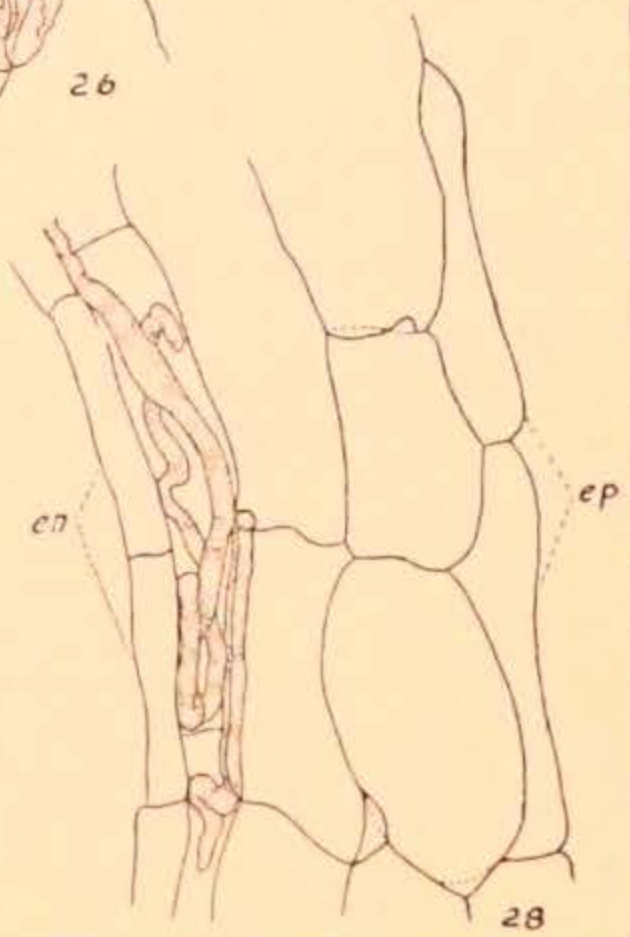
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PLATE VI

Fig. 29. *Ostrya virginiana.* Cross section of short, thick, lateral root, showing outer pseudoparenchymous layer formed by the weft of fungus hyphæ.

Fig. 30. Longitudinal section of root of same plant showing on external epidermal surface the type of fungus hyphæ which forms the weft in fig. 29.

Fig. 31. External surface view of hyphæ forming weft on roots of *Ostrya*.

Fig. 32. Cells in longitudinal section of *Ostrya* root showing hyphæ of ectotroph and haustoria between epidermal cells. Oil immersion; original magnification approximately x 1400.

Fig. 33. Hyphal character of fungus forming weft in fig. 32.

Fig. 34. Longitudinal section of *Ostrya* root some distance from tip showing same fungus as an ectotroph and an endotroph; *ep*, epidermis.

Fig. 35. *Populus deltoides.* Cross section of short lateral root showing pseudoparenchymous layer of hyphæ of fungus ectotroph; *en*, endodermis.

Fig. 36. Longitudinal section of root of another cottonwood seedling showing endotroph in cells next to endodermis *en*. This condition present mostly some distance from root tip. The ectotroph of fig. 35 was also present on this root at tip.

Fig. 37. *Fraxinus pennsylvanica* var. *lanceolata.* Longitudinal section of lateral root showing endotrophic hyphæ in cortical cells near root tip.

Fig. 38. Longitudinal section of root tip of *Quercus macrocarpa* showing ectotrophic fungus hyphæ. Note resemblance to same condition in *Ostrya* (fig. 29), *Populus* (fig. 35), and *Onoclea* (figs. 12 and 13).

Fig. 39. Diagram of median longitudinal section of same root tip to illustrate relative thickness of fungus weft to other parts of root. Root 0.12 mm. in diameter; average thickness of fungus weft 20 microns; *st*, stele; *ctx*, cortex; and *fw*, fungus weft. (Outlined with camera lucida, L.p.—original magnification approximately x 125.)

PLATE VI

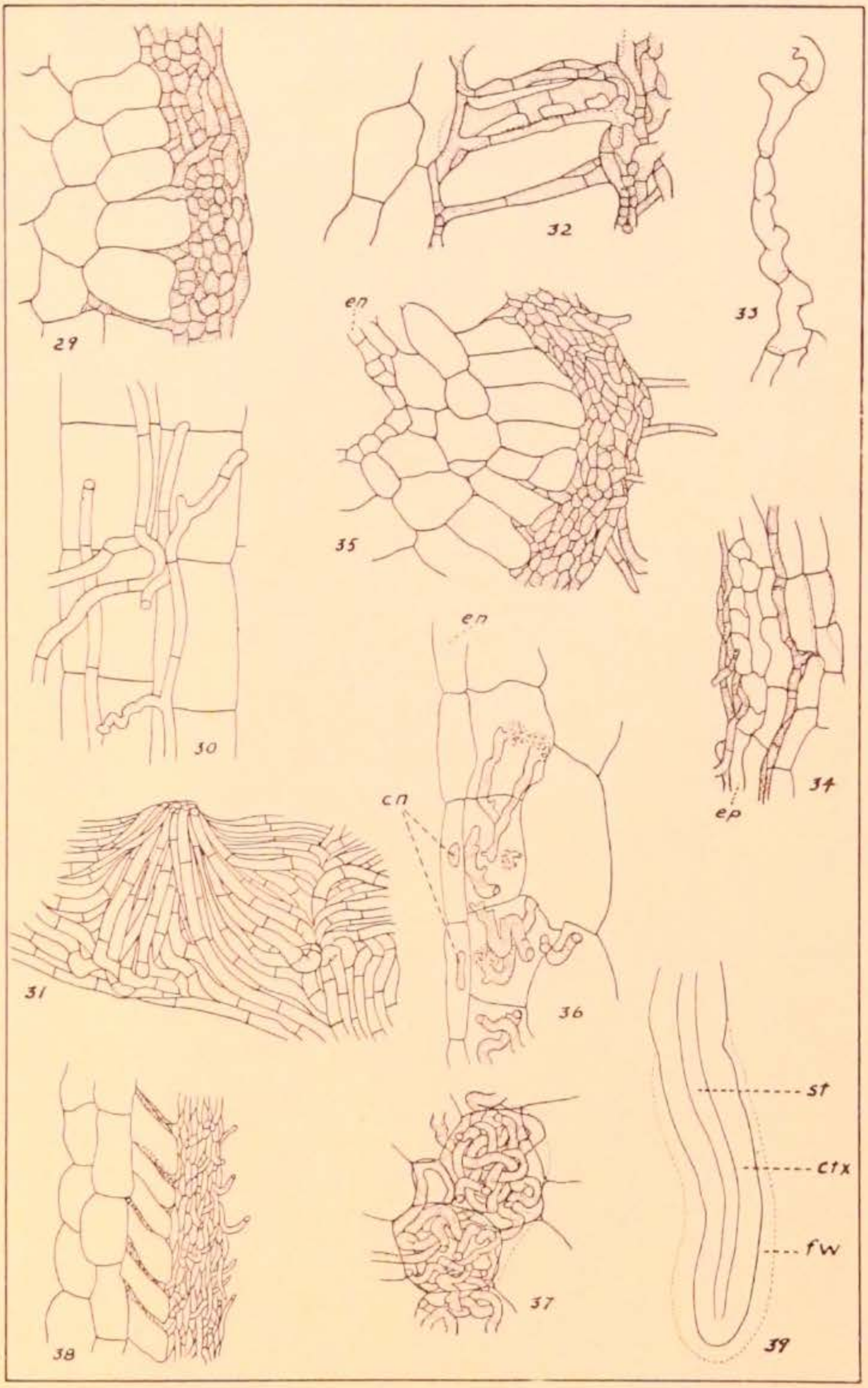

PLATE VII

Fig. 40. *Uvularia perfoliata.* Middle cortical cells near root tip in longitudinal section showing hyphal characters of endotroph with several arbuscles; *en*, cell nucleus.

Fig. 41. *Smilacina racemosa.* Longitudinal section showing hyphæ forming arbuscles in inner cortical cells about 1 cm. from root tip. Hyphæ present in epidermal cell and hyphal branches have entered two cortical cells within. External hyphæ of same character are pressed against the epidermal cells, *ep*. Root hairs were present on this root; *rh*, base of root hair; *en*, cell nucleus.

Fig. 42. *Podophyllum peltatum.* Semi-diagrammatic drawing of cross section of root about 1 cm. from tip with shaded cells to show region and relative abundance of fungus endotroph. (Outlined with camera lucida l.p. Original magnification approximately x 125.)

Fig. 43. Longitudinal section of another root of same plant showing hyphæ and digestion of hyphæ in cortical cells 1 to 2 cm. from root tip. Note starch grains in normal cell below, and in cells showing hyphal digestion; *en*, cell nucleus.

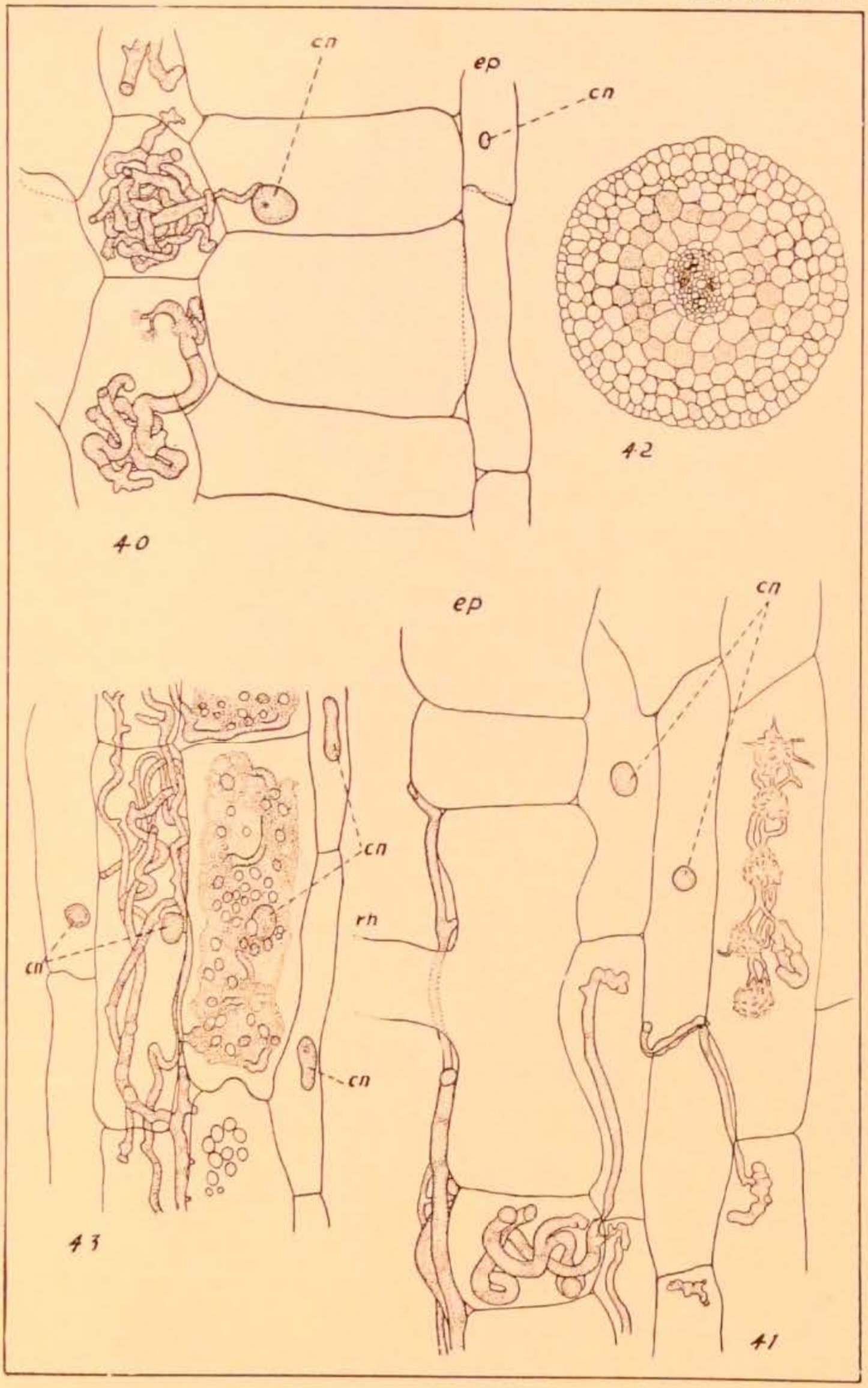
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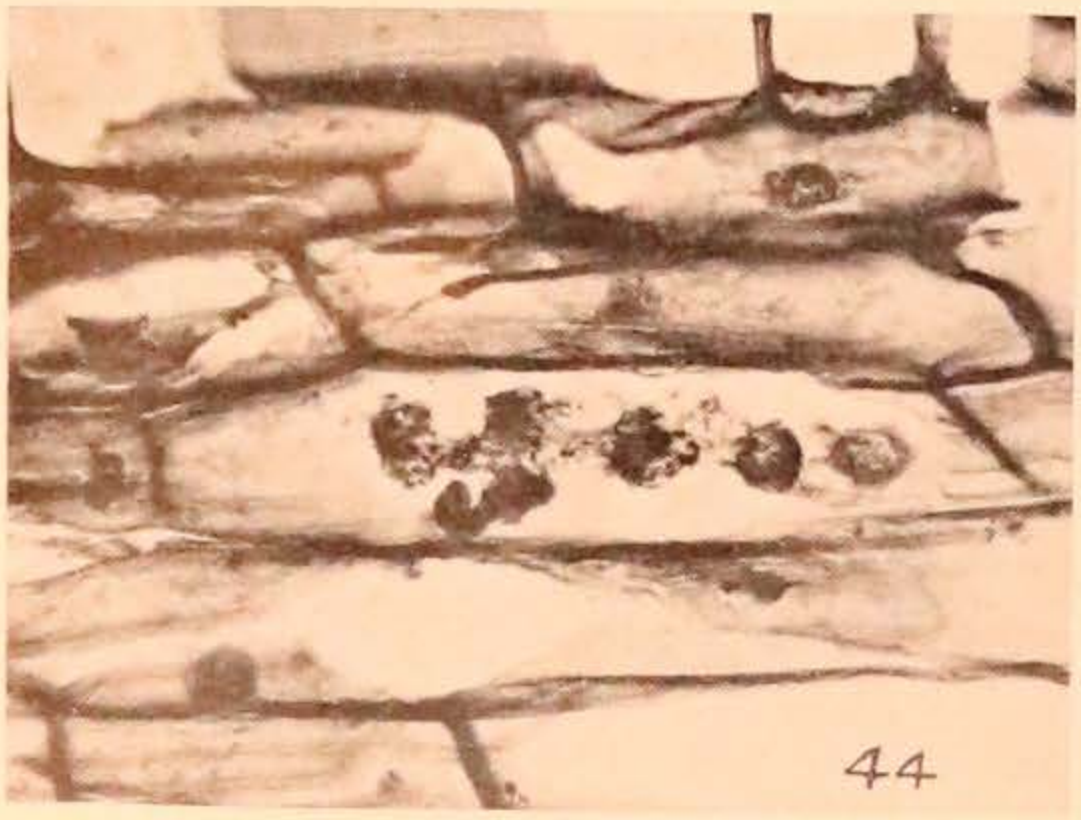
PLATE VIII

Fig. 44. *Smilacina racemosa*; photomicrograph of longitudinal section showing arbuscle formation in inner cortical cells about 1 cm. from tip of root. (See pl. 7, fig. 41).

Fig. 45. *Adiantum pedatum*; photomicrograph of longitudinal section showing vesicle formation by fungus endotroph about 1 cm. from tip of root. (See pl. 1).

Fig. 46. *Quercus macrocarpa*; photomicrograph of cross section of short lateral root showing weft of ectotrophic fungus hyphae forming a pseudoparenchymous-like layer. (See pl. 6, figs. 38 and 39).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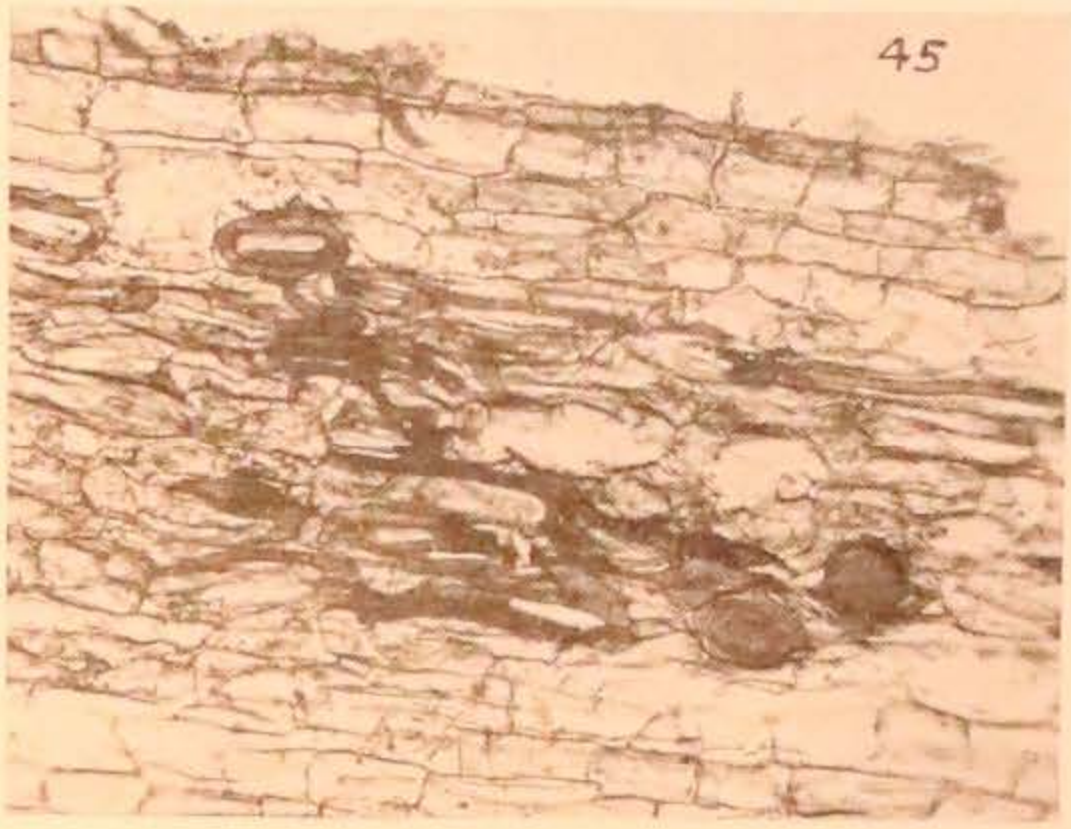
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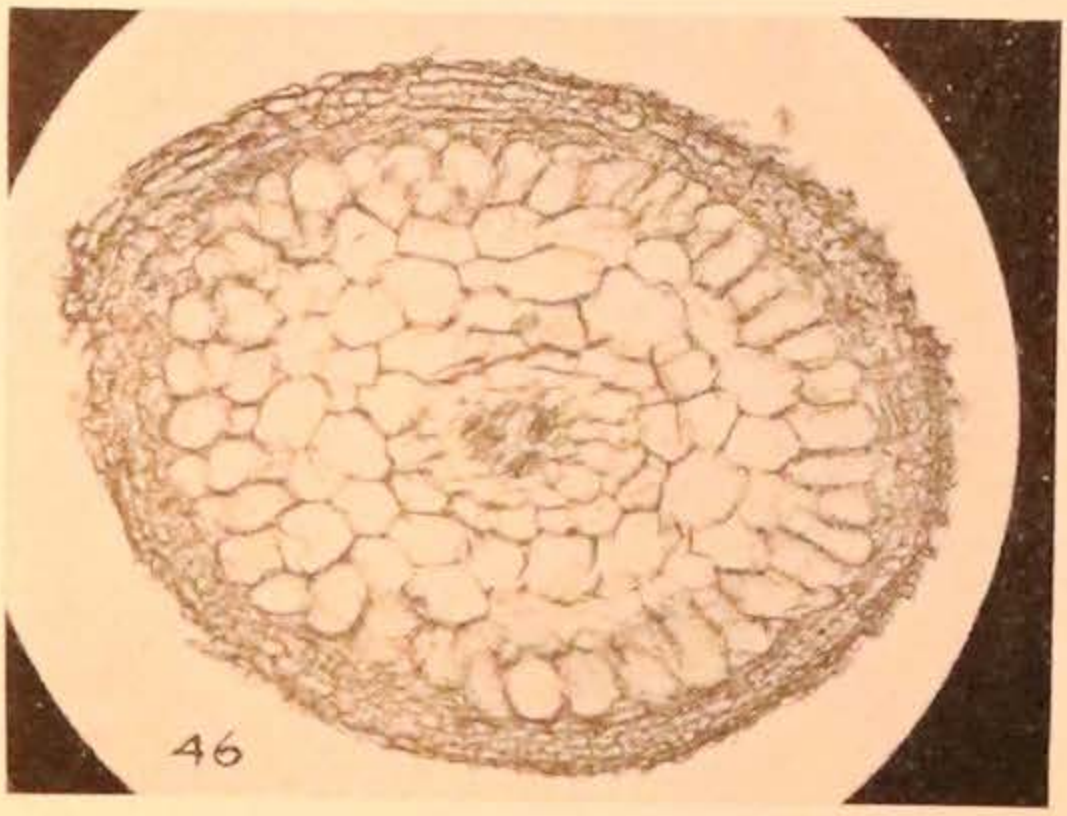
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