

THE MYCORRHIZAE OF CORYLUS AND JUGLANS

by

WILLIAM EVENDEN

A THESIS

submitted to the


OREGON STATE COLLEGE

in partial fulfillment of
the requirements for the
degree of


MASTER OF SCIENCE

May 1942

APPROVED:



Professor of Plant Physiology
In Charge of Major



Plant Pathologist, Agricultural Experiment
Station

In Charge of Major



Head of Department of Botany



Chairman of School Graduate Committee



Chairman of State College Graduate Council

ACKNOWLEDGEMENTS

The material presented in the following pages was accumulated by the writer during research conducted by Mr. C. E. Schuster, Pomologist, United States Department of Agriculture, in studies of filbert and walnut roots. The writer wishes to thank Mr. Schuster who has generously allowed him to present this material as a thesis topic. He also wishes to thank Drs. A. B. Hatch, S. M. Zeller, W. M. Atwood and C. E. Owens for their assistance. For bibliographies and abstracts of the literature the writer is greatly indebted to Drs. A. P. Kelley, M. C. Rayner and A. B. Hatch. The thousands of sections from which the histological data were taken were excellently done by Mrs. E. R. Geil and the writer is indebted to Mrs. E. A. Atwood and the United States Department of Agriculture for the excellent photographs.

TABLE OF CONTENTS

	Page
I. Introduction	1
II. Methods	3
III. Subject Material	4
A. Features of the Root System of Corylus from Macroscopic Examination	4
1. Frequency of Occurrence of Mycorrhizae and discussion	4
2. Specificity of Root Character to Mycorrhizae Formation and Discussion	4
3. Periods of Mycorrhizal Growth and Discussion	5
4. Form of Mycorrhizal Growth and Discussion	5
B. Study of the Ectotrophic Mycorrhizae of Corylus from Microscopic Exam- ination	5
1. Life History of the Mycorrhizal Condition and Discussion	6
2. The Effects and Characteristics of Association	6
a. The Fungus Constituent of the Mycorrhizae and Discussion	6
b. The Plant Constituent of the Mycorrhizae and Discussion	7
3. Discussion of the Difficulties of Determining the Role of Ectotrophic Mycorrhizae	9
C. Features of the Root System of Juglans from Macroscopic Examination and Discussion	10

	Page
1. Frequency of Occurrence of Mycorrhizae and Discussion	10
2. Specificity of Root Character to Mycorrhizae Formation and Discussion	11
3. Periods of Mycorrhizal Growth and Discussion	11
4. Form of Mycorrhizal Growth and Discussion	12
D. Study of the Endotrophic Mycorrhizae of Juglans from Microscopic Examination	12
1. Life History of the Mycorrhizal Condition and Discussion	12
2. The Effects and Characteristics of Association	13
a. The Fungus Constituent of the Mycorrhizae and Discussion	13
b. The Plant Constituent of the Mycorrhizae and Discussion	14
3. Discussion of the Difficulties of Determining the Role of Endotrophic Mycorrhizae	15
IV. Summary	17
V. Bibliography	18

Plates

	Page
Plate I The ectotrophic mycorrhizal roots of <u>Corylus avellana</u> , L.	19
Plate II The endotrophic mycorrhizal roots of <u>Juglans hindsii</u> , Sarg.	20
Plate III Photomicrographs of the mycorrhizae of <u>Corylus avellana</u> , L. and <u>Juglans hindsii</u> , Sarg.	21
Plate IV Semidiagrammatic illustration of an ectotrophic mycorrhiza of <u>Corylus</u> <u>avellana</u> , L.	22
Plate V Semidiagrammatic illustration of an endotrophic mycorrhiza of <u>Juglans</u> <u>hindsii</u> , Sarg.	23

THE MYCORRHIZAE OF CORYLUS AND JUGLANS

Introduction

The following represents a survey study dealing with the ectotrophic mycorrhizae of the cultivated filbert (Corylus avellana, L.) and the endotrophic mycorrhizae of the rootstock of the cultivated Persian or English walnut (Juglans hindsii, Sarg.).

The vast literature on mycorrhizae contains only a few references to the mycorrhizae of *Corylus* and *Juglans* and these apparently only mention occurrence. The subject of mycorrhizae cannot be adequately reviewed here (*).

In the study of mycorrhizae, there is apparently a definite trend toward acquiring data regarding the physiological functions of mycorrhizae. To indicate this, the following work may be briefly mentioned.

Hatch (1) indicates a relation between soil quality and the production of ectotrophic mycorrhizae of certain pines.

Mitchell, Finn and Rosendahl (3) substantiate Hatch's work.

(*) The world's literature upon the subject of mycorrhizae has been made available by Dr. A. P. Kelley in "The Literature of Mycorrhizae", a manuscript, as yet unpublished, a copy of which is held by the United States Department of Agriculture Library, Washington, D. C. The writer was fortunate to obtain a copy on loan through cooperation of the Oregon State College Library.

Knudsen (2) indicates the role of endotrophic mycorrhizae in the provision of food materials to certain species of orchid seedlings.

Features observed in the study will be given as observations followed by brief discussion in which the various observations are presented as evidence of physiological function.

It is realized, of course, that many concepts herewith presented may be subsequently modified with further studies.

Methods

Root samples were collected from the upper two feet of soil in twelve different walnut (*Juglans*) orchards and nine different filbert (*Corylus*) orchards in the Willamette Valley, Oregon.

Collections and microscopic examinations were made at various intervals during the years of 1935 and 1936 and again in 1939. Field observations were made intermittently from 1935 until 1942.

Microscopic detail was obtained from thousands of prepared sections. The usual histological procedure was, in general, used:

After the material was thoroughly washed, fixation was made in formalin-acetic-alcohol. Dehydration was accomplished with a graded series of dioxan to butyl alcohol and thence to paraffin (plus small amount of paraffin oil). Sections were cut eight to ten microns thick and stained in Delafield's haematoxylin.

Subject Material

A. Features of the Root System of *Corylus* from Macroscopic Examination

The general appearance of the mycorrhizal roots of *Corylus* is illustrated in Plate I.

1. Frequency of Occurrence of Mycorrhizae

Observations: In the upper soil (0-2 feet) of the orchards examined, the roots of *Corylus* are predominantly mycorrhizal.

Long roots (non-mycotrophic) are infrequently produced.

Discussion: Long roots apparently offer the chief means of root extension and later apparently serve as the main trunk roots.

2. Specificity of Root Character to Mycorrhizae Formation

Observations: Lateral growth from non-mycotrophic roots is transformed to mycorrhizae.

Long or non-mycotrophic roots are most often produced from the termini of larger roots.

Discussion: It appears that production of mycorrhizae is dependent in part, at least, upon the character of the roots. The use of the word "transformed" in the above is probably inaccurate since lateral growth may be highly specific to the formation of mycorrhizae.

3. Periods of Mycorrhizal Growth

Observations: Mycorrhizal growth occurs chiefly in the spring and to a lesser extent in the fall after the first rains.

Discussion: From the above it is apparent that any mycorrhizal root may be capable of growth for at least one year and perhaps longer. This may be, however, no indication of the length of function of the mycorrhizae.

4. Form of Mycorrhizal Growth

Observations: Mycorrhizal growth is distributed along the non-mycotrophic trunk or parent roots. Mycorrhizal roots are excessively branched and possess two distinct and extreme types of branching. In compact soils concentrated branching results in mats or clumps of mycorrhizae. Loose soils allow a dispersed type of branching.

Discussion: The above indicates that more factors than the relations of plant and fungus are concerned in the structure of mycorrhizae.

B. Study of the Ectotrophic Mycorrhizae of *Corylus* from Microscopic Examination

The microscopic appearance of *Corylus* mycorrhizae is shown in A of Plate III, and Plate IV.

1. Life History of the Mycorrhizal Condition

Observations: Initial association occurs in young laterals arising from mycorrhizal or long roots where intercellular penetration of the outer cortex is apparently begun by fungous hyphae. When mature, the mycorrhizae consist of the typically modified root structure covered by the mantle and having its outer cortical cells contained in the Hartig net. In completed association, the mantle has largely decayed and cork formation has occurred in the cortex.

Discussion: The relationship of fungus and plant is unknown but it is probably changing from the beginning to the end of association.

2. The Effects and Characteristics of Association

a. The Fungous Constituent of the Mycorrhizae

Observations: The mantle is composed of cells, resembling, in most cases, the tissue of the higher Basidiomycetes.

The cells of the Hartig net vary in appearance from the mantle cells.

The mantle occasionally possesses setae projecting from the exterior of the mantle.

The mantle varies in staining reactions and in cell structure.

The amount of fungous hyphae projecting from the mantle into the soil varies.

Discussion: Apparently there are at least three specialized tissues of the fungous constituent of the association. Fungous hyphae attached to the exterior probably have a direct relation to the soil. The mantle tissue may be a center of fungous metabolism, while the function of the Hartig net is in a more direct relation to the plant. The variance in staining reactions and cell structure of the mantle, and the occasional possession of setae indicate the possibility of different fungi in association and, of course, indicate differences in fungus-plant relation. The variance in the amount of hyphae attached to the mantle exterior would make hazardous any speculation regarding increase or decrease of absorbing surface when compared to roots which possess only root hairs. It would also appear unwise to compare root hair surface area with that of fungous hyphae when their relative efficiency per unit area is unknown.

b. The Plant Constituent of the Mycorrhizae

Observations: The outer cells of the cortex, containing the Hartig net, are radially elongated.

A characteristic precipitate occurs between the plant cells containing the Hartig net and the mantle (see Plate IV, a).

Cell contents of the cortex are seemingly high in the amount of protoplasmic material.

The vascular cylinder is of normal appearance.

Discussion: The outermost cells of the cortex, surrounded on its four radial sides by the Hartig net, are evidently different in function from the inner cortical cells. From the apparent high protoplasmic content of the cortical cells may be presumed a high metabolic activity. (These cells have the appearance of cells of meristematic tissue.)

The presence of a "precipitate" between the mantle and outer cortical cells may be an incompatibility response.

The normal appearance of the vascular cylinder provides evidence of one root function which is not directly affected.

The plant constituent of mycorrhizae can hardly be assumed to have the same functions as a "root hair" root. The fungus in mycotrophy undoubtedly acquires manufactured foods from the root, which fact would add at least one function to the others of its organization.

3. Discussion of the Difficulties of Determining the Role of Ectotrophic Mycorrhizae

The complexity of the soil environment and the intimate relation of the fungus with it probably makes a complete explanation of the role of ectotrophic mycorrhizae impossible.

To illustrate this point:

Supposing that a fungus is in ectotrophic association with a plant in a natural soil habitat whose mycorrhizosphere contains organisms (bacteria, fungi, etc.) C, D, E, F, etc. and colloidal systems N and solutions M, etc. Supposing also that we wish to know the effect of the fungus on the plant and we are able to attain the ultimate in technique by eliminating the fungus from the plant and its natural soil habitat without harming the plant or altering the soil environment. We may then compare the controls with the plant and environment minus the fungus. If the plant minus the fungus plus the remainder of the natural soil environment is less healthy than the plant plus the fungus plus the remainder of the environment, we may then say that under these conditions the fungus is of benefit to the plant. However, we may not with accuracy state that the fungus is of benefit to the plant by any one direct reason, for among other reasons, the beneficial effect

of the fungus may be indirect and the fungus itself actually detrimental. Stated in another way, the benefit of the fungus to the plant may be considered as an algebraic summation resulting in a positive (beneficial) effect.

Another difficulty in determining the role of ectotrophic mycorrhizae lies in the probability that solution for one mycorrhizal condition is inaccurate when applied to other cases of this type of mycotrophy. The variance in plant and fungus character or, in another sense, the varying speed of the processes of evolution of the relationship may have produced widely different results.

C. Features of the Root System of Juglans from Macroscopic Examination

The general appearance of the mycorrhizal roots of Juglans is illustrated in Plate II.

1. Frequency of Occurrence of Mycorrhizae

Observations: In the upper soil (0-2 feet) of the orchards examined, the roots of Juglans are commonly mycorrhizal.

Long roots (non-mycotrophic) are quite frequently produced.

The amount of mycorrhizal formation is correlated with the quality of the soil, apparently in a direct relation.

Discussion: Just as in the ectotrophic mycorrhizae of *Corylus*, long roots apparently offer the chief means of root extension and later apparently serve as trunk roots.

Since association is irregular and dependent upon soil character, the question of obligate relation constitutes a controversial question.

2. Specificity of Root Character to Mycorrhizae Formation

Observations: Lateral growth from the mycorrhizae or non-mycotrophic roots is usually transformed to mycorrhizae.

Young laterals are more often changed to mycorrhizae in poor than in good soils.

Discussion: Root and soil character are apparently two factors in mycorrhizae formation.

3. Periods of Mycorrhizal Growth

Observations: Mycorrhizal growth occurs chiefly during a period in the spring.

Discussion: The production of new growth from mycorrhizae is seemingly an uncertain indication of either the length of functional life or of the ability of the mycorrhizae to branch, since association areas are quite irregular and intermittent in occurrence.

4. Form of Mycorrhizal Growth

Observations: Mycorrhizal roots are decidedly clubbed and shortened if association occurs at an early stage in their development.

If association occurs at a later stage, or is less complete, they are of greater length but clubbed and distorted at the points of association.

Discussion: Association apparently occurs early enough to affect the meristems or possibly produces a new meristem. This latter possibility is doubtful, however, since the association tissue does not appear gall-like.

D. Study of the Endotrophic Mycorrhizae of Juglans from Microscopic Examination.

The microscopic appearance of Juglans mycorrhizae is shown in B of Plate III, and Plate V.

1. Life History of the Mycorrhizal Condition

Observations: The beginning of association occurs in young laterals emerging from the parent root, or may occur after emergence during the early growth period. The manner of fungus entry was not observed in thousands of sections.

In the first stages of association, intracellular hyphae are coiled in the middle cortex.

In the later stages, the hyphae have disintegrated, leaving typical tree-like bodies in the cell

cavities and oil-like drops in the exterior cortex.

Discussion: Lack of observed entry points of the fungus indicates little or no direct contact or relation with the soil. Lack of entry points also suggests possible carrying over of mycotrophy in an unknown form or location.

It is very evident that relations between plant and fungus must be in a constant state of change.

2. The Effects and Characteristics of Association

a. The Fungous Constituent of the Mycorrhizae

Observations: The fungus hyphae are large, non-septate and characteristically coiled.

There is a minimum of cell wall penetration by fungus hyphae in comparison to the quantity of fungus hyphae within the cell.

The fungous disintegration products are highly stainable with Delafield's haematoxylin.

Discussion: Lack of septation and the large size of the hyphae indicate that the fungus in association is a Phycomycete.

The characteristic coiling, and the size of hyphae indicate abundant nutrient and water supply and a continuance of a plentiful supply of both over a period of time. This would indicate that the fungous metabolism is not typically destructive, although this action might be

delayed and the cell contents killed later, as is evidenced by the very abnormal cell contents in the late stages. The apparent lack of cell wall penetration by the hyphae possibly indicates a lack of enzymes specific to this function and this fact also implies lack of highly destructive qualities.

The disintegration products stained by Delafield's haematoxylin point to the possibility of carbohydrates. It is unknown what part of these disintegration products are fungal.

b. The Plant Constituent of the Mycorrhizae

Observations: Points of association are regions of moderate radial hypertrophy and longitudinal hypotrophy.

The region of association in the middle cortex is quite definite.

Cork formation is apparently prevented in associated roots.

Root hair production is prevented in the large majority of cases of mycorrhizae formation.

Oil-like products are characteristically produced in the outer cortex in the later stages of association.

Discussion: The definite region of association implies a differentiation of tissue in the cor-

tex. Whether this differentiation is a natural feature of the cortex or due to the formation of mycorrhizae is, of course, unknown.

Radial hypertrophy is doubtless an effect on the meristem, which may possibly tend to derive its excess growth in part from a pronounced hypotrophy of longitudinally directed growth.

Normal suberization of root cells in cork formation is apparently prevented by association, and, of course, implies a change of function in cortical regions.

The presence of oil-like products in the outer cortex indicates, definitely, only a probable change in function of this region. To interpret the significance of these oil-like products and disintegration bodies would seem to need a vast amount of study.

3. Discussion of the Difficulties of Determining the Role of Endotrophic Mycorrhizae

The "within the plant" nature of endotrophic mycorrhizae does not apparently offer the same complications with the soil environment as does the ectotrophic type. However, the chief difficulty in the study of the role of endotrophic mycorrhizae has been the difficulty of isolation and culture of these apparently highly specific fungi. Here, it seems,

lies an endless problem for an advanced study of technique and the physiology of fungi in culture.

The inaccuracy of predicting similar relations between two different sets of plant-fungus associations was mentioned previously in the corresponding discussion regarding ectotrophic mycorrhizae and applies here.

Summary

The writer has presented a survey study regarding the root and mycorrhizal features of Corylus avellana, L. and Juglans hindsii, Sarg.

The subject material consists of observations from macro- and microscopic examination of the above, followed by discussion.

The discussion proposes interpretation of the observations upon a physiological basis. Each interpretation is offered as an introduction to further study, rather than as an explanation.

The observations and discussions have been condensed to comply with the "survey" purpose of the study. Since observations are diverse and the corresponding discussions are already condensed, an attempt to offer a summary regarding detail will not be made.

Bibliography

1. Hatch, A. B. The physical basis of mycotrophy in Pinus, 1937. (Black Rock Forest Bulletin, No. 6)
2. Knudson, L. Nonsymbiotic germination of orchid seeds. Botanical Gazette 73:1-25, 1922.
3. Mitchell, H. L., Finn, R. F. and Rosendahl, R. O. The relation between mycorrhizae and the growth and nutrient absorption of coniferous seedlings in nursery beds. Black Rock Forest Papers 1:58-77, 1937.

Plate I

This plate illustrates the appearance
of the ectotrophic mycorrhizal roots
of Corylus avellana, L. X 5.



Plate II

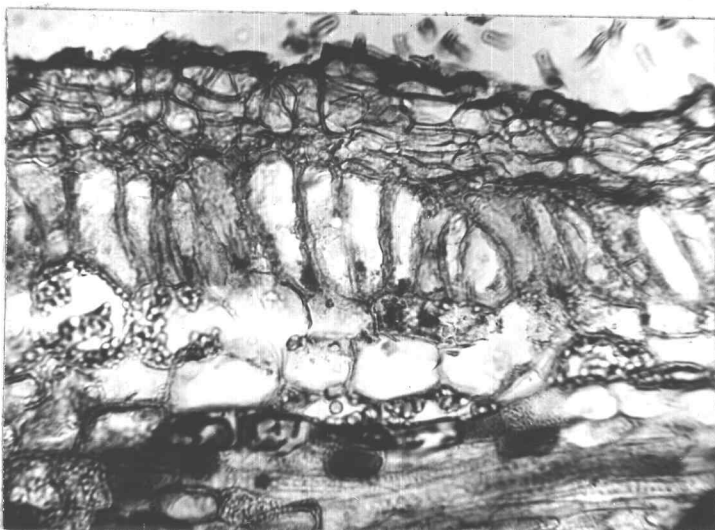
This plate illustrates the appearance
of the endotrophic mycorrhizal roots
of Juglans hindsii, Sarg. X 5.



Plate III

A of this plate presents a photomicrograph of a portion of a longitudinal section of an ectotrophic mycorrhiza of Corylus avellana, L. Compare with Plate IV.

B of this plate presents a photomicrograph of a portion of a longitudinal section of an endotrophic mycorrhiza of Juglans hindsii, Sarg. Compare with Plate V.



A



B

Plate IV

The top figure represents an outline diagram of a longitudinal section of an ectotrophic mycorrhiza of Corylus avelana, L. indicating the position of the section enlarged in semidiagrammatic form below.

The titles in the border indicate the various regions of the mycorrhizal structure. The blackened region, denoted by "a", represents a characteristic precipitate described on page 8.

The enlarged semidiagram is drawn to scale. The enlargement is approximately 200 diameters.

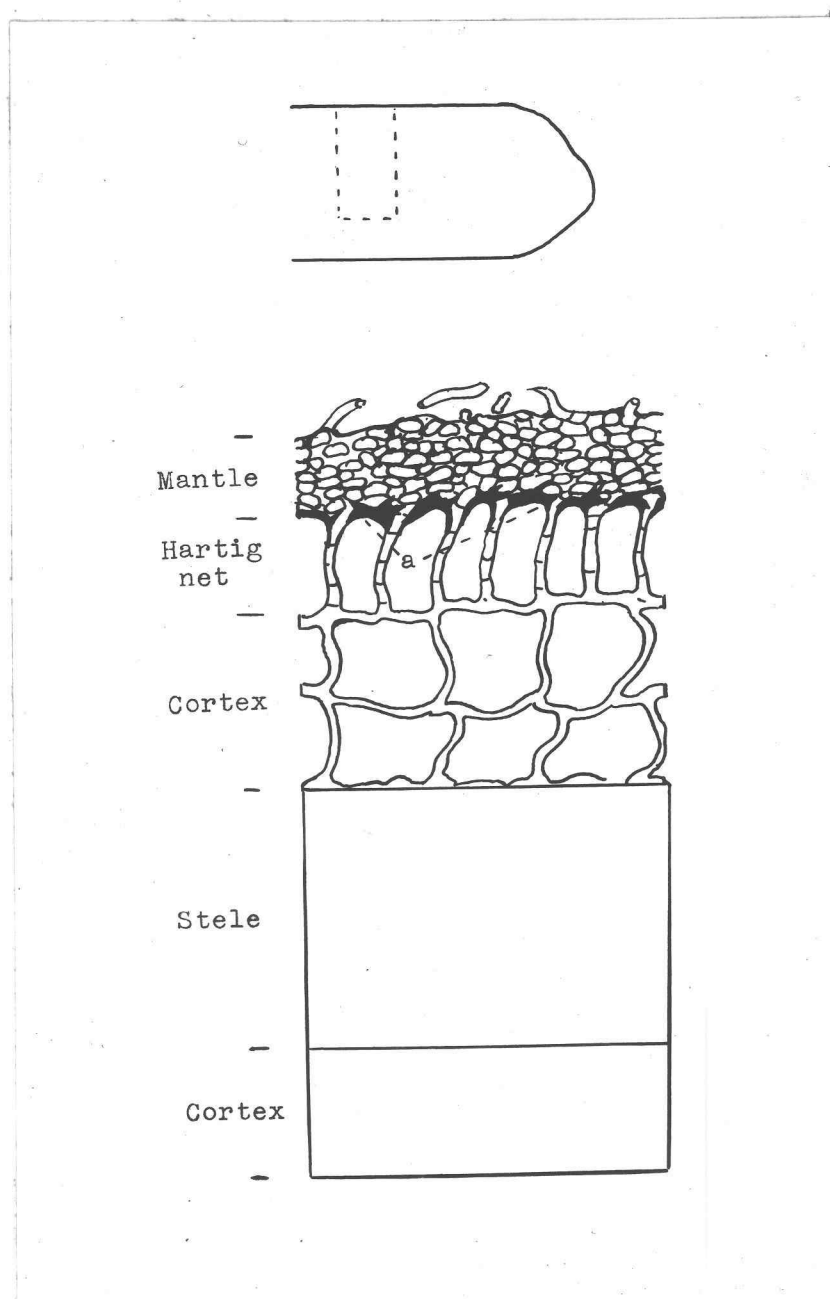


Plate V

The top figure represents an outline diagram of a longitudinal section of an endotrophic mycorrhiza of Juglans hindsii, Sarg., indicating the position of the sections enlarged in semidiagrammatic form below.

Section A, nearest the mycorrhizal tip, illustrates an early stage of mycotrophy.

Section B, farthest from the mycorrhizal tip, illustrates a late stage in mycotrophy.

The enlarged semidiagram is drawn to scale. The enlargement is approximately 300 diameters.

